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Inventor(s) Thompson; Brian et al.

Fusion proteins, recombinant bacteria, and methods for using recombinant bacteria

Abstract

Fusion proteins containing a targeting sequence, an exosporium protein, or an exosporium protein fragment that targets the fusion protein to the exosporium of a *Bacillus cereus* family member are provided. Recombinant *Bacillus cereus* family members expressing such fusion proteins are also provided. Genetically inactivated *Bacillus cereus* family members and recombinant *Bacillus cereus* family members that overexpress exosporium proteins are also provided. Seeds coated with the recombinant *Bacillus cereus* family members and methods for using the recombinant *Bacillus cereus* family members (e.g., for stimulating plant growth) are also provided. Various modifications of the recombinant *Bacillus cereus* family members that express the fusion proteins are further provided. Fusion proteins comprising a spore coat protein and a protein or peptide of interest, recombinant bacteria that express such fusion proteins, seeds coated with such recombinant bacteria, and methods for using such recombinant bacteria (e.g., for stimulating plant growth) are also provided.

Inventors: **Thompson; Brian (Creve Coeur, MO), Siegel; Ashley (St. Louis, MO)**

Applicant: **Spogen Biotech Inc.** (St. Louis, MO)

Family ID: **1000008762971**

Assignee: **Spogen Biotech Inc. (St. Louis, MO)**

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Primary Examiner: Rao; Manjunath N

Assistant Examiner: Xu; Qing

Attorney, Agent or Firm: Dentons US LLP

Background/Summary

CROSS REFERENCE TO RELATED APPLICATIONS (1) This application is a divisional of U.S. Non-Provisional patent application Ser. No. 17/079,942, filed on Oct. 26, 2020, which is a continuation of U.S. Non-Provisional patent application Ser. No. 16/563,086, filed on Sep. 6, 2019 and issued as U.S. Pat. No. 10,836,800 on Nov. 17, 2020, which is a divisional of U.S. Non-Provisional patent application Ser. No. 15/842,062, filed Dec. 14, 2017 and issued as U.S. Pat. No. 10,407,472 on Sep. 10, 2019, which is a continuation of U.S. Non-Provisional patent application Ser. No. 14/857,606, filed Sep. 17, 2015 and issued as U.S. Pat. No. 9,845,342 on Dec. 19, 2017, which claims priority to U.S. Provisional Application No. 62/051,885, filed Sep. 17, 2014. Each of the above-cited applications is incorporated herein by reference in its entirety.

REFERENCE TO SEQUENCE LISTING SUBMITTED ELECTRONICALLY
(1) A sequence listing contained in the file named “LMNE105USC3D1_ST26.xml” which is 348 kilobytes (measured in MS-Windows®) and created on Dec. 20, 2023, and comprises 313 sequences, is incorporated herein by reference in its entirety.
FIELD OF THE INVENTION
(2) The present invention generally relates to fusion proteins containing a targeting sequence, an exosporium protein, or an exosporium protein fragment that targets the fusion protein to the exosporium of a *Bacillus cereus* family member. The invention also relates to recombinant *Bacillus cereus* family members expressing such fusion proteins, formulations containing the recombinant *Bacillus cereus* family members, seeds coated with the recombinant *Bacillus cereus* family members, and methods for using the recombinant *Bacillus cereus* family members (e.g., for stimulating plant growth, protecting a plant from a pathogen, enhancing stress resistance in a plant, immobilizing a recombinant *Bacillus cereus* family member spore on a plant, stimulating germination of plant seeds, and delivering nucleic acids to plants). The invention additionally relates to recombinant *Bacillus cereus* family members that overexpress a protease or a nuclease, wherein overexpression of the protease or nuclease partially or completely inactivates spores of the *Bacillus cereus* family member or renders the spores more susceptible to physical or chemical inactivation. The present invention further relates to recombinant *Bacillus cereus* family members that overexpress exosporium proteins, seeds coated with such recombinant *Bacillus cereus* family members, and methods of using such recombinant *Bacillus cereus* family members (e.g., for stimulating plant growth, enhancing stress resistance in plants, and protecting plants from pathogens).
(3) The invention further relates to various modifications of the recombinant *Bacillus cereus* family members that express the fusion proteins, including: (i) overexpression of modulator proteins that modulate the expression of the fusion protein in the recombinant *Bacillus cereus* members; (ii) genetic inactivation of the recombinant *Bacillus cereus* family members; and (iii) mutations or other genetic alterations of the recombinant *Bacillus cereus* family members that allow for the collection of exosporium fragments containing the fusion protein. The invention also relates to various methods for using the exosporium fragments.
(4) The invention further relates to fusion proteins comprising a spore coat protein and a protein or peptide of interest, recombinant bacteria that express such fusion proteins, seeds coated with such recombinant bacteria, and methods for using such recombinant bacteria (e.g., for stimulating plant growth, protecting a plant from a pathogen, enhancing stress resistance in a plant, immobilizing a recombinant bacterial spore on a plant, stimulating germination of plant seeds, and delivering nucleic acids to plants).

(5) The present invention further relates to biologically pure bacterial cultures of novel strains of bacteria.
(6) The present invention additionally relates to plant seeds coated with an enzyme that catalyzes the production of nitric oxide or a superoxide dismutase, or with a recombinant spore-forming bacterium that overexpresses an enzyme that catalyzes the production of nitric oxide or a superoxide dismutase.
(7) The invention also relates to methods for delivering beneficial bacteria and enzymes or vaccines to animals, and other methods of use.

BACKGROUND OF THE INVENTION

(8) Within the zone surrounding a plant's roots is a region called the rhizosphere. In the rhizosphere, bacteria, fungi, and other organisms compete for nutrients and for binding to the root structures of the plant. Both detrimental and beneficial bacteria and fungi can occupy the rhizosphere. The bacteria, fungi, and the root system of the plant can all be influenced by the actions of peptides, enzymes, and other proteins in the rhizosphere. Augmentation of soil or treatment of plants with certain of these peptides, enzymes, or other proteins would have beneficial effects on the overall populations of beneficial soil bacteria and fungi, create a healthier overall soil environment for plant growth, improve plant growth, and provide for the protection of plants against certain bacterial and fungal pathogens. However, previous attempts to introduce peptides, enzymes, and other proteins into soil to induce such beneficial effects on plants have been hampered by the low survival of enzymes, proteins, and peptides in soil. Additionally, the prevalence of proteases naturally present in the soil leads to degradation of the proteins in the soil. The environment around the roots of a plant (the rhizosphere) is a unique mixture of bacteria, fungi, nutrients, and roots that has different qualities than that of native soil. The symbiotic relationship between these organisms is unique, and could be altered for the better with inclusion of exogenous proteins. The high concentration of fungi and bacteria in the rhizosphere causes even greater degradation of proteins due to abnormally high levels of proteases and other elements detrimental to proteins in the soil. In addition, enzymes and other proteins introduced into soil can dissipate away from plant roots quickly.
(9) Thus, there exists a need in the art for a method for effectively delivering peptides, enzymes, and other proteins to plants (e.g., to plant root systems) and for extending the period of time during which such molecules remain active. Furthermore, there exists a need in the art for a method of selectively targeting such peptides, enzymes, and proteins to the rhizosphere and to plant leaves and plant roots in particular.

SUMMARY OF THE INVENTION

(10) The features of the invention are defined in the appended claims. Other objects and features will be in part apparent and in part pointed out hereinafter.

Description

BRIEF DESCRIPTION OF THE DRAWINGS

(1) FIGS. **1**A and **1**B show alignments of the amino acid sequence of an amino-terminal portion of *Bacillus anthracis* Sterne strain BclA and with the corresponding region from various exosporium proteins from *Bacillus cereus* family members.
(2) FIG. **2** shows exemplary fluorescent microscopy results for the expression of fusion proteins containing various exosporium proteins linked to an mCherry reporter on the exosporium of a recombinant *Bacillus cereus* family member.
(3) FIG. **3** provides data showing to recombinant *Bacillus thuringiensis* BT013A spores expressing a fusion protein comprising a DNA binding protein.
(4) FIG. **4** is a transmission electron micrograph showing exosporium fragments and a *Bacillus cereus* family member spore from which the exosporium has been lost, generated using a recombinant *Bacillus cereus* family member having a knock-out mutation of its CotE gene.
(5) FIG. **5** is a photograph of an SDS-PAGE gel showing a protein marker standard (lane 1) and proteins from exosporium fragments generated using a recombinant *Bacillus cereus* family member having a knock-out mutation of its CotE gene (lane 2).
(6) FIG. **6** provides data illustrating enzyme activity of an acid phosphatase in exosporium fragments derived from a *Bacillus cereus* family member having a knock-out mutation of its CotE gene.
(7) FIG. **7** provides data illustrating that *Bacillus cereus* family member EE349 reduces the inhibitory effects of herbicide on root length in lentils.
(8) FIG. **8** provides data illustrating increased phosphatase activity in a *Bacillus cereus* family member modified to overexpress acid phosphatase (AcpC).
(9) FIG. **9** provides data showing the endoglucanase activity of recombinant *Bacillus thuringiensis* spores expressing a CotC-endoglucanase fusion protein.
(10) FIG. **10** provides bright-field and fluorescence microscopy images showing detection of RNA on the surface of recombinant *B. thuringiensis* spores expressing a fusion protein comprising amino acids 20-35 of SEQ ID NO: 1

and SspC bound to either single-stranded RNA (ssRNA) or double-stranded RNA (dsRNA).
(11) FIG. **11** provides a photograph showing the effects of the microRNA MIR319 on soy height and root development, following delivery to soybean plants using recombinant *B. thuringiensis* spores expressing a fusion protein comprising amino acids 20-35 of SEQ ID NO: 1 and SspC bound to MIR319.
(12) FIG. **12** provides bright-field and fluorescence microscopy images showing detection of GFP and mCherry in the gut of nematodes fed normal OP50 *E. coli* bacterial food (two right-hand panels) or nematodes fed *B. thuringiensis* spores expressing a fusion protein comprising amino acids 20-35 of SEQ ID NO: 1 and either GFP or mCherry (three left-hand panels).
(13) FIG. **13** provides a fluorescence microscopy image showing detection of endophytic bacteria isolated from inside of corn plants treated with *Bacillus thuringiensis* EE-B00184 expressing a fusion protein comprising amino acids 20-35 of SEQ ID NO: 1 and GFP. Arrows denote single spores.
(14) FIG. **14** provides a photograph showing fluorescence of bacterial colonies containing recombinant *Bacillus cereus* family members expressing a fusion protein comprising amino acids 20-35 of SEQ ID NO: 1 and GFP, isolated from inside of corn plants grown from seeds coated with the recombinant bacteria.
(15) FIG. **15** provides a transmission electron micrographs showing: (A) intact spores of *Bacillus thuringiensis* BT013A surrounded by attached exosporium; (B) spores of CotE knockout strain of *Bacillus thuringiensis* BT013A, with detached exosporium; and (C) a purified exosporium fragment preparation of exosporium fragments derived from a CotE knockout strain of *Bacillus thuringiensis* BT013A.
DEFINITIONS
(16) When the articles “a,” “an,” “one,” “the,” and “said” are used herein, the mean “at least one” or “one or more” unless otherwise indicated.
(17) The terms “agriculturally acceptable carrier” and “carrier” are used interchangeably herein.
(18) The term “animal” encompasses any non-human animal as well as humans. For example, where the term “animal” is used herein, the animal can be a mammal (e.g., a human, a sheep, goat, cow, pig, deer, alpaca, bison, camel, donkey, horse, mule, llama, rabbit, dog, or cat), a bird (e.g., a chicken, turkey, duck, goose, quail, or pheasant), a fish (e.g., almon, trout, tilapia, tuna, catfish, or a carp), or a crustacean (e.g., a shrimp, prawn, lobster, crab, or crayfish).
(19) A “biologically pure bacterial culture” refers to a culture of bacteria containing no other bacterial species in quantities sufficient to interfere with the replication of the culture or be detected by normal bacteriological techniques. Stated another way, it is a culture wherein virtually all of the bacterial cells present are of the selected strain.
(20) The terms “comprising,” “including,” and “having” are intended to be inclusive and mean that there may be additional elements other than the listed elements.
(21) The term “bioactive peptide” refers to any peptide that exerts a biological activity. “Bioactive peptides” can be generated, for example, via the cleavage of a protein, peptide, proprotein, or preproprotein by a protease or peptidase.
(22) The term “effective amount” refers to a quantity which is sufficient to result in a statistically significant increase of growth and/or of protein yield and/or of grain yield of a plant as compared to the growth, protein yield and grain yield of the control-treated plant.
(23) An “enzyme involved in the production or activation of a plant growth stimulating compound” includes any enzyme that catalyzes any step in a biological synthesis pathway for a compound that stimulates plant growth or alters plant structure, or any enzyme that catalyzes the conversion of an inactive or less active derivative of a compound that stimulates plant growth or alters plant structure to an active or more active form of the compound. Such compounds include, for example, but are not limited to, small molecule plant hormones such as auxins and cytokinins, bioactive peptides, and small plant growth stimulating molecules synthesized by bacteria or fungi in the rhizosphere (e.g., 2,3-butanediol).
(24) The term “fusion protein” as used herein refers to a protein having a polypeptide sequence that comprises sequences derived from two or more separate proteins. A fusion protein can be generated by joining together a nucleic acid molecule that encodes all or part of a first polypeptide with a nucleic acid molecule that encodes all or part of a second polypeptide to create a nucleic acid sequence which, when expressed, yields a single polypeptide having functional properties derived from each of the original proteins.
(25) The term “germination rate” as used herein refers to the number of seeds that germinate during a particular time period. For example, a germination rate of 85% indicates that 85 out of 100 seeds germinate during a given time period.
(26) The term “inactivate” or “inactivation” as used herein in reference to the inactivation of spores of a recombinant *Bacillus cereus* family member or a recombinant spore-forming bacterium means that the spores are unable to germinate, or that the spores can germinate, but are damaged such that germination does not result in a living bacterium. The terms “partially inactivate” or “partial inactivation” mean that a percentage of the spores are

inactivated, but that some spores retain the ability to germinate and return to a live, replicating state. The term "genetic inactivation" refers to inactivation of spores a recombinant *Bacillus cereus* family member or recombinant spore-forming bacterium by a mutation of the spore's DNA that results in complete or partial inactivation of the spore. The terms "physical inactivation" and "chemical inactivation refer to inactivation of spores using any physical or chemical means, e.g., by heat treatment, gamma irradiation, x-ray irradiation, UV-A irradiation, UV-B irradiation, or treatment with a solvent such as gluteraldehyde, formaldehyde, hydrogen peroxide, acetic acid, bleach, chloroform, or phenol, or any combination thereof.

(27) The terms "immobilizing a recombinant *Bacillus cereus* family member spore on a plant" and "immobilizing a spore of a recombinant spore-forming bacterium on a plant" refers to the binding of a recombinant *Bacillus cereus* family member spore or a spore of a recombinant spore-forming bacterium to plant, e.g., to a root of a plant or to an aerial portion of a plant such as a leaf, stem, flower, or fruit, such that the spore is maintained at the plant's root structure or aerial portion instead of dissipating into the plant growth medium or into the environment surrounding the aerial portions of the plant.

(28) The term "inoculant" as described in this invention is defined in several Federal, or State regulations as (1) "soil or plant inoculants shall include any carrier or culture of a specific micro-organism or mixture of micro-organisms represented to improve the soil or the growth, quality, or yield of plants, and shall also include any seed or fertilizer represented to be inoculated with such a culture" (New York State 10-A Consolidated Law); (2) "substances other than fertilizers, manufactured, sold or represented for use in the improvement of the physical condition of the soil or to aid plant growth or crop yields" (Canada Fertilizers Act); (3) "a formulation containing pure or predetermined mixtures of living bacteria, fungi or virus particles for the treatment of seed, seedlings or other plant propagation material for the purpose of enhancing the growth capabilities or disease resistance or otherwise altering the properties of the eventual plants or crop" (Ad hoc European Working Group, 1997) or (4) "meaning any chemical or biological substance of mixture of substances or device distributed in this state to be applied to soil, plants or seeds for soil corrective purposes; or which is intended to improve germination, growth, quality, yield, product quality, reproduction, flavor, or other desirable characteristics of plants or which is intended to produce any chemical, biochemical, biological or physical change in soil" (Section 14513 of the California Food and Agriculture Code).

(29) A "modulator protein" includes any protein that, when overexpressed in a *Bacillus cereus* family member expressing any of the fusion proteins described herein, modulates expression of the fusion protein, such that the expression of the fusion protein is increased or decreased as compared to expression of the fusion protein in a *Bacillus cereus* family member that does not overexpress the modulator protein.

(30) A "plant growth medium" includes any material that is capable of supporting the growth of a plant.

(31) A "plant immune system enhancer protein or peptide" as used herein includes any protein or peptide that has a beneficial effect on the immune system of a plant.

(32) The term "plant growth stimulating protein or peptide" as used herein includes any protein or peptide that increases plant growth in a plant exposed to the protein or peptide.

(33) The term "probiotic" as used herein refers to microorganisms (e.g., bacteria) that provide health benefits when consumed by or administered to an animal.

(34) The terms "promoting plant growth" and "stimulating plant growth" are used interchangeably herein, and refer to the ability to enhance or increase at least one of the plant's height, weight, leaf size, root size, or stem size, to increase protein yield from the plant or to increase grain yield of the plant.

(35) A "protein or peptide that protects a plant from a pathogen" as used herein includes any protein or peptide that makes a plant exposed to the protein or peptide less susceptible to infection with a pathogen.

(36) A "protein or peptide that enhances stress resistance in a plant" as used herein includes any protein or peptide that makes a plant exposed to the protein or peptide more resistant to stress.

(37) The term "plant binding protein or peptide" refers to any peptide or protein capable of specifically or non-specifically binding to any part of a plant (e.g., roots or aerial portions of a plant such as leaves foliage, stems, flowers, or fruits) or to plant matter.

(38) The term "pyrethrinase" refers to any enzyme that degrades a pyrethrin or a pyrethroid.

(39) The term "rhizosphere" is used interchangeably with "root zone" to denote that segment of the soil that surrounds the roots of a plant and is influenced by them.

(40) The term "targeting sequence" as used herein refers to a polypeptide sequence that, when present as part of a longer polypeptide or a protein, results in the localization of the longer polypeptide or the protein to a specific subcellular location. The targeting sequences described herein result in localization of proteins to the exosporium of a *Bacillus cereus* family member.

DESCRIPTION OF THE INVENTION

I. Fusion Proteins for Expression in *Bacillus Cereus* Family Members and Recombinant *Bacillus Cereus* Family Members Expressing Such Fusion Proteins

(41) The present invention relates to fusion proteins comprising a targeting sequence, an exosporium protein, or an exosporium protein fragment targets the fusion protein to the exosporium of a *Bacillus cereus* family member and at least one protein or peptide of interest. When expressed in *Bacillus cereus* family member bacteria, these fusion proteins are targeted to the exosporium layer of the spore and are physically oriented such that the protein or peptide of interest is displayed on the outside of the spore.
(42) This *Bacillus* exosporium display (BEMD) system can be used to deliver peptides, enzymes, and other proteins to plants (e.g., to plant foliage, fruits, flowers, stems, or roots) or to a plant growth medium such as soil. Peptides, enzymes, and proteins delivered to the soil or another plant growth medium in this manner persist and exhibit activity in the soil for extended periods of time. Introduction of recombinant *Bacillus cereus* family member bacteria expressing the fusion proteins described herein into soil or the rhizosphere of a plant leads to a beneficial enhancement of plant growth in many different soil conditions. The use of the BEMD to create these enzymes allows them to continue to exert their beneficial results to the plant and the rhizosphere over the first months of a plants life.
(43) A. Targeting Sequences, Exosporium Proteins, and Exosporium Protein Fragments for Targeting Proteins or Peptides of Interest to the Exosporium of a *Bacillus cereus* Family Member
(44) For ease of reference, descriptions of the amino acid sequences for the targeting sequences, exosporium proteins, and exosporium protein fragments that can be used for targeting of proteins or peptides of interest to the exosporium of a *Bacillus cereus* family members, are provided in Table 1 together with their SEQ ID NOs.
(45) TABLE-US-00001 TABLE 1 Peptide and protein sequences used for targeting of proteins or peptides of interest to the exosporium of *Bacillus cereus* family members Protein, protein fragment, or targeting sequence SEQ ID NO. AA 1-41 of BclA (*B. anthracis* Sterne) 1* Full length BclA (*B. anthracis* Sterne) 2* AA 1-33 of BetA/BAS3290 (*B. anthracis* Sterne) 3 Full length BetA/BAS3290 (*B. anthracis* Sterne) 4 Met + AA 2-43 of BAS4623 (*B. anthracis* Sterne) 5 Full length BAS4623 (*B. anthracis* Sterne) 6 AA 1-34 of BclB (*B. anthracis* Sterne) 7 Full length BclB (*B. anthracis* Sterne) 8 AA 1-30 of BAS1882 (*B. anthracis* Sterne) 9 Full length BAS1882 (*B. anthracis* Sterne) 10 AA 1-39 of gene 2280 (*B. weihenstephensis* KBAB4) 11 Full length KBAB4 gene 2280 (*B. weihenstephensis* KBAB4) 12 AA 1-39 of gene 3572 (*B. weihenstephensis* KBAB4) 13 Full Length KBAB4 gene 3572 (*B. weihenstephensis* KBAB4) 14 AA 1-49 of Exosporium Leader Peptide (*B. cereus* VD200) 15 Full Length Exosporium Leader Peptide (*B. cereus* VD200) 16 AA 1-33 of Exosporium Leader Peptide (*B. cereus* VD166) 17 Full Length Exosporium Leader Peptide (*B. cereus* VD166) 18 AA 1-39 of hypothetical protein IKG_04663 (*B. cereus* VD200) 19 Hypothetical protein IKG_04663, partial (*B. cereus* VD200) 20 AA 1-39 of YVTN β-propeller protein (*B. weihenstephensis* KBAB4) 21 Full length YVTN β-propeller protein (*B. weihenstephensis* KBAB4) 22 AA 1-30 of hypothetical protein bcerkbab4_2363 23 (*B. weihenstephensis* KBAB4) Full length hypothetical protein bcerkbab4_2363 24 (*B. weihenstephensis* KBAB4) AA 1-30 of hypothetical protein bcerkbab4_2131 25 (*B. weihenstephensis* KBAB4) Full length hypothetical protein bcerkbab4_2131 26 (*B. weihenstephensis* KBAB4) AA 1-36 of triple helix repeat containing collagen 27 (*B. weihenstephensis* KBAB4) Full length triple helix repeat-containing collagen KBAB4 28 (*B. weihenstephensis* KBAB4) AA 1-39 of hypothetical protein bmyco0001_21660 (*B. mycoides* 2048) 29 Full length hypothetical protein bmyco0001_21660 (*B. mycoides* 2048) 30 AA 1-30 of hypothetical protein bmyc0001_22540 (*B. mycoides* 2048) 31 Full length hypothetical protein bmyc0001_22540 (*B. mycoides* 20481 32 AA 1-21 of hypothetical protein bmyc0001_21510 (*B. mycoides* 2048) 33 Full length hypothetical protein bmyc0001_21510 (*B. mycoides* 2048) 34 AA 1-22 of collagen triple helix repeat protein (*B. thuringiensis* 35646) 35 Full length collagen triple helix repeat protein (*B. thuringiensis* 35646) 36 AA 1-35 of hypothetical protein WP_69652 (*B. cereus)* 43 Full length hypothetical protein WP_69652 (*B. cereus)* 44 AA 1-41 of exosporium leader WP016117717 (*B. cereus)* 45 Full length exosporium leader WP016117717 (*B. cereus)* 46 AA 1-49 of exosporium peptide WP002105192 (*B. cereus)* 47 Full length exosporium peptide WP002105192 (*B. cereus)* 48 AA 1-38 of hypothetical protein WP87353 (*B. cereus)* 49 Full length hypothetical protein WP87353 (*B. cereus)* 50 AA 1-39 of exosporium peptide 02112369 (*B. cereus)* 51 Full length exosporium peptide 02112369 (*B. cereus)* 52 AA 1-39 of exosporium protein WP016099770 (*B. cereus)* 53 Full length exosporium protein WP016099770 (*B. cereus)* 54 AA 1-36 of hypothetical protein YP006612525 (*B. thuringiensis)* 55 Full length hypothetical protein YP006612525 (*B. thuringiensis)* 56 AA 1-136 of hypothetical protein TIGR03720 (*B. mycoides)* 57** Full length hypothetical protein TIGR03720 (*B. mycoides)* 58** AA 1-36 of collagen triple helix repeat domain protein 59 (*B. cereus* ATCC 10987) Full length collagen triple helix repeat domain protein 60 (*B. cereus* ATCC 10987) AA 1-39 of collagen-like protein (*B. cereus* E33L) 61 Full length collagen-like protein (*B. cereus* E33L) 62 AA 1-41 of triple helix repeat-containing collagen 63 (*B. weihenstephensis* KBAB4) Full length triple helix repeat-containing collagen 64 (*B. weihenstephensis* KBAB4) AA 1-30 of hypothetical protein BALH_2230 65 (*B. thuringiensis* str. Al Hakam) Full length hypothetical protein BALH_2230 66 (*B. thuringiensis* str. Al Hakam) AA 1-33 of triple helix repeat-containing collagen (*B. cereus* ATCC 14579) 67 Full length triple helix repeat-containing collagen (*B. cereus* ATCC 14579) 68 AA 1-44 of collagen triple helix repeat (*B. cereus)* 69 Full

length collagen triple helix repeat (*B. cereus)* 70 AA 1-38 of triple helix repeat-containing collagen (*B. cereus* ATCC 14579) 71 Full length triple helix repeat-containing collagen (*B. cereus* ATCC 14579) 72 AA 1-30 of hypothetical protein BCZK1835 (*B. cereus* E33L) 73 Full length hypothetical protein BCZK1835 (*B. cereus* E33L) 74 AA 1-48 of triple helix repeat-containing collagen 75 (*B. weihenstephensis* KBAB4) Full length triple helix repeat-containing collagen 76 (*B. weihenstephensis* KBAB4) AA 1-30 of triple helix repeat-containing collagen (*B. cereus* ATCC 14579) 77 Full length triple helix repeat-containing collagen (*B. cereus* ATCC 14579) 78 AA 1-39 of hypothetical protein BC4725 (*B. cereus* ATCC 14579) 79 Full length hypothetical protein BC4725 (*B. cereus* ATCC 14579) 80 AA 1-44 of hypothetical protein BCZK4476 (*B. cereus* E33L) 81 Full length hypothetical protein BCZK4476 (*B. cereus* E33L) 82 AA 1-40 of triple helix repeat-containing collagen 83 (*B. anthracis* str. 'Ames Ancestor') Full length triple helix repeat-containing collagen 84 (*B. anthracis* str. 'Ames Ancestor') AA 1-34 of BclA protein (*B. thuringiensis* serovar konkukian str. 97-27) 85 Full length BclA protein (*B. thuringiensis* serovar konkukian str. 97-27) 86 AA 1-34 of conserved hypothetical protein (*B. cereus* ATCC 10987) 87 Full length conserved hypothetical protein (*B. cereus* ATCC 10987) 88 AA 1-34 of triple helix repeat-containing collagen (*B. cereus* ATCC 14579) 89 Full length triple helix repeat-containing collagen (*B. cereus* ATCC 14579) 90 AA 1-99 of exosporium leader peptide partial sequence (*B. cereus)* 91 Exosporium leader peptide partial sequence (*B. cereus)* 92 AA 1-136 of hypothetical protein ER45 27600, partial sequence 93 (*B. weihenstephensis)* Hypothetical protein ER45 27600, partial sequence (*B. weihenstephensis)* 94 AA 1-196 of BclA (*B. anthracis* Sterne) 95* Met + AA 20-35 of BclA (*B. anthracis* Sterne) 96 Met + AA 12-27 of BetA/BAS3290 (*B. anthracis* Sterne) 97 Met + AA 18-33 of gene 2280 (*B. weihenstephensis* KBAB4) 98 Met + AA 18-33 of gene 3572 (*B. weihenstephensis* KBAB4) 99 Met + AA 12-27 of Exosporium Leader Peptide (*B. cereus* VD166) 100 Met + AA 18-33 of YVTN β-propeller protein 101 (*B. weihenstephensis* KBAB4) Met + AA 9-24 of hypothetical protein bcerkbab4_2363 102 (*B. weihenstephensis* KBAB4) Met + AA 9-24 of hypothetical protein bcerkbab4_2131 103 (*B. weihenstephensis* KBAB4) Met + AA 9-24 of hypothetical protein bmyc0001_22540 104 (*B. mycoides* 2048) Met + AA 9-24 of BAS1882 (*B. anthracis* Sterne) 105 Met + AA 20-35 of exosporium leader WP016117717 (*B. cereus)* 106 Met + AA 9-24 of hypothetical protein BALH_2230 107 (*B. thuringiensis* str. Al Hakam) Full length InhA (*B. mycoides)* 108 Full length BAS1141 (ExsY) (*B. anthracis* Sterne) 109 Full length BAS1144 (BxpB/ExsFA) (*B. anthracis* Sterne) 110 Full length BAS1145 (CotY) (*B. anthracis* Sterne) 111 Full length BAS1140 (*B. anthracis* Sterne) 112 Full length ExsFB (*B. anthracis* H9401) 113 Full length InhA1 (*B. thuringiensis* HD74) 114 Full length ExsJ (*B. cereus* ATCC 10876) 115 Full length ExsH (*B. cereus)* 116 Full length YjcA (*B. anthracis* Ames) 117 Full length YjcB (*B. anthracis)* 118 Full length BclC (*B. anthracis* Sterne) 119 Full length acid phosphatase 120 (*Bacillus thuringiensis* serovar konkukian str. 97-27) Full length InhA2 (*B. thuringiensis* HD74) 121 Full length InhA3 (*B. mycoides)* 122 AA = amino acids **B. anthracis* Sterne strain BclA has 100% sequence identity with *B. thuringiensis* BclA. Thus, SEQ ID NOs: 1, 2, and 95 also represent amino acids 1-41 of *B. thuringiensis* BclA, full length *B. thuringiensis* BclA, and amino acids 1-196 of *B. thuringiensis* BclA, respectively. Likewise, SEQ ID NO: 96 also represents a methionine residue plus amino acids 20-35 of *B. thuringiensis* BclA. ***B. mycoides* hypothetical protein TIGR03720 has 100% sequence identity with *B. mycoides* hypothetical protein WP003189234. Thus, SEQ ID NOs: 57 and 58 also represent amino acids 1-136 of *B. mycoides* hypothetical protein WP003189234 and full length *B. mycoides* hypothetical protein WP003189234, respectively.

(46) *Bacillus* is a genus of rod-shaped bacteria. The *Bacillus cereus* family of bacteria includes any *Bacillus* species that is capable of producing an exosporium. Thus, the *Bacillus cereus* family of bacteria includes the species *Bacillus anthracis, Bacillus cereus, Bacillus thuringiensis, Bacillus mycoides, Bacillus pseudomycoides, Bacillus samanii, Bacillus gaemokensis, Bacillus weihenstephensis*, and *Bacillus toyoiensis*. Under stressful environmental conditions, *Bacillus cereus* family bacteria undergo sporulation and form oval endospores that can stay dormant for extended periods of time. The outermost layer of the endospores is known as the exosporium and comprises a basal layer surrounded by an external nap of hair-like projections. Filaments on the hair-like nap are predominantly formed by the collagen-like glycoprotein BclA, while the basal layer is comprised of a number of different proteins. Another collagen-related protein, BclB, is also present in the exosporium and exposed on endospores of *Bacillus cereus* family members. BclA, the major constituent of the surface nap, has been shown to be attached to the exosporium with its amino-terminus (N-terminus) positioned at the basal layer and its carboxy-terminus (C-terminus) extending outward from the spore.

(47) It was previously discovered that certain sequences from the N-terminal regions of BclA and BclB could be used to target a peptide or protein to the exosporium of a *Bacillus cereus* endospore (see U.S. Patent Application Publication Nos. 2010/0233124 and 2011/0281316, and Thompson et al., Targeting of the BclA and BclB proteins to the *Bacillus anthracis* spore surface, Molecular Microbiology 70(2):421-34 (2008)). It was also found that the BetA/BAS3290 protein of *Bacillus anthracis* localized to the exosporium.

(48) In particular, amino acids 20-35 of BclA from *Bacillus anthracis* Sterne strain have been found to be sufficient for targeting to the exosporium. A sequence alignment of amino acids 1-41 of BclA (SEQ ID NO: 1) with the corresponding N-terminal regions of several other *Bacillus cereus* family exosporium proteins and *Bacillus cereus*

family proteins having related sequences is shown in FIGS. **1**A and **1**B. As can be seen from FIGS. **1**A and **1**B, there is a region of high-homology among all of the proteins in the region corresponding to amino acids 20-41 of BclA. However, in these sequences, the amino acids corresponding to amino acids 36-41 of BclA contain secondary structure and are not necessary for fusion protein localization to the exosporium. The conserved targeting sequence region of BclA (amino acids 20-35 of SEQ ID NO: 1) is shown in bold in FIGS. **1**A and **1**B and corresponds to the minimal targeting sequence needed for localization to the exosporium. A more highly conserved region spanning amino acids 25-35 of BclA within the targeting sequence is underlined in the sequences in FIGS. **1**A and **1**B, and is the recognition sequence for ExsFA/BxpB/ExsFB and homologs, which direct and assemble the described proteins on the surface of the exosporium. The amino acid sequences of SEQ ID NOs. 3, 5, and 7 in FIG. **1**A are amino acids 1-33 of *Bacillus anthracis* Sterne strain BetA/BAS3290, a methionine followed by amino acids 2-43 of *Bacillus anthracis* Sterne strain BAS4623, and amino acids 1-34 of *Bacillus anthracis* Sterne strain BclB, respectively. (For BAS4623, it was found that replacing the valine present at position 1 in the native protein with a methionine resulted in better expression.) As can be seen from FIG. **1**A, each of these sequences contains a conserved region corresponding to amino acids 20-35 of BclA (SEQ ID NO: 1; shown in bold), and a more highly conserved region corresponding to amino acids 20-35 of BclA (underlined).

(49) Additional proteins from *Bacillus cereus* family members also contain the conserved targeting region. In particular, in FIGS. **1**A and **1**B, SEQ ID NO: 9 is amino acids 1-30 of *Bacillus anthracis* Sterne strain BAS1882, SEQ ID NO: 11 is amino acids 1-39 of the *Bacillus weihenstephensis* KBAB4 2280 gene product, SEQ ID NO: 13 is amino acids 1-39 of the *Bacillus weihenstephensis* KBAB4 3572 gene product, SEQ ID NO: 15 is amino acids 1-49 of *Bacillus cereus* VD200 exosporium leader peptide, SEQ ID NO: 17 is amino acids 1-33 of *Bacillus cereus* VD166 exosporium leader peptide, SEQ ID NO: 19 is amino acids 1-39 of *Bacillus cereus* VD200 hypothetical protein IKG_04663, SEQ ID NO: 21 is amino acids 1-39 of *Bacillus weihenstephensis* KBAB4 YVTN β-propeller protein, SEQ ID NO: 23 is amino acids 1-30 of *Bacillus weihenstephensis* KBAB4 hypothetical protein bcerkbab4_2363, SEQ ID NO: 25 is amino acids 1-30 of *Bacillus weihenstephensis* KBAB4 hypothetical protein bcerkbab4_2131, SEQ ID NO: 27 is amino acids 1-36 of *Bacillus weihenstephensis* KBAB4 triple helix repeat containing collagen, SEQ ID NO: 29 is amino acids 1-39 of *Bacillus mycoides* 2048 hypothetical protein bmyco0001_21660, SEQ ID NO: 31 is amino acids 1-30 of *Bacillus mycoides* 2048 hypothetical protein bmyc0001_22540, SEQ ID NO: 33 is amino acids 1-21 of *Bacillus mycoides* 2048 hypothetical protein bmyc0001_21510, SEQ ID NO: 35 is amino acids 1-22 of *Bacillus thuringiensis* 35646 collagen triple helix repeat protein, SEQ ID NO: 43 is amino acids 1-35 of *Bacillus cereus* hypothetical protein WP_69652, SEQ ID NO: 45 is amino acids 1-41 of *Bacillus cereus* exosporium leader WP016117717, SEQ ID NO: 47 is amino acids 1-49 of *Bacillus cereus* exosporium peptide WP002105192, SEQ ID NO: 49 is amino acids 1-38 of *Bacillus cereus* hypothetical protein WP87353, SEQ ID NO: 51 is amino acids 1-39 of *Bacillus cereus* exosporium peptide 02112369, SEQ ID NO: 53 is amino acids 1-39 of *Bacillus cereus* exosporium protein WP016099770, SEQ ID NO: 55 is amino acids 1-36 of *Bacillus thuringiensis* hypothetical protein YP006612525, SEQ ID NO: 57 is amino acids 1-136 of *Bacillus mycoides* hypothetical protein TIGR03720, SEQ ID NO: 59 is amino acids 1-36 of *B. cereus* ATCC 10987 collagen triple helix repeat domain protein, SEQ ID NO: 61 is amino acids 1-39 of *B. cereus* E33L collagen-like protein, SEQ ID NO: 63 is amino acids 1-41 of *B. weihenstephanensis* KBAB4 triple helix repeat-containing collagen, SEQ ID NO: 65 is amino acids 1-30 of *B. thuringiensis* str. Al Hakam hypothetical protein BALH_2230, SEQ ID NO: 67 is amino acids 1-33 of *B. cereus* ATCC 14579 triple helix repeat-containing collagen, SEQ ID NO: 69 is amino acids 1-44 of *B. cereus* collagen triple helix repeat, SEQ ID NO: 71 is amino acids 1-38 of *B. cereus* ATCC 14579 triple helix repeat-containing collagen, SEQ ID NO: 73 is amino acids 1-30 of *B. cereus* E33L hypothetical protein BCZK1835, SEQ ID NO: 75 is amino acids 1-48 of *B. weihenstephanensis* KBAB4 triple helix repeat-containing collagen, SEQ ID NO: 77 is amino acids 1-30 of *B. cereus* ATCC 14579 triple helix repeat-containing collagen, SEQ ID NO: 79 is amino acids 1-39 of *B. cereus* ATCC 14579 hypothetical protein BC4725, SEQ ID NO: 81 is amino acids 1-44 of *B. cereus* E33L hypothetical protein BCZK4476, SEQ ID NO: 83 is amino acids 1-40 of *B. anthracis* str. 'Ames Ancestor' triple helix repeat-containing collagen, SEQ ID NO: 85 is amino acids 1-34 of *B. thuringiensis* serovar konkukian str. 97-27 BclA protein, SEQ ID NO: 87 is amino acids 1-34 of *B. cereus* ATCC 10987 conserved hypothetical protein, SEQ ID NO: 89 is amino acids 1-34 of *B. cereus* ATCC 14579 triple helix repeat-containing collagen, SEQ ID NO: 91 is amino acids 1-99 of *B. cereus* exosporium leader peptide partial sequence, and SEQ ID NO: 93 is amino acids 1-136 of *B. weihenstephanensis* hypothetical protein ER45_27600. As shown in FIGS. **1**A and **1**B, each of the N-terminal regions of these proteins contains a region that is conserved with amino acids 20-35 of BclA (SEQ ID NO: 1), and a more highly conserved region corresponding to amino acids 25-35 of BclA.

(50) Any portion of BclA which includes amino acids 20-35 can be used as to target a fusion protein to the exosporium. In addition, full-length exosporium proteins or exosporium protein fragments can be used for targeting the fusion proteins to the exosporium. Thus, full-length BclA or a fragment of BclA that includes amino acids 20-35 can be used for targeting to the exosporium. For example, full length BclA (SEQ ID NO: 2) or a midsized fragment

of BclA that lacks the carboxy-terminus such as SEQ ID NO: 95 (amino acids 1-196 of BclA) can be used to target the fusion proteins to the exosporium. Midsized fragments such as the fragment of SEQ ID NO: 95 have less secondary structure than full length BclA and has been found to be suitable for use as a targeting sequence. The targeting sequence can also comprise much shorter portions of BclA which include amino acids 20-35, such as SEQ ID NO: 1 (amino acids 1-41 of BclA), amino acids 1-35 of SEQ ID NO: 1, amino acids 20-35 of SEQ ID NO: 1, or SEQ ID NO: 96 (a methionine residue linked to amino acids 20-35 of BclA). Even shorter fragments of BclA which include only some of amino acids 20-35 also exhibit the ability to target fusion proteins to the exosporium. For example, the targeting sequence can comprise amino acids 22-31 of SEQ ID NO: 1, amino acids 22-33 of SEQ ID NO: 1, or amino acids 20-31 of SEQ ID NO: 1.

(51) Alternatively, any portion of BetA/BAS3290, BAS4623, BclB, BAS1882, the KBAB4 2280 gene product, the KBAB4 3572 gene product, *B. cereus* VD200 exosporium leader peptide, *B. cereus* VD166 exosporium leader peptide, *B. cereus* VD200 hypothetical protein IKG_04663, *B. weihenstephensis* KBAB4 YVTN β-propeller protein, *B. weihenstephensis* KBAB4 hypothetical protein bcerkbab4_2363, *B. weihenstephensis* KBAB4 hypothetical protein bcerkbab4_2131, *B. weihenstephensis* KBAB4 triple helix repeat containing collagen, *B. mycoides* 2048 hypothetical protein bmyco0001_21660, *B. mycoides* 2048 hypothetical protein bmyc0001_22540, *B. mycoides* 2048 hypothetical protein bmyc0001_21510, *B. thuringiensis* 35646 collagen triple helix repeat protein, *B. cereus* hypothetical protein WP_69652, *B. cereus* exosporium leader WP016117717, *B. cereus* exosporium peptide WP002105192, *B. cereus* hypothetical protein WP87353, *B. cereus* exosporium peptide 02112369, *B. cereus* exosporium protein WP016099770, *B. thuringiensis* hypothetical protein YP006612525, *B. mycoides* hypothetical protein TIGR03720, *B. cereus* ATCC 10987 collagen triple helix repeat domain protein, *B. cereus* E33L collagen-like protein, *B. weihenstephanensis* KBAB4 triple helix repeat-containing collagen, *B. thuringiensis* str. Al Hakam hypothetical protein BALH_2230, *B. cereus* ATCC 14579 triple helix repeat-containing collagen, *B. cereus* collagen triple helix repeat, *B. cereus* ATCC 14579 triple helix repeat-containing collagen, *B. cereus* E33L hypothetical protein BCZK1835, *B. weihenstephanensis* KBAB4 triple helix repeat-containing collagen, *B. cereus* ATCC 14579 triple helix repeat-containing collagen, *B. cereus* ATCC 14579 hypothetical protein BC4725, *B. cereus* E33L hypothetical protein BCZK4476, *B. anthracis* str. 'Ames Ancestor' triple helix repeat-containing collagen, *B. thuringiensis* serovar konkukian str. 97-27 BclA protein, *B. cereus* ATCC 10987 conserved hypothetical protein, *B. cereus* ATCC 14579 triple helix repeat-containing collagen, *B. cereus* exosporium leader peptide partial sequence, or *B. weihenstephanensis* hypothetical protein ER45_27600 which includes the amino acids corresponding to amino acids 20-35 of BclA can serve as the targeting sequence.

(52) As can be seen from FIG. **1**A, amino acids 12-27 of BetA/BAS3290, amino acids 23-38 of BAS4623, amino acids 13-28 of BclB, amino acids 9-24 of BAS1882, amino acids 18-33 of KBAB4 2280 gene product, amino acids 18-33 of KBAB4 3572 gene product, amino acids 28-43 of *B. cereus* VD200 exosporium leader peptide, amino acids 12-27 of *B. cereus* VD166 exosporium leader peptide, amino acids 18-33 of *B. cereus* VD200 hypothetical protein IKG_04663, amino acids 18-33 *B. weihenstephensis* KBAB4 YVTN β-propeller protein, amino acids 9-24 of *B. weihenstephensis* KBAB4 hypothetical protein berkbab4_2363, amino acids 9-24 of *B. weihenstephensis* KBAB4 hypothetical protein bcerkbab4_2131, amino acids 15-30 of *B. weihenstephensis* KBAB4 triple helix repeat containing collagen, amino acids 18-33 of *B. mycoides* 2048 hypothetical protein bmyco0001_21660, amino acids 9-24 of *B. mycoides* 2048 hypothetical protein bmyc0001_22540, amino acids 1-15 of *B. mycoides* 2048 hypothetical protein bmyc0001_21510, amino acids 1-16 of *B. thuringiensis* 35646 collagen triple helix repeat protein, amino acids 14-29 of *B. cereus* hypothetical protein WP_69652, amino acids 20-35 of *B. cereus* exosporium leader WP016117717, amino acids 28-43 of *B. cereus* exosporium peptide WP002105192, amino acids 17-32 of *B. cereus* hypothetical protein WP87353, amino acids 18-33 of *B. cereus* exosporium peptide 02112369, amino acids 18-33 of *B. cereus* exosporium protein WP016099770, amino acids 15-30 of *B. thuringiensis* hypothetical protein YP006612525, and amino acids 115-130 of *B. mycoides* hypothetical protein TIGR03720 correspond to amino acids 20-35 of BclA. As can be seen from FIG. **1**B, amino acids 15-30 of *B. cereus* ATCC 10987 collagen triple helix repeat domain protein, amino acids 18-33 of *B. cereus* E33L collagen-like protein, amino acids 20-35 of *B. weihenstephanensis* KBAB4 triple helix repeat-containing collagen, amino acids 9-24 of *B. thuringiensis* str. Al Hakam hypothetical protein BALH_2230, amino acids 12-27 of *B. cereus* ATCC 14579 triple helix repeat-containing collagen, amino acids 23-38 of *B. cereus* collagen triple helix repeat, amino acids 17-32 of *B. cereus* ATCC 14579 triple helix repeat-containing collagen, amino acids 9-24 of *B. cereus* E33L hypothetical protein BCZK1835, amino acids 27-42 of *B. weihenstephanensis* KBAB4 triple helix repeat-containing collagen, amino acids 9-24 of *B. cereus* ATCC 14579 triple helix repeat-containing collagen, amino acids 18-33 of *B. cereus* ATCC 14579 hypothetical protein BC4725, amino acids 23-38 of *B. cereus* E33L hypothetical protein BCZK4476, amino acids 19-34 *B. anthracis* str. 'Ames Ancestor' triple helix repeat-containing collagen, amino acids 13-28 of *B. thuringiensis* serovar konkukian str. 97-27 BclA protein, amino acids 13-28 of *B. cereus* ATCC 10987 conserved hypothetical protein, amino acids 13-28 of *B. cereus* ATCC 14579 triple helix repeat-containing collagen, amino acids 78-93 of *B. cereus* exosporium leader peptide partial sequence, and amino acids 115-130 of *B.*

weihenstephanensis hypothetical protein ER45_27600 correspond to amino acids 20-35 of BclA. Thus, any portion of these proteins that includes the above-listed corresponding amino acids can serve as the targeting sequence.
(53) Furthermore, any amino acid sequence comprising amino acids 20-35 of BclA, or any of the above-listed corresponding amino acids can serve as the targeting sequence.
(54) Thus, the targeting sequence can comprise amino acids 1-35 of SEQ ID NO: 1, amino acids 20-35 of SEQ ID NO: 1, SEQ ID NO: 1, SEQ ID NO: 96, amino acids 22-31 of SEQ ID NO: 1, amino acids 22-33 of SEQ ID NO: 1, or amino acids 20-31 of SEQ ID NO: 1. Alternatively, the targeting sequence consists of amino acids 1-35 of SEQ ID NO: 1, amino acids 20-35 of SEQ ID NO: 1, SEQ ID NO: 1, or SEQ ID NO: 96. Alternatively, the targeting sequence can consist of amino acids 22-31 of SEQ ID NO: 1, amino acids 22-33 of SEQ ID NO: 1, or amino acids 20-31 of SEQ ID NO: 1. Alternatively, the exosporium protein can comprise full length BclA (SEQ ID NO: 2), or the exosporium protein fragment can comprise a midsized fragment of BclA that lacks the carboxy-terminus, such as SEQ ID NO: 59 (amino acids 1-196 of BclA). Alternatively, the exosporium protein fragment can consist of SEQ ID NO: 59.
(55) The targeting sequence can comprise amino acids 2-35 of SEQ ID NO: 1; amino acids 5-35 of SEQ ID NO: 1; amino acids 8-35 of SEQ ID NO: 1; amino acids 10-35 of SEQ ID NO: 1; or amino acids 15-35 of SEQ ID NO: 1.
(56) The targeting sequence can also comprise amino acids 1-27 of SEQ ID NO: 3, amino acids 12-27 of SEQ ID NO: 3, or SEQ ID NO: 3, or the exosporium protein can comprise full length BetA/BAS3290 (SEQ ID NO: 4). It has also been found that a methionine residue linked to amino acids 12-27 of BetA/BAS3290 can be used as a targeting sequence. Thus, the targeting sequence can comprise SEQ ID NO: 97. The targeting sequence can also comprise amino acids 14-23 of SEQ ID NO: 3, amino acids 14-25 of SEQ ID NO: 3, or amino acids 12-23 of SEQ ID NO: 3.
(57) The targeting sequence can comprise amino acids 2-27 of SEQ ID NO: 3; amino acids 5-27 of SEQ ID NO: 3; amino acids 8-27 of SEQ ID NO: 3; or amino acids 10-27 of SEQ ID NO: 3.
(58) The targeting sequence can also comprise amino acids 1-38 of SEQ ID NO: 5, amino acids 23-38 of SEQ ID NO: 5, or SEQ ID NO: 5, or the exosporium protein can comprise full length BAS4623 (SEQ ID NO: 6).
(59) The targeting sequence can comprise amino acids 2-38 of SEQ ID NO: 5; amino acids 5-38 of SEQ ID NO: 5; amino acids 8-38 of SEQ ID NO: 5; amino acids 10-38 of SEQ ID NO: 5; amino acids 15-38 of SEQ ID NO: 5; or amino acids 20-38 of SEQ ID NO: 5.
(60) Alternatively, the targeting sequence can comprise amino acids 1-28 of SEQ ID NO: 7, amino acids 13-28 of SEQ ID NO: 7, or SEQ ID NO: 7, or the exosporium protein can comprise full length BclB (SEQ ID NO:8).
(61) The targeting sequence can comprise amino acids 2-28 of SEQ ID NO: 7; amino acids 5-28 of SEQ ID NO: 7; amino acids 8-28 of SEQ ID NO: 7; or amino acids 10-28 of SEQ ID NO: 7.
(62) The targeting sequence can also comprise amino acids 1-24 of SEQ ID NO: 9, amino acids 9-24 of SEQ ID NO: 9, or SEQ ID NO: 9, or the exosporium protein can comprise full length BAS1882 (SEQ ID NO: 10). A methionine residue linked to amino acids 9-24 of BAS1882 can also be used as a targeting sequence. Thus, the targeting sequence can comprise SEQ ID NO: 105.
(63) The targeting sequence can comprise amino acids 2-24 of SEQ ID NO: 9; amino acids 5-24 of SEQ ID NO: 9; or amino acids 8-24 of SEQ ID NO: 9.
(64) The targeting sequence can also comprise amino acids 1-33 of SEQ ID NO:11, amino acids 18-33 of SEQ ID NO: 11, or SEQ ID NO: 11, or the exosporium protein can comprise the full length *B. weihenstephensis* KBAB4 2280 gene product (SEQ ID NO: 12). A methionine residue linked to amino acids 18-33 of the *B. weihenstephensis* KBAB4 2280 gene product can also be used as a targeting sequence. Thus, the targeting sequence can comprise SEQ ID NO: 98.
(65) The targeting sequence can comprise amino acids 2-33 of SEQ ID NO: 11; amino acids 5-33 of SEQ ID NO: 11; amino acids 8-33 of SEQ ID NO: 11; amino acids 10-33 of SEQ ID NO: 11; or amino acids 15-33 of SEQ ID NO: 11.
(66) The targeting sequence can also comprise amino acids 1-33 of SEQ ID NO: 13, amino acids 18-33 of SEQ ID NO: 13, or SEQ ID NO:13, or the exosporium protein can comprise the full length *B. weihenstephensis* KBAB4 3572 gene product (SEQ ID NO:14). A methionine residue linked to amino acids 18-33 of the *B. weihenstephensis* KBAB4 3572 gene product can also be used as a targeting sequence. Thus, the targeting sequence can comprise SEQ ID NO: 99.
(67) The targeting sequence can comprise amino acids 2-33 of SEQ ID NO: 13; amino acids 5-33 of SEQ ID NO: 13; amino acids 8-33 of SEQ ID NO: 13; amino acids 10-33 of SEQ ID NO: 13; or amino acids 15-33 of SEQ ID NO: 13;
(68) Alternatively, the targeting sequence can comprise amino acids 1-43 of SEQ ID NO: 15, amino acids 28-43 of SEQ ID NO: 15, or SEQ ID NO:15, or the exosporium protein can comprise full length *B. cereus* VD200 exosporium leader peptide (SEQ ID NO:16).
(69) The targeting sequence can comprise amino acids 2-43 of SEQ ID NO: 15; amino acids 5-43 of SEQ ID NO:

15; amino acids 8-43 of SEQ ID NO: 15; amino acids 10-43 of SEQ ID NO: 15; amino acids 15-43 of SEQ ID NO: 15; amino acids 20-43 of SEQ ID NO: 15; or amino acids 25-43 of SEQ ID NO: 15.
(70) The targeting sequence can also comprise amino acids 1-27 of SEQ ID NO: 17, amino acids 12-27 of SEQ ID NO: 17, or SEQ ID NO: 17, or the exosporium protein can comprise full-length *B. cereus* VD166 exosporium leader peptide (SEQ ID NO:18). A methionine residue linked to amino acids 12-27 of the *B. cereus* VD166 exosporium leader peptide can also be used as a targeting sequence. Thus, the targeting sequence can comprise SEQ ID NO: 100.
(71) The targeting sequence can comprise amino acids 2-27 of SEQ ID NO: 17; amino acids 5-27 of SEQ ID NO: 17; amino acids 8-27 of SEQ ID NO: 17; or amino acids 10-27 of SEQ ID NO: 17.
(72) The targeting sequence can also comprise amino acids 1-33 of SEQ ID NO: 19, amino acids 18-33 of SEQ ID NO: 19, or SEQ ID NO:19, or the exosporium protein can comprise full length *B. cereus* VD200 hypothetical protein IKG_04663 (SEQ ID NO:20).
(73) The targeting sequence can comprise amino acids 2-33 of SEQ ID NO: 19; amino acids 5-33 of SEQ ID NO: 19; amino acids 8-33 of SEQ ID NO: 19; amino acids 10-33 of SEQ ID NO: 19; or amino acids 15-33 of SEQ ID NO: 19.
(74) Alternatively, the targeting sequence comprises amino acids 1-33 of SEQ ID NO: 21, amino acids 18-33 of SEQ ID NO: 21, or SEQ ID NO:21, or the exosporium protein can comprise full length *B. weihenstephensis* KBAB4 YVTN β-propeller protein (SEQ ID NO:22). A methionine residue linked to amino acids 18-33 of the *B. weihenstephensis* KBAB4 YVTN β-propeller protein can also be used as a targeting sequence. Thus, the targeting sequence can comprise SEQ ID NO: 101.
(75) The targeting sequence can comprise amino acids 2-33 of SEQ ID NO: 21; amino acids 5-33 of SEQ ID NO: 21; amino acids 8-33 of SEQ ID NO: 21; amino acids 10-33 of SEQ ID NO: 21; or amino acids 15-33 of SEQ ID NO: 21.
(76) The targeting sequence can also comprise amino acids 1-24 of SEQ ID NO: 23, amino acids 9-24 of SEQ ID NO: 23, or SEQ ID NO:23, or the exosporium protein can comprise full length *B. weihenstephensis* KBAB4 hypothetical protein bcerkbab4_2363 (SEQ ID NO:24). A methionine residue linked to amino acids 9-24 of *B. weihenstephensis* KBAB4 hypothetical protein bcerkbab4_2363 can also be used as a targeting sequence. Thus, the targeting sequence can comprise SEQ ID NO: 102.
(77) The targeting sequence can comprise amino acids 2-24 of SEQ ID NO:23; amino acids 5-24 of SEQ ID NO: 23; or amino acids 8-24 of SEQ ID NO: 23.
(78) The targeting sequence comprise amino acids 1-24 of SEQ ID NO: 25, amino acids 9-24 of SEQ ID NO: 25, or SEQ ID NO: 25, or the exosporium protein can comprise full length *B. weihenstephensis* KBAB4 hypothetical protein bcerkbab4_2131 (SEQ ID NO:26). A methionine residue linked to amino acids 9-24 of *B. weihenstephensis* KBAB4 hypothetical protein bcerkbab4_2131 can also be used as a targeting sequence. Thus, the targeting sequence can comprise SEQ ID NO: 103.
(79) The targeting sequence can comprise amino acids 2-24 of SEQ ID NO: 25; amino acids 5-24 of SEQ ID NO: 25; or amino acids 8-24 of SEQ ID NO: 25.
(80) Alternatively, the targeting sequence comprises amino acids 1-30 of SEQ ID NO: 27, amino acids 15-30 of SEQ ID NO: 27, or SEQ ID NO:27, or the exosporium protein can comprise full length *B. weihenstephensis* KBAB4 triple helix repeat containing collagen (SEQ ID NO:28).
(81) The targeting sequence can comprise amino acids 2-30 of SEQ ID NO: 27; amino acids 5-30 of SEQ ID NO: 27; amino acids 8-30 of SEQ ID NO: 27; or amino acids 10-30 of SEQ ID NO: 27.
(82) The targeting sequence can also comprise amino acids 1-33 of SEQ ID NO: 29, amino acids 18-33 of SEQ ID NO: 29, or SEQ ID NO:29, or the exosporium protein can comprise full length *B. mycoides* 2048 hypothetical protein bmyco0001_21660 (SEQ ID NO:30).
(83) The targeting sequence can comprise amino acids 2-33 of SEQ ID NO: 29; amino acids 5-33 of SEQ ID NO: 29; amino acids 8-33 of SEQ ID NO: 29; amino acids 10-33 of SEQ ID NO: 29; or amino acids 15-33 of SEQ ID NO: 29.
(84) The targeting sequence can also comprise amino acids 1-24 of SEQ ID NO: 31, amino acids 9-24 of SEQ ID NO: 31, or SEQ ID NO:31, or the exosporium protein can comprise full length *B. mycoides* 2048 hypothetical protein bmyc0001_22540 (SEQ ID NO:32). A methionine residue linked to amino acids 9-24 of *B. mycoides* 2048 hypothetical protein bmyc0001_22540 can also be used as a targeting sequence. Thus, the targeting sequence can comprise SEQ ID NO: 104.
(85) The targeting sequence can comprise amino acids 2-24 of SEQ ID NO: 31; amino acids 5-24 of SEQ ID NO: 31; or amino acids 8-24 of SEQ ID NO: 31.
(86) Alternatively, the targeting sequence comprises amino acids 1-15 of SEQ ID NO: 33, SEQ ID NO:33, or the exosporium protein comprises full length *B. mycoides* 2048 hypothetical protein bmyc0001_21510 (SEQ ID NO:34).

(87) The targeting sequence can also comprise amino acids 1-16 of SEQ ID NO: 35, SEQ ID NO:35, or the exosporium protein can comprise full length *B. thuringiensis* 35646 collagen triple helix repeat protein (SEQ ID NO:36).
(88) The targeting sequence can comprise amino acids 1-29 of SEQ ID NO:43, amino acids 14-29 of SEQ ID NO: 43, or SEQ ID NO: 43, or the exosporium protein can comprise full length *B. cereus* hypothetical protein WP_69652 (SEQ ID NO: 44).
(89) The targeting sequence can comprise amino acids 2-29 of SEQ ID NO: 43; amino acids 5-29 of SEQ ID NO: 43; amino acids 8-29 of SEQ ID NO: 43; or amino acids 10-29 of SEQ ID NO: 43.
(90) Alternatively, the targeting sequence can comprise amino acids 1-35 of SEQ ID NO: 45, amino acids 20-35 of SEQ ID NO: 45, or SEQ ID NO: 45, or the exosporium protein can comprise full length *B. cereus* exosporium leader WP016117717 (SEQ ID NO: 46). A methionine residue linked to amino acids 20-35 of *B. cereus* exosporium leader WP016117717 can also be used as a targeting sequence. Thus, the targeting sequence can comprise SEQ ID NO: 106.
(91) The targeting sequence can comprise amino acids 2-35 of SEQ ID NO: 45; amino acids 5-35 of SEQ ID NO: 45; amino acids 8-35 of SEQ ID NO: 45; amino acids 10-35 of SEQ ID NO: 45; or amino acids 15-35 of SEQ ID NO: 45.
(92) The targeting sequence can comprise amino acids 1-43 of SEQ ID NO: 47, amino acids 28-43 of SEQ ID NO: 47, or SEQ ID NO: 47, or the exosporium protein can comprise full length *B. cereus* exosporium peptide WP002105192 (SEQ ID NO: 48).
(93) The targeting sequence can comprise amino acids 2-43 of SEQ ID NO: 47; amino acids 5-43 of SEQ ID NO: 47; amino acids 8-43 of SEQ ID NO: 47; amino acids 10-43 of SEQ ID NO: 47; amino acids 15-43 of SEQ ID NO: 47; amino acids 20-43 of SEQ ID NO: 47; or amino acids 25-43 of SEQ ID NO: 47.
(94) The targeting sequence can comprise amino acids 1-32 of SEQ ID NO: 49, amino acids 17-32 of SEQ ID NO: 49, or SEQ ID NO: 49, or the exosporium protein can comprise full length *B. cereus* hypothetical protein WP87353 (SEQ ID NO: 50).
(95) The targeting sequence can comprise amino acids 2-32 of SEQ ID NO: 49; amino acids 5-32 of SEQ ID NO: 49; amino acids 8-32 of SEQ ID NO: 49; amino acids 10-32 of SEQ ID NO: 49; or amino acids 15-32 of SEQ ID NO: 49.
(96) Alternatively, the targeting sequence can comprise amino acids 1-33 of SEQ ID NO: 51, amino acids 18-33 of SEQ ID NO: 51, or SEQ ID NO: 51, or the exosporium protein can comprise full length *B. cereus* exosporium peptide 02112369 (SEQ ID NO: 52).
(97) The targeting sequence can comprise amino acids 2-33 of SEQ ID NO: 51; amino acids 5-33 of SEQ ID NO: 51; amino acids 8-33 of SEQ ID NO: 51; amino acids 10-33 of SEQ ID NO: 51; or amino acids 15-33 of SEQ ID NO: 51;
(98) The targeting sequence can comprise amino acids 1-33 of SEQ ID NO: 53, amino acids 18-33 of SEQ ID NO: 53, or SEQ ID NO: 53, or the exosporium protein can comprise full length *B. cereus* exosporium protein WP016099770 (SEQ ID NO: 54).
(99) The targeting sequence can comprise amino acids 2-33 of SEQ ID NO: 53; amino acids 5-33 of SEQ ID NO: 53; amino acids 8-33 of SEQ ID NO: 53; amino acids 10-33 of SEQ ID NO: 53; or amino acids 15-33 of SEQ ID NO: 53.
(100) Alternatively, the targeting sequence can comprise acids 1-30 of SEQ ID NO: 55, amino acids 15-30 of SEQ ID NO: 55, or SEQ ID NO: 55, or the exosporium protein can comprise full length *B. thuringiensis* hypothetical protein YP006612525 (SEQ ID NO: 56).
(101) The targeting sequence can comprise amino acids 2-30 of SEQ ID NO: 55; amino acids 5-30 of SEQ ID NO: 55; amino acids 8-30 of SEQ ID NO: 55; or amino acids 10-30 of SEQ ID NO: 55.
(102) The targeting sequence can also comprise amino acids 1-130 of SEQ ID NO: 57, amino acids 115-130 of SEQ ID NO: 57, or SEQ ID NO: 57, or the exosporium protein can comprise full length *B. mycoides* hypothetical protein TIGR03720 (SEQ ID NO: 58).
(103) The targeting sequence can comprise amino acids 2-130 of SEQ ID NO: 57; amino acids 5-130 of SEQ ID NO: 57; amino acids 10-130 of SEQ ID NO: 57; amino acids 20-130 of SEQ ID NO: 57; amino acids 30-130 of SEQ ID NO: 57; amino acids 40-130 of SEQ ID NO: 57; amino acids 50-130 of SEQ ID NO: 57; amino acids 60-130 of SEQ ID NO: 57; amino acids 70-130 of SEQ ID NO: 57; amino acids 80-130 of SEQ ID NO: 57; amino acids 90-130 of SEQ ID NO: 57; amino acids 100-130 of SEQ ID NO: 57; or amino acids 110-130 of SEQ ID NO: 57.
(104) The targeting sequence can comprise amino acids 1-30 of SEQ ID NO: 59; or SEQ ID NO: 59; or the exosporium protein can comprise full length *B. cereus* ATCC 10987 collagen triple helix repeat domain protein (SEQ ID NO: 60).
(105) The targeting sequence can comprise amino acids 2-30 of SEQ ID NO: 59; amino acids 4-30 of SEQ ID NO:

59; or amino acids 6-30 of SEQ ID NO: 59.
(106) The targeting sequence can comprise amino acids 1-33 of SEQ ID NO: 61; amino acids 18-33 of SEQ ID NO: 61; or SEQ ID NO: 61; or the exosporium protein can comprise full length *B. cereus* E33L collagen-like protein (SEQ ID NO: 62).
(107) The targeting sequence can comprise amino acids 2-33 of SEQ ID NO: 61; amino acids 5-33 of SEQ ID NO: 61; amino acids 10-33 of SEQ ID NO: 61; or amino acids 15-33 of SEQ ID NO: 61.
(108) The targeting sequence can comprise amino acids 1-35 of SEQ ID NO: 63; or SEQ ID NO: 63; or the exosporium protein can comprise full length *B. weihenstephanensis* KBAB4 triple helix repeat-containing collagen (SEQ ID NO: 64).
(109) The targeting sequence can comprise amino acids 2-35 of SEQ ID NO: 63; amino acids 5-35 of SEQ ID NO: 63; amino acids 8-35 of SEQ ID NO: 63; amino acids 10-35 of SEQ ID NO: 63; or amino acids 15-35 of SEQ ID NO: 63.
(110) The targeting sequence can comprise amino acids 1-24 of SEQ ID NO: 65; acids 9-24 of SEQ ID NO: 65; SEQ ID NO: 65; or SEQ ID NO: 107; or the exosporium protein can comprise full length *B. thuringiensis* str. Al Hakam hypothetical protein BALH_2230 (SEQ ID NO: 66).
(111) The targeting sequence can comprise amino acids 2-24 of SEQ ID NO: 65; or amino acids 5-24 of SEQ ID NO: 65.
(112) The targeting sequence can comprise acids 1-27 of SEQ ID NO: 67; amino acids 12-27 of SEQ ID NO: 67; or SEQ ID NO: 67; or the exosporium protein can comprise full length *B. cereus* ATCC 14579 triple helix repeat-containing collagen (SEQ ID NO: 68).
(113) The targeting sequence can comprise amino acids 2-27 of SEQ ID NO: 67; amino acids 5-27 of SEQ ID NO: 67; or amino acids 10-27 of SEQ ID NO: 67.
(114) The targeting sequence can comprise amino acids 1-38 of SEQ ID NO: 69; amino acids 23-38 of SEQ ID NO: 69; or SEQ ID NO: 69; or the exosporium protein can comprise full length *B. cereus* collagen triple helix repeat (SEQ ID NO: 70).
(115) The targeting sequence can comprise amino acids 2-38 of SEQ ID NO: 69; amino acids 5-38 of SEQ ID NO: 69; amino acids 10-38 of SEQ ID NO: 69; or amino acids 15-38 of SEQ ID NO: 69.
(116) The exosporium protein can comprise full length *B. cereus* ATCC 14579 triple helix repeat-containing collagen (SEQ ID NO: 72).
(117) The targeting sequence can comprise SEQ ID NO: 73, or the exosporium protein can comprise full length *B. cereus* E33L hypothetical protein BCZK1835 (SEQ ID NO: 74).
(118) The targeting sequence can comprise amino acids 1-42 of SEQ ID NO: 75; amino acids 27-42 of SEQ ID NO: 75; or SEQ ID NO: 75; or the exosporium protein can comprise full length *B. weihenstephanensis* KBAB4 triple helix repeat-containing collagen (SEQ ID NO: 76).
(119) The targeting sequence can comprise amino acids 2-42 of SEQ ID NO: 75; amino acids 5-42 of SEQ ID NO: 75; amino acids 10-42 of SEQ ID NO: 75; amino acids 15-42 of SEQ ID NO: 75; amino acids 20-42 of SEQ ID NO: 75; or amino acids 25-42 of SEQ ID NO: 75.
(120) The targeting sequence can comprise amino acids 1-24 of SEQ ID NO: 77; amino acids 9-24 of SEQ ID NO: 77; or SEQ ID NO: 77; or the exosporium protein can comprise full length *B. cereus* ATCC 14579 triple helix repeat-containing collagen (SEQ ID NO: 78).
(121) The targeting sequence can comprise amino acids 2-24 of SEQ ID NO: 77; or amino acids 5-24 of SEQ ID NO: 77;
(122) The exosporium protein can comprise full length *B. cereus* ATCC 14579 hypothetical protein BC4725 (SEQ ID NO: 80).
(123) The targeting sequence can comprise amino acids 1-38 of SEQ ID NO: 81; amino acids 23-38 of SEQ ID NO: 81; or SEQ ID NO: 81; or the exosporium protein can comprise full length *B. cereus* E33L hypothetical protein BCZK4476 (SEQ ID NO: 82).
(124) The targeting sequence can comprise amino acids 2-38 of SEQ ID NO: 81; acids 5-38 of SEQ ID NO: 81; amino acids 10-38 of SEQ ID NO: 81; amino acids 15-38 of SEQ ID NO: 81; or amino acids 20-38 of SEQ ID NO: 81.
(125) The targeting sequence can comprise amino acids 1-34 of SEQ ID NO: 83; or SEQ ID NO: 83; or the exosporium protein can comprise full length *B. anthracis* str. ‘Ames Ancestor’ triple helix repeat-containing collagen (SEQ ID NO: 84).
(126) The exosporium protein can comprise full length *B. thuringiensis* serovar konkukian str. 97-27 BclA protein (SEQ ID NO: 86).
(127) The targeting sequence can comprise amino acids 1-28 of SEQ ID NO: 87; amino acids 13-28 of SEQ ID NO: 87; or SEQ ID NO: 87; or the exosporium protein can comprise full length *B. cereus* ATCC 10987 conserved hypothetical protein (SEQ ID NO: 88).

(128) The targeting sequence can comprise amino acids 2-28 of SEQ ID NO: 87; amino acids 5-28 of SEQ ID NO: 87; or amino acids 10-28 of SEQ ID NO: 87.
(129) The targeting sequence can comprise amino acids 1-28 of SEQ ID NO: 89; or SEQ ID NO: 89; or the exosporium protein can comprise full length *B. cereus* ATCC 14579 triple helix repeat-containing collagen (SEQ ID NO: 90).
(130) The targeting sequence can comprise amino acids 2-28 of SEQ ID NO: 89; amino acids 5-28 of SEQ ID NO: 89; or amino acids 10-28 of SEQ ID NO: 89
(131) The targeting sequence can comprise amino acids 1-93 of SEQ ID NO: 91; or SEQ ID NO: 91; or the exosporium protein can comprise *B. cereus* exosporium leader peptide partial sequence (SEQ ID NO: 92).
(132) The targeting sequence can comprise amino acids 2-93 of SEQ ID NO: 91; amino acids 10-93 of SEQ ID NO: 91; amino acids 20-93 of SEQ ID NO: 91; amino acids 30-93 of SEQ ID NO: 91; amino acids 40-93 of SEQ ID NO: 91; amino acids 50-93 of SEQ ID NO: 91; or amino acids 60-93 of SEQ ID NO: 91.
(133) The targeting sequence can comprise amino acids 1-130 of SEQ ID NO: 93; or SEQ ID NO: 93; or the exosporium protein can comprise *B. weihenstephanensis*) hypothetical protein ER45_27600, partial sequence (SEQ ID NO: 94).
(134) The targeting sequence can comprise amino acids 2-130 of SEQ ID NO: 93; amino acids 10-130 of SEQ ID NO: 93; amino acids 20-130 of SEQ ID NO: 93; or amino acids 30-130 of SEQ ID NO: 93.
(135) Furthermore, as illustrated in the Examples provided hereinbelow, it has been found that sequences shorter than amino acids 20-35 of BclA can be used to target a fusion protein to the exosporium of a recombinant *Bacillus cereus* family member. In particular, amino acids 20-33 of BclA, amino acids 20-31 of BclA, amino acids 21-33 of BclA, or amino acids 23-31 of BclA can be used to target a fusion protein to the exosporium of a recombinant *Bacillus cereus* family member. Thus, the targeting sequence can consist of amino acids 20-33 of SEQ ID NO: 1, amino acids 20-31 of SEQ ID NO: 1, amino acids 21-33 of SEQ ID NO: 1, or amino acids 23-31 of SEQ ID NO: 1. The corresponding regions of any of the SEQ ID NOs. shown in FIGS. **1**A and **1**B can also be used to target a fusion protein to the exosporium of a recombinant *Bacillus cereus* family member. By "corresponding regions," it is meant that when the sequences are aligned with SEQ ID NO: 1, as shown in FIGS. **1**A and **1**B, the regions of the other amino acid sequences that align with the amino acids of SEQ ID NO: are the "corresponding regions" of those sequences. Thus, for example, amino acids 12-25 of SEQ ID NO: 3, amino acids 23-36 of SEQ ID NO: 5, amino acids 13-26 of SEQ ID NO: 7, etc. can be used to target a fusion protein to the exosporium of a recombinant *Bacillus cereus* family member, since these regions align with amino acids 20-33 of SEQ ID NO: 1 as shown in FIG. **1**A.
(136) Even shorter regions within amino acids 20-35 of BclA can also be used for targeting a fusion protein to the exosporium of a recombinant *Bacillus cereus* family member. In particular, any amino acid sequence that includes amino acids 25-30 of SEQ ID NO: 1 or the corresponding amino acids from any of the sequences shown in FIGS. **1**A and **1**B can be used. A skilled person will recognize that starting with amino acids 25-30 of SEQ ID NO: 1 or the corresponding region of any of the sequences shown in FIGS. **1**A and **1**B, additional amino acids can be added to the amino-terminus, the carboxy terminus, or both the amino- and carboxy termini to create a targeting sequence that will be effective for targeting a fusion protein to the exosporium of a recombinant *Bacillus cereus* family member.
(137) In addition, it can readily be seen from the sequence alignment in FIGS. **1**A and **1**B that while amino acids 20-35 of BclA are conserved, and amino acids 25-35 are more conserved, some degree of variation can occur in this region without affecting the ability of the targeting sequence to target a protein to the exosporium. FIGS. **1**A and **1**B list the percent identity of each of corresponding amino acids of each sequence to amino acids 20-35 of BclA ("20-35% Identity") and to amino acids 25-35 of BclA ("25-35% Identity"). Thus, for example, as compared to amino acids 20-35 of BclA, the corresponding amino acids of BetA/BAS3290 are about 81.3% identical, the corresponding amino acids of BAS4623 are about 50.0% identical, the corresponding amino acids of BclB are about 43.8% identical, the corresponding amino acids of BAS1882 are about 62.5% identical, the corresponding amino acids of the KBAB4 2280 gene product are about 81.3% identical, and the corresponding amino acids of the KBAB4 3572 gene product are about 81.3% identical. The sequence identities over this region for the remaining sequences are listed in FIGS. **1**A and **1**B.
(138) With respect to amino acids 25-35 of BclA, the corresponding amino acids of BetA/BAS3290 are about 90.9% identical, the corresponding amino acids of BAS4623 are about 72.7% identical, the corresponding amino acids of BclB are about 54.5% identical, the corresponding amino acids of BAS1882 are about 72.7% identical, the corresponding amino acids of the KBAB4 2280 gene product are about 90.9% identical, and the corresponding amino acids of the KBAB4 3572 gene product are about 81.8% identical. The sequence identities over this region for the remaining sequences are listed in FIGS. **1**A and **1**B.
(139) Thus, the targeting sequence can comprise an amino acid sequence having at least about 43% identity with amino acids 20-35 of SEQ ID NO: 1, wherein the identity with amino acids 25-35 is at least about 54%.

Alternatively, the targeting sequence consists of an amino acid sequence consisting of 16 amino acids and having at least about 43% identity with amino acids 20-35 of SEQ ID NO: 1, wherein the identity with amino acids 25-35 is at least about 54%.

(140) The targeting sequence can also comprise an amino acid sequence having at least about 50% identity with amino acids 20-35 of SEQ ID NO: 1, wherein the identity with amino acids 25-35 is at least about 63%. Alternatively the targeting sequence consists of an amino acid sequence consisting of 16 amino acids and having at least about 50% identity with amino acids 20-35 of SEQ ID NO: 1, wherein the identity with amino acids 25-35 is at least about 63%.

(141) The targeting sequence can also comprise an amino acid sequence having at least about 50% identity with amino acids 20-35 of SEQ ID NO: 1, wherein the identity with amino acids 25-35 is at least about 72%. Alternatively, the targeting sequence consists of an amino acid sequence consisting of 16 amino acids and having at least about 50% identity with amino acids 20-35 of SEQ ID NO: 1, wherein the identity with amino acids 25-35 is at least about 72%.

(142) The targeting sequence can also comprise an amino acid sequence having at least about 56% identity with amino acids 20-35 of SEQ ID NO: 1, wherein the identity with amino acids 25-35 is at least about 63%. Alternatively, the targeting sequence consists of an amino acid sequence consisting of 16 amino acids and having at least about 56% identity with amino acids 20-35 of SEQ ID NO: 1, wherein the identity with amino acids 25-35 is at least about 63%.

(143) Alternatively, the targeting sequence can comprise an amino sequence having at least about 62% identity with amino acids 20-35 of SEQ ID NO: 1, wherein the identity with amino acids 25-35 is at least about 72%. The targeting sequence can also consist of an amino acid sequence consisting of 16 amino acids and having at least about 62% identity with amino acids 20-35 of SEQ ID NO: 1, wherein the identity with amino acids 25-35 of SEQ ID NO: 1 is at least about 72%.

(144) The targeting sequence can comprise an amino acid sequence having at least 68% identity with amino acids 20-35 of SEQ ID NO: 1, wherein the identity with amino acids 25-35 is at least about 81%. Alternatively, the targeting sequence consists of an amino acid sequence consisting of 16 amino acids and having at least 68% identity with amino acids 20-35 of SEQ ID NO: 1, wherein the identity with amino acids 25-35 is at least about 81%.

(145) The targeting sequence can also comprises an amino sequence having at least about 75% identity with amino acids 20-35 of SEQ ID NO: 1, wherein the identity with amino acids 25-35 is at least about 72%. Alternatively, the targeting sequence consists of an amino acid sequence consisting of 16 amino acids and having at least about 75% identity with amino acids 20-35 of SEQ ID NO: 1, wherein the identity with amino acids 25-35 of SEQ ID NO:1 is at least about 72%.

(146) The targeting sequence can also comprise an amino sequence having at least about 75% identity with amino acids 20-35 of SEQ ID NO: 1, wherein the identity with amino acids 25-35 is at least about 81%. Alternatively, the targeting sequence consists of an amino acid sequence consisting of 16 amino acids and having at least about 75% identity with amino acids 20-35 of SEQ ID NO: 1, wherein the identity with amino acids 25-35 of SEQ ID NO: 1 is at least about 81%.

(147) The targeting sequence can also comprise an amino acid sequence having at least about 81% identity with amino acids 20-35 of SEQ ID NO:1, wherein the identity with amino acids 25-35 is at least about 81%. Alternatively, the targeting sequence consists of an amino acid sequence consisting of 16 amino acids and having at least about 81% identity with amino acids 20-35 of SEQ ID NO:1, wherein the identity with amino acids 25-35 is at least about 81%.

(148) The targeting sequence can comprise an amino acid sequence having at least about 81% identity with amino acids 20-35 of SEQ ID NO: 1, wherein the identity with amino acids 25-35 is at least about 90%. Alternatively, the targeting sequence consists of an amino acid sequence consisting of 16 amino acids and having at least about 81% identity with amino acids 20-35 of SEQ ID NO: 1, wherein the identity with amino acids 25-35 is at least about 90%.

(149) The skilled person will recognize that variants of the above sequences can also be used as targeting sequences, so long as the targeting sequence comprises amino acids 20-35 of BclA, the corresponding amino acids of BetA/BAS3290, BAS4263, BclB, BAS1882, the KBAB4 2280 gene product, or the KBAB 3572 gene product, or a sequence comprising any of the above noted sequence identities to amino acids 20-35 and 25-35 of BclA is present.

(150) Certain *Bacillus cereus* family exosporium proteins which lack regions having homology to amino acids 25-35 of BclA can also be used to target a peptide or protein to the exosporium of a *Bacillus cereus* family member. In particular, the fusion proteins can comprise an exosporium protein comprising SEQ ID NO: 108 (*B. mycoides* InhA), an exosporium protein comprising SEQ ID NO: 109 (*B. anthracis* Sterne BAS1141 (ExsY)), an exosporium protein comprising SEQ ID NO: 110 (*B. anthracis* Sterne BAS1144 (BxpB/ExsFA)), an exosporium protein comprising SEQ ID NO: 111 (*B. anthracis* Sterne BAS1145 (CotY)), an exosporium protein comprising SEQ ID NO: 112 (*B. anthracis* Sterne BAS1140), an exosporium protein comprising SEQ ID NO: 113 (*B. anthracis* H9401

ExsFB), an exosporium protein comprising SEQ ID NO: 114 (*B. thuringiensis* HD74 InhA1), an exosporium protein comprising SEQ ID NO: 115 (*B. cereus* ATCC 10876 ExsJ), an exosporium protein comprising SEQ ID NO: 116 (*B. cereus* ExsH), an exosporium protein comprising SEQ ID NO: 117 (*B. anthracis* Ames YjcA), an exosporium protein comprising SEQ ID NO: 118 (*B. anthracis* YjcB), an exosporium protein comprising SEQ ID NO: 119 (*B. anthracis* Sterne BclC), an exosporium protein comprising SEQ ID NO: 120 (*Bacillus thuringiensis* serovar konkukian str. 97-27 acid phosphatase), an exosporium protein comprising SEQ ID NO: 121 (*B. thuringiensis* HD74 InhA2), or an exosporium protein comprising SEQ ID NO: 122 (*B. mycoides* InhA3). Inclusion of an exosporium protein comprising any of SEQ ID NOs: 108-122 in the fusion proteins described herein will result in targeting to the exosporium of a *B. cereus* family member.

(151) Moreover, exosporium proteins having a high degree of sequence identity with any of the full-length exosporium proteins or the exosporium protein fragments described above can also be used to target a peptide or protein to the exosporium of a *Bacillus cereus* family member. Thus, the fusion protein can comprise an exosporium protein or exosporium protein fragment comprising an amino acid sequence having at least 85% identity with any one of SEQ ID NOs: 2, 4, 6, 8, 10, 12, 14, 16, 18, 20, 22, 24, 26, 28, 30, 32, 34, 36, 44, 46, 48, 50, 52, 54, 56, 58, 60, 62, 64, 66, 68, 70, 72, 74, 76, 78, 80, 82, 84, 86, 88, 90, 92, 94, 95, 108, 109, 110, 111, 112, 113, 114, 115, 116, 117, 118, 119, 120, 121, and 122. Alternatively, the fusion protein can comprise an exosporium protein having at least 90%, at least 95%, at least 98%, at least 99%, or 100% identity with any one of SEQ ID NOs: 2, 4, 6, 8, 10, 12, 14, 16, 18, 20, 22, 24, 26, 28, 30, 32, 34, 36, 44, 46, 48, 50, 52, 54, 56, 58, 60, 62, 64, 66, 68, 70, 72, 74, 76, 78, 80, 82, 84, 86, 88, 90, 92, 94, 95, 108, 109, 110, 111, 112, 113, 114, 115, 116, 117, 118, 119, 120, 121, and 122.

(152) During sporulation of a recombinant *Bacillus cereus* family member expressing any of the fusion proteins described herein, the targeting motif, exosporium protein, or exosporium protein fragment is recognized by the spore exosporium assembly machinery and directed to the exosporium, resulting in display of the protein or peptide of interest portion of the fusion protein on the outside of the spore.

(153) As illustrated further by the Examples provided hereinbelow, the use of different targeting sequences allows for control of the expression level of the fusion protein on the surface of the *Bacillus cereus* family member spore. Use of certain of the targeting sequences described herein will result in a higher level of expression of the fusion protein, whereas use of others of the targeting sequences will result in lower levels of expression of the fusion protein on the surface of the spore.

(154) In any of the fusion proteins described herein, the targeting sequence, exosporium protein, or exosporium protein fragment can comprise the amino acid sequence GXT at its carboxy terminus, wherein X is any amino acid.

(155) In any of the fusion proteins described herein, the targeting sequence, exosporium protein, or exosporium protein fragment, can comprise an alanine residue at the position of the targeting sequence that corresponds to amino acid 20 of SEQ ID NO: 1.

(156) In any of the fusion proteins described herein, the targeting sequence, exosporium protein, or exosporium protein fragment can further comprise a methionine, serine, or threonine residue at the amino acid position immediately preceding the first amino acid of the targeting sequence, exosporium protein, or exosporium protein fragment or at the position of the targeting sequence that corresponds to amino acid 20 of SEQ ID NO: 1.

(157) B. Fusion Proteins for Expression in Recombinant *Bacillus cereus* Family Members

(158) The present invention relates to fusion proteins comprising at least one protein or peptide of interest and a targeting sequence or exosporium protein. When the protein or peptide of interest is any protein or peptide of interest, the fusion protein can comprise: (1) a targeting sequence comprising amino acids 1-30 of SEQ ID NO: 59; (2) a targeting sequence comprising SEQ ID NO: 59; (3) an exosporium protein comprising an amino acid sequence having at least 85% identity with SEQ ID NO: 60; (4) a targeting sequence comprising amino acids 2-30 of SEQ ID NO: 59; (5) a targeting sequence comprising amino acids 4-30 of SEQ ID NO: 59; (6) a targeting sequence comprising amino acids 6-30 of SEQ ID NO: 59; (7) a targeting sequence comprising amino acids 1-33 of SEQ ID NO: 61; (8) a targeting sequence comprising amino acids 18-33 of SEQ ID NO: 61; (9) a targeting sequence comprising SEQ ID NO: 61; (10) an exosporium protein comprising an amino acid sequence having at least 85% sequence identity with SEQ ID NO: 62; (11) a targeting sequence comprising amino acids 2-33 of SEQ ID NO: 61; (12) a targeting sequence comprising amino acids 5-33 of SEQ ID NO: 61; (13) a targeting sequence comprising amino acids 10-33 of SEQ ID NO: 61; (14) a targeting sequence comprising amino acids 15-33 of SEQ ID NO: 61; (15) a targeting sequence comprising amino acids 1-35 of SEQ ID NO: 63; (16) a targeting sequence comprising SEQ ID NO: 63; (17) an exosporium protein comprising an amino acid sequence having at least 85% identity with SEQ ID NO: 64; (18) a targeting sequence comprising amino acids 2-35 of SEQ ID NO: 63; (19) a targeting sequence comprising amino acids 5-35 of SEQ ID NO: 63; (20) a targeting sequence comprising amino acids 8-35 of SEQ ID NO: 63; (21) a targeting sequence comprising amino acids 10-35 of SEQ ID NO: 63; (22) a targeting sequence comprising amino acids 15-35 of SEQ ID NO: 63; (23) a targeting sequence comprising amino acids 1-24 of SEQ ID NO: 65; (24) a targeting sequence comprising amino acids 9-24 of SEQ ID NO: 65; (25) a targeting sequence comprising SEQ ID NO: 65; (26) an exosporium protein comprising an amino acid sequence having at

least 85% identity with SEQ ID NO: 66; (27) a targeting sequence comprising SEQ ID NO: 107; (28) a targeting sequence comprising amino acids 2-24 of SEQ ID NO: 65; (29) a targeting sequence comprising amino acids 5-24 of SEQ ID NO: 65; (30) a targeting sequence comprising amino acids 1-27 of SEQ ID NO: 67; (31) a targeting sequence comprising amino acids 12-27 of SEQ ID NO: 67; (32) a targeting sequence comprising SEQ ID NO: 67; (33) an exosporium protein comprising an amino acid sequence having at least 85% identity with SEQ ID NO: 68; (34) an targeting sequence comprising amino acids 2-27 of SEQ ID NO: 67; (35) a targeting sequence comprising amino acids 5-27 of SEQ ID NO: 67; (36) a targeting sequence comprising amino acids 10-27 of SEQ ID NO: 67; (37) a targeting sequence comprising amino acids 1-38 of SEQ ID NO: 69; (38) a targeting sequence comprising amino acids 23-38 of SEQ ID NO: 69; (39) a targeting sequence comprising SEQ ID NO: 69; (40) an exosporium protein comprising an amino acid sequence having at least 85% identity with SEQ ID NO: 70; (41) a targeting sequence comprising amino acids 2-38 of SEQ ID NO: 69; (42) a targeting sequence comprising amino acids 5-38 of SEQ ID NO: 69; (43) a targeting sequence comprising amino acids 10-38 of SEQ ID NO: 69; (44) a targeting sequence comprising amino acids 15-38 of SEQ ID NO: 69; (45) an exosporium protein comprising SEQ ID NO: 72; (46) a targeting sequence comprising SEQ ID NO: 73; (47) an exosporium protein comprising an amino acid sequence having at least 95% identity with SEQ ID NO: 74; (48) a targeting sequence comprising amino acids 1-42 of SEQ ID NO: 75; (49) a targeting sequence comprising amino acids 27-42 of SEQ ID NO: 75; (50) a targeting sequence comprising SEQ ID NO: 75; (51) an exosporium protein comprising an amino acid sequence having at least 85% identity with SEQ ID NO: 76; (52) a targeting sequence comprising amino acids 2-42 of SEQ ID NO: 75; (53) a targeting sequence comprising amino acids 5-42 of SEQ ID NO: 75; (54) a targeting sequence comprising amino acids 10-42 of SEQ ID NO: 75; (55) a targeting sequence comprising amino acids 15-42 of SEQ ID NO: 75; (56) a targeting sequence comprising amino acids 20-42 of SEQ ID NO: 75; (57) a targeting sequence comprising amino acids 25-42 of SEQ ID NO: 75; (58) a targeting sequence comprising amino acids 1-24 of SEQ ID NO: 77; (59) a targeting sequence comprising amino acids 9-24 of SEQ ID NO: 77; (60) a targeting sequence comprising SEQ ID NO: 77; (61) an exosporium protein comprising an amino acid sequence having at least 85% identity with SEQ ID NO: 78; (62) a targeting sequence comprising amino acids 2-24 of SEQ ID NO: 77; (63) a targeting sequence comprising amino acids 5-24 of SEQ ID NO: 77; (64) an exosporium protein comprising an amino acid sequence having at least 85% identity with SEQ ID NO: 80; (65) a targeting sequence comprising amino acids 1-38 of SEQ ID NO: 81; (66) a targeting sequence comprising amino acids 23-38 of SEQ ID NO: 81; (67) a targeting sequence comprising SEQ ID NO: 81; (68) an exosporium protein comprising an amino acid sequence having at least 85% identity with SEQ ID NO: 82; (69) a targeting sequence comprising amino acids 2-38 of SEQ ID NO: 81; (70) a targeting sequence comprising amino acids 5-38 of SEQ ID NO: 81; (71) a targeting sequence comprising amino acids 10-38 of SEQ ID NO: 81; (72) a targeting sequence comprising amino acids 15-38 of SEQ ID NO: 81; (73) a targeting sequence comprising amino acids 20-38 of SEQ ID NO: 81; (74) a targeting sequence comprising amino acids 1-34 of SEQ ID NO: 83; (75) a targeting sequence comprising SEQ ID NO: 83; (76) an exosporium protein comprising an amino acid sequence having at least 85% identity with SEQ ID NO: 84; (77) an exosporium protein comprising an amino acid sequence having at least 85% identity with SEQ ID NO: 86; (78) a targeting sequence comprising amino acids 1-28 of SEQ ID NO: 87; (79) a targeting sequence comprising amino acids 13-28 of SEQ ID NO: 87; (80) a targeting sequence comprising SEQ ID NO: 87; (81) an exosporium protein comprising an amino acid sequence having at least 85% identity with SEQ ID NO: 88; (82) a targeting sequence comprising amino acids 2-28 of SEQ ID NO: 87; (83) a targeting sequence comprising amino acids 5-28 of SEQ ID NO: 87; (84) a targeting sequence comprising amino acids 10-28 of SEQ ID NO: 87; (85) a targeting sequence comprising amino acids 1-28 of SEQ ID NO: 89; (86) a targeting sequence comprising SEQ ID NO: 89; (87) an exosporium protein comprising an amino acid sequence having at least 85% identity with SEQ ID NO: 90; (88) a targeting sequence comprising amino acids 2-28 of SEQ ID NO: 89; (89) a targeting sequence comprising amino acids 5-28 of SEQ ID NO: 89; (90) a targeting sequence comprising amino acids 10-28 of SEQ ID NO: 89; (91) a targeting sequence comprising amino acids 1-93 of SEQ ID NO: 91; (92) a targeting sequence comprising SEQ ID NO: 91; (93) an exosporium protein comprising an amino acid sequence having at least 85% identity with SEQ ID NO: 92; (94) a targeting sequence comprising amino acids 2-93 of SEQ ID NO: 91; (95) a targeting sequence comprising amino acids 10-93 of SEQ ID NO: 91; (96) a targeting sequence comprising amino acids 20-93 of SEQ ID NO: 91; (97) a targeting sequence comprising amino acids 30-93 of SEQ ID NO: 91; (98) a targeting sequence comprising amino acids 40-93 of SEQ ID NO: 91; (99) a targeting sequence comprising amino acids 50-93 of SEQ ID NO: 91; (100) a targeting sequence comprising amino acids 60-93 of SEQ ID NO: 91; (101) a targeting sequence comprising amino acids 1-130 of SEQ ID NO: 93; (102) a targeting sequence comprising SEQ ID NO: 93; (103) an exosporium protein comprising an amino acid sequence having at least 85% identity with SEQ ID NO: 94; (104) a targeting sequence comprising amino acids 2-130 of SEQ ID NO: 93; (105) a targeting sequence comprising amino acids 10-130 of SEQ ID NO: 93; (106) a targeting sequence comprising amino acids 20-130 of SEQ ID NO: 93; (107) a targeting sequence comprising amino acids 30-130 of SEQ ID NO: 93; or (108) an exosporium protein comprising an amino acid sequence having at least 85% sequence identity with SEQ ID NO: 122.

(159) For example, when the protein or peptide of interest is any protein or peptide of interest, the fusion protein can comprise: (1) a targeting sequence comprising amino acids 2-30 of SEQ ID NO: 59; (2) a targeting sequence comprising amino acids 4-30 of SEQ ID NO: 59; (3) a targeting sequence comprising amino acids 6-30 of SEQ ID NO: 59; (4) a targeting sequence comprising amino acids 2-33 of SEQ ID NO: 61; (5) a targeting sequence comprising amino acids 5-33 of SEQ ID NO: 61; (6) a targeting sequence comprising amino acids 10-33 of SEQ ID NO: 61; (7) a targeting sequence comprising amino acids 15-33 of SEQ ID NO: 61; (8) a targeting sequence comprising amino acids 2-35 of SEQ ID NO: 63; (9) a targeting sequence comprising amino acids 5-35 of SEQ ID NO: 63; (10) a targeting sequence comprising amino acids 8-35 of SEQ ID NO: 63; (11) a targeting sequence comprising amino acids 10-35 of SEQ ID NO: 63; (12) a targeting sequence comprising amino acids 15-35 of SEQ ID NO: 63; (13) a targeting sequence comprising amino acids 2-24 of SEQ ID NO: 65; (14) a targeting sequence comprising amino acids 5-24 of SEQ ID NO: 65; (15) a targeting sequence comprising amino acids 2-27 of SEQ ID NO: 67; (16) a targeting sequence comprising amino acids 5-27 of SEQ ID NO: 67; (17) a targeting sequence comprising amino acids 10-27 of SEQ ID NO: 67; (18) a targeting sequence comprising amino acids 2-38 of SEQ ID NO: 69; (19) a targeting sequence comprising amino acids 5-38 of SEQ ID NO: 69; (20) a targeting sequence comprising amino acids 10-38 of SEQ ID NO: 69; (21) a targeting sequence comprising amino acids 15-38 of SEQ ID NO: 69; (22) a targeting sequence comprising amino acids 2-42 of SEQ ID NO: 75; (23) a targeting sequence comprising amino acids 5-42 of SEQ ID NO: 75; (24) a targeting sequence comprising amino acids 10-42 of SEQ ID NO: 75; (25) a targeting sequence comprising amino acids 15-42 of SEQ ID NO: 75; (26) a targeting sequence comprising amino acids 20-42 of SEQ ID NO: 75; (27) a targeting sequence comprising amino acids 25-42 of SEQ ID NO: 75; (28) a targeting sequence comprising amino acids 2-24 of SEQ ID NO: 77; (29) a targeting sequence comprising amino acids 5-24 of SEQ ID NO: 77; (30) a targeting sequence comprising amino acids 2-38 of SEQ ID NO: 81; (31) a targeting sequence comprising amino acids 5-38 of SEQ ID NO: 81; (32) a targeting sequence comprising amino acids 10-38 of SEQ ID NO: 81; (33) a targeting sequence comprising amino acids 15-38 of SEQ ID NO: 81; (34) a targeting sequence comprising amino acids 20-38 of SEQ ID NO: 81; (35) a targeting sequence comprising amino acids 2-28 of SEQ ID NO: 87; (36) a targeting sequence comprising amino acids 5-28 of SEQ ID NO: 87; (37) a targeting sequence comprising amino acids 10-28 of SEQ ID NO: 87; (38) a targeting sequence comprising amino acids 2-28 of SEQ ID NO: 89; (39) a targeting sequence comprising amino acids 5-28 of SEQ ID NO: 89; (40) a targeting sequence comprising amino acids 10-28 of SEQ ID NO: 89; (41) a targeting sequence comprising amino acids 2-93 of SEQ ID NO: 91; (42) a targeting sequence comprising amino acids 10-93 of SEQ ID NO: 91; (43) a targeting sequence comprising amino acids 20-93 of SEQ ID NO: 91; (44) a targeting sequence comprising amino acids 30-93 of SEQ ID NO: 91; (45) a targeting sequence comprising amino acids 40-93 of SEQ ID NO: 91; (46) a targeting sequence comprising amino acids 50-93 of SEQ ID NO: 91; (47) a targeting sequence comprising amino acids 60-93 of SEQ ID NO: 91; (48) a targeting sequence comprising amino acids 2-130 of SEQ ID NO: 93; (49) a targeting sequence comprising amino acids 10-130 of SEQ ID NO: 93; (50) a targeting sequence comprising amino acids 20-130 of SEQ ID NO: 93; or (51) a targeting sequence comprising amino acids 30-130 of SEQ ID NO: 93.

(160) Alternatively, when the protein or peptide of interest is any protein or peptide of interest, the fusion protein can comprise: (1) a targeting sequence consisting of amino acids 20-33 of SEQ ID NO: 1; (2) a targeting sequence consisting of amino acids 21-33 of SEQ ID NO: 1; (3) a targeting sequence consisting of amino acids 23-31 of SEQ ID NO: 1; (4) a targeting sequence consisting of amino acids 1-15 of SEQ ID NO: 96; (5) a targeting sequence consisting of amino acids 1-13 of SEQ ID NO: 96; (6) a targeting sequence consisting of amino acids 12-25 of SEQ ID NO: 3; (7) a targeting sequence consisting of amino acids 13-25 of SEQ ID NO: 3; (8) a targeting sequence consisting of amino acids 15-23 of SEQ ID NO: 3; (9) a targeting sequence consisting of amino acids 1-15 of SEQ ID NO: 97; (10) a targeting sequence consisting of amino acids 1-13 of SEQ ID NO: 98; (11) a targeting sequence consisting of amino acids 23-36 of SEQ ID NO: 5; (12) a targeting sequence consisting of amino acids 23-34 of SEQ ID NO: 5; (13) a targeting sequence consisting of amino acids 24-36 of SEQ ID NO: 5; (14) a targeting sequence consisting of amino acids 26-34 of SEQ ID NO: 5; (15) a targeting sequence consisting of amino acids 13-26 of SEQ ID NO: 7; (16) a targeting sequence consisting of amino acids 13-24 of SEQ ID NO: 7; (17) a targeting sequence consisting of amino acids 14-26 of SEQ ID NO: 7; (18) a targeting sequence consisting of amino acids 16-24 of SEQ ID NO: 7; (19) a targeting sequence consisting of amino acids 9-22 of SEQ ID NO: 9; (20) a targeting sequence consisting of amino acids 9-20 of SEQ ID NO: 9; (21) a targeting sequence consisting of amino acids 10-22 of SEQ ID NO: 9; (22) a targeting sequence consisting of amino acids 12-20 of SEQ ID NO: 9; (23) a targeting sequence consisting of amino acids 1-15 of SEQ ID NO: 105; (24) a targeting sequence consisting of amino acids 1-13 of SEQ ID NO: 105; (25) a targeting sequence consisting of amino acids 18-31 of SEQ ID NO: 11; (26) a targeting sequence consisting of amino acids 18-29 of SEQ ID NO: 11; (27) a targeting sequence consisting of amino acids 19-31 of SEQ ID NO: 11; (28) a targeting sequence consisting of amino acids 1-15 of SEQ ID NO: 98; (29) a targeting sequence consisting of amino acids 1-13 of SEQ ID NO: 98; (30) a targeting sequence consisting of amino acids 18-31 of SEQ ID NO: 13; (31) a targeting sequence consisting of amino acids 18-29 of SEQ ID NO:

13; (32) a targeting sequence consisting of amino acids 19-31 of SEQ ID NO: 13; (33) a targeting sequence consisting of amino acids 21-29 of SEQ ID NO: 13; (34) a targeting sequence consisting of amino acids 1-15 of SEQ ID NO: 99; (35) a targeting sequence consisting of amino acids 1-13 of SEQ ID NO: 99; (36) a targeting sequence consisting of amino acids 28-41 of SEQ ID NO: 15; (37) a targeting sequence consisting of amino acids 28-39 of SEQ ID NO: 15; (38) a targeting sequence consisting of amino acids 29-41 of SEQ ID NO: 15; (39) a targeting sequence consisting of amino acids 31-39 of SEQ ID NO: 15; (40) a targeting sequence consisting of amino acids 12-25 of SEQ ID NO: 17; (41) a targeting sequence consisting of amino acids 13-25 of SEQ ID NO: 17; (42) a targeting sequence consisting of amino acids 1-15 of SEQ ID NO: 100; (43) a targeting sequence consisting of amino acids 18-31 of SEQ ID NO: 19; (44) a targeting sequence consisting of amino acids 18-29 of SEQ ID NO: 19; (45) a targeting sequence consisting of amino acids 19-31 of SEQ ID NO: 19; (46) a targeting sequence consisting of amino acids 21-29 of SEQ ID NO: 19; (47) a targeting sequence consisting of amino acids 18-31 of SEQ ID NO: 21; (48) a targeting sequence consisting of amino acids 18-29 of SEQ ID NO: 21; (49) a targeting sequence consisting of amino acids 19-31 of SEQ ID NO: 21; (50) a targeting sequence consisting of amino acids 21-29 of SEQ ID NO: 21; (51) a targeting sequence consisting of amino acids 1-15 of SEQ ID NO: 101; (52) a targeting sequence consisting of amino acids 1-13 of SEQ ID NO: 101; (53) a targeting sequence consisting of amino acids 9-22 of SEQ ID NO: 23; (54) a targeting sequence consisting of amino acids 9-20 of SEQ ID NO: 23; (55) a targeting sequence consisting of amino acids 10-22 of SEQ ID NO: 23; (56) a targeting sequence consisting of amino acids 12-20 of SEQ ID NO: 23; (57) a targeting sequence consisting of amino acids 1-15 of SEQ ID NO: 102; (58) a targeting sequence consisting of amino acids 1-13 of SEQ ID NO: 102; (59) a targeting sequence consisting of amino acids 9-22 of SEQ ID NO: 25; (60) a targeting sequence consisting of amino acids 9-20 of SEQ ID NO: 25; (61) a targeting sequence consisting of amino acids 10-22 of SEQ ID NO: 25; (62) a targeting sequence consisting of amino acids 12-20 of SEQ ID NO: 25; (63) a targeting sequence consisting of amino acids 1-15 of SEQ ID NO: 103; (64) a targeting sequence consisting of amino acids 1-13 of SEQ ID NO: 103; (65) a targeting sequence consisting of amino acids 15-28 of SEQ ID NO: 27; (66) a targeting sequence consisting of amino acids 15-26 of SEQ ID NO: 27; (67) a targeting sequence consisting of amino acids 16-28 of SEQ ID NO: 27; (68) a targeting sequence consisting of amino acids 18-26 of SEQ ID NO: 27; (69) a targeting sequence consisting of amino acids 1-15 of SEQ ID NO: 104; (70) a targeting sequence consisting of amino acids 1-13 of SEQ ID NO: 104; (71) a targeting sequence consisting of amino acids 1-13 of SEQ ID NO: 33; (72) a targeting sequence consisting of amino acids 1-11 of SEQ ID NO: 33; (73) a targeting sequence consisting of amino acids 3-11 of SEQ ID NO: 33; (74) a targeting sequence consisting of amino acids 1-14 of SEQ ID NO: 35; (75) a targeting sequence consisting of amino acids 1-12 of SEQ ID NO: 35; (76) a targeting sequence consisting of amino acids 2-14 of SEQ ID NO: 35; (77) a targeting sequence consisting of amino acids 14-27 of SEQ ID NO: 43; (78) a targeting sequence consisting of amino acids 14-25 of SEQ ID NO: 43; (79) a targeting sequence consisting of amino acids 15-27 of SEQ ID NO: 43; (80) a targeting sequence consisting of amino acids 20-33 of SEQ ID NO: 45; (81) a targeting sequence consisting of amino acids 20-31 of SEQ ID NO: 45; (82) a targeting sequence consisting of amino acids 21-33 of SEQ ID NO: 45; (83) a targeting sequence consisting of amino acids 1-15 of SEQ ID NO: 106; (84) a targeting sequence consisting of amino acids 1-13 of SEQ ID NO: 106; (85) a targeting sequence consisting of amino acids 28-41 of SEQ ID NO: 47; (86) a targeting sequence consisting of amino acids 28-39 of SEQ ID NO: 47; (87) a targeting sequence consisting of amino acids 18-31 of SEQ ID NO: 53; (88) a targeting sequence consisting of amino acids 18-29 of SEQ ID NO: 53; (89) a targeting sequence consisting of amino acids 19-31 of SEQ ID NO: 53; (90) a targeting sequence comprising amino acids 18-31 of SEQ ID NO: 61; (91) a targeting sequence comprising amino acids 18-29 of SEQ ID NO: 61; (92) a targeting sequence comprising amino acids 19-31 of SEQ ID NO: 61; (93) a targeting sequence comprising amino acids 9-22 of SEQ ID NO: 65; (94) a targeting sequence comprising amino acids 9-20 of SEQ ID NO: 65; (95) a targeting sequence comprising amino acids 10-22 of SEQ ID NO: 65; (96) a targeting sequence comprising amino acids 1-15 of SEQ ID NO: 107; (97) a targeting sequence comprising amino acids 1-13 of SEQ ID NO: 107; (98) a targeting sequence comprising amino acids 12-25 of SEQ ID NO: 67; (99) a targeting sequence comprising amino acids 12-23 of SEQ ID NO: 67; (100) a targeting sequence comprising amino acids 13-25 of SEQ ID NO: 67; (101) a targeting sequence comprising amino acids 15-23 of SEQ ID NO: 67; (102) a targeting sequence comprising amino acids 23-36 of SEQ ID NO: 69; (103) a targeting sequence comprising amino acids 23-34 of SEQ ID NO: 69; (104) a targeting sequence comprising amino acids 24-36 of SEQ ID NO: 69; (105) a targeting sequence comprising amino acids 26-34 of SEQ ID NO: 69; (106) a targeting sequence comprising amino acids 27-40 of SEQ ID NO: 75; (107) a targeting sequence comprising amino acids 27-38 of SEQ ID NO: 75; (108) a targeting sequence comprising amino acids 9-22 of SEQ ID NO: 77; (109) a targeting sequence comprising amino acids 9-20 of SEQ ID NO: 77; (110) a targeting sequence comprising amino acids 10-22 of SEQ ID NO: 77; (111) a targeting sequence comprising amino acids 12-20 of SEQ ID NO: 77; (112) a targeting sequence comprising amino acids 23-36 of SEQ ID NO: 81; (113) a targeting sequence comprising amino acids 23-34 of SEQ ID NO: 81; (114) a targeting sequence comprising amino acids 24-36 of SEQ ID NO: 81; (115) a targeting sequence comprising amino acids 26-34 of SEQ ID NO: 81; (116) a targeting sequence

comprising amino acids 13-26 of SEQ ID NO: 87; (117) a targeting sequence comprising amino acids 13-24 of SEQ ID NO: 87; or (118) a targeting sequence comprising amino acids 14-26 of SEQ ID NO: 87. The targeting sequence can also consist of any of these sequences.

(161) The present invention relates to fusion proteins comprising at least one protein or peptide of interest and a targeting sequence, exosporium protein, or exosporium protein fragment. The protein or peptide of interest can be an enzyme that catalyzes the production of nitric oxide or a nucleic acid binding protein or peptide. When the protein or peptide of interest comprises an enzyme that catalyzes the production of nitric oxide or a nucleic acid binding protein or peptide, the targeting sequence, exosporium protein, or exosporium protein fragment can be any targeting sequence, exosporium protein, or exosporium protein fragment that targets the fusion protein to the exosporium of a recombinant *Bacillus cereus* family member. For example, the targeting sequence exosporium protein or exosporium protein fragment can be any of the targeting sequences, exosporium proteins, or exosporium protein fragments listed herein above for use with any protein or peptide of interest or: (1) a targeting sequence comprising an amino acid sequence having at least about 43% identity with amino acids 20-35 of SEQ ID NO: 1, wherein the identity with amino acids 25-35 is at least about 54%; (2) a targeting sequence comprising amino acids 1-35 of SEQ ID NO: 1; (3) a targeting sequence comprising amino acids 20-35 of SEQ ID NO: 1; (4) a targeting sequence comprising SEQ ID NO: 1; (5) an exosporium protein comprising an amino acid sequence having at least 85% identity with SEQ ID NO: 2; (6) a targeting sequence comprising amino acids 2-35 of SEQ ID NO: 1; (7) a targeting sequence comprising amino acids 5-35 of SEQ ID NO: 1; (8) a targeting sequence comprising amino acids 8-35 of SEQ ID NO: 1; (9) a targeting sequence comprising amino acids 10-35 of SEQ ID NO: 1; (10) a targeting sequence comprising amino acids 15-35 of SEQ ID NO: 1; (11) a targeting sequence comprising amino acids 1-27 of SEQ ID NO: 3; (12) a targeting sequence comprising amino acids 12-27 of SEQ ID NO: 3; (13) a targeting sequence comprising SEQ ID NO: 3; (14) an exosporium protein comprising an amino acid sequence having at least 85% identity with SEQ ID NO: 4; (15) a targeting sequence comprising amino acids 2-27 of SEQ ID NO: 3; (16) a targeting sequence comprising amino acids 5-27 of SEQ ID NO: 3; (17) a targeting sequence comprising amino acids 8-27 of SEQ ID NO: 3; (18) a targeting sequence comprising amino acids 10-27 of SEQ ID NO: 3; (19) a targeting sequence comprising amino acids 1-38 of SEQ ID NO: 5; (20) a targeting sequence comprising amino acids 23-38 of SEQ ID NO: 5; (21) a targeting sequence comprising SEQ ID NO: 5; (22) an exosporium protein comprising an amino acid sequence having at least 85% identity with SEQ ID NO: 6; (23) a targeting sequence comprising amino acids 2-38 of SEQ ID NO: 5; (24) a targeting sequence comprising amino acids 5-38 of SEQ ID NO: 5; (25) a targeting sequence comprising amino acids 8-38 of SEQ ID NO: 5; (26) a targeting sequence comprising amino acids 10-38 of SEQ ID NO: 5; (27) a targeting sequence comprising amino acids 15-38 of SEQ ID NO: 5; (28) a targeting sequence comprising amino acids 20-38 of SEQ ID NO: 5; (29) a targeting sequence comprising amino acids 1-28 of SEQ ID NO: 7; (30) a targeting sequence comprising amino acids 13-28 of SEQ ID NO: 7; (31) a targeting sequence comprising SEQ ID NO: 7; (32) an exosporium protein comprising an amino acid sequence having at least 85% identity with SEQ ID NO: 8; (33) a targeting sequence comprising amino acids 2-28 of SEQ ID NO: 7; (34) a targeting sequence comprising amino acids 5-28 of SEQ ID NO: 7; (35) a targeting sequence comprising amino acids 8-28 of SEQ ID NO: 7; (36) a targeting sequence comprising amino acids 10-28 of SEQ ID NO: 7; (37) a targeting sequence comprising amino acids 1-24 of SEQ ID NO: 9; (38) a targeting sequence comprising amino acids 9-24 of SEQ ID NO: 9; (39) a targeting sequence comprising SEQ ID NO: 9; (40) an exosporium protein comprising an amino acid sequence having at least 85% identity with SEQ ID NO: 10; (41) a targeting sequence comprising amino acids 2-24 of SEQ ID NO: 9; (42) a targeting sequence comprising amino acids 5-24 of SEQ ID NO: 9; (43) a targeting sequence comprising amino acids 8-24 of SEQ ID NO: 9; (44) a targeting sequence comprising amino acids 1-33 of SEQ ID NO:11; (45) a targeting sequence comprising amino acids 18-33 of SEQ ID NO: 11; (46) a targeting sequence comprising SEQ ID NO: 11; (47) an exosporium protein comprising an amino acid sequence having at least 85% identity with SEQ ID NO: 12; (48) a targeting sequence comprising amino acids 2-33 of SEQ ID NO: 11; (49) a targeting sequence comprising amino acids 5-33 of SEQ ID NO: 11; (50) a targeting sequence comprising amino acids 8-33 of SEQ ID NO: 11; (51) a targeting sequence comprising amino acids 10-33 of SEQ ID NO: 11; (52) a targeting sequence comprising amino acids 15-33 of SEQ ID NO: 11; (53) a targeting sequence comprising amino acids 1-33 of SEQ ID NO: 13; (54) a targeting sequence comprising amino acids 18-33 of SEQ ID NO: 13; (55) a targeting sequence comprising SEQ ID NO: 13; (56) an exosporium protein comprising an amino acid sequence having at least 85% identity with SEQ ID NO:14; (57) a targeting sequence comprising amino acids 2-33 of SEQ ID NO: 13; (58) a targeting sequence comprising amino acids 5-33 of SEQ ID NO: 13; (59) a targeting sequence comprising amino acids 8-33 of SEQ ID NO: 13; (60) a targeting sequence comprising amino acids 10-33 of SEQ ID NO: 13; (61) a targeting sequence comprising amino acids 15-33 of SEQ ID NO: 13; (62) a targeting sequence comprising amino acids 1-43 of SEQ ID NO: 15; (63) a targeting sequence comprising amino acids 28-43 of SEQ ID NO: 15; (64) a targeting sequence comprising SEQ ID NO: 15; (65) an exosporium protein comprising an amino acid sequence having at least 85% identity with SEQ ID NO:16; (66) a targeting sequence comprising amino acids 2-43 of SEQ ID NO: 15; (67) a targeting sequence

comprising amino acids 5-43 of SEQ ID NO: 15; (68) a targeting sequence comprising amino acids 8-43 of SEQ ID NO: 15; (69) a targeting sequence comprising amino acids 10-43 of SEQ ID NO: 15; (70) a targeting sequence comprising amino acids 15-43 of SEQ ID NO: 15; (71) a targeting sequence comprising amino acids 20-43 of SEQ ID NO: 15; (72) a targeting sequence comprising amino acids 25-43 of SEQ ID NO: 15; (73) a targeting sequence comprising amino acids 1-27 of SEQ ID NO: 17; (74) a targeting sequence comprising amino acids 12-27 of SEQ ID NO: 17; (75) a targeting sequence comprising SEQ ID NO:17; (76) an exosporium protein comprising an amino acid sequence having at least 85% identity with SEQ ID NO:18; (77) a targeting sequence comprising amino acids 2-27 of SEQ ID NO: 17; (78) a targeting sequence comprising amino acids 5-27 of SEQ ID NO: 17; (79) a targeting sequence comprising amino acids 8-27 of SEQ ID NO: 17; (80) a targeting sequence comprising amino acids 10-27 of SEQ ID NO: 17; (81) a targeting sequence comprising amino acids 1-33 of SEQ ID NO: 19; (82) a targeting sequence comprising amino acids 18-33 of SEQ ID NO: 19; (83) a targeting sequence comprising SEQ ID NO:19; (84) an exosporium protein comprising an amino acid sequence having at least 85% identity with SEQ ID NO:20; (85) a targeting sequence comprising amino acids 2-33 of SEQ ID NO: 19; (86) a targeting sequence comprising amino acids 5-33 of SEQ ID NO: 19; (87) a targeting sequence comprising amino acids 8-33 of SEQ ID NO: 19; (88) a targeting sequence comprising amino acids 10-33 of SEQ ID NO: 19; (89) a targeting sequence comprising amino acids 15-33 of SEQ ID NO: 19; (90) a targeting sequence comprising amino acids 1-33 of SEQ ID NO: 21; (91) a targeting sequence comprising amino acids 18-33 of SEQ ID NO: 21; (92) a targeting sequence comprising SEQ ID NO:21; (93) an exosporium protein comprising an amino acid sequence having at least 85% identity with SEQ ID NO:22; (94) a targeting sequence comprising amino acids 2-33 of SEQ ID NO: 21; (95) a targeting sequence comprising amino acids 5-33 of SEQ ID NO: 21; (96) a targeting sequence comprising amino acids 8-33 of SEQ ID NO: 21; (97) a targeting sequence comprising amino acids 10-33 of SEQ ID NO: 21; (98) a targeting sequence comprising amino acids 15-33 of SEQ ID NO: 21; (99) a targeting sequence comprising amino acids 1-24 of SEQ ID NO: 23; (100) a targeting sequence comprising amino acids 9-24 of SEQ ID NO: 23; (101) a targeting sequence comprising SEQ ID NO:23; (102) an exosporium protein comprising an amino acid sequence having at least 85% identity with SEQ ID NO:24; (103) a targeting sequence comprising amino acids 2-24 of SEQ ID NO:23; (104) a targeting sequence comprising amino acids 5-24 of SEQ ID NO: 23; (105) a targeting sequence comprising amino acids 8-24 of SEQ ID NO: 23; (106) a targeting sequence comprising amino acids 1-24 of SEQ ID NO: 25; (107) a targeting sequence comprising amino acids 9-24 of SEQ ID NO: 25; (108) a targeting sequence comprising SEQ ID NO:25; (109) an exosporium protein comprising an amino acid sequence having at least 85% identity with SEQ ID NO:26; (110) a targeting sequence comprising amino acids 2-24 of SEQ ID NO: 25; (111) a targeting sequence comprising amino acids 5-24 of SEQ ID NO: 25; (112) a targeting sequence comprising amino acids 8-24 of SEQ ID NO: 25; (113) a targeting sequence comprising amino acids 1-30 of SEQ ID NO: 27; (114) a targeting sequence comprising amino acids 15-30 of SEQ ID NO: 27; (115) a targeting sequence comprising SEQ ID NO:27; (116) an exosporium protein comprising an amino acid sequence having at least 85% identity with SEQ ID NO:28; (117) a targeting sequence comprising amino acids 2-30 of SEQ ID NO: 27; (118) a targeting sequence comprising amino acids 5-30 of SEQ ID NO: 27; (119) a targeting sequence comprising amino acids 8-30 of SEQ ID NO: 27; (120) a targeting sequence comprising amino acids 10-30 of SEQ ID NO: 27; (121) a targeting sequence comprising amino acids 1-33 of SEQ ID NO: 29; (122) a targeting sequence comprising amino acids 18-33 of SEQ ID NO: 29; (123) a targeting sequence comprising SEQ ID NO:29; (124) an exosporium protein comprising an amino acid sequence having at least 85% identity with SEQ ID NO:30; (125) a targeting sequence comprising amino acids 2-33 of SEQ ID NO: 29; (126) a targeting sequence comprising amino acids 5-33 of SEQ ID NO: 29; (127) a targeting sequence comprising amino acids 8-33 of SEQ ID NO: 29; (128) a targeting sequence comprising amino acids 10-33 of SEQ ID NO: 29; (129) a targeting sequence comprising amino acids 15-33 of SEQ ID NO: 29; (130) a targeting sequence comprising amino acids 1-24 of SEQ ID NO: 31; (131) a targeting sequence comprising amino acids 9-24 of SEQ ID NO: 31; (132) a targeting sequence comprising SEQ ID NO:31; (133) an exosporium protein comprising an amino acid sequence having at least 85% identity with SEQ ID NO:32; (134) a targeting sequence comprising amino acids 2-24 of SEQ ID NO: 31; (135) a targeting sequence comprising amino acids 5-24 of SEQ ID NO: 31; (136) a targeting sequence comprising amino acids 8-24 of SEQ ID NO: 31; (137) a targeting sequence comprising amino acids 1-15 of SEQ ID NO: 33; (138) a targeting sequence comprising SEQ ID NO:33; (139) an exosporium protein comprising an amino acid sequence having at least 85% identity with SEQ ID NO:34; (140) a targeting sequence comprising amino acids 1-16 of SEQ ID NO: 35; (141) a targeting sequence comprising SEQ ID NO:35; (142) an exosporium protein comprising an amino acid sequence having at least 85% identity with SEQ ID NO:36; (143) a targeting sequence comprising amino acids 1-29 of SEQ ID NO:43; (144) a targeting sequence comprising amino acids 14-29 of SEQ ID NO: 43; (145) a targeting sequence comprising SEQ ID NO: 43; (146) an exosporium protein comprising an amino acid sequence having at least 85% identity with SEQ ID NO: 44; (147) a targeting sequence comprising amino acids 2-29 of SEQ ID NO: 43; (148) a targeting sequence comprising amino acids 5-29 of SEQ ID NO: 43; (149) a targeting sequence comprising amino acids 8-29 of SEQ ID NO: 43; (150) a targeting sequence comprising amino acids 10-29 of SEQ ID NO: 43; (151) a targeting sequence comprising amino

acids 1-35 of SEQ ID NO: 45; (152) a targeting sequence comprising amino acids 20-35 of SEQ ID NO: 45; (153) a targeting sequence comprising SEQ ID NO: 45; (154) an exosporium protein comprising an amino acid sequence having at least 85% identity with SEQ ID NO: 46; (155) a targeting sequence comprising amino acids 2-35 of SEQ ID NO: 45; (156) a targeting sequence comprising amino acids 5-35 of SEQ ID NO: 45; (157) a targeting sequence comprising amino acids 8-35 of SEQ ID NO: 45; (158) a targeting sequence comprising amino acids 10-35 of SEQ ID NO: 45; (159) a targeting sequence comprising amino acids 15-35 of SEQ ID NO: 45; (160) a targeting sequence comprising amino acids 1-43 of SEQ ID NO: 47; (161) a targeting sequence comprising amino acids 28-43 of SEQ ID NO: 47; (162) a targeting sequence comprising SEQ ID NO: 47; (163) an exosporium protein comprising an amino acid sequence having at least 85% identity with SEQ ID NO: 48; (164) a targeting sequence comprising amino acids 2-43 of SEQ ID NO: 47; (165) a targeting sequence comprising amino acids 5-43 of SEQ ID NO: 47; (166) a targeting sequence comprising amino acids 8-43 of SEQ ID NO: 47; (167) a targeting sequence comprising amino acids 10-43 of SEQ ID NO: 47; (168) a targeting sequence comprising amino acids 15-43 of SEQ ID NO: 47; (169) a targeting sequence comprising amino acids 20-43 of SEQ ID NO: 47; (170) a targeting sequence comprising amino acids 25-43 of SEQ ID NO: 47; (171) a targeting sequence comprising amino acids 1-32 of SEQ ID NO: 49; (172) a targeting sequence comprising amino acids 17-32 of SEQ ID NO: 49; (173) a targeting sequence comprising SEQ ID NO: 49; (174) an exosporium protein comprising an amino acid sequence having at least 85% identity with SEQ ID NO: 50; (175) a targeting sequence comprising amino acids 2-32 of SEQ ID NO: 49; (176) a targeting sequence comprising amino acids 5-32 of SEQ ID NO: 49; (177) a targeting sequence comprising amino acids 8-32 of SEQ ID NO: 49; (178) a targeting sequence comprising amino acids 10-32 of SEQ ID NO: 49; (179) a targeting sequence comprising amino acids 15-32 of SEQ ID NO: 49; (180) a targeting sequence comprising amino acids 1-33 of SEQ ID NO: 51; (181) a targeting sequence comprising amino acids 18-33 of SEQ ID NO: 51; (182) a targeting sequence comprising SEQ ID NO: 51; (183) an exosporium protein comprising an amino acid sequence having at least 85% identity with SEQ ID NO: 52; (184) a targeting sequence comprising amino acids 2-33 of SEQ ID NO: 51; (185) a targeting sequence comprising amino acids 5-33 of SEQ ID NO: 51; (186) a targeting sequence comprising amino acids 8-33 of SEQ ID NO: 51; (187) a targeting sequence comprising amino acids 10-33 of SEQ ID NO: 51; (188) a targeting sequence comprising amino acids 15-33 of SEQ ID NO: 51; (189) a targeting sequence comprising amino acids 1-33 of SEQ ID NO: 53; (190) a targeting sequence comprising amino acids 18-33 of SEQ ID NO: 53; (191) a targeting sequence comprising SEQ ID NO: 53; (192) an exosporium protein comprising an amino acid sequence having at least 85% identity with SEQ ID NO: 54; (193) a targeting sequence comprising amino acids 2-33 of SEQ ID NO: 53; (194) a targeting sequence comprising amino acids 5-33 of SEQ ID NO: 53; (195) a targeting sequence comprising amino acids 8-33 of SEQ ID NO: 53; (196) a targeting sequence comprising amino acids 10-33 of SEQ ID NO: 53; (197) a targeting sequence comprising amino acids 15-33 of SEQ ID NO: 53; (198) a targeting sequence comprising amino acids 1-30 of SEQ ID NO: 55; (199) a targeting sequence comprising amino acids 15-30 of SEQ ID NO: 55; (200) a targeting sequence comprising SEQ ID NO: 55; (201) an exosporium protein comprising an amino acid sequence having at least 85% identity with SEQ ID NO: 56; (202) a targeting sequence comprising amino acids 2-30 of SEQ ID NO: 55; (203) a targeting sequence comprising amino acids 5-30 of SEQ ID NO: 55; (204) a targeting sequence comprising amino acids 8-30 of SEQ ID NO: 55; (205) a targeting sequence comprising amino acids 10-30 of SEQ ID NO: 55; (206) a targeting sequence comprising amino acids 1-130 of SEQ ID NO: 57; (207) a targeting sequence comprising amino acids 115-130 of SEQ ID NO: 57; (208) a targeting sequence comprising SEQ ID NO: 57; (209) an exosporium protein comprising an amino acid sequence having at least 85% identity with SEQ ID NO: 58; (210) a targeting sequence comprising amino acids 2-130 of SEQ ID NO: 57; (211) a targeting sequence comprising amino acids 5-130 of SEQ ID NO: 57; (212) a targeting sequence comprising amino acids 10-130 of SEQ ID NO: 57; (213) a targeting sequence comprising amino acids 20-130 of SEQ ID NO: 57; (214) a targeting sequence comprising amino acids 30-130 of SEQ ID NO: 57; (215) a targeting sequence comprising amino acids 40-130 of SEQ ID NO: 57; (216) a targeting sequence comprising amino acids 50-130 of SEQ ID NO: 57; (217) a targeting sequence comprising amino acids 60-130 of SEQ ID NO: 57; (218) a targeting sequence comprising amino acids 70-130 of SEQ ID NO: 57; (219) a targeting sequence comprising amino acids 80-130 of SEQ ID NO: 57; (220) a targeting sequence comprising amino acids 90-130 of SEQ ID NO: 57; (221) a targeting sequence comprising amino acids 100-130 of SEQ ID NO: 57; (222) a targeting sequence comprising amino acids 110-130 of SEQ ID NO: 57; (223) an exosporium protein fragment comprising an amino acid sequence having at least 85% identity with SEQ ID NO: 95; (224) a targeting sequence comprising SEQ ID NO: 96; (225) a targeting sequence comprising SEQ ID NO: 97; (226) a targeting sequence comprising SEQ ID NO: 98; (227) a targeting sequence comprising SEQ ID NO: 99; (228) a targeting sequence comprising SEQ ID NO: 100; (229) a targeting sequence comprising SEQ ID NO: 101; (230) a targeting sequence comprising SEQ ID NO: 102; (231) a targeting sequence comprising SEQ ID NO: 103; (232) a targeting sequence comprising SEQ ID NO: 104; (233) a targeting sequence comprising SEQ ID NO: 105; (234) a targeting sequence comprising SEQ ID NO: 106; (235) an exosporium protein comprising an amino acid sequence having at least 85% identity with SEQ ID NO: 108; (236) an exosporium protein comprising an amino acid sequence having at least

85% identity with SEQ ID NO: 109; (237) an exosporium protein comprising an amino acid sequence having at least 85% identity with SEQ ID NO: 110; (238) an exosporium protein comprising an amino acid sequence having at least 85% identity with SEQ ID NO: 111; (239) an exosporium protein comprising an amino acid sequence having at least 85% identity with SEQ ID NO: 112; (240) an exosporium protein comprising an amino acid sequence having at least 85% identity with SEQ ID NO: 113; (241) an exosporium protein comprising an amino acid sequence having at least 85% identity with SEQ ID NO: 114; (242) an exosporium protein comprising an amino acid sequence having at least 85% identity with SEQ ID NO: 115; (243) an exosporium protein comprising an amino acid sequence having at least 85% identity with SEQ ID NO: 116; (244) an exosporium protein comprising an amino acid sequence having at least 85% identity with SEQ ID NO: 117; (245) an exosporium protein comprising an amino acid sequence having at least 85% identity with SEQ ID NO: 118; (246) an exosporium protein comprising an amino acid sequence having at least 85% identity with SEQ ID NO: 119; (247) an exosporium protein comprising an amino acid sequence having at least 85% identity with SEQ ID NO: 120; (248) an exosporium protein comprising an amino acid sequence having at least 85% identity with SEQ ID NO: 121; (249) a targeting sequence comprising amino acids 22-31 of SEQ ID NO: 1; (250) a targeting sequence comprising amino acids 22-33 of SEQ ID NO: 1; (251) a targeting sequence comprising amino acids 20-31 of SEQ ID NO: 1; (252) a targeting sequence comprising amino acids 14-23 of SEQ ID NO: 3; (253) a targeting sequence comprising amino acids 14-25 of SEQ ID NO: 3; or (254) a targeting sequence comprising amino acids 12-23 of SEQ ID NO: 3.

(162) For example, when the protein or peptide of interest comprises an enzyme that catalyzes the production of nitric oxide or a nucleic acid binding protein or peptide, the targeting sequence, exosporium protein, or exosporium protein fragment can be: (1) a targeting sequence comprising amino acids 2-35 of SEQ ID NO: 1; (2) a targeting sequence comprising amino acids 5-35 of SEQ ID NO: 1; (3) a targeting sequence comprising amino acids 8-35 of SEQ ID NO: 1; (4) a targeting sequence comprising amino acids 10-35 of SEQ ID NO: 1; (5) a targeting sequence comprising amino acids 15-35 of SEQ ID NO: 1; (6) a targeting sequence comprising amino acids 2-27 of SEQ ID NO: 3; (7) a targeting sequence comprising amino acids 5-27 of SEQ ID NO: 3; (8) a targeting sequence comprising amino acids 8-27 of SEQ ID NO: 3; (9) a targeting sequence comprising amino acids 10-27 of SEQ ID NO: 3; (10) a targeting sequence comprising amino acids 2-38 of SEQ ID NO: 5; (11) a targeting sequence comprising amino acids 5-38 of SEQ ID NO: 5; (12) a targeting sequence comprising amino acids 8-38 of SEQ ID NO: 5; (13) a targeting sequence comprising amino acids 10-38 of SEQ ID NO: 5; (14) a targeting sequence comprising amino acids 15-38 of SEQ ID NO: 5; (15) a targeting sequence comprising amino acids 20-38 of SEQ ID NO: 5; (16) a targeting sequence comprising amino acids 2-28 of SEQ ID NO: 7; (17) a targeting sequence comprising amino acids 5-28 of SEQ ID NO: 7; (18) a targeting sequence comprising amino acids 8-28 of SEQ ID NO: 7; (19) a targeting sequence comprising amino acids 10-28 of SEQ ID NO: 7; (20) a targeting sequence comprising amino acids 2-24 of SEQ ID NO: 9; (21) a targeting sequence comprising amino acids 5-24 of SEQ ID NO: 9; (22) a targeting sequence comprising amino acids 8-24 of SEQ ID NO: 9; (23) a targeting sequence comprising amino acids 2-33 of SEQ ID NO: 11; (24) a targeting sequence comprising amino acids 5-33 of SEQ ID NO: 11; (25) a targeting sequence comprising amino acids 8-33 of SEQ ID NO: 11; (26) a targeting sequence comprising amino acids 10-33 of SEQ ID NO: 11; (27) a targeting sequence comprising amino acids 15-33 of SEQ ID NO: 11; (28) a targeting sequence comprising amino acids 2-33 of SEQ ID NO: 13; (29) a targeting sequence comprising amino acids 5-33 of SEQ ID NO: 13; (30) a targeting sequence comprising amino acids 8-33 of SEQ ID NO: 13; (31) a targeting sequence comprising amino acids 10-33 of SEQ ID NO: 13; (32) a targeting sequence comprising amino acids 15-33 of SEQ ID NO: 13; (33) a targeting sequence comprising amino acids 2-43 of SEQ ID NO: 15; (34) a targeting sequence comprising amino acids 5-43 of SEQ ID NO: 15; (35) a targeting sequence comprising amino acids 8-43 of SEQ ID NO: 15; (36) a targeting sequence comprising amino acids 10-43 of SEQ ID NO: 15; (37) a targeting sequence comprising amino acids 15-43 of SEQ ID NO: 15; (38) a targeting sequence comprising amino acids 20-43 of SEQ ID NO: 15; (39) a targeting sequence comprising amino acids 25-43 of SEQ ID NO: 15; (40) a targeting sequence comprising amino acids 2-27 of SEQ ID NO: 17; (41) a targeting sequence comprising amino acids 5-27 of SEQ ID NO: 17; (42) a targeting sequence comprising amino acids 8-27 of SEQ ID NO: 17; (43) a targeting sequence comprising amino acids 10-27 of SEQ ID NO: 17; (44) a targeting sequence comprising amino acids 2-33 of SEQ ID NO: 19; (45) a targeting sequence comprising amino acids 5-33 of SEQ ID NO: 19; (46) a targeting sequence comprising amino acids 8-33 of SEQ ID NO: 19; (47) a targeting sequence comprising amino acids 10-33 of SEQ ID NO: 19; (48) a targeting sequence comprising amino acids 15-33 of SEQ ID NO: 19; (49) a targeting sequence comprising amino acids 2-33 of SEQ ID NO: 21; (50) a targeting sequence comprising amino acids 5-33 of SEQ ID NO: 21; (51) a targeting sequence comprising amino acids 8-33 of SEQ ID NO: 21; (52) a targeting sequence comprising amino acids 10-33 of SEQ ID NO: 21; (53) a targeting sequence comprising amino acids 15-33 of SEQ ID NO: 21; (54) a targeting sequence comprising amino acids 2-24 of SEQ ID NO:23; (55) a targeting sequence comprising amino acids 5-24 of SEQ ID NO: 23; (56) a targeting sequence comprising amino acids 8-24 of SEQ ID NO: 23; (57) a targeting sequence comprising amino acids 2-24 of SEQ ID NO: 25; (58) a

targeting sequence comprising amino acids 5-24 of SEQ ID NO: 25; (59) a targeting sequence comprising amino acids 8-24 of SEQ ID NO: 25; (60) a targeting sequence comprising amino acids 2-30 of SEQ ID NO: 27; (61) a targeting sequence comprising amino acids 5-30 of SEQ ID NO: 27; (62) a targeting sequence comprising amino acids 8-30 of SEQ ID NO: 27; (63) a targeting sequence comprising amino acids 10-30 of SEQ ID NO: 27; (64) a targeting sequence comprising amino acids 2-33 of SEQ ID NO: 29; (65) a targeting sequence comprising amino acids 5-33 of SEQ ID NO: 29; (66) a targeting sequence comprising amino acids 8-33 of SEQ ID NO: 29; (67) a targeting sequence comprising amino acids 10-33 of SEQ ID NO: 29; (68) a targeting sequence comprising amino acids 15-33 of SEQ ID NO: 29; (69) a targeting sequence comprising amino acids 2-24 of SEQ ID NO: 31; (70) a targeting sequence comprising amino acids 5-24 of SEQ ID NO: 31; (71) a targeting sequence comprising amino acids 8-24 of SEQ ID NO: 31; (72) a targeting sequence comprising amino acids 2-29 of SEQ ID NO: 43; (73) a targeting sequence comprising amino acids 5-29 of SEQ ID NO: 43; (74) a targeting sequence comprising amino acids 8-29 of SEQ ID NO: 43; (75) a targeting sequence comprising amino acids 10-29 of SEQ ID NO: 43; (76) a targeting sequence comprising amino acids 2-35 of SEQ ID NO: 45; (77) a targeting sequence comprising amino acids 5-35 of SEQ ID NO: 45; (78) a targeting sequence comprising amino acids 8-35 of SEQ ID NO: 45; (79) a targeting sequence comprising amino acids 10-35 of SEQ ID NO: 45; (80) a targeting sequence comprising amino acids 15-35 of SEQ ID NO: 45; (81) a targeting sequence comprising amino acids 2-43 of SEQ ID NO: 47; (82) a targeting sequence comprising amino acids 5-43 of SEQ ID NO: 47; (83) a targeting sequence comprising amino acids 8-43 of SEQ ID NO: 47; (84) a targeting sequence comprising amino acids 10-43 of SEQ ID NO: 47; (85) a targeting sequence comprising amino acids 15-43 of SEQ ID NO: 47; (86) a targeting sequence comprising amino acids 20-43 of SEQ ID NO: 47; (87) a targeting sequence comprising amino acids 25-43 of SEQ ID NO: 47; (88) a targeting sequence comprising amino acids 2-32 of SEQ ID NO: 49; (89) a targeting sequence comprising amino acids 5-32 of SEQ ID NO: 49; (90) a targeting sequence comprising amino acids 8-32 of SEQ ID NO: 49; (91) a targeting sequence comprising amino acids 10-32 of SEQ ID NO: 49; (92) a targeting sequence comprising amino acids 15-32 of SEQ ID NO: 49; (93) a targeting sequence comprising amino acids 2-33 of SEQ ID NO: 51; (94) a targeting sequence comprising amino acids 5-33 of SEQ ID NO: 51; (95) a targeting sequence comprising amino acids 8-33 of SEQ ID NO: 51; (96) a targeting sequence comprising amino acids 10-33 of SEQ ID NO: 51; (97) a targeting sequence comprising amino acids 15-33 of SEQ ID NO: 51; (98) a targeting sequence comprising amino acids 2-33 of SEQ ID NO: 53; (99) a targeting sequence comprising amino acids 5-33 of SEQ ID NO: 53; (100) a targeting sequence comprising amino acids 8-33 of SEQ ID NO: 53; (101) a targeting sequence comprising amino acids 10-33 of SEQ ID NO: 53; (102) a targeting sequence comprising amino acids 15-33 of SEQ ID NO: 53; (103) a targeting sequence comprising amino acids 2-30 of SEQ ID NO: 55; (104) a targeting sequence comprising amino acids 5-30 of SEQ ID NO: 55; (105) a targeting sequence comprising amino acids 8-30 of SEQ ID NO: 55; (106) a targeting sequence comprising amino acids 10-30 of SEQ ID NO: 55; (107) a targeting sequence comprising amino acids 2-130 of SEQ ID NO: 57; (108) a targeting sequence comprising amino acids 5-130 of SEQ ID NO: 57; (109) a targeting sequence comprising amino acids 10-130 of SEQ ID NO: 57; (110) a targeting sequence comprising amino acids 20-130 of SEQ ID NO: 57; (111) a targeting sequence comprising amino acids 30-130 of SEQ ID NO: 57; (112) a targeting sequence comprising amino acids 40-130 of SEQ ID NO: 57; (113) a targeting sequence comprising amino acids 50-130 of SEQ ID NO: 57; (114) a targeting sequence comprising amino acids 60-130 of SEQ ID NO: 57; (115) a targeting sequence comprising amino acids 70-130 of SEQ ID NO: 57; (116) a targeting sequence comprising amino acids 80-130 of SEQ ID NO: 57; (117) a targeting sequence comprising amino acids 90-130 of SEQ ID NO: 57; (118) a targeting sequence comprising amino acids 100-130 of SEQ ID NO: 57; or (119) a targeting sequence comprising amino acids 110-130 of SEQ ID NO: 57.

(163) A fusion protein is provided which comprises an antigen or a remediation enzyme and a targeting sequence or exosporium protein. The targeting sequence or exosporium protein can comprise any of the targeting sequences or exosporium proteins listed herein above for use with any protein or peptide of interest or: (1) a targeting sequence comprising amino acids 2-35 of SEQ ID NO: 1; (2) a targeting sequence comprising amino acids 5-35 of SEQ ID NO: 1; (3) a targeting sequence comprising amino acids 8-35 of SEQ ID NO: 1; (4) a targeting sequence comprising amino acids 10-35 of SEQ ID NO: 1; (5) a targeting sequence comprising amino acids 15-35 of SEQ ID NO: 1; (6) a targeting sequence comprising amino acids 22-31 of SEQ ID NO: 1; (7) a targeting sequence comprising amino acids 22-33 of SEQ ID NO: 1; (8) a targeting sequence comprising amino acids 20-31 of SEQ ID NO: 1; (9) a targeting sequence comprising amino acids 2-27 of SEQ ID NO: 3; (10) a targeting sequence comprising amino acids 5-27 of SEQ ID NO: 3; (11) a targeting sequence comprising amino acids 8-27 of SEQ ID NO: 3; (12) a targeting sequence comprising amino acids 10-27 of SEQ ID NO: 3; (13) a targeting sequence comprising amino acids 14-23 of SEQ ID NO: 3; (14) a targeting sequence comprising amino acids 14-25 of SEQ ID NO: 3; (15) a targeting sequence comprising amino acids 12-23 of SEQ ID NO: 3; (16) a targeting sequence comprising amino acids 2-38 of SEQ ID NO: 5; (17) a targeting sequence comprising amino acids 5-38 of SEQ ID NO: 5; (18) a targeting sequence comprising amino acids 8-38 of SEQ ID NO: 5; (19) a targeting sequence comprising amino acids 10-38 of SEQ ID NO: 5; (20) a targeting sequence comprising amino acids 15-38 of SEQ ID NO: 5; (21) a

targeting sequence comprising amino acids 20-38 of SEQ ID NO: 5; (22) a targeting sequence comprising amino acids 2-28 of SEQ ID NO: 7; (23) a targeting sequence comprising amino acids 5-28 of SEQ ID NO: 7; (24) a targeting sequence comprising amino acids 8-28 of SEQ ID NO: 7; (25) a targeting sequence comprising amino acids 10-28 of SEQ ID NO: 7; (26) a targeting sequence comprising amino acids 1-24 of SEQ ID NO: 9; (27) a targeting sequence comprising amino acids 9-24 of SEQ ID NO: 9; (28) a targeting sequence comprising SEQ ID NO: 9; (29) an exosporium protein comprising an amino acid sequence having at least 85% identity with SEQ ID NO: 10; (30) a targeting sequence comprising amino acids 2-24 of SEQ ID NO: 9; (31) a targeting sequence comprising amino acids 5-24 of SEQ ID NO: 9; (32) a targeting sequence comprising amino acids 8-24 of SEQ ID NO: 9; (33) a targeting sequence comprising amino acids 1-33 of SEQ ID NO:11; (34) a targeting sequence comprising amino acids 18-33 of SEQ ID NO: 11; (35) a targeting sequence comprising SEQ ID NO: 11; (36) an exosporium protein comprising an amino acid sequence having at least 85% identity with SEQ ID NO: 12; (37) a targeting sequence comprising amino acids 2-33 of SEQ ID NO: 11; (38) a targeting sequence comprising amino acids 5-33 of SEQ ID NO: 11; (39) a targeting sequence comprising amino acids 8-33 of SEQ ID NO: 11; (40) a targeting sequence comprising amino acids 10-33 of SEQ ID NO: 11; (41) a targeting sequence comprising amino acids 15-33 of SEQ ID NO: 11; (42) a targeting sequence comprising amino acids 1-33 of SEQ ID NO: 13; (43) a targeting sequence comprising amino acids 18-33 of SEQ ID NO: 13; (44) a targeting sequence comprising SEQ ID NO:13; (45) an exosporium protein comprising an amino acid sequence having at least 85% identity with SEQ ID NO: 14; (46) a targeting sequence comprising amino acids 2-33 of SEQ ID NO: 13; (47) a targeting sequence comprising amino acids 5-33 of SEQ ID NO: 13; (48) a targeting sequence comprising amino acids 8-33 of SEQ ID NO: 13; (49) a targeting sequence comprising amino acids 10-33 of SEQ ID NO: 13; (50) a targeting sequence comprising amino acids 15-33 of SEQ ID NO: 13; (51) a targeting sequence comprising amino acids 1-43 of SEQ ID NO: 15; (52) a targeting sequence comprising amino acids 28-43 of SEQ ID NO: 15; (53) a targeting sequence comprising SEQ ID NO:15; (54) an exosporium protein comprising an amino acid sequence having at least 85% identity with SEQ ID NO: 16; (55) a targeting sequence comprising amino acids 2-43 of SEQ ID NO: 15; (56) a targeting sequence comprising amino acids 5-43 of SEQ ID NO: 15; (57) a targeting sequence comprising amino acids 8-43 of SEQ ID NO: 15; (58) a targeting sequence comprising amino acids 10-43 of SEQ ID NO: 15; (59) a targeting sequence comprising amino acids 15-43 of SEQ ID NO: 15; (60) a targeting sequence comprising amino acids 20-43 of SEQ ID NO: 15; (61) a targeting sequence comprising amino acids 25-43 of SEQ ID NO: 15; (62) a targeting sequence comprising amino acids 1-27 of SEQ ID NO: 17; (63) a targeting sequence comprising amino acids 12-27 of SEQ ID NO: 17; (64) a targeting sequence comprising SEQ ID NO:17; (65) an exosporium protein comprising an amino acid sequence having at least 85% identity with SEQ ID NO: 18; (66) a targeting sequence comprising amino acids 2-27 of SEQ ID NO: 17; (67) a targeting sequence comprising amino acids 5-27 of SEQ ID NO: 17; (68) a targeting sequence comprising amino acids 8-27 of SEQ ID NO: 17; (69) a targeting sequence comprising amino acids 10-27 of SEQ ID NO: 17; (70) a targeting sequence comprising amino acids 1-33 of SEQ ID NO: 19; (71) a targeting sequence comprising amino acids 18-33 of SEQ ID NO: 19; (72) a targeting sequence comprising SEQ ID NO:19; (73) an exosporium protein comprising an amino acid sequence having at least 85% identity with SEQ ID NO:20; (74) a targeting sequence comprising amino acids 2-33 of SEQ ID NO: 19; (75) a targeting sequence comprising amino acids 5-33 of SEQ ID NO: 19; (76) a targeting sequence comprising amino acids 8-33 of SEQ ID NO: 19; (77) a targeting sequence comprising amino acids 10-33 of SEQ ID NO: 19; (78) a targeting sequence comprising amino acids 15-33 of SEQ ID NO: 19; (79) a targeting sequence comprising amino acids 1-33 of SEQ ID NO: 21; (80) a targeting sequence comprising amino acids 18-33 of SEQ ID NO: 21; (81) a targeting sequence comprising SEQ ID NO:21; (82) an exosporium protein comprising an amino acid sequence having at least 85% identity with SEQ ID NO:22; (83) a targeting sequence comprising amino acids 2-33 of SEQ ID NO: 21; (84) a targeting sequence comprising amino acids 5-33 of SEQ ID NO: 21; (85) a targeting sequence comprising amino acids 8-33 of SEQ ID NO: 21; (86) a targeting sequence comprising amino acids 10-33 of SEQ ID NO: 21; (87) a targeting sequence comprising amino acids 15-33 of SEQ ID NO: 21; (88) a targeting sequence comprising amino acids 1-24 of SEQ ID NO: 23; (89) a targeting sequence comprising amino acids 9-24 of SEQ ID NO: 23; (90) a targeting sequence comprising SEQ ID NO:23; (91) an exosporium protein comprising an amino acid sequence having at least 85% identity with SEQ ID NO:24; (92) a targeting sequence comprising amino acids 2-24 of SEQ ID NO:23; (93) a targeting sequence comprising amino acids 5-24 of SEQ ID NO: 23; (94) a targeting sequence comprising amino acids 8-24 of SEQ ID NO: 23; (95) a targeting sequence comprising amino acids 1-24 of SEQ ID NO: 25; (96) a targeting sequence comprising amino acids 9-24 of SEQ ID NO: 25; (97) a targeting sequence comprising SEQ ID NO:25; (98) an exosporium protein comprising an amino acid sequence having at least 85% identity with SEQ ID NO:26; (99) a targeting sequence comprising amino acids 2-24 of SEQ ID NO: 25; (100) a targeting sequence comprising amino acids 5-24 of SEQ ID NO: 25; (101) a targeting sequence comprising amino acids 8-24 of SEQ ID NO: 25; (102) a targeting sequence comprising amino acids 1-30 of SEQ ID NO: 27; (103) a targeting sequence comprising amino acids 15-30 of SEQ ID NO: 27; (104) a targeting sequence comprising SEQ ID NO:27; (105) an exosporium protein comprising an amino acid sequence having at least 85% identity with

SEQ ID NO:28; (106) a targeting sequence comprising amino acids 2-30 of SEQ ID NO: 27; (107) a targeting sequence comprising amino acids 5-30 of SEQ ID NO: 27; (108) a targeting sequence comprising amino acids 8-30 of SEQ ID NO: 27; (109) a targeting sequence comprising amino acids 10-30 of SEQ ID NO: 27; (110) a targeting sequence comprising amino acids 1-33 of SEQ ID NO: 29; (111) a targeting sequence comprising amino acids 18-33 of SEQ ID NO: 29; (112) a targeting sequence comprising SEQ ID NO:29; (113) an exosporium protein comprising an amino acid sequence having at least 85% identity with SEQ ID NO:30; (114) a targeting sequence comprising amino acids 2-33 of SEQ ID NO: 29; (115) a targeting sequence comprising amino acids 5-33 of SEQ ID NO: 29; (116) a targeting sequence comprising amino acids 8-33 of SEQ ID NO: 29; (117) a targeting sequence comprising amino acids 10-33 of SEQ ID NO: 29; (118) a targeting sequence comprising amino acids 15-33 of SEQ ID NO: 29; (119) a targeting sequence comprising amino acids 1-24 of SEQ ID NO: 31; (120) a targeting sequence comprising amino acids 9-24 of SEQ ID NO: 31; (121) a targeting sequence comprising SEQ ID NO:31; (122) an exosporium protein comprising an amino acid sequence having at least 85% identity with SEQ ID NO:32; (123) a targeting sequence comprising amino acids 2-24 of SEQ ID NO: 31; (124) a targeting sequence comprising amino acids 5-24 of SEQ ID NO: 31; (125) a targeting sequence comprising amino acids 8-24 of SEQ ID NO: 31; (126) a targeting sequence comprising amino acids 1-15 of SEQ ID NO: 33; (127) a targeting sequence comprising SEQ ID NO:33; (128) an exosporium protein comprising an amino acid sequence having at least 85% identity with SEQ ID NO:34; (129) a targeting sequence comprising amino acids 1-16 of SEQ ID NO: 35; (130) a targeting sequence comprising SEQ ID NO:35; (131) an exosporium protein comprising an amino acid sequence having at least 85% identity with SEQ ID NO:36; (132) a targeting sequence comprising amino acids 1-29 of SEQ ID NO:43; (133) a targeting sequence comprising amino acids 14-29 of SEQ ID NO: 43; (134) a targeting sequence comprising SEQ ID NO: 43; (135) an exosporium protein comprising an amino acid sequence having at least 85% identity with SEQ ID NO: 44; (136) a targeting sequence comprising amino acids 2-29 of SEQ ID NO: 43; (137) a targeting sequence comprising amino acids 5-29 of SEQ ID NO: 43; (138) a targeting sequence comprising amino acids 8-29 of SEQ ID NO: 43; (139) a targeting sequence comprising amino acids 10-29 of SEQ ID NO: 43; (140) a targeting sequence comprising amino acids 1-35 of SEQ ID NO: 45; (141) a targeting sequence comprising amino acids 20-35 of SEQ ID NO: 45; (142) a targeting sequence comprising SEQ ID NO: 45; (143) an exosporium protein comprising an amino acid sequence having at least 85% identity with SEQ ID NO: 46; (144) a targeting sequence comprising amino acids 2-35 of SEQ ID NO: 45; (145) a targeting sequence comprising amino acids 5-35 of SEQ ID NO: 45; (146) a targeting sequence comprising amino acids 8-35 of SEQ ID NO: 45; (147) a targeting sequence comprising amino acids 10-35 of SEQ ID NO: 45; (148) a targeting sequence comprising amino acids 15-35 of SEQ ID NO: 45; (149) a targeting sequence comprising amino acids 1-43 of SEQ ID NO: 47; (150) a targeting sequence comprising amino acids 28-43 of SEQ ID NO: 47; (151) a targeting sequence comprising SEQ ID NO: 47; (152) an exosporium protein comprising an amino acid sequence having at least 85% identity with SEQ ID NO: 48; (153) a targeting sequence comprising amino acids 2-43 of SEQ ID NO: 47; (154) a targeting sequence comprising amino acids 5-43 of SEQ ID NO: 47; (155) a targeting sequence comprising amino acids 8-43 of SEQ ID NO: 47; (156) a targeting sequence comprising amino acids 10-43 of SEQ ID NO: 47; (157) a targeting sequence comprising amino acids 15-43 of SEQ ID NO: 47; (158) a targeting sequence comprising amino acids 20-43 of SEQ ID NO: 47; (159) a targeting sequence comprising amino acids 25-43 of SEQ ID NO: 47; (160) a targeting sequence comprising amino acids 1-32 of SEQ ID NO: 49; (161) a targeting sequence comprising amino acids 17-32 of SEQ ID NO: 49; (162) a targeting sequence comprising SEQ ID NO: 49; (163) an exosporium protein comprising an amino acid sequence having at least 85% identity with SEQ ID NO: 50; (164) a targeting sequence comprising amino acids 2-32 of SEQ ID NO: 49; (165) a targeting sequence comprising amino acids 5-32 of SEQ ID NO: 49; (166) a targeting sequence comprising amino acids 8-32 of SEQ ID NO: 49; (167) a targeting sequence comprising amino acids 10-32 of SEQ ID NO: 49; (168) a targeting sequence comprising amino acids 15-32 of SEQ ID NO: 49; (169) a targeting sequence comprising amino acids 1-33 of SEQ ID NO: 51; (170) a targeting sequence comprising amino acids 18-33 of SEQ ID NO: 51; (171) a targeting sequence comprising SEQ ID NO: 51; (172) an exosporium protein comprising an amino acid sequence having at least 85% identity with SEQ ID NO: 52; (173) a targeting sequence comprising amino acids 2-33 of SEQ ID NO: 51; (174) a targeting sequence comprising amino acids 5-33 of SEQ ID NO: 51; (175) a targeting sequence comprising amino acids 8-33 of SEQ ID NO: 51; (176) a targeting sequence comprising amino acids 10-33 of SEQ ID NO: 51; (177) a targeting sequence comprising amino acids 15-33 of SEQ ID NO: 51; (178) a targeting sequence comprising amino acids 1-33 of SEQ ID NO: 53; (179) a targeting sequence comprising amino acids 18-33 of SEQ ID NO: 53; (180) a targeting sequence comprising SEQ ID NO: 53; (181) an exosporium protein comprising an amino acid sequence having at least 85% identity with SEQ ID NO: 54; (182) a targeting sequence comprising amino acids 2-33 of SEQ ID NO: 53; (183) a targeting sequence comprising amino acids 5-33 of SEQ ID NO: 53; (184) a targeting sequence comprising amino acids 8-33 of SEQ ID NO: 53; (185) a targeting sequence comprising amino acids 10-33 of SEQ ID NO: 53; (186) a targeting sequence comprising amino acids 15-33 of SEQ ID NO: 53; (187) a targeting sequence comprising amino acids 1-30 of SEQ ID NO: 55; (188) a targeting sequence comprising amino acids 15-30 of SEQ ID NO: 55; (189) a targeting sequence

comprising SEQ ID NO: 55; (190) an exosporium protein comprising an amino acid sequence having at least 85% identity with SEQ ID NO: 56; (191) a targeting sequence comprising amino acids 2-30 of SEQ ID NO: 55; (192) a targeting sequence comprising amino acids 5-30 of SEQ ID NO: 55; (193) a targeting sequence comprising amino acids 8-30 of SEQ ID NO: 55; (194) a targeting sequence comprising amino acids 10-30 of SEQ ID NO: 55; (195) a targeting sequence comprising amino acids 1-130 of SEQ ID NO: 57; (196) a targeting sequence comprising amino acids 115-130 of SEQ ID NO: 57; (197) a targeting sequence comprising SEQ ID NO: 57; (198) an exosporium protein comprising an amino acid sequence having at least 85% identity with SEQ ID NO: 58; (199) a targeting sequence comprising amino acids 2-130 of SEQ ID NO: 57; (200) a targeting sequence comprising amino acids 5-130 of SEQ ID NO: 57; (201) a targeting sequence comprising amino acids 10-130 of SEQ ID NO: 57; (202) a targeting sequence comprising amino acids 20-130 of SEQ ID NO: 57; (203) a targeting sequence comprising amino acids 30-130 of SEQ ID NO: 57; (204) a targeting sequence comprising amino acids 40-130 of SEQ ID NO: 57; (205) a targeting sequence comprising amino acids 50-130 of SEQ ID NO: 57; (206) a targeting sequence comprising amino acids 60-130 of SEQ ID NO: 57; (207) a targeting sequence comprising amino acids 70-130 of SEQ ID NO: 57; (208) a targeting sequence comprising amino acids 80-130 of SEQ ID NO: 57; (209) a targeting sequence comprising amino acids 90-130 of SEQ ID NO: 57; (210) a targeting sequence comprising amino acids 100-130 of SEQ ID NO: 57; (211) a targeting sequence comprising amino acids 110-130 of SEQ ID NO: 57; (212) a targeting sequence comprising SEQ ID NO: 97; (213) a targeting sequence comprising SEQ ID NO: 98; (214) a targeting sequence comprising SEQ ID NO: 99; (215) a targeting sequence comprising SEQ ID NO: 100; (216) a targeting sequence comprising SEQ ID NO: 101; (217) a targeting sequence comprising SEQ ID NO: 102; (218) a targeting sequence comprising SEQ ID NO: 103; (219) a targeting sequence comprising SEQ ID NO: 104; (220) a targeting sequence comprising SEQ ID NO: 105; (221) a targeting sequence comprising SEQ ID NO: 106; (222) an exosporium protein comprising an amino acid sequence having at least 85% identity with SEQ ID NO: 108; (223) an exosporium protein comprising an amino acid sequence having at least 85% identity with SEQ ID NO: 109; (224) an exosporium protein comprising an amino acid sequence having at least 85% identity with SEQ ID NO: 110; (225) an exosporium protein comprising an amino acid sequence having at least 85% identity with SEQ ID NO: 111; (226) an exosporium protein comprising an amino acid sequence having at least 85% identity with SEQ ID NO: 112; (227) an exosporium protein comprising an amino acid sequence having at least 85% identity with SEQ ID NO: 113; (228) an exosporium protein comprising an amino acid sequence having at least 85% identity with SEQ ID NO: 114; (229) an exosporium protein comprising an amino acid sequence having at least 85% identity with SEQ ID NO: 115; (230) an exosporium protein comprising an amino acid sequence having at least 85% identity with SEQ ID NO: 116; (231) an exosporium protein comprising an amino acid sequence having at least 85% identity with SEQ ID NO: 117; (232) an exosporium protein comprising an amino acid sequence having at least 85% identity with SEQ ID NO: 118; (233) an exosporium protein comprising an amino acid sequence having at least 85% identity with SEQ ID NO: 119; (234) an exosporium protein comprising an amino acid sequence having at least 85% identity with SEQ ID NO: 120; or (235) an exosporium protein comprising an amino acid sequence having at least 85% identity with SEQ ID NO: 121.

(164) A fusion protein is provided which comprises an enzyme suitable for breaking an emulsion or gel in a hydraulic fracturing fluid or an antibacterial protein or peptide and a targeting sequence, exosporium protein, or exosporium protein fragment. The targeting sequence, exosporium protein, or exosporium protein fragment can comprise any of the targeting sequences or exosporium proteins listed herein above for use with any protein or peptide of interest or: (1) a targeting sequence comprising an amino acid sequence having at least about 43% identity with amino acids 20-35 of SEQ ID NO: 1, wherein the identity with amino acids 25-35 is at least about 54%; (2) a targeting sequence comprising amino acids 1-35 of SEQ ID NO: 1; (3) a targeting sequence comprising amino acids 20-35 of SEQ ID NO: 1; (4) a targeting sequence comprising SEQ ID NO: 1; (5) an exosporium protein comprising an amino acid sequence having at least 85% identity with SEQ ID NO: 2; (6) a targeting sequence comprising amino acids 2-35 of SEQ ID NO: 1; (7) a targeting sequence comprising amino acids 5-35 of SEQ ID NO: 1; (8) a targeting sequence comprising amino acids 8-35 of SEQ ID NO: 1; (9) a targeting sequence comprising amino acids 10-35 of SEQ ID NO: 1; (10) a targeting sequence comprising amino acids 15-35 of SEQ ID NO: 1; (11) a targeting sequence comprising amino acids 1-27 of SEQ ID NO: 3; (12) a targeting sequence comprising amino acids 12-27 of SEQ ID NO: 3; (13) a targeting sequence comprising SEQ ID NO: 3; (14) an exosporium protein comprising an amino acid sequence having at least 85% identity with SEQ ID NO: 4; (15) a targeting sequence comprising amino acids 2-27 of SEQ ID NO: 3; (16) a targeting sequence comprising amino acids 5-27 of SEQ ID NO: 3; (17) a targeting sequence comprising amino acids 8-27 of SEQ ID NO: 3; (18) a targeting sequence comprising amino acids 10-27 of SEQ ID NO: 3; (19) a targeting sequence comprising amino acids 1-38 of SEQ ID NO: 5; (20) a targeting sequence comprising amino acids 23-38 of SEQ ID NO: 5; (21) a targeting sequence comprising SEQ ID NO: 5; (22) an exosporium protein comprising an amino acid sequence having at least 85% identity with SEQ ID NO: 6; (23) a targeting sequence comprising amino acids 2-38 of SEQ ID NO: 5; (24) a targeting sequence comprising amino acids 5-38 of SEQ ID NO: 5; (25) a targeting sequence comprising amino

acids 8-38 of SEQ ID NO: 5; (26) a targeting sequence comprising amino acids 10-38 of SEQ ID NO: 5; (27) a targeting sequence comprising amino acids 15-38 of SEQ ID NO: 5; (28) a targeting sequence comprising amino acids 20-38 of SEQ ID NO: 5; (29) a targeting sequence comprising amino acids 1-28 of SEQ ID NO: 7; (30) a targeting sequence comprising amino acids 13-28 of SEQ ID NO: 7; (31) a targeting sequence comprising SEQ ID NO: 7; (32) an exosporium protein comprising an amino acid sequence having at least 85% identity with SEQ ID NO: 8; (33) a targeting sequence comprising amino acids 2-28 of SEQ ID NO: 7; (34) a targeting sequence comprising amino acids 5-28 of SEQ ID NO: 7; (35) a targeting sequence comprising amino acids 8-28 of SEQ ID NO: 7; (36) a targeting sequence comprising amino acids 10-28 of SEQ ID NO: 7; (37) a targeting sequence comprising amino acids 1-24 of SEQ ID NO: 9; (38) a targeting sequence comprising amino acids 9-24 of SEQ ID NO: 9; (39) a targeting sequence comprising SEQ ID NO: 9; (40) an exosporium protein comprising an amino acid sequence having at least 85% identity with SEQ ID NO: 10; (41) a targeting sequence comprising amino acids 2-24 of SEQ ID NO: 9; (42) a targeting sequence comprising amino acids 5-24 of SEQ ID NO: 9; (43) a targeting sequence comprising amino acids 8-24 of SEQ ID NO: 9; (44) a targeting sequence comprising amino acids 1-33 of SEQ ID NO: 11; (45) a targeting sequence comprising amino acids 18-33 of SEQ ID NO: 11; (46) a targeting sequence comprising SEQ ID NO: 11; (47) an exosporium protein comprising an amino acid sequence having at least 85% identity with SEQ ID NO: 12; (48) a targeting sequence comprising amino acids 2-33 of SEQ ID NO: 11; (49) a targeting sequence comprising amino acids 5-33 of SEQ ID NO: 11; (50) a targeting sequence comprising amino acids 8-33 of SEQ ID NO: 11; (51) a targeting sequence comprising amino acids 10-33 of SEQ ID NO: 11; (52) a targeting sequence comprising amino acids 15-33 of SEQ ID NO: 11; (53) a targeting sequence comprising amino acids 1-33 of SEQ ID NO: 13; (54) a targeting sequence comprising amino acids 18-33 of SEQ ID NO: 13; (55) a targeting sequence comprising SEQ ID NO: 13; (56) an exosporium protein comprising an amino acid sequence having at least 85% identity with SEQ ID NO:14; (57) a targeting sequence comprising amino acids 2-33 of SEQ ID NO: 13; (58) a targeting sequence comprising amino acids 5-33 of SEQ ID NO: 13; (59) a targeting sequence comprising amino acids 8-33 of SEQ ID NO: 13; (60) a targeting sequence comprising amino acids 10-33 of SEQ ID NO: 13; (61) a targeting sequence comprising amino acids 15-33 of SEQ ID NO: 13; (62) a targeting sequence comprising amino acids 1-43 of SEQ ID NO: 15; (63) a targeting sequence comprising amino acids 28-43 of SEQ ID NO: 15; (64) a targeting sequence comprising SEQ ID NO:15; (65) an exosporium protein comprising an amino acid sequence having at least 85% identity with SEQ ID NO: 16; (66) a targeting sequence comprising amino acids 2-43 of SEQ ID NO: 15; (67) a targeting sequence comprising amino acids 5-43 of SEQ ID NO: 15; (68) a targeting sequence comprising amino acids 8-43 of SEQ ID NO: 15; (69) a targeting sequence comprising amino acids 10-43 of SEQ ID NO: 15; (70) a targeting sequence comprising amino acids 15-43 of SEQ ID NO: 15; (71) a targeting sequence comprising amino acids 20-43 of SEQ ID NO: 15; (72) a targeting sequence comprising amino acids 25-43 of SEQ ID NO: 15; (73) a targeting sequence comprising amino acids 1-27 of SEQ ID NO: 17; (74) a targeting sequence comprising amino acids 12-27 of SEQ ID NO: 17; (75) a targeting sequence comprising SEQ ID NO:17; (76) an exosporium protein comprising an amino acid sequence having at least 85% identity with SEQ ID NO:18; (77) a targeting sequence comprising amino acids 2-27 of SEQ ID NO: 17; (78) a targeting sequence comprising amino acids 5-27 of SEQ ID NO: 17; (79) a targeting sequence comprising amino acids 8-27 of SEQ ID NO: 17; (80) a targeting sequence comprising amino acids 10-27 of SEQ ID NO: 17; (81) a targeting sequence comprising amino acids 1-33 of SEQ ID NO: 19; (82) a targeting sequence comprising amino acids 18-33 of SEQ ID NO: 19; (83) a targeting sequence comprising SEQ ID NO: 19; (84) an exosporium protein comprising an amino acid sequence having at least 85% identity with SEQ ID NO: 20; (85) a targeting sequence comprising amino acids 2-33 of SEQ ID NO: 19; (86) a targeting sequence comprising amino acids 5-33 of SEQ ID NO: 19; (87) a targeting sequence comprising amino acids 8-33 of SEQ ID NO: 19; (88) a targeting sequence comprising amino acids 10-33 of SEQ ID NO: 19; (89) a targeting sequence comprising amino acids 15-33 of SEQ ID NO: 19; (90) a targeting sequence comprising amino acids 1-33 of SEQ ID NO: 21; (91) a targeting sequence comprising amino acids 18-33 of SEQ ID NO: 21; (92) a targeting sequence comprising SEQ ID NO:21; (93) an exosporium protein comprising an amino acid sequence having at least 85% identity with SEQ ID NO:22; (94) a targeting sequence comprising amino acids 2-33 of SEQ ID NO: 21; (95) a targeting sequence comprising amino acids 5-33 of SEQ ID NO: 21; (96) a targeting sequence comprising amino acids 8-33 of SEQ ID NO: 21; (97) a targeting sequence comprising amino acids 10-33 of SEQ ID NO: 21; (98) a targeting sequence comprising amino acids 15-33 of SEQ ID NO: 21; (99) a targeting sequence comprising amino acids 1-24 of SEQ ID NO: 23; (100) a targeting sequence comprising amino acids 9-24 of SEQ ID NO: 23; (101) a targeting sequence comprising SEQ ID NO:23; (102) an exosporium protein comprising an amino acid sequence having at least 85% identity with SEQ ID NO: 24; (103) a targeting sequence comprising amino acids 2-24 of SEQ ID NO: 23; (104) a targeting sequence comprising amino acids 5-24 of SEQ ID NO: 23; (105) a targeting sequence comprising amino acids 8-24 of SEQ ID NO: 23; (106) a targeting sequence comprising amino acids 1-24 of SEQ ID NO: 25; (107) a targeting sequence comprising amino acids 9-24 of SEQ ID NO: 25; (108) a targeting sequence comprising SEQ ID NO:25; (109) an exosporium protein comprising an amino acid sequence having at least 85% identity with SEQ ID NO:26; (110) a targeting

sequence comprising amino acids 2-24 of SEQ ID NO: 25; (111) a targeting sequence comprising amino acids 5-24 of SEQ ID NO: 25; (112) a targeting sequence comprising amino acids 8-24 of SEQ ID NO: 25; (113) a targeting sequence comprising amino acids 1-30 of SEQ ID NO: 27; (114) a targeting sequence comprising amino acids 15-30 of SEQ ID NO: 27; (115) a targeting sequence comprising SEQ ID NO:27; (116) an exosporium protein comprising an amino acid sequence having at least 85% identity with SEQ ID NO:28; (117) a targeting sequence comprising amino acids 2-30 of SEQ ID NO: 27; (118) a targeting sequence comprising amino acids 5-30 of SEQ ID NO: 27; (119) a targeting sequence comprising amino acids 8-30 of SEQ ID NO: 27; (120) a targeting sequence comprising amino acids 10-30 of SEQ ID NO: 27; (121) a targeting sequence comprising amino acids 1-33 of SEQ ID NO: 29; (122) a targeting sequence comprising amino acids 18-33 of SEQ ID NO: 29; (123) a targeting sequence comprising SEQ ID NO:29; (124) an exosporium protein comprising an amino acid sequence having at least 85% identity with SEQ ID NO:30; (125) a targeting sequence comprising amino acids 2-33 of SEQ ID NO: 29; (126) a targeting sequence comprising amino acids 5-33 of SEQ ID NO: 29; (127) a targeting sequence comprising amino acids 8-33 of SEQ ID NO: 29; (128) a targeting sequence comprising amino acids 10-33 of SEQ ID NO: 29; (129) a targeting sequence comprising amino acids 15-33 of SEQ ID NO: 29; (130) a targeting sequence comprising amino acids 1-24 of SEQ ID NO: 31; (131) a targeting sequence comprising amino acids 9-24 of SEQ ID NO: 31; (132) a targeting sequence comprising SEQ ID NO:31; (133) an exosporium protein comprising an amino acid sequence having at least 85% identity with SEQ ID NO:32; (134) a targeting sequence comprising amino acids 2-24 of SEQ ID NO: 31; (135) a targeting sequence comprising amino acids 5-24 of SEQ ID NO: 31; (136) a targeting sequence comprising amino acids 8-24 of SEQ ID NO: 31; (137) a targeting sequence comprising amino acids 1-15 of SEQ ID NO: 33; (138) a targeting sequence comprising SEQ ID NO:33; (139) an exosporium protein comprising an amino acid sequence having at least 85% identity with SEQ ID NO:34; (140) a targeting sequence comprising amino acids 1-16 of SEQ ID NO: 35; (141) a targeting sequence comprising SEQ ID NO:35; (142) an exosporium protein comprising an amino acid sequence having at least 85% identity with SEQ ID NO:36; (143) a targeting sequence comprising amino acids 1-29 of SEQ ID NO:43; (144) a targeting sequence comprising amino acids 14-29 of SEQ ID NO: 43; (145) a targeting sequence comprising SEQ ID NO: 43; (146) an exosporium protein comprising an amino acid sequence having at least 85% identity with SEQ ID NO: 44; (147) a targeting sequence comprising amino acids 2-29 of SEQ ID NO: 43; (148) a targeting sequence comprising amino acids 5-29 of SEQ ID NO: 43; (149) a targeting sequence comprising amino acids 8-29 of SEQ ID NO: 43; (150) a targeting sequence comprising amino acids 10-29 of SEQ ID NO: 43; (151) a targeting sequence comprising amino acids 1-35 of SEQ ID NO: 45; (152) a targeting sequence comprising amino acids 20-35 of SEQ ID NO: 45; (153) a targeting sequence comprising SEQ ID NO: 45; (154) an exosporium protein comprising an amino acid sequence having at least 85% identity with SEQ ID NO: 46; (155) a targeting sequence comprising amino acids 2-35 of SEQ ID NO: 45; (156) a targeting sequence comprising amino acids 5-35 of SEQ ID NO: 45; (157) a targeting sequence comprising amino acids 8-35 of SEQ ID NO: 45; (158) a targeting sequence comprising amino acids 10-35 of SEQ ID NO: 45; (159) a targeting sequence comprising amino acids 15-35 of SEQ ID NO: 45; (160) a targeting sequence comprising amino acids 1-43 of SEQ ID NO: 47; (161) a targeting sequence comprising amino acids 28-43 of SEQ ID NO: 47; (162) a targeting sequence comprising SEQ ID NO: 47; (163) an exosporium protein comprising an amino acid sequence having at least 85% identity with SEQ ID NO: 48; (164) a targeting sequence comprising amino acids 2-43 of SEQ ID NO: 47; (165) a targeting sequence comprising amino acids 5-43 of SEQ ID NO: 47; (166) a targeting sequence comprising amino acids 8-43 of SEQ ID NO: 47; (167) a targeting sequence comprising amino acids 10-43 of SEQ ID NO: 47; (168) a targeting sequence comprising amino acids 15-43 of SEQ ID NO: 47; (169) a targeting sequence comprising amino acids 20-43 of SEQ ID NO: 47; (170) a targeting sequence comprising amino acids 25-43 of SEQ ID NO: 47; (171) a targeting sequence comprising amino acids 1-32 of SEQ ID NO: 49; (172) a targeting sequence comprising amino acids 17-32 of SEQ ID NO: 49; (173) a targeting sequence comprising SEQ ID NO: 49; (174) an exosporium protein comprising an amino acid sequence having at least 85% identity with SEQ ID NO: 50; (175) a targeting sequence comprising amino acids 2-32 of SEQ ID NO: 49; (176) a targeting sequence comprising amino acids 5-32 of SEQ ID NO: 49; (177) a targeting sequence comprising amino acids 8-32 of SEQ ID NO: 49; (178) a targeting sequence comprising amino acids 10-32 of SEQ ID NO: 49; (179) a targeting sequence comprising amino acids 15-32 of SEQ ID NO: 49; (180) a targeting sequence comprising amino acids 1-33 of SEQ ID NO: 51; (181) a targeting sequence comprising amino acids 18-33 of SEQ ID NO: 51; (182) a targeting sequence comprising SEQ ID NO: 51; (183) an exosporium protein comprising an amino acid sequence having at least 85% identity with SEQ ID NO: 52; (184) a targeting sequence comprising amino acids 2-33 of SEQ ID NO: 51; (185) a targeting sequence comprising amino acids 5-33 of SEQ ID NO: 51; (186) a targeting sequence comprising amino acids 8-33 of SEQ ID NO: 51; (187) a targeting sequence comprising amino acids 10-33 of SEQ ID NO: 51; (188) a targeting sequence comprising amino acids 15-33 of SEQ ID NO: 51; (189) a targeting sequence comprising amino acids 1-33 of SEQ ID NO: 53; (190) a targeting sequence comprising amino acids 18-33 of SEQ ID NO: 53; (191) a targeting sequence comprising SEQ ID NO: 53; (192) an exosporium protein comprising an amino acid sequence having at least 85% identity with SEQ ID NO: 54; (193) a targeting sequence comprising amino acids 2-33 of SEQ ID NO: 53; (194) a

targeting sequence comprising amino acids 5-33 of SEQ ID NO: 53; (195) a targeting sequence comprising amino acids 8-33 of SEQ ID NO: 53; (196) a targeting sequence comprising amino acids 10-33 of SEQ ID NO: 53; (197) a targeting sequence comprising amino acids 15-33 of SEQ ID NO: 53; (198) a targeting sequence comprising amino acids 1-30 of SEQ ID NO: 55; (199) a targeting sequence comprising amino acids 15-30 of SEQ ID NO: 55; (200) a targeting sequence comprising SEQ ID NO: 55; (201) an exosporium protein comprising an amino acid sequence having at least 85% identity with SEQ ID NO: 56; (202) a targeting sequence comprising amino acids 2-30 of SEQ ID NO: 55; (203) a targeting sequence comprising amino acids 5-30 of SEQ ID NO: 55; (204) a targeting sequence comprising amino acids 8-30 of SEQ ID NO: 55; (205) a targeting sequence comprising amino acids 10-30 of SEQ ID NO: 55; (206) a targeting sequence comprising amino acids 1-130 of SEQ ID NO: 57; (207) a targeting sequence comprising amino acids 115-130 of SEQ ID NO: 57; (208) a targeting sequence comprising SEQ ID NO: 57; (209) an exosporium protein comprising an amino acid sequence having at least 85% identity with SEQ ID NO: 58; (210) a targeting sequence comprising amino acids 2-130 of SEQ ID NO: 57; (211) a targeting sequence comprising amino acids 5-130 of SEQ ID NO: 57; (212) a targeting sequence comprising amino acids 10-130 of SEQ ID NO: 57; (213) a targeting sequence comprising amino acids 20-130 of SEQ ID NO: 57; (214) a targeting sequence comprising amino acids 30-130 of SEQ ID NO: 57; (215) a targeting sequence comprising amino acids 40-130 of SEQ ID NO: 57; (216) a targeting sequence comprising amino acids 50-130 of SEQ ID NO: 57; (217) a targeting sequence comprising amino acids 60-130 of SEQ ID NO: 57; (218) a targeting sequence comprising amino acids 70-130 of SEQ ID NO: 57; (219) a targeting sequence comprising amino acids 80-130 of SEQ ID NO: 57; (220) a targeting sequence comprising amino acids 90-130 of SEQ ID NO: 57; (221) a targeting sequence comprising amino acids 100-130 of SEQ ID NO: 57; (222) a targeting sequence comprising amino acids 110-130 of SEQ ID NO: 57; (223) an exosporium protein fragment comprising an amino acid sequence having at least 85% identity with SEQ ID NO: 95; (224) a targeting sequence comprising SEQ ID NO: 96; (225) a targeting sequence comprising SEQ ID NO: 97; (226) a targeting sequence comprising SEQ ID NO: 98; (227) a targeting sequence comprising SEQ ID NO: 99; (228) a targeting sequence comprising SEQ ID NO: 100; (229) a targeting sequence comprising SEQ ID NO: 101; (230) a targeting sequence comprising SEQ ID NO: 102; (231) a targeting sequence comprising SEQ ID NO: 103; (232) a targeting sequence comprising SEQ ID NO: 104; (233) a targeting sequence comprising SEQ ID NO: 105; (234) a targeting sequence comprising SEQ ID NO: 106; (235) an exosporium protein comprising an amino acid sequence having at least 85% identity with SEQ ID NO: 108; (236) an exosporium protein comprising an amino acid sequence having at least 85% identity with SEQ ID NO: 109; (237) an exosporium protein comprising an amino acid sequence having at least 85% identity with SEQ ID NO: 110; (238) an exosporium protein comprising an amino acid sequence having at least 85% identity with SEQ ID NO: 111; (239) an exosporium protein comprising an amino acid sequence having at least 85% identity with SEQ ID NO: 112; (240) an exosporium protein comprising an amino acid sequence having at least 85% identity with SEQ ID NO: 113; (241) an exosporium protein comprising an amino acid sequence having at least 85% identity with SEQ ID NO: 114; (242) an exosporium protein comprising an amino acid sequence having at least 85% identity with SEQ ID NO: 115; (243) an exosporium protein comprising an amino acid sequence having at least 85% identity with SEQ ID NO: 116; (244) an exosporium protein comprising an amino acid sequence having at least 85% identity with SEQ ID NO: 117; (245) an exosporium protein comprising an amino acid sequence having at least 85% identity with SEQ ID NO: 118; (246) an exosporium protein comprising an amino acid sequence having at least 85% identity with SEQ ID NO: 119; (247) an exosporium protein comprising an amino acid sequence having at least 85% identity with SEQ ID NO: 120; (248) an exosporium protein comprising an amino acid sequence having at least 85% identity with SEQ ID NO: 121; (249) a targeting sequence comprising amino acids 22-31 of SEQ ID NO: 1; (250) a targeting sequence comprising amino acids 22-33 of SEQ ID NO: 1; (251) a targeting sequence comprising amino acids 20-31 of SEQ ID NO: 1; (252) a targeting sequence comprising amino acids 14-23 of SEQ ID NO: 3; (253) a targeting sequence comprising amino acids 14-25 of SEQ ID NO: 3; or (254) a targeting sequence comprising amino acids 12-23 of SEQ ID NO: 3.

(165) When the protein or peptide of interest comprises an antigen, a remediation enzyme, an enzyme suitable for breaking an emulsion or gel in a hydraulic fracturing fluid or an antibacterial protein or peptide, preferably, the targeting sequence or exosporium protein comprises any of the targeting sequences or exosporium proteins listed herein above for use with any protein or peptide of interest or: (1) a targeting sequence comprising amino acids 2-35 of SEQ ID NO: 1; (2) a targeting sequence comprising amino acids 5-35 of SEQ ID NO: 1; (3) a targeting sequence comprising amino acids 8-35 of SEQ ID NO: 1; (4) a targeting sequence comprising amino acids 10-35 of SEQ ID NO: 1; (5) a targeting sequence comprising amino acids 15-35 of SEQ ID NO: 1; (6) a targeting sequence comprising amino acids 2-27 of SEQ ID NO: 3; (7) a targeting sequence comprising amino acids 5-27 of SEQ ID NO: 3; (8) a targeting sequence comprising amino acids 8-27 of SEQ ID NO: 3; (9) a targeting sequence comprising amino acids 10-27 of SEQ ID NO: 3; (10) a targeting sequence comprising amino acids 2-38 of SEQ ID NO: 5; (11) a targeting sequence comprising amino acids 5-38 of SEQ ID NO: 5; (12) a targeting sequence comprising amino acids 8-38 of SEQ ID NO: 5; (13) a targeting sequence comprising amino acids 10-38 of SEQ ID NO: 5; (14) a

targeting sequence comprising amino acids 15-38 of SEQ ID NO: 5; (15) a targeting sequence comprising amino acids 20-38 of SEQ ID NO: 5; (16) a targeting sequence comprising amino acids 2-28 of SEQ ID NO: 7; (17) a targeting sequence comprising amino acids 5-28 of SEQ ID NO: 7; (18) a targeting sequence comprising amino acids 8-28 of SEQ ID NO: 7; (19) a targeting sequence comprising amino acids 10-28 of SEQ ID NO: 7; (20) a targeting sequence comprising amino acids 2-24 of SEQ ID NO: 9; (21) a targeting sequence comprising amino acids 5-24 of SEQ ID NO: 9; (22) a targeting sequence comprising amino acids 8-24 of SEQ ID NO: 9; (23) a targeting sequence comprising amino acids 2-33 of SEQ ID NO: 11; (24) a targeting sequence comprising amino acids 5-33 of SEQ ID NO: 11; (25) a targeting sequence comprising amino acids 8-33 of SEQ ID NO: 11; (26) a targeting sequence comprising amino acids 10-33 of SEQ ID NO: 11; (27) a targeting sequence comprising amino acids 15-33 of SEQ ID NO: 11; (28) a targeting sequence comprising amino acids 2-33 of SEQ ID NO: 13; (29) a targeting sequence comprising amino acids 5-33 of SEQ ID NO: 13; (30) a targeting sequence comprising amino acids 8-33 of SEQ ID NO: 13; (31) a targeting sequence comprising amino acids 10-33 of SEQ ID NO: 13; (32) a targeting sequence comprising amino acids 15-33 of SEQ ID NO: 13; (33) a targeting sequence comprising amino acids 2-43 of SEQ ID NO: 15; (34) a targeting sequence comprising amino acids 5-43 of SEQ ID NO: 15; (35) a targeting sequence comprising amino acids 8-43 of SEQ ID NO: 15; (36) a targeting sequence comprising amino acids 10-43 of SEQ ID NO: 15; (37) a targeting sequence comprising amino acids 15-43 of SEQ ID NO: 15; (38) a targeting sequence comprising amino acids 20-43 of SEQ ID NO: 15; (39) a targeting sequence comprising amino acids 25-43 of SEQ ID NO: 15; (40) a targeting sequence comprising amino acids 2-27 of SEQ ID NO: 17; (41) a targeting sequence comprising amino acids 5-27 of SEQ ID NO: 17; (42) a targeting sequence comprising amino acids 8-27 of SEQ ID NO: 17; (43) a targeting sequence comprising amino acids 10-27 of SEQ ID NO: 17; (44) a targeting sequence comprising amino acids 2-33 of SEQ ID NO: 19; (45) a targeting sequence comprising amino acids 5-33 of SEQ ID NO: 19; (46) a targeting sequence comprising amino acids 8-33 of SEQ ID NO: 19; (47) a targeting sequence comprising amino acids 10-33 of SEQ ID NO: 19; (48) a targeting sequence comprising amino acids 15-33 of SEQ ID NO: 19; (49) a targeting sequence comprising amino acids 2-33 of SEQ ID NO: 21; (50) a targeting sequence comprising amino acids 5-33 of SEQ ID NO: 21; (51) a targeting sequence comprising amino acids 8-33 of SEQ ID NO: 21; (52) a targeting sequence comprising amino acids 10-33 of SEQ ID NO: 21; (53) a targeting sequence comprising amino acids 15-33 of SEQ ID NO: 21; (54) a targeting sequence comprising amino acids 2-24 of SEQ ID NO:23; (55) a targeting sequence comprising amino acids 5-24 of SEQ ID NO: 23; (56) a targeting sequence comprising amino acids 8-24 of SEQ ID NO: 23; (57) a targeting sequence comprising amino acids 2-24 of SEQ ID NO: 25; (58) a targeting sequence comprising amino acids 5-24 of SEQ ID NO: 25; (59) a targeting sequence comprising amino acids 8-24 of SEQ ID NO: 25; (60) a targeting sequence comprising amino acids 2-30 of SEQ ID NO: 27; (61) a targeting sequence comprising amino acids 5-30 of SEQ ID NO: 27; (62) a targeting sequence comprising amino acids 8-30 of SEQ ID NO: 27; (63) a targeting sequence comprising amino acids 10-30 of SEQ ID NO: 27; (64) a targeting sequence comprising amino acids 2-33 of SEQ ID NO: 29; (65) a targeting sequence comprising amino acids 5-33 of SEQ ID NO: 29; (66) a targeting sequence comprising amino acids 8-33 of SEQ ID NO: 29; (67) a targeting sequence comprising amino acids 10-33 of SEQ ID NO: 29; (68) a targeting sequence comprising amino acids 15-33 of SEQ ID NO: 29; (69) a targeting sequence comprising amino acids 2-24 of SEQ ID NO: 31; (70) a targeting sequence comprising amino acids 5-24 of SEQ ID NO: 31; (71) a targeting sequence comprising amino acids 8-24 of SEQ ID NO: 31; (72) a targeting sequence comprising amino acids 2-29 of SEQ ID NO: 43; (73) a targeting sequence comprising amino acids 5-29 of SEQ ID NO: 43; (74) a targeting sequence comprising amino acids 8-29 of SEQ ID NO: 43; (75) a targeting sequence comprising amino acids 10-29 of SEQ ID NO: 43; (76) a targeting sequence comprising amino acids 2-35 of SEQ ID NO: 45; (77) a targeting sequence comprising amino acids 5-35 of SEQ ID NO: 45; (78) a targeting sequence comprising amino acids 8-35 of SEQ ID NO: 45; (79) a targeting sequence comprising amino acids 10-35 of SEQ ID NO: 45; (80) a targeting sequence comprising amino acids 15-35 of SEQ ID NO: 45; (81) a targeting sequence comprising amino acids 2-43 of SEQ ID NO: 47; (82) a targeting sequence comprising amino acids 5-43 of SEQ ID NO: 47; (83) a targeting sequence comprising amino acids 8-43 of SEQ ID NO: 47; (84) a targeting sequence comprising amino acids 10-43 of SEQ ID NO: 47; (85) a targeting sequence comprising amino acids 15-43 of SEQ ID NO: 47; (86) a targeting sequence comprising amino acids 20-43 of SEQ ID NO: 47; (87) a targeting sequence comprising amino acids 25-43 of SEQ ID NO: 47; (88) a targeting sequence comprising amino acids 2-32 of SEQ ID NO: 49; (89) a targeting sequence comprising amino acids 5-32 of SEQ ID NO: 49; (90) a targeting sequence comprising amino acids 8-32 of SEQ ID NO: 49; (91) a targeting sequence comprising amino acids 10-32 of SEQ ID NO: 49; (92) a targeting sequence comprising amino acids 15-32 of SEQ ID NO: 49; (93) a targeting sequence comprising amino acids 2-33 of SEQ ID NO: 51; (94) a targeting sequence comprising amino acids 5-33 of SEQ ID NO: 51; (95) a targeting sequence comprising amino acids 8-33 of SEQ ID NO: 51; (96) a targeting sequence comprising amino acids 10-33 of SEQ ID NO: 51; (97) a targeting sequence comprising amino acids 15-33 of SEQ ID NO: 51; (98) a targeting sequence comprising amino acids 2-33 of SEQ ID NO: 53; (99) a targeting sequence comprising amino acids 5-33 of SEQ ID NO: 53; (100) a targeting sequence comprising amino acids 8-33 of SEQ ID NO: 53; (101) a

targeting sequence comprising amino acids 10-33 of SEQ ID NO: 53; (102) a targeting sequence comprising amino acids 15-33 of SEQ ID NO: 53; (103) a targeting sequence comprising amino acids 2-30 of SEQ ID NO: 55; (104) a targeting sequence comprising amino acids 5-30 of SEQ ID NO: 55; (105) a targeting sequence comprising amino acids 8-30 of SEQ ID NO: 55; (106) a targeting sequence comprising amino acids 10-30 of SEQ ID NO: 55; (107) a targeting sequence comprising amino acids 2-130 of SEQ ID NO: 57; (108) a targeting sequence comprising amino acids 5-130 of SEQ ID NO: 57; (109) a targeting sequence comprising amino acids 10-130 of SEQ ID NO: 57; (110) a targeting sequence comprising amino acids 20-130 of SEQ ID NO: 57; (111) a targeting sequence comprising amino acids 30-130 of SEQ ID NO: 57; (112) a targeting sequence comprising amino acids 40-130 of SEQ ID NO: 57; (113) a targeting sequence comprising amino acids 50-130 of SEQ ID NO: 57; (114) a targeting sequence comprising amino acids 60-130 of SEQ ID NO: 57; (115) a targeting sequence comprising amino acids 70-130 of SEQ ID NO: 57; (116) a targeting sequence comprising amino acids 80-130 of SEQ ID NO: 57; (117) a targeting sequence comprising amino acids 90-130 of SEQ ID NO: 57; (118) a targeting sequence comprising amino acids 100-130 of SEQ ID NO: 57; (119) a targeting sequence comprising amino acids 110-130 of SEQ ID NO: 57.

(166) When the protein or peptide of interest comprises an antigen, a remediation enzyme, an enzyme suitable for breaking an emulsion or gel in a hydraulic fracturing fluid or an antibacterial protein or peptide, more preferably, the targeting sequence or exosporium protein comprises any of the targeting sequences or exosporium proteins listed herein above for use with any protein or peptide of interest or: (1) a targeting sequence comprising amino acids 2-24 of SEQ ID NO: 9; (2) a targeting sequence comprising amino acids 5-24 of SEQ ID NO: 9; (3) a targeting sequence comprising amino acids 8-24 of SEQ ID NO: 9; (4) a targeting sequence comprising amino acids 2-33 of SEQ ID NO: 11; (5) a targeting sequence comprising amino acids 5-33 of SEQ ID NO: 11; (6) a targeting sequence comprising amino acids 8-33 of SEQ ID NO: 11; (7) a targeting sequence comprising amino acids 10-33 of SEQ ID NO: 11; (8) a targeting sequence comprising amino acids 15-33 of SEQ ID NO: 11; (9) a targeting sequence comprising amino acids 2-33 of SEQ ID NO: 13; (10) a targeting sequence comprising amino acids 5-33 of SEQ ID NO: 13; (11) a targeting sequence comprising amino acids 8-33 of SEQ ID NO: 13; (12) a targeting sequence comprising amino acids 10-33 of SEQ ID NO: 13; (13) a targeting sequence comprising amino acids 15-33 of SEQ ID NO: 13; (14) a targeting sequence comprising amino acids 2-43 of SEQ ID NO: 15; (15) a targeting sequence comprising amino acids 5-43 of SEQ ID NO: 15; (16) a targeting sequence comprising amino acids 8-43 of SEQ ID NO: 15; (17) a targeting sequence comprising amino acids 10-43 of SEQ ID NO: 15; (18) a targeting sequence comprising amino acids 15-43 of SEQ ID NO: 15; (19) a targeting sequence comprising amino acids 20-43 of SEQ ID NO: 15; (20) a targeting sequence comprising amino acids 25-43 of SEQ ID NO: 15; (21) a targeting sequence comprising amino acids 2-27 of SEQ ID NO: 17; (22) a targeting sequence comprising amino acids 5-27 of SEQ ID NO: 17; (23) a targeting sequence comprising amino acids 8-27 of SEQ ID NO: 17; (24) a targeting sequence comprising amino acids 10-27 of SEQ ID NO: 17; (25) a targeting sequence comprising amino acids 2-33 of SEQ ID NO: 19; (26) a targeting sequence comprising amino acids 5-33 of SEQ ID NO: 19; (27) a targeting sequence comprising amino acids 8-33 of SEQ ID NO: 19; (28) a targeting sequence comprising amino acids 10-33 of SEQ ID NO: 19; (29) a targeting sequence comprising amino acids 15-33 of SEQ ID NO: 19; (30) a targeting sequence comprising amino acids 2-33 of SEQ ID NO: 21; (31) a targeting sequence comprising amino acids 5-33 of SEQ ID NO: 21; (32) a targeting sequence comprising amino acids 8-33 of SEQ ID NO: 21; (33) a targeting sequence comprising amino acids 10-33 of SEQ ID NO: 21; (34) a targeting sequence comprising amino acids 15-33 of SEQ ID NO: 21; (35) a targeting sequence comprising amino acids 2-24 of SEQ ID NO:23; (36) a targeting sequence comprising amino acids 5-24 of SEQ ID NO: 23; (37) a targeting sequence comprising amino acids 8-24 of SEQ ID NO: 23; (38) a targeting sequence comprising amino acids 2-24 of SEQ ID NO: 25; (39) a targeting sequence comprising amino acids 5-24 of SEQ ID NO: 25; (40) a targeting sequence comprising amino acids 8-24 of SEQ ID NO: 25; (41) a targeting sequence comprising amino acids 2-30 of SEQ ID NO: 27; (42) a targeting sequence comprising amino acids 5-30 of SEQ ID NO: 27; (43) a targeting sequence comprising amino acids 8-30 of SEQ ID NO: 27; (44) a targeting sequence comprising amino acids 10-30 of SEQ ID NO: 27; (45) a targeting sequence comprising amino acids 2-33 of SEQ ID NO: 29; (46) a targeting sequence comprising amino acids 5-33 of SEQ ID NO: 29; (47) a targeting sequence comprising amino acids 8-33 of SEQ ID NO: 29; (48) a targeting sequence comprising amino acids 10-33 of SEQ ID NO: 29; (49) a targeting sequence comprising amino acids 15-33 of SEQ ID NO: 29; (50) a targeting sequence comprising amino acids 2-24 of SEQ ID NO: 31; (51) a targeting sequence comprising amino acids 5-24 of SEQ ID NO: 31; (52) a targeting sequence comprising amino acids 8-24 of SEQ ID NO: 31; (53) a targeting sequence comprising amino acids 2-29 of SEQ ID NO: 43; (54) a targeting sequence comprising amino acids 5-29 of SEQ ID NO: 43; (55) a targeting sequence comprising amino acids 8-29 of SEQ ID NO: 43; (56) a targeting sequence comprising amino acids 10-29 of SEQ ID NO: 43; (57) a targeting sequence comprising amino acids 2-35 of SEQ ID NO: 45; (58) a targeting sequence comprising amino acids 5-35 of SEQ ID NO: 45; (59) a targeting sequence comprising amino acids 8-35 of SEQ ID NO: 45; (60) a targeting sequence comprising amino acids 10-35 of SEQ ID NO: 45; (61) a targeting sequence comprising amino acids 15-

35 of SEQ ID NO: 45; (62) a targeting sequence comprising amino acids 2-43 of SEQ ID NO: 47; (63) a targeting sequence comprising amino acids 5-43 of SEQ ID NO: 47; (64) a targeting sequence comprising amino acids 8-43 of SEQ ID NO: 47; (65) a targeting sequence comprising amino acids 10-43 of SEQ ID NO: 47; (66) a targeting sequence comprising amino acids 15-43 of SEQ ID NO: 47; (67) a targeting sequence comprising amino acids 20-43 of SEQ ID NO: 47; (68) a targeting sequence comprising amino acids 25-43 of SEQ ID NO: 47; (69) a targeting sequence comprising amino acids 2-32 of SEQ ID NO: 49; (70) a targeting sequence comprising amino acids 5-32 of SEQ ID NO: 49; (71) a targeting sequence comprising amino acids 8-32 of SEQ ID NO: 49; (72) a targeting sequence comprising amino acids 10-32 of SEQ ID NO: 49; (73) a targeting sequence comprising amino acids 15-32 of SEQ ID NO: 49; (74) a targeting sequence comprising amino acids 2-33 of SEQ ID NO: 51; (75) a targeting sequence comprising amino acids 5-33 of SEQ ID NO: 51; (76) a targeting sequence comprising amino acids 8-33 of SEQ ID NO: 51; (77) a targeting sequence comprising amino acids 10-33 of SEQ ID NO: 51; (78) a targeting sequence comprising amino acids 15-33 of SEQ ID NO: 51; (79) a targeting sequence comprising amino acids 2-33 of SEQ ID NO: 53; (80) a targeting sequence comprising amino acids 5-33 of SEQ ID NO: 53; (81) a targeting sequence comprising amino acids 8-33 of SEQ ID NO: 53; (82) a targeting sequence comprising amino acids 10-33 of SEQ ID NO: 53; (83) a targeting sequence comprising amino acids 15-33 of SEQ ID NO: 53; (84) a targeting sequence comprising amino acids 2-30 of SEQ ID NO: 55; (85) a targeting sequence comprising amino acids 5-30 of SEQ ID NO: 55; (86) a targeting sequence comprising amino acids 8-30 of SEQ ID NO: 55; (87) a targeting sequence comprising amino acids 10-30 of SEQ ID NO: 55; (88) a targeting sequence comprising amino acids 2-130 of SEQ ID NO: 57; (89) a targeting sequence comprising amino acids 5-130 of SEQ ID NO: 57; (90) a targeting sequence comprising amino acids 10-130 of SEQ ID NO: 57; (91) a targeting sequence comprising amino acids 20-130 of SEQ ID NO: 57; (92) a targeting sequence comprising amino acids 30-130 of SEQ ID NO: 57; (93) a targeting sequence comprising amino acids 40-130 of SEQ ID NO: 57; (94) a targeting sequence comprising amino acids 50-130 of SEQ ID NO: 57; (95) a targeting sequence comprising amino acids 60-130 of SEQ ID NO: 57; (96) a targeting sequence comprising amino acids 70-130 of SEQ ID NO: 57; (97) a targeting sequence comprising amino acids 80-130 of SEQ ID NO: 57; (98) a targeting sequence comprising amino acids 90-130 of SEQ ID NO: 57; (99) a targeting sequence comprising amino acids 100-130 of SEQ ID NO: 57; (100) a targeting sequence comprising amino acids 110-130 of SEQ ID NO: 57.

(167) When the protein or peptide of interest comprises an antigen, a remediation enzyme, an enzyme suitable for breaking an emulsion or gel in a hydraulic fracturing fluid or an antibacterial protein or peptide, even more preferably, the targeting sequence or exosporium protein comprises: (1) a targeting sequence comprising amino acids 5-24 of SEQ ID NO: 9; (2) a targeting sequence comprising amino acids 8-24 of SEQ ID NO: 9; (3) a targeting sequence comprising amino acids 5-33 of SEQ ID NO: 11; (4) a targeting sequence comprising amino acids 8-33 of SEQ ID NO: 11; (5) a targeting sequence comprising amino acids 10-33 of SEQ ID NO: 11; (6) a targeting sequence comprising amino acids 15-33 of SEQ ID NO: 11; (7) a targeting sequence comprising amino acids 5-33 of SEQ ID NO: 13; (8) a targeting sequence comprising amino acids 8-33 of SEQ ID NO: 13; (9) a targeting sequence comprising amino acids 10-33 of SEQ ID NO: 13; (10) a targeting sequence comprising amino acids 15-33 of SEQ ID NO: 13; (11) a targeting sequence comprising amino acids 5-43 of SEQ ID NO: 15; (12) a targeting sequence comprising amino acids 8-43 of SEQ ID NO: 15; (13) a targeting sequence comprising amino acids 10-43 of SEQ ID NO: 15; (14) a targeting sequence comprising amino acids 15-43 of SEQ ID NO: 15; (15) a targeting sequence comprising amino acids 20-43 of SEQ ID NO: 15; (16) a targeting sequence comprising amino acids 25-43 of SEQ ID NO: 15; (17) a targeting sequence comprising amino acids 5-27 of SEQ ID NO: 17; (18) a targeting sequence comprising amino acids 8-27 of SEQ ID NO: 17; (19) a targeting sequence comprising amino acids 10-27 of SEQ ID NO: 17; (20) a targeting sequence comprising amino acids 5-33 of SEQ ID NO: 19; (21) a targeting sequence comprising amino acids 8-33 of SEQ ID NO: 19; (22) a targeting sequence comprising amino acids 10-33 of SEQ ID NO: 19; (23) a targeting sequence comprising amino acids 15-33 of SEQ ID NO: 19; (24) a targeting sequence comprising amino acids 5-33 of SEQ ID NO: 21; (25) a targeting sequence comprising amino acids 8-33 of SEQ ID NO: 21; (26) a targeting sequence comprising amino acids 10-33 of SEQ ID NO: 21; (27) a targeting sequence comprising amino acids 15-33 of SEQ ID NO: 21; (28) a targeting sequence comprising amino acids 5-24 of SEQ ID NO: 23; (29) a targeting sequence comprising amino acids 8-24 of SEQ ID NO: 23; (30) a targeting sequence comprising amino acids 5-24 of SEQ ID NO: 25; (31) a targeting sequence comprising amino acids 8-24 of SEQ ID NO: 25; (32) a targeting sequence comprising amino acids 5-30 of SEQ ID NO: 27; (33) a targeting sequence comprising amino acids 8-30 of SEQ ID NO: 27; (34) a targeting sequence comprising amino acids 10-30 of SEQ ID NO: 27; (35) a targeting sequence comprising amino acids 5-33 of SEQ ID NO: 29; (36) a targeting sequence comprising amino acids 8-33 of SEQ ID NO: 29; (37) a targeting sequence comprising amino acids 10-33 of SEQ ID NO: 29; (38) a targeting sequence comprising amino acids 15-33 of SEQ ID NO: 29; (39) a targeting sequence comprising amino acids 5-24 of SEQ ID NO: 31; (40) a targeting sequence comprising amino acids 8-24 of SEQ ID NO: 31; (41) a targeting sequence comprising amino acids 5-29 of SEQ ID NO: 43; (42) a targeting sequence comprising amino acids 8-29 of SEQ ID NO: 43; (43) a targeting sequence comprising amino

acids 10-29 of SEQ ID NO: 43; (44) a targeting sequence comprising amino acids 5-35 of SEQ ID NO: 45; (45) a targeting sequence comprising amino acids 8-35 of SEQ ID NO: 45; (46) a targeting sequence comprising amino acids 10-35 of SEQ ID NO: 45; (47) a targeting sequence comprising amino acids 15-35 of SEQ ID NO: 45; (48) a targeting sequence comprising amino acids 5-43 of SEQ ID NO: 47; (49) a targeting sequence comprising amino acids 8-43 of SEQ ID NO: 47; (50) a targeting sequence comprising amino acids 10-43 of SEQ ID NO: 47; (51) a targeting sequence comprising amino acids 15-43 of SEQ ID NO: 47; (52) a targeting sequence comprising amino acids 20-43 of SEQ ID NO: 47; (53) a targeting sequence comprising amino acids 25-43 of SEQ ID NO: 47; (54) a targeting sequence comprising amino acids 5-32 of SEQ ID NO: 49; (55) a targeting sequence comprising amino acids 8-32 of SEQ ID NO: 49; (56) a targeting sequence comprising amino acids 10-32 of SEQ ID NO: 49; (57) a targeting sequence comprising amino acids 15-32 of SEQ ID NO: 49; (58) a targeting sequence comprising amino acids 5-33 of SEQ ID NO: 51; (59) a targeting sequence comprising amino acids 8-33 of SEQ ID NO: 51; (60) a targeting sequence comprising amino acids 10-33 of SEQ ID NO: 51; (61) a targeting sequence comprising amino acids 15-33 of SEQ ID NO: 51; (62) a targeting sequence comprising amino acids 5-33 of SEQ ID NO: 53; (63) a targeting sequence comprising amino acids 8-33 of SEQ ID NO: 53; (64) a targeting sequence comprising amino acids 10-33 of SEQ ID NO: 53; (65) a targeting sequence comprising amino acids 15-33 of SEQ ID NO: 53; (66) a targeting sequence comprising amino acids 5-30 of SEQ ID NO: 55; (67) a targeting sequence comprising amino acids 8-30 of SEQ ID NO: 55; (68) a targeting sequence comprising amino acids 10-30 of SEQ ID NO: 55; (69) a targeting sequence comprising amino acids 5-130 of SEQ ID NO: 57; (70) a targeting sequence comprising amino acids 10-130 of SEQ ID NO: 57; (71) a targeting sequence comprising amino acids 20-130 of SEQ ID NO: 57; (72) a targeting sequence comprising amino acids 30-130 of SEQ ID NO: 57; (73) a targeting sequence comprising amino acids 40-130 of SEQ ID NO: 57; (74) a targeting sequence comprising amino acids 50-130 of SEQ ID NO: 57; (75) a targeting sequence comprising amino acids 60-130 of SEQ ID NO: 57; (76) a targeting sequence comprising amino acids 70-130 of SEQ ID NO: 57; (77) a targeting sequence comprising amino acids 80-130 of SEQ ID NO: 57; (78) a targeting sequence comprising amino acids 90-130 of SEQ ID NO: 57; (79) a targeting sequence comprising amino acids 100-130 of SEQ ID NO: 57; (80) a targeting sequence comprising amino acids 110-130 of SEQ ID NO: 57; (81) a targeting sequence comprising amino acids 4-30 of SEQ ID NO: 59; (82) a targeting sequence comprising amino acids 6-30 of SEQ ID NO: 59; (83) a targeting sequence comprising amino acids 5-33 of SEQ ID NO: 61; (84) a targeting sequence comprising amino acids 10-33 of SEQ ID NO: 61; (85) a targeting sequence comprising amino acids 15-33 of SEQ ID NO: 61; (86) a targeting sequence comprising amino acids 5-35 of SEQ ID NO: 63; (87) a targeting sequence comprising amino acids 8-35 of SEQ ID NO: 63; (88) a targeting sequence comprising amino acids 10-35 of SEQ ID NO: 63; (89) a targeting sequence comprising amino acids 15-35 of SEQ ID NO: 63; (90) a targeting sequence comprising amino acids 5-24 of SEQ ID NO: 65; (91) a targeting sequence comprising amino acids 5-27 of SEQ ID NO: 67; (92) a targeting sequence comprising amino acids 10-27 of SEQ ID NO: 67; (93) a targeting sequence comprising amino acids 5-38 of SEQ ID NO: 69; (94) a targeting sequence comprising amino acids 10-38 of SEQ ID NO: 69; (95) a targeting sequence comprising amino acids 15-38 of SEQ ID NO: 69; (96) a targeting sequence comprising amino acids 5-42 of SEQ ID NO: 75; (97) a targeting sequence comprising amino acids 10-42 of SEQ ID NO: 75; (98) a targeting sequence comprising amino acids 15-42 of SEQ ID NO: 75; (99) a targeting sequence comprising amino acids 20-42 of SEQ ID NO: 75; (100) a targeting sequence comprising amino acids 25-42 of SEQ ID NO: 75; (101) a targeting sequence comprising amino acids 5-24 of SEQ ID NO: 77; (102) a targeting sequence comprising amino acids 5-38 of SEQ ID NO: 81; (103) a targeting sequence comprising amino acids 10-38 of SEQ ID NO: 81; (104) a targeting sequence comprising amino acids 15-38 of SEQ ID NO: 81; (105) a targeting sequence comprising amino acids 20-38 of SEQ ID NO: 81; (106) a targeting sequence comprising amino acids 5-28 of SEQ ID NO: 87; (107) a targeting sequence comprising amino acids 10-28 of SEQ ID NO: 87; (108) a targeting sequence comprising amino acids 5-28 of SEQ ID NO: 89; (109) a targeting sequence comprising amino acids 10-28 of SEQ ID NO: 89; (110) a targeting sequence comprising amino acids 10-93 of SEQ ID NO: 91; (111) a targeting sequence comprising amino acids 20-93 of SEQ ID NO: 91; (112) a targeting sequence comprising amino acids 30-93 of SEQ ID NO: 91; (113) a targeting sequence comprising amino acids 40-93 of SEQ ID NO: 91; (114) a targeting sequence comprising amino acids 50-93 of SEQ ID NO: 91; (115) a targeting sequence comprising amino acids 60-93 of SEQ ID NO: 91; (116) a targeting sequence comprising amino acids 10-130 of SEQ ID NO: 93; (117) a targeting sequence comprising amino acids 20-130 of SEQ ID NO: 93; or (118) a targeting sequence comprising amino acids 30-130 of SEQ ID NO: 93.

(168) The protein or peptide of interest of the fusion protein described above can comprise an antigen.

(169) The protein or peptide of interest of the fusion protein described above can comprise a remediation enzyme.

(170) The protein or peptide of interest of the fusion protein described above can comprise an enzyme suitable for breaking an emulsion or gel in a hydraulic fracturing fluid.

(171) The protein or peptide of interest of the fusion protein described above can comprise an antibacterial protein or peptide.

(172) C. Recombinant *Bacillus cereus* Family Members that Express Fusion Proteins

(173) The present invention further relates to recombinant *Bacillus cereus* family members that express a fusion protein. The fusion protein can be any of the fusion proteins described above in Section I.B.

II. Modulation of Fusion Protein Expression in Recombinant *Bacillus cereus* Family Members that Express a Fusion Protein by Co-Overexpression of Modulator Proteins

(174) Recombinant *Bacillus cereus* family members that express the fusion proteins described herein display the protein or peptide of interest portion of the fusion protein on the outside of their spores. It has been found that overexpression of certain exosporium proteins (referred to herein as “modulator proteins”) in a recombinant *Bacillus cereus* family member that also expresses a fusion protein allows for modulation (i.e., increasing or decreasing) the expression level of the fusion protein, thereby increasing or decreasing the amount of the protein or peptide of interest that is displayed on the outside of the spore. The ability to the control the amount of the protein or peptide of interest that is displayed on the outside of the spore is beneficial, since in some cases, it will be desirable to increase the amount of the protein or peptide of interest that is displayed. For example, where the protein of interest is an enzyme that degrades a plant nutrient source, it may be desirable to increase the amount of the enzyme displayed on the spore, such that greater enzymatic activity and greater stimulation of plant growth can be achieved upon introducing the spores into a plant growth medium or application of the spores to a plant or plant seed or an area surrounding a plant or a plant seed. In other instances, it will be desirable to decrease the amount of the protein or peptide of interest that is displayed. For example, where the protein or peptide of interest comprises a plant immune system enhancer protein or peptide, it may be desirable to decrease the amount of the protein or peptide displayed on the spore, since excess stimulation of a plant's immune system can lead to undesirable effects.

(175) As is described further hereinbelow, the recombinant *Bacillus cereus* family members that express a modulator protein can be used in any of the various fields and methods described herein, and for any of the uses described herein. For example, the recombinant *Bacillus cereus* family members that express a modulator protein can be used in methods for stimulating plant growth; methods for protecting a plant from a pathogen; methods for enhancing stress resistance in plants; methods for immobilizing recombinant *Bacillus cereus* family member spores on plants; methods for stimulating germination of a plant seed; methods for delivering nucleic acids to a plant; methods for delivering nucleic acids to animals, insects, worms (e.g., nematodes), fungi, or protozoans; methods for delivering enzymes to a plant; methods for altering a property of a plant; methods for delivering proteins or peptides to an animal; vaccines and methods of producing an immunogenic response in a subject; methods for reducing contaminants in an environment; methods for phytoremediation of contaminated soil; methods of treating a hydraulic fracturing fluid to break an emulsion or gel within the fluid; methods of disinfecting a surface; and for uses such as grease, oil, or fat treatment or degumming; leather hide processing; biofuel, biodiesel, or bioethanol formation; sugar processing or conversion; starch treatment; paper or linen processing; animal or fungal byproduct treatment or amino acid recovery; targeted digestion of facility wastes; feed or food additives; dietary supplements; animal nutrition; industrial cleaning; grain processing; cosmetic manufacturing; odor control; food or beverage processing; brewing enhancement or additives; detergent additives; or textile or yarn processing.

(176) For many applications of proteins (e.g., enzymes), there is a biological response curve wherein an optimal concentration of a protein or enzyme leads to the desired effect, and an excess of the protein or too small of an amount of the protein leads to undesirable or diminished effects. One example of this biological curve is the demonstration that a biological drug, such as the protein drug insulin for diabetes treatment, requires an optimum dose in order to reduce blood sugar levels in diabetic patients. Too little insulin leads to an insufficient response and maintenance of undesired elevated blood sugar levels and potential hyperkalemia. Too great of a dose of insulin leads to low blood sugar levels and potential hypokalemia and related morbidity.

(177) Similar biological response curves exist for many of the proteins and peptides of interest comprised within the fusion proteins described herein. Thus, for the various fields of use and methods for the recombinant *Bacillus cereus* family members described herein, it may be desirable to modulate the expression level of the protein or peptide of interest on the exosporium. By increasing or decreasing the expression levels of the protein or peptide of interest on the exosporium of the recombinant *Bacillus cereus* family member, expression levels can be optimized to maintain the overall expression level of the protein or peptide of interest at the most effective concentration.

(178) For example, it would be desirable to modulate expression levels of the fusion protein in cases where the protein or peptide of interest comprises a protein or peptide involved in direct signaling in plants, such as the flagellin peptide flg22, and the recombinant *Bacillus cereus* family member expressing the fusion protein is to be applied to a plant to provide a beneficial effect to the plant. Such modulation would be beneficial to avoid a signaling response that is great enough that it would lead to detrimental responses to the plant (e.g., too great of a response to flg22 can result in necrosis), or a signaling response that is low enough that it would yield a poor or insufficient response to the peptide.

(179) A biological response curve would also be relevant for recombinant *Bacillus cereus* family members expressing a fusion protein wherein the protein or peptide of interest comprises an antigen. In such cases, it would be desirable to modulate the expression level of the fusion protein comprising the antigen to achieve an optimal

range for generating a proper immune response in an animal. Too large of a dose could lead to injection site edema and unwanted inflammation, whereas too small of a dose could lead to insufficient vaccination or immune response.
(180) Modulation of the expression level of a fusion protein on the exosporium of a recombinant *Bacillus cereus* family member also provides benefits, for example, when the recombinant *Bacillus cereus* family member is used for breaking an emulsion or gel in a hydraulic fracturing fluid. Polysaccharide gels are frequently used in the hydraulic fracturing processing gels. These gels require breaking. When the gel solution is ready to break, the operator will desire that the break, which is an enzymatic reaction, happen at a particular optimized rate. Breaking the gel too quickly can lead to undesired side effects such as pooling of undigested gel fragments. On the other hand, breaking the gel too slowly leads to long wait times and increased expense. Using the techniques described hereinbelow, the enzyme levels on the exosporium of a recombinant *Bacillus cereus* family member expressing a fusion protein comprising an enzyme suitable for breaking an emulsion or gel in a hydraulic fracturing fluid can be modulated to ensure that an optimized level of enzyme is present for breaking gels, leading to preferred results when used in the field.
(181) A recombinant *Bacillus cereus* family member is provided that expresses: (i) a fusion protein comprising at least one protein or peptide of interest and a targeting sequence, exosporium protein, or exosporium protein fragment that targets the fusion protein to the exosporium of the recombinant *Bacillus cereus* family member; and (ii) a modulator protein, wherein the expression of the modulator protein is increased as compared to expression of the modulator protein in a wild-type *Bacillus cereus* family member under the same conditions. The modulator protein, when co-expressed with the fusion protein in the recombinant *Bacillus cereus* family member, results in increased or decreased expression of the fusion protein as compared to the expression level of the fusion protein in a recombinant *Bacillus cereus* family member that does not express the modulator protein at an increased level under the same conditions as compared to the expression of the modulator protein in a wild-type *Bacillus cereus* family member.
(182) The modulator protein can comprise an ExsY protein, an ExsFA/BxpB protein, a CotY protein, a CotO protein, an ExsFB protein, an InhA1 protein, an InhA2 protein, an ExsJ protein, an ExsH protein, a YjcA protein, a YjcB protein, a BclC protein, an AcpC protein, an InhA3 protein, an alanine racemase 1, an alanine racemase 2, a BclA protein, a BclB protein, a BxpA protein, a BclE protein, a BetA/BAS3290 protein, a CotE protein, an ExsA protein, an ExsK protein, an ExsB protein, a YabG protein, a Tgl protein, a SODA1 protein, a SODA2 protein, a variant of any thereof, or a combination of any thereof.
(183) For example, the modulator protein, when co-expressed in the recombinant *Bacillus cereus* family member with the fusion protein, results in increased expression of the fusion protein as compared to the expression level of the fusion protein in a recombinant *Bacillus cereus* family member that does not express the modulator protein at an increased level under the same conditions as compared to the expression of the modulator protein in a wild-type *Bacillus cereus* family member. Where the modulator protein, when co-expressed in the recombinant *Bacillus cereus* family member with the fusion protein, results in such increased expression of the fusion protein, the modulator protein can comprise a BclB protein, a CotE protein, a BxpB protein, a CotO protein, a BclA protein, a variant of any thereof, or a combination of any thereof
(184) Alternatively, the modulator protein, when co-expressed in the recombinant *Bacillus cereus* family member with the fusion protein, results in decreased expression of the fusion protein as compared to the expression level of the fusion protein in a recombinant *Bacillus cereus* family member that does not express the modulator protein at an increased level under the same conditions as compared to the expression of the modulator protein in a wild-type *Bacillus cereus* family member. Where the modulator protein, when co-expressed in the recombinant *Bacillus cereus* family member with the fusion protein, results in such decreased expression of the fusion protein, the modulator protein can comprise a BclC protein, an ApcC protein, a YjcB protein, a variant of any thereof, or a combination of any thereof.
(185) For example, the modulator protein can comprise a CotO protein, a BclB protein, an ExsFA/BxpB protein, a YjcB protein, a variant of any thereof, or a combination of any thereof.
(186) For ease of reference, descriptions of the modulator proteins and their SEQ ID NOs. are listed in Table 2 below.
(187) TABLE-US-00002 TABLE 2 Amino Acid Sequences for Modulator Proteins Modulator Protein SEQ ID NO. ExsY, *Bacillus thuringiensis* 123 ExsFA/BxpB, *Bacillus thuringiensis* 124 CotY, *Bacillus cereus* 125 CotO, *Bacillus anthracis* 126 ExsFB, Variant 1, *Bacillus cereus* 127 ExsFB, Variant 2, *Bacillus cereus* 128 InhA1, *Bacillus cereus* 129 InhA3, *Bacillus mycoides* 130 ExsJ, *Bacillus cereus* ATCC 10876 131 ExsH, *Bacillus cereus* 132 YjcA, *Bacillus cereus* 133 YjcB, Variant 1, *Bacillus cereus* 134 YjcB, Variant 2, *Bacillus cereus* 135 BclC, *Bacillus anthracis* 136 AcpC, *Bacillus cereus* 137 InhA2, *Bacillus cereus* 138 Alanine racemase 1, *Bacillus cereus* 139 Alanine racemase 2, *Bacillus cereus* 140 BclA, variant 1, *Bacillus anthracis* Sterne 141 BclA, variant 2, *Bacillus anthracis* 142 BclB, variant 1, *Bacillus anthracis* Sterne 143 BclB, variant 2, *Bacillus anthracis* Sterne 144 BxpA, *Bacillus anthracis* 145 BAS4623/BclE, variant 1, *Bacillus anthracis* Sterne 146 BAS4623/BclE, variant 2, *Bacillus*

anthracis Sterne 147 BetA/BAS3290, *Bacillus anthracis* 148 CotE, *Bacillus cereus* group 149 ExsA, *Bacillus cereus* 150 ExsK, *Bacillus cereus* AH187 151 ExsB, *Bacillus cereus* 152 YabG, *Bacillus cereus* 153 Tgl, *Bacillus cereus* group 154 SODA1, *Bacillus cereus* 155 SODA2, *Bacillus thuringiensis* 156

(188) Many of the modulator proteins have homologs, paralogs, or genetic rearrangements. Thus, many proteins that have at least 70% homology to any of the modulator sequences listed above in Table 2 will retain the ability to act as modulator proteins when overexpressed in a recombinant *Bacillus cereus* family member that also expresses any of the fusion proteins described herein. In addition, many of the modulator proteins (e.g., BclA, BclB, and BclE) have internal repeat regions that can differ significantly between strains. Additions or reductions in the number of repeats in the internal repeat region would affect overall sequence homology, but so long as the homology of amino- and carboxy-terminal regions of the protein retain at least 75% sequence identity to any of the amino acid sequences of the modulator proteins listed in the table above, such homologs would be expected to retain the ability to act as modulator proteins.

(189) Thus, for example, the modulator protein can comprise an amino acid sequence having at least 70% sequence identity with any of SEQ ID NOs: 123-156.

(190) The modulator protein can comprise an amino acid sequence having at least 75% sequence identity with any of SEQ ID NOs: 123-156.

(191) The modulator protein can comprise an amino acid sequence having at least 85% sequence identity with any of SEQ ID NOs: 123-156.

(192) The modulator protein can comprise an amino acid sequence having at least 90% sequence identity with any of SEQ ID NOs: 123-156.

(193) The modulator protein can comprise an amino acid sequence having at least 95% sequence identity with any of SEQ ID NOs: 123-156.

(194) The modulator protein can comprise an amino acid sequence having at least 98% sequence identity with any of SEQ ID NOs: 123-156.

(195) The modulator protein can comprise an amino acid sequence having at least 99% sequence identity with any of SEQ ID NOs: 123-156.

(196) The modulator protein can comprise an amino acid sequence having 100% sequence identity with any of SEQ ID NOs: 123-156.

(197) For example, the modulator protein can comprise SEQ ID NO: 124, 126, 134, 135, 143, or 144.

(198) The recombinant *Bacillus cereus* family members that express a modulator protein can comprise a vector encoding the modulator protein. For example, the vector can comprise a multicopy plasmid. Multicopy plasmids allow for high expression levels of the modulator protein.

III. Promoters for Expression of Fusion Proteins and/or Modulator Proteins in Recombinant *Bacillus cereus* Family Members

(199) When the fusion protein comprises a targeting sequence, exosporium protein, or exosporium protein fragment that targets the fusion protein to the exosporium of a *Bacillus cereus* family member, the DNA encoding the fusion protein is suitably under the control of a sporulation promoter which will cause expression of the fusion protein on the exosporium of a *B. cereus* family member endospore (e.g., a native bclA promoter from a *B. cereus* family member).

(200) Thus, any of the fusion proteins described above in Section 1.B can be expressed in the recombinant *Bacillus cereus* family member under the control of a sporulation promoter that is native to the targeting sequence, exosporium protein, or exosporium protein fragment of the fusion protein, or a portion of such a promoter.

(201) Similarly, any of the modulator proteins described above in Section II can be expressed under the control of its native promoter or a portion thereof.

(202) Any of the fusion proteins or modulator proteins can be expressed under the control of a high-expression sporulation promoter.

(203) The high-expression sporulation promoter comprises a sigma-K sporulation-specific polymerase promoter sequence.

(204) For case of reference, exemplary nucleotide sequences for promoters that can be used to express any of the fusion proteins or any of the modulator proteins in a recombinant *Bacillus cereus* family member are provided in Table 3 below, together with their SEQ ID NOs. Table 3 also provides exemplary minimal promoter sequences for many of the promoters. In Table 3, sigma-K sporulation-specific polymerase promoter sequences in the promoters are indicated by bold and underlined text. Several of the sequences have multiple sigma K sequences that overlap with one another. The overlaps are indicated by double underlining in the table. The promoter sequences are immediately upstream of the start codon for each of the indicated genes. In other words, in the sequences shown in Table 3 below, the last nucleotide of the promoter sequence immediately precedes the first nucleotide of the start codon for the coding region of the gene encoding the indicated protein.

(205) TABLE-US-00003 TABLE 3 Promoter Sequences for Expression of Fusion Proteins and

Modulator Proteins in Recombinant *Bacillus cereus* family members Promoter (SEQ ID NO) Promoter Sequence ExsY promoter TTTCTTAATCCTTTACCCTTTACTTTTGTAAAAGTTGATACACTT (*B. cereus* F837/76) CCATCCGGCTCTGTAATTTCTAATTCATCAATAAATGGTCTTCG (SEQ ID NO: 157) CAAAAAGCCTGTAATTTTATCATAAACAATTAAACGAGTGAGC
CTAAAAGCAGCTAACGCGAAAATAAAAAATAAAAGCCAGCTT
GTAAACAGCATAATTCCACCTTCCCTTATCCTCTTTCGCCTATT
TAAAAAAAGGTCTTGAGATTGTGACCAAATCTCCTCAACTCC**A**
ATATCTTATTAATGTAAATACAAACAAGAAGATAAGGA ExsY minimal promoter
ACCAAATCTCCTCAACTCC**AATATCTTA**TTAATGTAAATACAA (*B. cereus* F837/76)
ACAAGAAGATAAGGA (SEQ ID NO: 158) ExsFA/BxpB promoter
ACCACCTACCGACGATCCAATCTGTACATTCCTAGCTGTACCA (*B. anthracis* Sterne)
AATGCAAGATTAATATCGACTAACACTTGTCTTACTGTTGATTT (SEQ ID NO: 159)
AAGTTGCTTCTGTGCGATTCAATGCTTGCGTGATGTTACGATTT
AAAACTAAATAATGAGCTAAG**CATGGATTG**GGTGGCAGAATT
ATCTGCCACCCAATCCATGCTTAACGAGTATTATTATGTAAATT
TCTTAAAATTGGGAACTTGTCTAGAACATAGAACCTGTCCTTTT
CATTAACTGAAAGTAGAAACAGATAAAGGAGTGAAAAAC ExsFA/BxpB minimal
ACATAGAACCTGTCCTTTT**CATTAACTG**AAAGTAGAAACAGAT promoter (*B. anthracis*
AAAGGAGTGAAAAAC Sterne) (SEQ ID NO: 160) CotY/CotZ promoter (*B.*
TAGAAGAAGAACGCCGACTACTTTATGTCGCAATTACACGGGC *anthracis Sterne)*
GAAAGAAGAACTTTACATTTCCTCTCCGCAATTTTTTAGAGGA (SEQ ID NO: 161)
AAAAAATTAGATATATCTCGTTTTTTATACACTGTGCGAAAAG
ATTTACCTGAAAAGACATCCACTAAATAAGGATGTCTTTTTTTA
TATTGTATTATGTACATCCCTACTATATAAATTCCCTGCTTTTAT
CGTAAGAATTAACGTAATATCAACCATATCCCGTT**CATATTGT**
AGTAGTGTATGTCAGAACTCACGAGAAGGAGTGAACATA CotY/CotZ minimal
TCAACCATATCCCGTT**CATATTGTA**GTAGTGTATGTCAGAACT promoter (*B. anthracis*
CACGAGAAGGAGTGAACATA Sterne) (SEQ ID NO: 162) CotO promoter (*B. cereus*)
TAACTCAATCTTAAGAGAAATTGAGGAGCGCGCACCACTTCGT (SEQ ID NO: 163)
CGTACAACAACGCAAGAAGAAGTTGGGGATACAGCAGTATTCT
TATTCAGTGATTTAGCACGCGGCGTAACAGGAGAAAACATTCA
CGTTGATTCAGGGTAT**CATATCTTA**GGATA**AATATAATA**TTAA
TTTTAAAGGACAATCTCTACATGTTGAGATTGTCCTTTTTATTT
GTTCTTAGAAAGAACGATTTTTAACGAAAGTTCTTACCACGTTA
TGAATATAAGTATAATAGTACACGATTTATTCAGCTACGT CotO minimal promoter (*B.*
ACGTTGATTCAGGGTAT**CATATCTTA**GGATA**AATATAATA**TTA *cereus)*
ATTTTAAAGGACAATCTCTACATGTTGAGATTGTCCTTTTTATT (SEQ ID NO: 164)
TGTTCTTAGAAAGAACGATTTTTAACGAAAGTTCTTACCACGTT
ATGAATATAAGTATAATAGTACACGATTTATTCAGCTACGT ExsFB promoter (*B. cereus*
CATAAAAATCTACTTTTCTTGTCAAAGAGTATGCTTATATGCGT F837/76)
GCTCTTTTTATTTGGTTTTCTTTCATTTCTAAATAACATTTTCAA (SEQ ID NO: 165)
CTCTATTCATACTATTCTTTCAACTTTAGGTTACAAACTATTTCT
GTAAGCGTAGTGTTTCTTTTGTACTATAGGCAGTTAGTTTTATC
CATAACAGTACACCTCTGCACTATTCACTATAAATTTT**CATATA**
TTATATTGTGCTTGTCCAAAACATGTGGTTATTACTCACGCGAT CTAAATGAAAGAAAGGAGTGAAAAT
ExsFB minimal promoter (*B.* ACTATTCACTATAAATTTT**CATATATTA**TATTGTGCTTGTCCAA *cereus*
F837/76) AACATGTGGTTATTACTCACGCGATCTAAATGAAAGAAAGGAG (SEQ ID NO: 166)
TGAAAAT InhA1 promoter (*B.* AATACATGATAATGAAATCCGATTTTGTGTTTTATATAGTGAAT
thuringiensis serovar TATCAAATATTGTGTAGATGAAACAAAGATAAAATCCCCATTA *kurstaki* str. HD-
1) AACTCCCTCTATGGAAATTATAAATTGTTCGATAAAAACTTTCA (SEQ ID NO: 167)
ATATTTTCAGAAAACATTGTTGAATTGTGATATATTCGTATGCT
AACTATGAAATTTTTACAAATATATTAAAAACATTA**CATAATA**
TGACTAAATATTGAAAAAATATTGAATTTTTAATAAAATTTAA
TTTGTAATA**CATATTATT**TATTAGGGGAGGAAATAAGGG InhA1 minimal promoter (*B.*
AAAATTTAATTTGTAATA**CATATTATT**TATTAGGGGAGGAAAT *thuringiensis* serovar AAGGG kurstaki
str. HD-1) (SEQ ID NO: 168) InhA2 promoter (*B. mycoides*
AATTGTGCATATTGTCTTTTAAATTTTCTATCTAAGTTATTTAAT strain 219298)
ATATAATAAATAACTCTTTTTTGTGAGTTTTTTTGATACGAGGT (SEQ ID NO: 169)

AAATAATCAGTACAGGGTCTGACCAGAGGACTGGAGGGCATG
ATTCTATAAGGGAATATTTACTATTCCATGATTATAGAACTATG
TCTTTTTTATTGTATATAGAAGGGGGGATAGGTC**TATATTATA**
GAACTTATATATATTGTGCATTC**CATATTATC**AATTATCTAAAT
TTTAAGTCTTGTTACAATTAATAAGGGAGGAAATAGTA InhA2 minimal promoter (*B.*
ACTTATATATATTGTGCATTC**CATATTATC**AATTATCTAAATTT *mycoides* strain 219298)
TAAGTCTTGTTACAATTAATAAGGGAGGAAATAGTA (SEQ ID NO: 170) ExsJ promoter (*B.*
AATGACGTTTTCAAGTTTGATTATCATTCATGTTTCCTATTTTAA *thuringiensis* serovar
GAGAAACATATAACTCAACTACTTTTTTCAATGG**CATCTTTTA** *kurstaki*)
TAGTACTTAGAATAGGAAAACACTCAACTATAAGAAAAGTAA (SEQ ID NO: 171) GGAGGAAATAA
ExsJ minimal promoter (*B.* ACTACTTTTTTCAATGG**CATCTTTTA**TAGTACTTAGAATAGGA
thuringiensis serovar AAACACTCAACTATAAGAAAAGTAAGGAGGAAATAA *kurstaki*) (SEQ ID NO:
172) ExsH promoter (*B. cereus* ATATGCTAATGCTTAGTTTTTATACTCAAGTTAAAATGTGCTTT
F837/76) TGGACCTAAGAGATAAACGTGGAAA**AATAAAATA**AACTCTTA (SEQ ID NO: 173)
AGTTTAGGTGTTTAATCTAAGCAGTCAATTATTAAAAA**CATAT**
AATTAATATGTGAGTCATGAA**CATAATTAA**ATAATGTTTTCAA
GTTTAATTATCGTTCATGTTTCCTATTTTAAGCAGAACAAATAA
CTCAATTACTTTTTTCGATTGGATCTTTTTTAACTCTTATAATAG
GAAAACACTCAACTATAAAAATAAGTAAGGAGGAAATAA ExsH minimal promoter (*B.*
AATATGTGAGTCATGAA**CATAATTA**AATAATGTTTTCAAGTTT *cereus* F837/76)
AATTATCGTTCATGTTTCCTATTTTAAGCAGAACAAATAACTCA (SEQ ID NO: 174)
ATTACTTTTTTCGATTGGATCTTTTTTAACTCTTATAATAGGAA
AACACTCAACTATAAAAATAAGTAAGGAGGAAATAA YjcA promoter
TATAAAATAAAAGGGCGTGTATTTGCTACTGATGCAGTATTGT (B. *thuringiensis* serovar
GTGCGCCTAAAAATGGAATTTCACAACCAGATCCACATGTTGT *kurstaki* str. HD73)
TGTAGAACAATCTTGTAATTCATTGATGAATTTTACAACGTCAA (SEQ ID NO: 175)
CTACACAATGAGAAGAGCCATGGTGTTTATTTTCGTTACAACTC
ATTAATGTCACTCCTTATCTTCTTGTTTGTATTTACATT**AATAA**
GATATTGGAGTTGAGGAGATTTGGTCACAATCTCAAGACCTTT
TTTTTAAATAGGCGAAAGAGGATAAGGGAAGGTGGAATT YjcA minimal promoter
TCTTGTTTGTATTTACATT**AATAAGATA**TTGGAGTTGAGGAGAT (*B. thuringiensis* serovar
TTGGTCACAATCTCAAGACCTTTTTTTTAAATAGGCGAAAGAG *kurstaki* str. HD73)
GATAAGGGAAGGTGGAATT (SEQ ID NO: 176) YjcB promoter
ATCAACTTTTACAAAAGTAAAGGGTAAAGGATTAAGAAAGTG (*B. thuringiensis* serovar
GATTGGCGAATTATTAAGCTGTTATTGGTGTACAGGTGTATGG *kurstaki* str. HD73)
GTTAGTGCTTTTTTATTAGTTTTATATAATTGGATTCCGATCGTT (SEQ ID NO: 177)
GCAGAGCCGTTACTTGCATTATTAGCTATTGCAGGAGCAGCAG
CAATCATTGAAACGATTACAG**GATATTTTA**TGGGAG**AATAAT**
ATATTTTCATAATACGAGAAAAAGCGGAGTTTAAAAGAATGAG
GGAACGGAAATAAAGAGTTGTT**CATATAGTA**AATAGACAGAA YjcB minimal promoter
ACGGAAATAAAGAGTTGTT**CATATAGTA**AATAGACAGAA (*B. thuringiensis* serovar *kurstaki* str.
HD73) (SEQ ID NO: 178) BclC promoter
TGAAGTATCTAGAGCTAATTTACGCAAAGGAATCTCAGGACAA (*B. anthracis* Sterne)
CACTTTCGCAACACC**TATATTTTA**AATTTAATAAAAAAAGAGA (SEQ ID NO: 179)
CTCCGGAGTCAGAAATTATAAAGCTAGCTGGGTTCAAATCAAA
AATTTCACTAAAACGATATTATCAATACGCAGAAAATGGAAAA
AACGCCTTATCATAAGGCGTTTTTTCCATTTTTTCTTCAAACAA
ACGATTTTACTATGACCATTTAACTAATTTTTG**CATCTACTA**TG
ATGAGTTTCATTCACATTCTCATTAGAAAGGAGAGATTTA BclC minimal promoter
ACCATTTAACTAATTTTTG**CATCTACTA**TGATGAGTTTCATTCA (*B. anthracis* Sterne)
CATTCTCATTAGAAAGGAGAGATTTA (SEQ ID NO: 180) AcpC promoter (*B. cereus*
GACTATGTTTATTCAG**GATAAAATA**TAGCACTACACTCTCTCCT F837/76)
CTTATTATGTAGCATCTCTCTAATCCATCATTTGTTTCATTTAGT (SEQ ID NO: 181)
TAAAATTGTAAATAAAATCACATGATTTGTCAATTATAATTGTC
ATTTCGACAATTAAACTTGTCAAAATAATTCTCATCATTTTTTC
TCATCTTTCTAATATAGGACATACTACTATATATACAAAAGAC
AATATGCAAATGTT**CATACAAAA**AATATTATTTTTCGA**TATAT**
AATATTAACTGATTTTCTAACATCAAGGAGGGTACAT AcpC minimal promoter (*B.*

AGACAATATGCAAATGTT**CATACAAAA**AATATTATTTTTCGA**T** *cereus* F837/76)
ATATAATATTAACTGATTTTCTAACATCAAGGAGGGTACAT (SEQ ID NO: 182) InhA3 promoter
(*B.* ATAGTGAGTAATATGGTAATC**CATAGATTAAATAGTATA**GAA *thuringiensis* serovar
AATATTTAATTCTTATTTTTATTAAAAAAGCATGAATCCCAGAT *kurstaki* str. HD73)
TTACTGGGTTTTGATTGTAACTAAGAA**CATATAAAA**GTTCACT (SEQ ID NO: 183)
GTTATTTATAGGAGAGTCTGTTTGTTTT**TATATCTTA**TGTATTT
CACCCTG**CATAAAAAA**ATATTTCTCAACATTTTATTTGTTGAAA
AATATTGAATATTCGTATTATAACGAATATTATGTTGTTATCGG
CAAAAAACGATAATTTGCAGACACTGGGGAGGAAATACA InhA3 minimal promoter (*B.*
TCTTATGTATTTCACCCTG**CATAAAAAA**ATATTTCTCAACATTT *thuringiensis* serovar
TATTTGTTGAAAAATATTGAATATTCGTATTATAACGAATATTA *kurstaki* str. HD73)
TGTTGTTATCGGCAAAAAACGATAATTTGCAGACACTGGGGAG (SEQ ID NO: 184) GAAATACA
Alanine racemase 1 promoter CTTCGTCAGCAATAAGTGTGAGCGGAGAATTGGTTGATCTTGG (*B.*
cereus F837/76) CTTTACAATTGGAGCATTGACGAAAGACTCTTTAACGTGGTCG (SEQ ID NO: 185)
CATAACGGAGTAGAATATATGCTCGTGTCTAAAGGTTTAGAGC
CGAAGGAGCTATTAATGGTTGCTCGTTCAGTTACAGAGAAGCA
AGTGAAGTAAACTTCTTAGACGTGGTGATATATGTGCACCACG
TCTTTTCTTAGTTTGAAGGGTGGATTT**CATAAAAGA**AG**CATAT**
AAAAGAATAAGCTTCG**CATATCGTG**TATAAGGAAGTGTATTT Alanine racemase 1 minimal
ATAAAAGAATAAGCTTCG**CATATCGTG**TATAAGGAAGTGTAT promoter (*B. cereus* F837/76) TT
(SEQ ID NO: 186) Alanine racemase 2 promoter
CATTTCAAATAATGAACGCTTCGATTGAATCGGAGCTATTTTCA (*B. thuringiensis* serovar
AATCAATTTCAGTATATTGATCCAGCATTTGAATAGAAGTATC *kurstaki* str. HD73)
AACAGCAACTTTAAGTTGATGCAATGCAGATTGTACAAACATT (SEQ ID NO: 187)
GTAATTCTCCTCTTCTCCG**TATATAATA**GTTTCTTGAGGGTATT
ATATCATGCTCAAAATTCCGAAAATTCTAGTAGTTTGACTAG**C**
ATATTGAAAAGTAT**TATATTGTA**AAAGGT**CATATGAAA**CGTG
AAATAGAATGGAATGCAATTATTGAGTTAGGAGTTAGACCA Alanine racemase 2 minimal
T**TATATTGTA**AAAGGT**CATATGAAA**CGTGAAATAGAATGGAA promoter (*B. thuringiensis*
TGCAATTATTGAGTTAGGAGTTAGACCA serovar *kurstaki* str. HD73) (SEQ ID NO: 188) BclA
promoter (*B. cereus* ATCGATGGAACCTGTATCAACCACTATAATTTCATCCACAATTT F837/76)
TTTCAACTGAGTCTAAACAACGGGCTATTGTCTTCTCCTCATCT (SEQ ID NO: 189)
CGAACAAT**CATACATAAACTA**ATTGTAATTCCTTGCTTGTTCA
ACATAATCACCCTCTTCCAAATCAAT**CATATGTTA**TA**CATATA**
CTAAACTTTCCATTTTTTTAAATTGTTCAAGTAGTTTAAGATTT
CTTTTCAATAATTCAAATGTCCGTGTCATTTTCTTTCGGTTTTG**C**
ATCTACTATATAATGAACGCTTTATGGAGGTGAATTT BclA minimal promoter (*B.*
AATCAAT**CATATGTTA**TA**CATATACTA**AACTTTCCATTTTTTT *cereus* F837/76)
AAATTGTTCAAGTAGTTTAAGATTTCTTTTCAATAATTCAAATG (SEQ ID NO: 190)
TCCGTGTCATTTTCTTTCGGTTTTG**CATCTACTA**TATAATGAAC GCTTTATGGAGGTGAATTT BclB
promoter (*B.* GACCTGTAAGTCTGTAGGGAAGAATAATTTCAAGAGCCAGTGA *thuringiensis* serovar
TAATAGATTTTTTTGTTTTTTCATTCTTATCTTGAATATAAATCA *konkukian* str. 97-27)
CCT**CATCTTTTA**ATTAGAACGTAACCAATTTAGTATTTTGAAA (SEQ ID NO: 191)
TAGAGCTAT**CATTTTATA**ATATGAATACTACTAGTTATAGAAA
CGGCAAAAAGTTTAATATATGTAAAAATCATTTGGATATGAAA
AAAGTAGC**CATAGATTT**TTTCGAAATGATAAATGTTTTATTTT
GTTAATTAGGAAACAAAAATGTGGAATGAGGGGGATTTAA BclB minimal promoter (*B.*
ATATGAAAAAAGTAGC**CATAGATTT**TTTCGAAATGATAAATGT *thuringiensis* serovar
TTTATTTTGTTAATTAGGAAACAAAAATGTGGAATGAGGGGGA *konkukian* str. 97-27) TTTAA (SEQ
ID NO: 192) BxpA promoter (*B. anthracis*
TTTT**CATCTGCTA**CATCGTGAAGTAATGCTGCCATTTCAATTAT str. Sterne)
AAAACGATTTCCTCCTTCTTGCTCGGATAAAGAAATCGCCAGTT (SEQ ID NO: 193)
TATGTACACGCTC**AATATGATA**CCAATCATGCCCACTGGCATC
TTTTTCTAAAATATGTTTTACAAAAGTAATTGTTTTTTCTATCTT
TTCTTGTTTTGTCATTTTATCTTCACCCAGTTACTTATTGTAACA
CGCCCGCATTTTTTT**CATCACATATTTTC**TTGTCCGCC**CATACA**
CTAGGTGGTAGGCATCATCATGAAGGAGGAATAGAT BxpA minimal promoter (*B.*
ACATATTTTCTTGTCCGCC**CATACACTA**GGTGGTAGGCATCAT *anthracis* str. Sterne)

CATGAAGGAGGAATAGAT (SEQ ID NO: 194) BclE promoter (*B. anthracis*
GGTGACGACAA**CATATACAA**GAGGCACTCCTGCTGGTACTGTA ΔSterne)
ACAGGAACAAATATGGGGCAAAGTGTAAATA**CATCGGGTA**TA (SEQ ID NO: 195)
GCACAAGCTGTCCCGAATACA**GATAATATG**GATTCAACGGCG
GGACTCCCTTAAGAAATTAGGGGAGTCTTTATTTGGAAAAAGA
GCTTATGTTACATAAAAACAGGAGTAATTGTTTTAAAAGTAGT
ATTGGTGACGTTGTTAGAAAATACAATTTAAGTAGAAGGTGCG
TTTTTATATGA**AATATATTT**TATAGCTGTACTTTACCTTTCAAG BclE minimal promoter (*B.*
ACAAGCTGTCCCGAATACA**GATAATATG**GATTCAACGGCGGG *anthracis* ΔSterne)
ACTCCCTTAAGAAATTAGGGGAGTCTTTATTTGGAAAAAGAGC (SEQ ID NO: 196)
TTATGTTACATAAAAACAGGAGTAATTGTTTTAAAAGTAGTAT
TGGTGACGTTGTTAGAAAATACAATTTAAGTAGAAGGTGCGTT
TTTATATGA**AATATATTT**TATAGCTGTACTTTACCTTTCAAG BetA promoter
ATTTATTTCATTCAATTTTTCCTATTTAGTACCTACCGCACTCAC (*B. anthracis* Sterne)
AAAAAGCACCTCTCATTAATTTATATTATAGTCATTGAAATCTA (SEQ ID NO: 197)
ATTTAATGAAATCAT**CATACTATA**TGTTTTATAAGAAGTAAAG
GTAC**CATACTTAA**TTAATACATATCTATACACTTCAATATCAC
AGCATGCAGTTGAATTATATCCAACTTTCATTTCAAATTAAATA
AGTGCCTCCGCTATTGTGAATGTCATTTACTCTCCCTACTA**CAT**
TTAATAATTATGACAAGCAATCATAGGAGGTTACTAC BetA minimal promoter
TAAGAAGTAAAGGTAC**CATACTTAA**TTAATACATATCTATACA (*B. anthracis* Sterne)
CTTCAATATCACAGCATGCAGTTGAATTATATCCAACTTTCATT (SEQ ID NO: 198)
TCAAATTAAATAAGTGCCTCCGCTATTGTGAATGTCATTTACTC
TCCCTACTA**CATTTAATA**ATTATGACAAGCAATCATAGGAGGT TACTAC CotE promoter (*B. cereus*
AGTTGTACAAGAATTTAAATCTTCACAAA**CATATGTAA**ATGAC AH820)
TTACTACAGCTAGTTGCAAGTACGATTTCTAACAACGTAACAG (SEQ ID NO: 199)
ATGAAATATTAATTTCAACTAATGGCGATGTATTGAAGGGTGA
AACGGGCGCAGCGGTAGAAAGTAAAAAAGGAAATTGTGGTTG
TTAAAGAGATGTCGAAATGACATCTCTTTTTTTAGTGGATTAAA
CGTAAGTTCTTCTCAAAAAAAGAATGACACATTCCGCTATTGT
CACG**CATATGATT**AAGTGAATAGTGATTGAGGAGGGTTACGA CotE minimal promoter (*B.*
ACATTCCGCTATTGTCACG**CATATGATT**AAGTGAATAGTGATT *cereus* AH820) GAGGAGGGTTACGA
(SEQ ID NO: 200) ExsA promoter (*B. cereus*
AACGTTATTAGCGTAGACAAACAAGTAACGGCAGAAGCAGTTC strain ATCC 10876)
TTG**CATTAAATC**GTATGTTAGAGCGTGTGTAAAGCAACGGTAT (SEQ ID NO: 201)
TCCCGTTGCTTTTTTTCATA**CATATAATC**ATAACGAGAACGAA
ATGGG**CATACATTG**TTTTGAAGAAATCATTGTGGTTCTTTATG
CTTATTCCACTTCGAATGATATTGAAAATCGAAGAAGTGATAA
AAGTAAAAAGAAGTTAATGTTATTTAGAAAGAGTTACTTCATG
AGATTTGTTACTTATA**GATAAGTTA**TACAGGAGGGGGAAAAT ExsA minimal promoter (*B.*
TCATGAGATTTGTTACTTATA**GATAAGTTA**TACAGGAGGGGGA *cereus* strain ATCC 10876) AAAT
(SEQ ID NO: 202) ExsK promoter (*B.*
AAGCCGCGGTCAATGCTGTATATGCA**AATAAGATT**GCAGCTTT *thuringiensis* serovar
ACCTGAAGAAGAGCGT**GATAGCTTC**ATTGCTGAAAAACGAGA *konkukian* str. 97-27)
AGAGTATAAGAAAGATATTGATATTTACCATTTAGCATCAGAG (SEQ ID NO: 203)
ATGGTCATTGATGGTATTGTTCATCCAAACAATTTAAGAGAAG
AGTTAAAAGGACGATTCGAAATGTATATGAGTAAATATCAAGT
ATTTACGGATCGTAAA**CATCCTGTT**TATCCAGTTTAAAAGCCC
TATTTAGGGCTTTCTTGCTCAAAAAGTTAAGGAGGGGAAAACA ExsK minimal promoter (*B.*
TCAAGTATTTACGGATCGTAAA**CATCCTGTT**TATCCAGTTTAA *thuringiensis* serovar
AAGCCCTATTTAGGGCTTTCTTGCTCAAAAAGTTAAGGAGGGG *konkukian* str. 97-27) AAAACA
(SEQ ID NO: 204) ExsB promoter (*B. cereus*
AGGATTTCAGTGGGACGCCTCCTCTCTTCTTACATTAAATTAAT F837/76)
CATACTATAAAATGAAAGAAATGAAATGAAAAATAGCGGAAA (SEQ ID NO: 205)
AATCAGAAATTTTTTCTGGTAG**TATACAATATGTTA**CAATAAG
CTTTGTCAATGAAAGAAGGAATTCCGTGCAATGCACGGGAGAG
GTTCGCGAACTCCCTCTATAAAAAACTATGGAAACAAC**AATAT**
CTTTAGGTATTGTTTTGTTTTTTTATTGTGACAGTTCAAGAACG

TTCTTTCTTCTTATTCGTAGTAGAGAAGGAGAATGAGTGAA ExsB minimal promoter (*B.*
ACTATGGAAACAAC**AATATCTTT**AGGTATTGTTTTGTTTTTTTA *cereus* F837/76)
TTGTGACAGTTCAAGAACGTTCTTTCTTCTTATTCGTAGTAGAG (SEQ ID NO: 206)
AAGGAGAATGAGTGAA YabG promoter (*B. cereus*
TTTTGCACAACGCCGTAAAACTTTAATG**AATAATTTA**TCA**AAT** AH820)
AATTTAAATGGTTTCCCGAAAGATAAAGAGCTGTTGGATCGAA (SEQ ID NO: 207)
TTTTAACAGAAGTAGGAATTGATCCAAAACGAAGAGGCGAAA
CGCTATCTATCGAAGAGTTTGCGACATTAAGTAATGCATTAGTT
CTT**CATAAGTTA**TCATAAGAATACAAAAGGGACAGTTCAATTT
GAACTGTCCCTTTTGTCACCTTTCTCCTCCTAAATT**CATACTTT**
AAAAACAGGTAAGATGGCCTAACGAGTTTGGAGGTAGGAGA YabG minimal promoter (*B.*
TCTCCTCCTAAATT**CATACTTTA**AAAACAGGTAAGATGGCCTA *cereus* AH820)
ACGAGTTTGGAGGTAGGAGA (SEQ ID NO: 208) Tgl promoter (*B.*
GGAAACAGAAGTCATCCCATTTGAAAATGCAGCAGGTCGTATT *thuringiensis* serovar
ATAGCTGATTTCGTTATGGTTTATCCGCCAGGGATTCCAATCTT *konkukian* str. 97-27)
TACTCCGGGGGAAATTATTACACAAGACAACTTAGAGTATATT (SEQ ID NO: 209)
CGTAAAAACTTAGAAGCAGGTTTACCTGTACAAGGTCCTGAAG
ATATGACATTACAAACATTACGCGTGATCAAAGAGTACAAGCC
TATCAGTTGATAGGCTTTTTTTCACCCTTTTTCCCTTTTCT**CATA**
CGATATTATGTAATGTAACGTATAGGTGGGGATACTACT Tgl minimal promoter (*B.*
ACCCTTTTTCCCTTTTCT**CATACGATA**TTATGTAATGTAACGTA *thuringiensis* serovar
TAGGTGGGGATACTACT *konkukian* str. 97-27) (SEQ ID NO: 210) Superoxide dismutase
ATTGTGGACCCTTAGCTCAGCTGGTTAGAGCAGACGGCTCATA (SODA1) promoter (*B.*
ACCGTCCGGTCGTAGGTTCGAGTCCTACAGGGTCCATATCCATT *cereus* F837/76)
TCACATGTTTATTATGTCGGCAGGAAGCTTCCTTGTAGAAGGG (SEQ ID NO: 211)
AGCTTTTTTTATGAAATATATGAGCATTTTAATTGAAATGAAGT
GGGAATTTTGCTACTTTAATGATAGCAAGACAATGTGATTTATT
TGTTTGCACCCTATGGCAATTAGGGTAGAATGAAGTTGTATGT
CACTTAAGTGGCAATA**CATAAACTG**GGAGGAATATAACA Superoxide dismutase
ACTTAAGTGGCAATA**CATAAACTG**GGAGGAATATAACA (SODA1) minimal promoter (*B. cereus*
F837/76) (SEQ ID NO: 212) Superoxide dismutase
AATATAACAGAAAATTCTGATGTTTTTTCAAATCCTATAATAAG (SODA2) promoter (*B.*
GAGTGTTCCGTATGATGCCTT**TATATTTTC**CGGAAGATAAAAC *cereus* AH820)
AG**AATATATTA**TTCCAGGGATTGTTTGTGTTCTATTTATCATCG (SEQ ID NO: 213)
GTGCGATTGCTACGTGGCGTATGTTCATTCGTGTATCAAAACG
AGAAGCAGAGCGATTACAGAAAGTTGAAGAAAAGCTGTTAGC
TGAAAAGAAACAGTAACTCATTTTTGTATGTTTCCCTCTATGCT
CGGACAATCTAAGGGCAGAATGTATTTTGGAGGGAATGAA Superoxide dismutase
TCCGGAAGATAAAACAG**AATATATTA**TTCCAGGGATTGTTTGT (SODA2) minimal promoter
GTTCTATTTATCATCGGTGCGATTGCTACGTGGCGTATGTTCAT (*B. cereus* AH820)
TCGTGTATCAAAACGAGAAGCAGAGCGATTACAGAAAGTTGA (SEQ ID NO: 214)
AGAAAAGCTGTTAGCTGAAAAGAAACAGTAACTCATTTTTGTA
TGTTTCCCTCTATGCTCGGACAATCTAAGGGCAGAATGTATTTT GGAGGGAATGAA BclA promoter
TAATCACCCTCTTCCAAATCAAT**CATATGTTA**TA**CATATACTA** (*B. anthracis* Sterne)
AACTTTCCATTTTTTTAAATTGTTCAAGTAGTTTAAGATTTCTT (SEQ ID NO: 215)
TTCAATAATTCAAATGTCCGTGTCATTTTCTTTCGGTTTTG**CAT**
CTACTATATAATGAACGCTTTATGGAGGTGAATTT BAS 1882 promoter (*B.*
AATTACATAACAAGAACTACATTAGGGAGCAAGCAGTCTAGCG *anthracis* Sterne)
AAAGCTAACTGCTTTTTTATTAAATAACTATTTTATTAAATTTC (SEQ ID NO: 216)
ATATATACAATCGCTTGTCCATTTCATTTGGCTCTACCCACG**CA**
TTTACTATTAGTAATATGAATTTTTCAGAGGTGGATTTTATT Gene 3572 promoter
CTATGATTTAAGATACACAATAGCAAAAGAGAAA**CATATTAT** (*B. weihenstephensis*
ATAACGATAAATGAAACTTATGTATATGTATGGTAACTGTATA KBAB 4)
TATTACTACAATACAGTATACTCATAGGAGGTAGGT (SEQ ID NO: 217) YVTN β-propeller protein
GGTAGGTAGATTTGAAATATGATGAAGAAAAGGAATAACTAA promoter
AAGGAGTCGATATCCGACTCCTTTTAGTTATAAATAATGTGGA (*B. weihenstephensis*
ATTAGAGTATAATTTTATATAGGTATATTGTATTAGATGAACGC KBAB 4)
TTTATCCTTTAATTGTGATTAATGATGGATTGTAAGAGAAGGG (SEQ ID NO: 218)

GCTTACAGTCCTTTTTTTATGGTGTTCTATAAGCCTTTTTAAAA
GGGGTACCACCCCACACCCAAAAACAGGGGGGGTTATAACTA
CATATTGGATGTTTTGTAACGTACAAGAATCGGTATTAATTACC
CTGTAAATAAGTTATGTGTATATAAGGTAACTT**TATATATTC**T
CCTACAATAAAATAAAGGAGGTAATAAA Cry1A promoter
AACCCTTAATGCATTGGTTAAACATTGTAAAGTCTAAAGCATG (*B. thuringiensis* HD-73)
GATAATGGGCGAGAAGTAAGTAGATTGTTAACACCCTGGGTCA (SEQ ID NO: 219)
AAAATTGATATTTAGTAAAATTAGTTGCACTTTGTGCATTTTTT
CATAAGATGAGT**CATATGTTT**TAAATTGTAGTAATGAAAAAC
AGTAT**TATATCATAATGAA**TTGGTATCTTAATAAAAGAGATGG AGGTAACTTA ExsY promoter
TAATTCCACCTTCCCTTATCCTCTTTCGCCTATTTAAAAAAAGG (*B. thuringiensis* serovar
TCTTGAGATTGTGACCAAATCTCCTCAACTCC**AATATCTTA**TTA *konkukian* str. 97-27)
ATGTAAATACAAACAAGAAGATAAGGA (SEQ ID NO: 220) CotY promoter
AGGATGTCTTTTTTTATATTGTATTATGTACATCCCTACTATATA (*B. thuringiensis* Al Hakam)
AATTCCCTGCTTTTATCGTAAGAATTAACGTAATATCAACCATA (SEQ ID NO: 221)
TCCCGTT**CATATTGTA**GTAGTGTATGTCAGAACTCACGAGAAG GAGTGAACATAA YjcA promoter
TTAATGTCACTCCTTATCTTCTTGTTTGTATTTACATT**AATAAG** (*B. thuringiensis* serovar
ATATTGGAGTTGAGGAGATTTGGTCACAATCTCAAGACCTTTTT *kurstaki* str. HD73)
TTTAAATAGGCGAAAGAGGATAAGGGAAGGTGGAATT (SEQ ID NO: 222) YjcB promoter
ATATATTTTCATAATACGAGAAAAAGCGGAGTTTAAAAGAATG (*B. thuringiensis* serovar
AGGGAACGGAAATAAAGAGTTGTT**CATATAGTA**AATAGACAG *kurstaki* str. HD73) AA (SEQ ID
NO: 223) ExsFA/BxpB promoter AAACTAAATAATGAGCTAAGCATGGATTGGGTGGCAGAATTAT (*B. thuringiensis* Al Hakam*)* CTGCCACCCAATC**CATGCTTAA**CGAGTATTATTATGTAAATTT (SEQ ID
NO: 224) CTTAAAATTGGGAACTTGTCTAGAACATAGAACCTGTCCTTTT**C**
ATTAACTGAAAGTAGAAACAGATAAAGGAGTGAAAAAC Rhamnose promoter
ATTCACTACAACGGGGATGAGTTTGATGCGGATA**CATATGAG** (*B. thuringiensis* Al Hakam*)*
AAGTACCGGAAAGTGTTTGTAGAA**CATTACAA**AGATATATTAT (SEQ ID NO: 225)
CTCCATCATAAAGGAGAGATGCAAAG CotO promoter (*B. anthracis*
CGCGCACCACTTCGTCGTACAACAACGCAAGAAGAAGTTGGGG Sterne)
ATACAGCAGTATTCTTATTCAGTGATTTAGCACGCGGCGTAAC (SEQ ID NO: 226)
AGGAGAAAACATTCACGTTGATTCAGGGTAT**CATATCTTA**GGA
TAAATATAATATTAATTTTAAAGGACAATCTCTACATGTTGAG
ATTGTCCTTTTTATTTGTTCTTAGAAAGAACGATTTTTAACGAA
AGTTCTTACCACGTTATGAATATAAGTATAATAGTACACGATTT ATTCAGCTACGTA Sigma K promoter
TATATCATATGTAAAATTAGTTCTTATTCCCA**CATATCATA**TAG (*B. anthracis* Sterne)
AATCGC**CATATTATA**CATGCAGAAAACTAAGTATGGTATTATT (SEQ ID NO: 227)
CTTAAATTGTTTAGCACCTTCTAATATTACAGATAGAATCCGTC
ATTTTCAACAGTGAACATGGATTTCTTCTGAACACAACTCTTTT
TCTTTCCTTATTTCCAAAAAGAAAAGCAGCCCATTTTAAAATAC GGCTGCTTGTAATGTACATTA
InhA1 promoter TATCACATAACTCTTTATTTTTAATATTTCGA**CATAAAGTG**AAA (*B. thuringiensis*
Al Hakam*)* CTTTAATCAGTGGGGGCTTTGTTCATCCCCCCACTGATTATTAA (SEQ ID NO: 228)
TTGAACCAAGGGATAAAAAGATAGAGGGTCTGACCAGAAAAC
TGGAGGGCATGATTCTATAACAAAAAGCTTAATGTTTATAGAA
TTATGTCTTTTTATATAGGGAGGGTAGTAAACAGAGATTTGGA
CAAAAATGCACCGATTTATCTGAATTTTAAGTTTTATAAAGGG GAGAAATG BclA cluster glycosyl
ATTTTTTACTTAGCAGTAAAACTGATATCAGTTTTACTGCTTTTT transferase operon 1
CATTTTTAAATTCAATCATTAAATCTTCCTTTTCTACATAGT**CA** (*B. thuringiensis* serovar
TAATGTTGTATGACATTCCGTAGGAGGCACTTATA *konkukian* str. 97-27) (SEQ ID NO: 229)
BclA cluster glycosyl ACATAAATTCACCTCCATAAAGCGTTCATTATATAGTAGATGC transferase
operon 2 AAAACCGAAAGAAAATGACACGGACATTTGAATTATTGAAAA (*B. thuringiensis* serovar
GAAATCTTAAACTACTTGAACAATTTAAAAAAATGGAAAGTTT *kurstaki* str. HD73)
AGTATATGTATAA**CATATGATT**GATTTGGAAGAGGGTGATTA (SEQ ID NO: 230) Glycosyl
transferase TTCTATTTTCCAA**CATAACATG**CTACGATTAAATGGTTTTTTGC promoter
AAATGCCTTCTTGGGAAGAAGGATTAGAGCGTTTTTTTATAGA (*B. thuringiensis* Al Hakam*)*
AACCAAAAGTCATTAACAATTTTAAGTTAATGACTTTTTTGTTT (SEQ ID NO: 231)
GCCTTTAAGAGGTTTTATGTTACTATAATTATAGTATCAGGTAC
TAATAACAAGTATAAGTATTTCTGGGAGGATATATCA

(206) The sigma-K sporulation-specific polymerase promoter sequences in the promoter sequences shown in Table

3 result in high expression levels of the fusion protein or modulator protein during late sporulation. The consensus sequence for the sigma-K sporulation-specific polymerase promoter sequence is CATANNNTN; however, this sequence can comprise up to two mutations and still be functional. The sigma-K sporulation-specific polymerase promoter sequence is generally found upstream of the ribosome binding site (RBS).
(207) Promoters having a high degree of sequence identity to any of the sequences shown above in Table 3 can also be used to express the fusion proteins or the modulator proteins.
(208) For example, the fusion protein or modulator protein can be expressed under the control of a promoter comprising a nucleic acid sequence having at least 80% identity with a nucleic acid sequence of any one of SEQ ID NOs: 157-231.
(209) The fusion protein or modulator protein can be expressed under the control of a promoter comprising a nucleic acid sequence having at least 90% identity with a nucleic acid sequence of any one of SEQ ID NOs: 157-231.
(210) The fusion protein or modulator protein can be expressed under the control of a promoter comprising a nucleic acid sequence having at least 95% identity with a nucleic acid sequence of any one of SEQ ID NOs: 157-231.
(211) The fusion protein or modulator protein can be expressed under the control of a promoter comprising a nucleic acid sequence having at least 98% identity with a nucleic acid sequence of any one of SEQ ID NOs: 157-231.
(212) The fusion protein or modulator protein can be expressed under the control of a promoter comprising a nucleic acid sequence having at least 99% identity with a nucleic acid sequence of any one of SEQ ID NOs: 157-231.
(213) The fusion protein or modulator protein can be expressed under the control of a promoter comprising a nucleic acid sequence having 100% identity with a nucleic acid sequence of any one of SEQ ID NOs: 157-231.
(214) For example, the modulator protein or fusion protein can be expressed under the control of a BclA promoter (e.g., SEQ ID NO: 189, 190, 215, 229 or 230), a CotY promoter (e.g., SEQ ID NO: 161, 162 or 221), an ExsY promoter (e.g., SEQ ID NO: 157, 158 or 220), or a rhamnose promoter (e.g., SEQ ID NO: 225). For example, the fusion protein can be expressed under the control of a promoter comprising a nucleic acid sequence having at least 80% identity with a nucleic acid sequence of any one of SEQ ID NOs: 157, 158, 161, 162, 189, 190, 215, 220, 221, 225, 229, or 230.
(215) The fusion protein can be expressed under the control of a promoter comprising a nucleic acid sequence having at least 85% identity with a nucleic acid sequence of any one of SEQ ID NOs: 157, 158, 161, 162, 189, 190, 215, 220, 221, 225, 229, or 230.
(216) The fusion protein can be expressed under the control of a promoter comprising a nucleic acid sequence having at least 90% identity with a nucleic acid sequence of any one of SEQ ID NOs: 157, 158, 161, 162, 189, 190, 215, 220, 221, 225, 229, or 230.
(217) The fusion protein can be expressed under the control of a promoter comprising a nucleic acid sequence having at least 95% identity with a nucleic acid sequence of any one of SEQ ID NOs: 157, 158, 161, 162, 189, 190, 215, 220, 221, 225, 229, or 230.
(218) The fusion protein can be expressed under the control of a promoter comprising a nucleic acid sequence having at least 98% identity with a nucleic acid sequence of any one of SEQ ID NOs: 157, 158, 161, 162, 189, 190, 215, 220, 221, 225, 229, or 230.
(219) The fusion protein can be expressed under the control of a promoter comprising a nucleic acid sequence having at least 99% identity with a nucleic acid sequence of any one of SEQ ID NOs: 157, 158, 161, 162, 189, 190, 215, 220, 221, 225, 229, or 230.
(220) The fusion protein can be expressed under the control of a promoter comprising a nucleic acid sequence having 100% identity with a nucleic acid sequence of any one of SEQ ID NOs: 157, 158, 161, 162, 189, 190, 215, 220, 221, 225, 229, or 230.
(221) The fusion protein or modulator protein can be expressed under the control of a promoter comprising a sigma-K sporulation specific polymerase promoter sequence, wherein the sigma-K sporulation-specific polymerase promoter sequence or sequences have 100% identity with the corresponding nucleotides of any of SEQ ID NOs: 157-231.
(222) The fusion proteins can be expressed under the control of a promoter that is native to the targeting sequence, exosporium protein, or exosporium protein fragment of the fusion protein. Thus, for example, where the targeting sequence is derived from BclA, the fusion protein can be expressed under the control of a native BclA promoter (e.g., SEQ ID NO: 189, 190, 215, 229 or 230).
(223) The modulator proteins can be expressed under the control of their native promoters. Thus, for example, where the modulator protein comprises CotO, the CotO can be expressed under the control of a native CotO promoter (e.g., SEQ ID NO: 163 or 226). Native promoter sequences for each of the modulator proteins are

provided above in Table 3.
(224) Table 3 also provides exemplary minimal promoter sequences for each modulator protein. The modulator proteins and fusion proteins can be expressed under any of these minimal promoter sequences. For example, the modulator protein can be expressed under a minimal promoter that comprises a portion of the native promoter sequence. For instance, where the modulator protein comprises CotO, the CotO can be expressed under the minimal CotO promoter (SEQ ID NO: 164).
(225) Alternatively, the modulator proteins can be expressed under the control of any promoter comprising a sigma-K sporulation-specific polymerase promoter sequence, regardless of whether the promoter is the native promoter for the modulator protein. As can be seen from Table 3, each of the native promoters and the minimal promoters for the modulator proteins contains at least one sigma-K sporulation-specific polymerase promoter sequence. Thus, for example, where the modulator protein is BxpB, the BxpB can be expressed under the control of a BclA promoter (e.g., SEQ ID NO: 189, 190, 215, 229 or 230) or any of the other promoters listed in Table 3.
(226) Furthermore, the modulator protein or the fusion protein can be expressed under a portion of any of the promoters listed above in Table 3, so long as the portion of the promoter includes a sigma-K sporulation-specific polymerase promoter sequence. For example, the modulator protein can be expressed under a promoter region that comprises the first 25, 50, 100, 150, 200, 250, or 300 nucleotides upstream of the start codon, so long as that region comprises a sigma-K sporulation-specific polymerase promoter sequence.
IV. Mutations and Other Genetic Alterations to Recombinant *Bacillus cereus* Family Members that Allow for Collection of Free Exosporium
(227) As is described further hereinbelow, the recombinant *Bacillus cereus* family members that express fusion proteins comprising a protein or peptide of interest and a targeting sequence, an exosporium protein, or an exosporium protein fragment that targets the fusion protein to the exosporium of the recombinant *Bacillus cereus* family member can be used to deliver proteins or peptides of interest to plants, seeds, a plant growth medium, or an area surrounding a seed or a plant (e.g., via soil drench, foliar application, or as a seed treatment). In addition, the recombinant *Bacillus cereus* family members can be used to deliver nucleic acid molecules to animals, insects, worms (e.g., nematodes), fungi, and protozoans; to deliver proteins or peptides to an animal; in vaccines and for producing an immunogenic response; for remediation; for treating a hydraulic fracturing fluid to break an emulsion or gel within the fluid; for disinfection; and for various other uses described hereinbelow. However, in some cases, the presence of the living microorganisms may not be desirable, and instead, it would be desirable to separate the living spore from the fusion proteins in the exosporium on the outside surface of the spore. For example, in some applications it will be desirable to increase enzyme activity without concern for spore integrity. In such situations, the exosporium fragments may be preferred over living microorganisms having the enzyme on their exosporium.
(228) In addition, for some uses, it may be desirable to reduce the density of the product. In such instances, it would be desirable to separate the dense spore from the exosporium (containing the fusion proteins). In the field of vaccines, it may be desirable to separate the spore from the exosporium (containing fusion proteins that comprise an antigen) in order to remove potential antigens present on the spore itself from the vaccine preparation. Furthermore, under some circumstances the presence of live spores would lead to potential for bacterial growth in a product, which would be undesirable for some applications (e.g., animal feed supplementation and leather hide processing).
(229) Mutations or other genetic alterations (e.g., overexpression of a protein) can be introduced into the recombinant *Bacillus cereus* family members that allow free exosporium to be separated from spores of the recombinant *Bacillus cereus* family member. This separation process yields exosporium fragments that contain the fusion proteins but that are substantially free of the spores themselves. By “substantially free of spores” it is meant that once the free exosporium is separated from the spores, a preparation is obtained that contains less than 5% by volume of spores, preferably less than 3% by volume of spores, even more preferably less than 1% by volume of spores, and most preferably contains no spores or if spores are present, they are undetectable. These exosporium fragments can be used in place of the recombinant *Bacillus cereus* family members themselves and can be used to deliver proteins or peptides of interest to plants, seeds, a plant growth medium, or an area surrounding a seed or a plant, or for any of the other purposes described herein.
(230) Thus, a recombinant *Bacillus cereus* family member is provided that expresses a fusion protein comprising at least one protein or peptide of interest and a targeting sequence, exosporium protein, or exosporium protein fragment that targets the fusion protein to the exosporium of the recombinant *Bacillus cereus* family member. The recombinant *Bacillus cereus* family member comprises a mutation or expresses a protein, wherein the expression of the protein is increased as compared to the expression of the protein in a wild-type *Bacillus cereus* family member under the same conditions. The mutation or the increased expression of the protein results in *Bacillus cereus* spores having an exosporium that is easier to remove from the spore as compared to the exosporium of a wild-type spore.
(231) A further recombinant *Bacillus cereus* family member is provided that expresses a fusion protein comprising at least one protein or peptide of interest and a targeting sequence, exosporium protein, or exosporium protein fragment that targets the fusion protein to the exosporium of the recombinant *Bacillus cereus* family member. The

recombinant *Bacillus cereus* family member: (i) comprises a mutation in a CotE gene; (ii) expresses an ExsY protein, wherein the expression of the ExsY protein is increased as compared to the expression of the ExsY protein in a wild-type *Bacillus cereus* family member under the same conditions, and wherein the ExsY protein comprises a carboxy-terminal tag comprising a globular protein; (iii) expresses a BclB protein, wherein the expression of the BclB protein is increased as compared to the expression of the BclB protein in a wild-type *Bacillus cereus* family member under the same conditions; (iv) expresses a YjcB protein, wherein the expression of the YjcB protein is increased as compared to the expression of the YjcB protein in a wild-type *Bacillus cereus* family member under the same conditions; (v) comprises a mutation in an ExsY gene; (vi) comprises a mutation in a CotY gene; (vii) comprises a mutation in an ExsA gene; or (viii) comprises a mutation in a CotO gene.

(232) The recombinant *Bacillus cereus* family member can comprise a mutation in the CotE gene, such as a knock-out of the CotE gene or a dominant negative form of the CotE gene. The mutation in the CotE gene can partially or completely inhibit the ability of CotE to attach the exosporium to the spore.

(233) The recombinant *Bacillus cereus* family member can express an ExsY protein. The ExsY protein comprises a carboxy-terminal tag comprising a globular protein (e.g., a green fluorescent protein (GFP) or a variant thereof), and the expression of the ExsY protein is increased as compared to the expression of the ExsY protein in a wild-type *Bacillus cereus* family member under the same conditions. The globular protein can have a molecular weight of between 25 kDa and 100 kDa. Expression of the ExsY protein comprising the carboxy-terminal tag comprising a globular protein can also inhibit binding of the ExsY protein to its targets in the exosporium.

(234) The recombinant *Bacillus cereus* family member can express a BclB protein, which may result in the formation of a fragile exosporium. The expression of the BclB protein can be increased as compared to the expression of the BclB protein in a wild-type *Bacillus cereus* family member under the same conditions.

(235) The recombinant *Bacillus cereus* family member can express a YjcB protein, which may cause the exosporium to form in pieces rather than in a complete structure. The expression of the YjcB protein can be increased as compared to the expression of the YjcB protein in a wild-type *Bacillus cereus* family member under the same conditions.

(236) The recombinant *Bacillus cereus* family member can comprise a mutation an ExsY gene, such as a knock-out of the ExsY gene. The mutation in the ExsY gene can partially or completely inhibit the ability of ExsY to complete the formation of the exosporium or attach the exosporium to the spore.

(237) The recombinant *Bacillus cereus* family member can comprise a mutation a CotY gene, such as a knock-out of the CotY gene. The mutation in the CotY gene can result in the formation of a fragile exosporium.

(238) The recombinant *Bacillus cereus* family member can comprise a mutation an ExsA gene, such as a knock-out of the ExsA gene. The mutation in the ExsA gene can result in the formation of a fragile exosporium.

(239) The recombinant *Bacillus cereus* family member can comprise a mutation a CotO gene, such as a knock-out of the CotO gene or a dominant negative form of the CotO gene. The mutation in the CotO gene can cause the exosporium to form in strips.

(240) Exosporium fragments can be prepared from any of these recombinant *Bacillus cereus* family members and used for various purposes as described further hereinbelow. The exosporium fragments comprise the fusion proteins. Upon purification of the exosporium fragments that contain the fusion proteins from the spores, a cell-free protein preparation is obtained in which the fusion proteins are stabilized and supported through covalent bonds to the exosporium fragments.

(241) Due to the strong covalent bonds between the fusion proteins and the exosporium fragments, the fusion proteins become resistant to heat. The heat resistance of the fusion proteins bound to the exosporium fragments allows them to be used for applications that require heat-resistant proteins or enzymes (e.g., in feed additives).

V. Inactivation of Spores of *Bacillus* Genus Bacteria, Including Spores of Recombinant *Bacillus cereus* Family Members

(242) Spores of bacteria of the genus *Bacillus* can be genetically inactivated. Genetic inactivation of the spores can be advantageous, for example because it allows for delivery of spores to a plant or a plant growth medium while eliminating any detrimental effects that the live bacteria might have on a plant. In addition, use of inactivated spores can provide many of the same benefits (e.g., prevention of bacterial growth in a product) as discussed above in Section IV with respect to the use of exosporium fragments.

(243) A. Genetic Inactivation by Overexpression of a Protease or a Nuclease

(244) A recombinant bacterium of the genus *Bacillus* that expresses a protease or a nuclease is provided. The expression of the protease or nuclease is increased as compared to the expression of the protease or the nuclease in a wild-type bacterium of the genus *Bacillus* under the same conditions. The increased expression of the protease or the nuclease partially or completely inactivates spores of the recombinant bacterium of the genus *Bacillus* or renders spores of the recombinant bacterium of the genus *Bacillus* more susceptible to physical or chemical inactivation.

(245) The recombinant bacterium of the genus *Bacillus* is preferably a recombinant *Bacillus cereus* family member.

(246) The recombinant *Bacillus cereus* family member can also express a fusion protein comprising at least one protein or peptide of interest and a targeting sequence, exosporium protein, or exosporium protein fragment that targets the fusion protein to the exosporium of the recombinant *Bacillus cereus* family member.
(247) The recombinant bacterium of the genus *Bacillus* can express both a protease and a nuclease, wherein the expression of the protease is increased as compared to the expression of the protease in a wild-type bacterium of the genus *Bacillus* under the same conditions and the expression of the nuclease is increased as compared to the expression of the nuclease in a wild-type bacterium of the genus *Bacillus* under the same conditions.
(248) The protease of the recombinant bacterium can comprise a non-specific protease.
(249) The protease of the recombinant bacterium can comprise a serine protease, a threonine protease, a cysteine protease, an aspartate protease, a glutamic acid protease, an alkaline protease, a subtilisin, a histidine protease, or a metalloprotease.
(250) The protease of the recombinant bacterium can comprise a germination spore protease, such as a *Bacillus subtilis* germination spore protease, a *Bacillus mycoides* germination spore protease, or a *Bacillus thuringiensis* germination spore protease.
(251) The germination spore protease can comprise an active form of the germination spore protease. This protease is naturally inactive in the spore. Upon germination, the protease becomes active due to cleavage of the protease into a proprotein active form. Thus, the recombinant bacterium can comprise an active protease rather than the naturally inactive form. The active protease can digest the protective SASP proteins in the spore prior to germination.
(252) The nuclease of the recombinant bacterium can comprise an endonuclease or an exonuclease. The nuclease can comprise a non-specific endonuclease, such as *Bacillus subtilis* endonuclease 1. For example, the germination spore protease and endonuclease 1 can have the amino acid sequences listed below in Table 4.
(253) TABLE-US-00004 TABLE 4 Amino acid sequences of a germination spore protease and endonuclease 1 Protein SEQ ID NO. Endonuclease 1, *B. subtilis* 168 232 GPR Protease, *B. subtilis* 168 233 GPR Protease, *B. cereus* 234
(254) A protease or a nuclease having a high degree of amino acid identity to the sequences listed above in Table 4 can also be used.
(255) Thus, for example, the germination spore protease can comprise an amino acid sequence having at least 85% identity with SEQ ID NO: 233 or 234.
(256) The germination spore protease can comprise an amino acid sequence having at least 90% identity with SEQ ID NO: 233 or 234.
(257) The germination spore protease can comprise an amino acid sequence having at least 95% identity with SEQ ID NO: 233 or 234.
(258) The germination spore protease can comprise an amino acid sequence having at least 98% identity with SEQ ID NO: 233 or 234.
(259) The germination spore protease can comprise an amino acid sequence having at least 99% identity with SEQ ID NO: 233 or 234.
(260) The germination spore protease can comprise an amino acid sequence having 100% identity with SEQ ID NO: 233 or 234.
(261) Similarly, the non-specific endonuclease can comprise an amino acid having at least 85% identity with SEQ ID NO: 232.
(262) The non-specific endonuclease can comprise an amino acid having at least 90% identity with SEQ ID NO: 232.
(263) The non-specific endonuclease can comprise an amino acid having at least 95% identity with SEQ ID NO: 232.
(264) The non-specific endonuclease can comprise an amino acid having at least 98% identity with SEQ ID NO: 232.
(265) The non-specific endonuclease can comprise an amino acid having at least 99% identity with SEQ ID NO: 232.
(266) The non-specific endonuclease can comprise an amino acid having 100% identity with SEQ ID NO: 232.
(267) The protease or nuclease can be expressed under the control of a promoter comprising a sigma G promoter sequence. For example, the promoter can have one of the sequences shown in Table 5 below. The consensus sequence for binding of the sigma G transcription factor is CATNNTA, where N is any nucleotide. The sigma G promoter sequences in the promoters in Table 5 are indicated by bold and underlined text.
(268) TABLE-US-00005 TABLE 5 Promoter Sequences having sigma G sequences Promoter Nucleic Acid Sequence GPR Protease, *B. subtilis* 168 GTAACTAAAGCTTCTACAGTTTTAACAGCTGAACGCATGTCAGACTT GATAGAAGCGTTATGTGCACGACGCTCTTCGCTAAGTTTAGCGCGTT (SEQ ID NO: 235)

TGATAGCAGATTTAATGTTTGC**CATACTT**TTCACCTCCCTGGTGCGA
TCGAGTGACTCGATACTTA**CATAGAA**CAAGTGATATTCTATCAAACG
GAGAAGAGAATTGCAATAGCGAGATCAATGAAATTT**CATGTAA**AGG
AAAGAATGACCTTATATATTTTTGGGGAATCTAACTATATTTACTAT GAATTGCGGAGGAGATACG GPR
Protease GCAATAGCGAGATCAATGAAATTT**CATGTAA**AGGAAAGAATGACCT minimal promoter,
TATATATTTTTGGGGAATCTAACTATATTTACTATGAATTGCGGAGG *B. subtilis* 168 AGATACG (SEQ
ID NO: 236) GPR Protease, *B.*
TTTCACCTCCTAAGATACAACCTGTAGCACAGTGTCTTAAGGTTAAA *subtilis* 168
TCTTCTTCACAATAGAACAAATTGTATTCTATCAAACACACCTTTAG (SEQ ID NO: 237)
ATTGCAATATAAATGTAAAGTATTTTT**CATTGAA**GGTTCTCTTTTTAG
CATGATTTATTCAGCAAATGGCAACAATATAGGTACTTAATGTGAA GGAGGCCCCTGT GPR Protease
GAAGGTTCTCTTTTTAG**CATGATT**TATTCAGCAAATGGCAACAATAT minimal promoter,
AGGTACTTAATGTGAAGGAGGCCCCTGT *B. subtilis* 168 (SEQ ID NO: 238) SASPα, *B. subtilis*
GCTTTGTTGATTTCGAGCCGTATATTCAAGAAGCGGTAGATAACATT 168
GAGACAATGACCCTTTATAGCGAACAAGAAGCTAACGATAAATTCG (SEQ ID NO: 239)
CTGAACTCTTTTAAATCAATTTTCAGCTCCTGTATACAATTACCAAAG
TTTTTCTGAATGAAGCCATGTGTTTTGACA**CATTCTA**TACTCACAAG GAGGTGAGACAC SASPα
minimal GAATGAAGCCATGTGTTTTGACA**CATTCTA**TACTCACAAGGAGGTG promoter, *B. subtilis*
AGACAC 168 (SEQ ID NO: 240) SASPβ, *B. subtilis*
AAACGGCTAAGCTTTTTTTATTTCTCAAGATTTACCACACAATTCTCC 168
GCATGATTTTCCGGCCATTTTAACATAATACGTAGTAACAAGCCGGC (SEQ ID NO: 241)
AAAGCATTGGGTTACGCCGAGGCGGCAGTGACACCCGAGAAGGGTT
CACAGATTGGTGCAACTCCAGTTAACCCAAC**CATACTA**AATAAAAA GGAGATTTTACAC SASPβ
minimal GATTGGTGCAACTCCAGTTAACCCAAC**CATACTA**AATAAAAAGGAG promoter, *B. subtilis*
ATTTTACAC 168 (SEQ ID NO: 242) SASPγ, *B. subtilis*
TTCGCTTCTCCCACTTAATCTGATTTACATTCCAAGGAATCCAATGAT 168
TTATATGGAGATCTGAAACATAATCAATTTTCATTTTGTCTCCACCTT (SEQ ID NO: 243)
TCTTAATGAAAAATTTATTTCTTTGGCGTGTATAAATTAAAATAATCT
CTC**CATAATA**TGATTCAAACAAGCTTGTTTTCATTACACTTTAGGAG ATGAATAAG SASPγ minimal
GTATAAATTAAAATAATCTCTC**CATAATA**TGATTCAAACAAGCTTGT promoter, *B. subtilis*
TTTCATTACACTTTAGGAGATGAATAAG 168 (SEQ ID NO: 244) SASPδ, *B. subtilis*
TACAGTCCTCTCCATTTTGACATTCCATATTCAGGCAACCGCACATA 168
AAATGACAGCAGA**CATTCTA**TAGTCTGCGCCACCCCGGCTCAGAGG (SEQ ID NO: 245)
CCGGGGTTTTATTTTTCTCCACAACAATTGCCAGCATAAATAAACCC
CGTATATTTCAAACTAAATACGCGTTAAGAATTTCTTTATCGAAAAA GGAGATGAAAAAG SASPδ
minimal GCAACCGCACATAAAATGACAGCAGA**CATTCTA**TAGTCTGCGCCAC promoter, *B. subtilis*
CCCGGCTCAGAGGCCGGGGTTTTATTTTTCTCCACAACAATTGCCAG 168
CATAAATAAACCCCGTATATTTCAAACTAAATACGCGTTAAGAATTT (SEQ ID NO: 246)
CTTTATCGAAAAAGGAGATGAAAAAG

(269) Expression of a nuclease or protease under a sigma G promoter allows for site-specific expression of the nuclease or protease in the forespore, where the enzyme's activity is directed towards the forespore and, the region where the bacterial target DNA is located. Extensive cleavage of the forespore DNA is lethal to the bacterial spore when it begins to germinate.

(270) For example, as illustrated further in the Examples provided hereinbelow, overexpression of germination spore protease (GPR) in its active form in the forespore of a *Bacillus cereus* family member during sporulation results in proteolytic cleavage of proteins in the forespore and inactivation of the spore and/or renders the spore more sensitive to inactivation by ultraviolet or gamma irradiation. Similarly, overexpression of a non-specific endonuclease in the forespore during sporulation destroys the DNA in the spore, leading to a high number of inactivated spore particle. These methods for inactivating *Bacillus cereus* family member spores can be used separately or in conjunction with each other and/or with other spore inactivation methods.

(271) Expression of genes in *Bacillus* spores is tightly regulated by expression of specific sporulation sigma factors that direct the RNA polymerase to the genes that need to be expressed during each stage of sporulation. Late expression of genes in the forespore, where bacterial DNA and essential proteins are packaged, is regulated by the sigma factor sigma G. During late sporulation, the bacterial DNA is packaged with protective proteins called small acid soluble proteins (SASPs). These SASP proteins include SASPα, SASPβ, and SASPγ, among others. The SASP proteins protect the bacterial DNA from UV irradiation and other assaults. Upon germination, the proprotein germination spore protease is activated and digests these SASP proteins.

(272) By expressing a GPR under the control of a sigma G promoter, the GPR is expressed in the forespore and the

protective SASP proteins are degraded as sporulation commences, leaving the bacterial DNA more susceptible to degradation. Similarly, expression of a non-specific nuclease under the control of a sigma G promoter leads to digestion of the host DNA. Since the spore is unable to repair the large scale damage to its DNA, this ultimately leads to killing of the spore. Overexpression of a GPR and a non-specific endonuclease can be used together to both degrade the protective SASP proteins and the host DNA.
(273) The protease or the nuclease can be expressed under the control of any promoter comprising a sigma G promoter sequence.
(274) Thus, the protease or nuclease can be expressed under the control of any of the promoters listed in Table 5 above. In addition, the protease or nuclease can be expressed under the control of a promoter having a high degree of sequence identity with any of the promoter sequences listed above in Table 5.
(275) For example, the promoter can comprise a nucleic acid sequence having at least 95% identity with a nucleic acid sequence of any of SEQ ID NOs: 235-246.
(276) The promoter can comprise a nucleic acid sequence having at least 98% identity with a nucleic acid sequence of any of SEQ ID NOs: 235-246.
(277) The promoter can comprise a nucleic acid sequence having at least 99% identity with a nucleic acid sequence of any of SEQ ID NOs: 235-246.
(278) The promoter can comprise a nucleic acid sequence having 100% identity with a nucleic acid sequence of any of SEQ ID NOs: 235-246.
(279) In any of the recombinant bacteria of the genus *Bacillus* that express a protease or a nuclease, spores of the recombinant bacterium of the genus *Bacillus* can be more susceptible to inactivation, for example, by ultraviolet irradiation, gamma irradiation, or by treatment with bleach, hydrogen peroxide, chloroform, phenol, or acetic acid, as compared to the same spores that do not expresses the protease or the nuclease at an increased level as compared to expression of the protease or the nuclease in a wild-type bacterium of the genus *Bacillus*, treated under the same conditions.
(280) B. Genetic Inactivation by Mutation of a Gene Encoding a Germination Receptor, a Spore Core Lytic Enzyme, a Small Acid-Soluble Spore Protein (SASP), or a Spore Coat Protein
(281) Spores of any of the recombinant *Bacillus cereus* family member spores that express a fusion protein comprising a targeting sequence, an exosporium protein, or an exosporium protein fragment that targets the fusion protein to the exosporium of the recombinant *Bacillus cereus* family member can also be genetically inactivated or rendered more susceptible to physical or chemical inactivation by modification of the *Bacillus cereus* family member to comprise a mutation.
(282) Such mutations include knock-out or other inactivating mutations in one or more genes encoding a germination receptor. The germination receptor genes include, for example, GerA, GerB, GerK, GerH, GerI, GerG, GerL, GerQ, GerR, GerS, GerN, GerU, or GerX.
(283) Such mutations also include knock-out or other inactivating mutations in spore cortex lytic enzymes. For example, the spore cortex lytic enzymes SleB and CwJ can be mutated to inactivate spores. Such mutations prevent outgrowth of the spore upon germination and effectively inactivate the spores.
(284) Such mutations further include knock-out or other inactivating mutations of SASP genes (e.g., SASPα, SASPβ, or SASPγ). Such mutations eliminate the UV protection of the spores and render them more susceptible to inactivation by ultraviolet irradiation and other methods.
(285) Such methods also include making knock-out or other inactivating mutations in genes encoding spore coat or cortex proteins (e.g., CotA, CotB, or CotC). Such mutations render the spores more susceptible to inactivation by physical or chemical methods such as exposure to ultraviolet irradiation, gamma irradiation, or treatment with solvents such as bleach, hydrogen peroxide, chloroform, phenol, or acetic acid.
(286) Thus, the present invention relates to a recombinant *Bacillus cereus* family member that expresses a fusion protein comprising at least one protein or peptide of interest and a targeting sequence, exosporium protein, or exosporium protein fragment that targets the fusion protein to the exosporium of the recombinant *Bacillus cereus* family member. The recombinant *Bacillus cereus* family member comprises a mutation that partially or completely inactivates spores of the recombinant *Bacillus cereus* family member or renders spores of the recombinant *Bacillus cereus* family more susceptible to physical or chemical inactivation as compared to the same spores that do not comprise the mutation. The mutation comprises a mutation in a gene encoding a germination receptor, a mutation in a gene encoding a spore cortex lytic enzyme, a mutation in a gene encoding a small acid-soluble spore protein (SASP), or a mutation in a gene encoding a spore coat or cortex protein.
(287) The present invention further relates to a recombinant *Bacillus cereus* family member that expresses a fusion protein as described in Section I above. The recombinant *Bacillus cereus* family member comprises a mutation that partially or completely inactivates spores of the recombinant *Bacillus cereus* family member or renders spores of the recombinant *Bacillus cereus* family more susceptible to physical or chemical inactivation as compared to the same spores that do not comprise the mutation.

(288) Any of the recombinant *Bacillus cereus* family members described above in Section V.A that express a protease or a nuclease can also comprise a mutation that partially or completely inactivates spores of the recombinant *Bacillus cereus* family member or renders spores of the recombinant *Bacillus cereus* family more susceptible to physical or chemical inactivation as compared to the same spores that do not comprise the mutation. For example, the mutation can comprise a mutation in a gene encoding a germination receptor, a mutation in a gene encoding a spore cortex lytic enzyme, a mutation in a gene encoding a small acid-soluble spore protein (SASP), or a mutation in a gene encoding a spore coat or cortex protein.
(289) For example, the mutation can comprise a mutation in a gene encoding a germination receptor, such as a knock-out mutation of the gene encoding the germination receptor. The germination receptor can comprise GerA, GerB, GerK, GerH, GerI, GerG, GerL, GerQ, GerR, GerS, GerN, GerU, or GerX.
(290) For example, the mutation can comprise a mutation in a gene encoding a spore cortex lytic enzyme, such as a knock-out mutation of the gene encoding the spore cortex lytic enzyme. The spore cortex lytic enzyme can comprise SleB or CwlJ.
(291) For example, the mutation can comprise a mutation in a gene encoding a SASP, such as a mutation in a SspA gene, a mutation in a SspB gene, a mutation in a SspC gene, a mutation in a SspD gene, a mutation in a SspE gene, a mutation in a SspF gene, a mutation in a SspG gene, a mutation in a SspH gene, a mutation in a SspI gene, a mutation in a SspJ gene, a mutation in a SspK gene, a mutation in a SspL gene, a mutation in a SspM gene, a mutation in a SspN gene, a mutation in a SspO gene, a mutation in a SspP gene, or a combination thereof. The SASP can comprise SASPα, SASPβ, or SASPγ. The spores of the recombinant *Bacillus cereus* family member may be more susceptible to inactivation by ultraviolet irradiation or gamma irradiation as compared to the same spores that do not comprise the mutation in the gene encoding the SASP.
(292) For example, the mutation can comprise a mutation in a gene encoding a spore coat or cortex protein, such as a knock-out mutation of the gene encoding the spore coat or cortex protein. The spore coat or cortex protein can comprise CotA, CotB, or CotC. The spores of the recombinant *Bacillus cereus* family member may be more susceptible to inactivation by ultraviolet irradiation, gamma irradiation or by treatment with bleach, hydrogen peroxide, chloroform, phenol, or acetic acid, as compared to the same spores that do not comprise the mutation in the spore coat or cortex protein, treated under the same conditions.
VI. Recombinant *Bacillus cereus* Family Members that Overexpress Exosporium Enzymes that have Beneficial Effects on Plants or Delay Germination of *Bacillus cereus* Family Member Spores
(293) Recombinant *Bacillus cereus* family members that overexpress various exosporium proteins to provide beneficial effects on plants or delay spore germination are also provided.
(294) A recombinant *Bacillus cereus* family member that expresses an exosporium protein is provided, wherein the expression of the exosporium protein is increased as compared to the expression of the exosporium protein in a wild-type *Bacillus cereus* family member under the same conditions. The exosporium protein can comprise an exosporium enzyme, wherein the exosporium enzyme comprises an enzyme involved in nutrient solubilization, an inosine-uridine hydrolase, a protease, an enzyme that catalyzes the degradation of a free radical, an arginase, or an alanine racemase. Alternatively, the exosporium protein can comprise a BclA protein, a BclB protein, a CotE protein a CotO protein, an ExsY protein, an ExsFA/BxpB protein, a CotY protein, an ExsFB protein, an ExsJ protein, an ExsH protein, a YjcA protein, a YjcB protein, a BclC protein, a BxpA protein, a BclE protein, a BetA/BAS3290 protein, an ExsA protein, an ExsK protein, an ExsB protein, a YabG protein, or a Tgl protein.
(295) The exosporium protein is preferably not part of a fusion protein.
(296) Exemplary amino acid sequences for AcpC, InhA1, InhA2, InhA3, SODA1, and SODA2 are provided above in Tables 1 and 2. Exemplary sequences for alanine racemase 1, alanine racemase 2, arginase, IunH1, and IunH2 are provided by the SEQ ID NOs. referenced in Table 6 below.
(297) TABLE-US-00006 TABLE 6 Exemplary amino acid sequences for exosporium enzymes Protein and Strain SEQ ID NO. Alanine Racemase 1, *B. anthracis* ΔSterne 247 Alanine Racemase 2, *Bacillus cereus* F837/78 248 Arginase, *Bacillus thuringiensis* pondicheriensis 4BA1 249 IunH1, *B. cereus* Str. CI 250 IunH2, *Bacillus thuringiensis* 251
(298) Overexpression of inosine-uridine hydrolases and alanine racemases hinders the ability of spores to germinate and thereby maintains the spores in a dormant stage and increases the stability of the spores.
(299) The SODA enzymes and arginase degrade free radicals. Spores that overexpress these enzymes have increased resistance to stress caused by free radicals.
(300) Where the exosporium protein comprises an exosporium enzyme, and the exosporium enzyme comprises an enzyme involved in nutrient solubilization, the enzyme involved in nutrient solubilization can comprise an enzyme involved in phosphate solubilization, such as an acid phosphatase (e.g., AcpC). The acid phosphatase can comprise an amino acid sequence having at least 90% identity with SEQ ID NO: 137.
(301) The acid phosphatase can comprise an amino acid sequence having at least 95% identity with SEQ ID NO: 137.

(302) The acid phosphatase can comprise an amino acid sequence having at least 98% identity with SEQ ID NO: 137.
(303) The acid phosphatase can comprise an amino acid sequence having at least 99% identity with SEQ ID NO: 137.
(304) The acid phosphatase can comprise an amino acid sequence having 100% identity with SEQ ID NO: 137.
(305) Where the exosporium protein comprises an exosporium enzyme, and the exosporium enzyme comprises an inosine-uridine hydrolase, the inosine-uridine hydrolase can comprise IunH1 or IunH2. The inosine-uridine hydrolase can comprise an amino acid sequence having at least 85% identity with SEQ ID NO: 250 or 251.
(306) The inosine-uridine hydrolase can comprise an amino acid sequence having at least 90% identity with SEQ ID NO: 250 or 251.
(307) The inosine-uridine hydrolase can comprise an amino acid sequence having at least 95% identity with SEQ ID NO: 250 or 251.
(308) The inosine-uridine hydrolase can comprise an amino acid sequence having at least 98% identity with SEQ ID NO: 250 or 251.
(309) The inosine-uridine hydrolase can comprise an amino acid sequence having at least 99% identity with SEQ ID NO: 250 or 251.
(310) The inosine-uridine hydrolase can comprise an amino acid sequence having 100% identity with SEQ ID NO: 250 or 251.
(311) Where the exosporium protein comprises an exosporium enzyme, and the exosporium enzyme comprises a protease, the protease can be a metalloprotease (e.g., InhA1, InhA2, or InhA3). The metalloprotease can comprise an amino acid sequence having at least 85% identity with SEQ ID NO: 114, 121, 122, 129, 130, or 138.
(312) The metalloprotease can comprise an amino acid sequence having at least 85% identity with SEQ ID NO: 114, 121, 122, 129, 130, or 138.
(313) The metalloprotease can comprise an amino acid sequence having at least 90% identity with SEQ ID NO: 114, 121, 122, 129, 130, or 138.
(314) The metalloprotease can comprise an amino acid sequence having at least 95% identity with SEQ ID NO: 114, 121, 122, 129, 130, or 138.
(315) The metalloprotease can comprise an amino acid sequence having at least 98% identity with SEQ ID NO: 114, 121, 122, 129, 130, or 138.
(316) The metalloprotease can comprise an amino acid sequence having at least 99% identity with SEQ ID NO: 114, 121, 122, 129, 130, or 138.
(317) The metalloprotease can comprise an amino acid sequence having 100% identity with SEQ ID NO: 114, 121, 122, 129, 130, or 138.
(318) Where the exosporium protein comprises an exosporium enzyme, and the exosporium enzyme comprises an enzyme that catalyzes the degradation of a free radical, the enzyme that catalyzes the degradation of a free radical can comprise a superoxide dismutase (e.g., superoxide dismutase 1 (SODA1) or superoxide dismutase 2 (SODA2)). The superoxide dismutase can comprise an amino acid sequence having at least 85% identity with SEQ ID NO: 155 or 156.
(319) The superoxide dismutase can comprise an amino acid sequence having at least 90% identity with SEQ ID NO: 155 or 156.
(320) The superoxide dismutase can comprise an amino acid sequence having at least 95% identity with SEQ ID NO: 155 or 156.
(321) The superoxide dismutase can comprise an amino acid sequence having at least 98% identity with SEQ ID NO: 155 or 156.
(322) The superoxide dismutase can comprise an amino acid sequence having at least 99% identity with SEQ ID NO: 155 or 156.
(323) The superoxide dismutase can comprise an amino acid sequence having 100% identity with SEQ ID NO: 155 or 156.
(324) Where the exosporium protein comprises an exosporium enzyme, and the exosporium enzyme comprises an arginase, the arginase can comprise a *Bacillus thuringiensis* arginase. The arginase can comprise an amino acid sequence having at least 85% identity with SEQ ID NO: 249.
(325) The arginase can comprise an amino acid sequence having at least 90% identity with SEQ ID NO: 249.
(326) The arginase can comprise an amino acid sequence having at least 95% identity with SEQ ID NO: 249.
(327) The arginase can comprise an amino acid sequence having at least 98% identity with SEQ ID NO: 249.
(328) The arginase can comprise an amino acid sequence having at least 99% identity with SEQ ID NO: 249.
(329) The arginase can comprise an amino acid sequence having 100% identity with SEQ ID NO: 249.
(330) Where the exosporium protein comprises an exosporium enzyme, and the exosporium enzyme comprises an alanine racemase, the alanine racemase can comprise alanine racemase 1 (ALR1) or alanine racemase 2 (ALR2).

The alanine racemase can comprise an amino acid sequence having at least 85% identity with SEQ ID NO: 247 or 248.
(331) The alanine racemase can comprise an amino acid sequence having at least 90% identity with SEQ ID NO: 247 or 248.
(332) The alanine racemase can comprise an amino acid sequence having at least 95% identity with SEQ ID NO: 247 or 248.
(333) The alanine racemase can comprise an amino acid sequence having at least 98% identity with SEQ ID NO: 247 or 248.
(334) The alanine racemase can comprise an amino acid sequence having at least 99% identity with SEQ ID NO: 247 or 248.
(335) The alanine racemase can comprise an amino acid sequence having 100% identity with SEQ ID NO: 247 or 248.
(336) The exosporium protein can comprise a BclA protein, a BclB protein, a CotE protein a CotO protein, an ExsY protein, an ExsFA/BxpB protein, a CotY protein, an ExsFB protein, an ExsJ protein, an ExsH protein, a YjcA protein, a YjcB protein, a BclC protein, a BxpA protein, a BclE protein, a BetA/BAS3290 protein, an ExsA protein, an ExsK protein, an ExsB protein, a YabG protein, or a Tgl protein. The exosporium protein preferably comprises a BclA protein, a BclB protein, a CotE protein, or a CotO protein. Exemplary amino acid sequences for these exosporium proteins can be found in Table 2 above.
(337) The exosporium protein can comprise a BclA protein. The BclA protein can comprise an amino acid sequence having at least 85% identity with SEQ ID NO: 141 or 142.
(338) The BclA protein can comprise an amino acid sequence having at least 90% identity with SEQ ID NO: 141 or 142.
(339) The BclA protein can comprise an amino acid sequence having at least 95% identity with SEQ ID NO: 141 or 142.
(340) The BclA protein can comprise an amino acid sequence having at least 98% identity with SEQ ID NO: 141 or 142.
(341) The BclA protein can comprise an amino acid sequence having at least 99% identity with SEQ ID NO: 141 or 142.
(342) The BclA protein can comprise an amino acid sequence having 100% identity with SEQ ID NO: 141 or 142.
(343) The exosporium protein can comprise a BclB protein. The BclB protein can comprise an amino acid sequence having at least 85% identity with SEQ ID NO: 143 or 144.
(344) The BclB protein can comprise an amino acid sequence having at least 90% identity with SEQ ID NO: 143 or 144.
(345) The BclB protein can comprise an amino acid sequence having at least 95% identity with SEQ ID NO: 143 or 144.
(346) The BclB protein can comprise an amino acid sequence having at least 98% identity with SEQ ID NO: 143 or 144.
(347) The BclB protein can comprise an amino acid sequence having at least 99% identity with SEQ ID NO: 143 or 144.
(348) The BclB protein can comprise an amino acid sequence having 100% identity with SEQ ID NO: 143 or 144.
(349) The exosporium protein can comprise a CotE protein. The CotE protein can comprise an amino acid sequence having at least 85% identity with SEQ ID NO: 149.
(350) The CotE protein can comprise an amino acid sequence having at least 90% identity with SEQ ID NO: 149.
(351) The CotE protein can comprise an amino acid sequence having at least 95% identity with SEQ ID NO: 149.
(352) The CotE protein can comprise an amino acid sequence having at least 98% identity with SEQ ID NO: 149.
(353) The CotE protein can comprise an amino acid sequence having at least 99% identity with SEQ ID NO: 149.
(354) The CotE protein can comprise an amino acid sequence having 100% identity with SEQ ID NO: 149.
(355) The exosporium protein can comprise a CotO protein. The CotO protein can comprise an amino acid sequence having at least 85% identity with SEQ ID NO: 126.
(356) The CotO protein can comprise an amino acid sequence having at least 90% identity with SEQ ID NO: 126.
(357) The CotO protein can comprise an amino acid sequence having at least 95% identity with SEQ ID NO: 126.
(358) The CotO protein can comprise an amino acid sequence having at least 98% identity with SEQ ID NO: 126.
(359) The CotO protein can comprise an amino acid sequence having at least 99% identity with SEQ ID NO: 126.
(360) The CotO protein can comprise an amino acid sequence having at least 100% identity with SEQ ID NO: 126.
(361) The exosporium protein can comprise an ExsY protein. The ExsY protein can comprise an amino acid sequence having at least 85%, at least 90%, at least 95%, at least 98%, at least 99%, or 100% identity with SEQ ID NO: 123.
(362) The exosporium protein can comprise an ExsFA/BxpB protein. The ExsFA/BxpB protein can comprise an

amino acid sequence having at least 85%, at least 90%, at least 95%, at least 98%, at least 99%, or 100% identity with SEQ ID NO:124.
(363) The exosporium protein can comprise a CotY protein. The CotY protein can comprise an amino acid sequence having at least 85%, at least 90%, at least 95%, at least 98%, at least 99%, or 100% identity with SEQ ID NO: 125.
(364) The exosporium protein can comprise an ExsFB protein. The ExsFB protein can comprise an amino acid sequence having at least 85%, at least 90%, at least 95%, at least 98%, at least 99%, or 100% identity with SEQ ID NO: 127 or 128.
(365) The exosporium protein can comprise an ExsJ protein. The ExJ protein can comprise an amino acid sequence having at least 85%, at least 90%, at least 95%, at least 98%, at least 99%, or 100% identity with SEQ ID NO: 131.
(366) The exosporium protein can comprise an ExsH protein. The ExsH protein can comprise an amino acid sequence having at least 85%, at least 90%, at least 95%, at least 98%, at least 99%, or 100% identity with SEQ ID NO: 132.
(367) The exosporium protein can comprise a YjcA protein. The YjcA protein can comprise an amino acid sequence having at least 85%, at least 90%, at least 95%, at least 98%, at least 99%, or 100% identity with SEQ ID NO: 133.
(368) The exosporium protein can comprise a YjcB protein. The YjcB protein can comprise an amino acid sequence having at least 85%, at least 90%, at least 95%, at least 98%, at least 99%, or 100% identity with SEQ ID NO: 134 or 135.
(369) The exosporium protein can comprise a BclC protein. The BclC protein can comprise an amino acid sequence having at least 85%, at least 90%, at least 95%, at least 98%, at least 99%, or 100% with SEQ ID NO: 136.
(370) The exosporium protein can comprise a BxpA protein. The BxpA protein can comprise an amino acid sequence having at least 85%, at least 90%, at least 95%, at least 98%, at least 99%, or 100% with SEQ ID NO: 145.
(371) The exosporium protein can comprise a BclE protein. The BclE protein can comprise an amino acid sequence having at least 85%, at least 90%, at least 95%, at least 98%, at least 99%, or 100% identity with SEQ ID NO: 146 or 147.
(372) The exosporium protein can comprise a BetA/BAS3290 protein. The BetA/BAS3290 protein can comprise an amino acid sequence having at least 85%, at least 90%, at least 95%, at least 98%, at least 99%, or 100% identity with SEQ ID NO: 148.
(373) The exosporium protein can comprise an ExsA protein. The ExsA protein can comprise an amino acid sequence having at least 85%, at least 90%, at least 95%, at least 98%, at least 99%, or 100% identity with SEQ ID NO: 150.
(374) The exosporium protein can comprise an ExsK protein. The ExsK protein can comprise an amino acid sequence having at least 85%, at least 90%, at least 95%, at least 98%, at least 99%, or 100% identity with SEQ ID NO: 151.
(375) The exosporium protein can comprise an ExsB protein. The ExsB protein can comprise an amino acid sequence having at least 85%, at least 90%, at least 95%, at least 98%, at least 99%, or 100% identity with SEQ ID NO: 152.
(376) The exosporium protein can comprise a YabG protein. The YabG protein can comprise an amino acid sequence having at least 85%, at least 90%, at least 95%, at least 98%, at least 99%, or 100% identity with SEQ ID NO: 153.
(377) The exosporium protein can comprise a Tgl protein. The Tjl protein can comprise an amino acid sequence having at least 85%, at least 90%, at least 95%, at least 98%, at least 99%, or 100% identity with SEQ ID NO: 156.
(378) The recombinant *Bacillus cereus* family member can also express a fusion protein comprising at least one protein or peptide of interest and a targeting sequence, exosporium protein, or exosporium protein fragment that targets the fusion protein to the exosporium of the recombinant *Bacillus cereus* family member.

VII. Expression of Fusion Proteins in Endophytic *Bacillus cereus* Family Members, in *Bacillus cereus* Family Members Capable of Degrading Herbicides or Pesticides, or in Probiotic *Bacillus cereus* Family Members

(379) Any of the fusion proteins comprising a protein or peptide of interest and a targeting sequence, an exosporium protein, or an exosporium protein fragment that targets the fusion protein to the exosporium of a recombinant *Bacillus cereus* family member, can be expressed an endophytic *Bacillus cereus* family member, a strain of bacteria that is capable of degrading an herbicide or a pesticide, or a probiotic strain of bacteria.
(380) The expression of the fusion proteins in an endophytic strain of bacteria provides the ability to deliver the protein or peptide of interest into the plant itself. The endophytic strains can be delivered to plants using various methods, e.g., the endophytic strains can be delivered via seed treatment, treatment of the plant growth medium (e.g., soil), irrigation, application to the plant itself (e.g., foliar application to the aerial portions of a plant). Once inside the plant, the bacteria multiply and colonize the internal tissues of the plant.
(381) As is explained further hereinbelow, probiotic strains of bacteria that express of the fusion proteins, and in particular strains that are both probiotic and endophytic that express the fusion proteins, are useful in methods for

delivering the proteins or peptides of interest (e.g., enzymes) to animals.
(382) While any of the fusion proteins comprising a protein or peptide of interest and a targeting sequence, an exosporium protein, or an exosporium protein fragment that targets the fusion protein to the exosporium of a recombinant *Bacillus cereus* family member can be expressed in *Bacillus cereus* family member strain that is capable of degrading an herbicide or a pesticide, as explained further hereinbelow, these strains are particularly useful in methods for decontamination of an environment contaminated with an herbicide and/or a pesticide.
(383) The present invention therefore relates to a recombinant *Bacillus cereus* family member that expresses a fusion protein comprising at least one protein or peptide of interest and a targeting sequence, exosporium protein, or exosporium protein fragment that targets the fusion protein to the exosporium of the recombinant *Bacillus cereus* family member, wherein the recombinant *Bacillus cereus* family member comprises an endophytic strain of bacteria, a strain of bacteria that is capable of degrading an herbicide or a pesticide, or a probiotic strain of bacteria.
(384) The endophytic strain of bacteria can comprise *Bacillus cereus* family member EE349, *Bacillus cereus* family member EE439, *Bacillus thuringiensis* EE417, *Bacillus cereus* EE444, or *Bacillus thuringiensis* EE319, *Bacillus thuringiensis* EE-B00184, *Bacillus cereus* family member EE-B00377, *Bacillus pseudomycoides* EE-B00366, or *Bacillus mycoides* EE-B00363.
(385) For example, the endophytic strain of bacteria can comprise *Bacillus cereus* family member EE439, *Bacillus thuringiensis* EE417, *Bacillus cereus* EE444, or *Bacillus thuringiensis* EE319, *Bacillus thuringiensis* EE-B00184, *Bacillus cereus* family member EE-B00377, *Bacillus pseudomycoides* EE-B00366, or *Bacillus mycoides* EE-B00363.
(386) The strain of bacteria that is capable of degrading an herbicide or a pesticide can comprise *Bacillus cereus* family member EE349, *Bacillus cereus* family member EE-B00377, *Bacillus pseudomycoides* EE-B00366, or *Bacillus mycoides* EE-B00363.
(387) The probiotic strain of bacteria can comprise *Bacillus cereus* family member EE349, *Bacillus cereus* family member EE439, *Bacillus thuringiensis* EE417, or *Bacillus cereus* EE444.
(388) The present invention further relates to a recombinant *Bacillus cereus* family member that expresses a fusion protein comprising at least one protein or peptide of interest and a targeting sequence, exosporium protein, or exosporium protein fragment that targets the fusion protein to the exosporium of the recombinant *Bacillus cereus* family member, wherein the recombinant *Bacillus cereus* family member comprises an endophytic strain of bacteria, and the fusion protein comprises any of the fusion proteins described in Section I above.
VIII. Targeting Sequences, Exosporium Proteins, and Exosporium Protein Fragments for Use in: (a) Recombinant *Bacillus cereus* Family Members that Express a Fusion Protein and Co-Overexpress a Modulator Protein; (b) Recombinant *Bacillus cereus* Family Members that Comprise a Mutation or Other Genetic Alteration that Allows for Collection of Free Exosporium; (c) Recombinant *Bacillus cereus* Family Members that Overexpress a Protease or a Nuclease; (d) Recombinant *Bacillus cereus* Family Members that Express a Fusion Protein and Overexpress an Exosporium Protein that has Beneficial Effects on Plants; or (e) or Endophytic Recombinant *Bacillus cereus* Family Members that Express Fusion Proteins
(389) Any of the targeting sequences, exosporium proteins, or exosporium proteins described in this section can be in any of the fusion proteins in: (a) any of the recombinant *Bacillus cereus* family members that express a fusion protein and overexpress a modulator protein, described in Section II above; (b) any of the recombinant *Bacillus cereus* family members that express a fusion protein and comprise a mutation or other genetic alteration that allows for collection of free exosporium, described in Section IV above; (c) any of the recombinant *Bacillus cereus* family members that expresses a fusion protein and overexpress a protease or a nuclease, described above in Section V.A; (d) any of the recombinant *Bacillus cereus* family members that express a fusion protein and overexpress an exosporium protein that has beneficial effects on plants, described in Section VI above; and (e) any of the endophytic recombinant *Bacillus cereus* family members that express a fusion protein, described in Section VII above.
(390) In any of the recombinant *Bacillus cereus* members (a) through (e), the targeting sequence, exosporium protein, or exosporium protein fragment can comprise: (1) a targeting sequence comprising an amino acid sequence having at least about 43% identity with amino acids 20-35 of SEQ ID NO: 1, wherein the identity with amino acids 25-35 is at least about 54%; (2) a targeting sequence comprising amino acids 1-35 of SEQ ID NO: 1; (3) a targeting sequence comprising amino acids 20-35 of SEQ ID NO: 1; (4) a targeting sequence comprising SEQ ID NO: 1; (5) an exosporium protein comprising an amino acid sequence having at least 85% identity with SEQ ID NO: 2; (6) a targeting sequence comprising amino acids 2-35 of SEQ ID NO: 1; (7) a targeting sequence comprising amino acids 5-35 of SEQ ID NO: 1; (8) a targeting sequence comprising amino acids 8-35 of SEQ ID NO: 1; (9) a targeting sequence comprising amino acids 10-35 of SEQ ID NO: 1; (10) a targeting sequence comprising amino acids 15-35 of SEQ ID NO: 1; (11) a targeting sequence comprising amino acids 1-27 of SEQ ID NO: 3; (12) a targeting sequence comprising amino acids 12-27 of SEQ ID NO: 3; (13) a targeting sequence comprising SEQ ID NO: 3; (14) an exosporium protein comprising an amino acid sequence having at least 85% identity with SEQ ID NO: 4;

(15) a targeting sequence comprising amino acids 2-27 of SEQ ID NO: 3; (16) a targeting sequence comprising amino acids 5-27 of SEQ ID NO: 3; (17) a targeting sequence comprising amino acids 8-27 of SEQ ID NO: 3; (18) a targeting sequence comprising amino acids 10-27 of SEQ ID NO: 3; (19) a targeting sequence comprising amino acids 1-38 of SEQ ID NO: 5; (20) a targeting sequence comprising amino acids 23-38 of SEQ ID NO: 5; (21) a targeting sequence comprising SEQ ID NO: 5; (22) an exosporium protein comprising an amino acid sequence having at least 85% identity with SEQ ID NO: 6; (23) a targeting sequence comprising amino acids 2-38 of SEQ ID NO: 5; (24) a targeting sequence comprising amino acids 5-38 of SEQ ID NO: 5; (25) a targeting sequence comprising amino acids 8-38 of SEQ ID NO: 5; (26) a targeting sequence comprising amino acids 10-38 of SEQ ID NO: 5; (27) a targeting sequence comprising amino acids 15-38 of SEQ ID NO: 5; (28) a targeting sequence comprising amino acids 20-38 of SEQ ID NO: 5; (29) a targeting sequence comprising amino acids 1-28 of SEQ ID NO: 7; (30) a targeting sequence comprising amino acids 13-28 of SEQ ID NO: 7; (31) a targeting sequence comprising SEQ ID NO: 7; (32) an exosporium protein comprising an amino acid sequence having at least 85% identity with SEQ ID NO: 8; (33) a targeting sequence comprising amino acids 2-28 of SEQ ID NO: 7; (34) a targeting sequence comprising amino acids 5-28 of SEQ ID NO: 7; (35) a targeting sequence comprising amino acids 8-28 of SEQ ID NO: 7; (36) a targeting sequence comprising amino acids 10-28 of SEQ ID NO: 7; (37) a targeting sequence comprising amino acids 1-24 of SEQ ID NO: 9; (38) a targeting sequence comprising amino acids 9-24 of SEQ ID NO: 9; (39) a targeting sequence comprising SEQ ID NO: 9; (40) an exosporium protein comprising an amino acid sequence having at least 85% identity with SEQ ID NO: 10; (41) a targeting sequence comprising amino acids 2-24 of SEQ ID NO: 9; (42) a targeting sequence comprising amino acids 5-24 of SEQ ID NO: 9; (43) a targeting sequence comprising amino acids 8-24 of SEQ ID NO: 9; (44) a targeting sequence comprising amino acids 1-33 of SEQ ID NO:11; (45) a targeting sequence comprising amino acids 18-33 of SEQ ID NO: 11; (46) a targeting sequence comprising SEQ ID NO: 11; (47) an exosporium protein comprising an amino acid sequence having at least 85% identity with SEQ ID NO: 12; (48) a targeting sequence comprising amino acids 2-33 of SEQ ID NO: 11; (49) a targeting sequence comprising amino acids 5-33 of SEQ ID NO: 11; (50) a targeting sequence comprising amino acids 8-33 of SEQ ID NO: 11; (51) a targeting sequence comprising amino acids 10-33 of SEQ ID NO: 11; (52) a targeting sequence comprising amino acids 15-33 of SEQ ID NO: 11; (53) a targeting sequence comprising amino acids 1-33 of SEQ ID NO: 13; (54) a targeting sequence comprising amino acids 18-33 of SEQ ID NO: 13; (55) a targeting sequence comprising SEQ ID NO:13; (56) an exosporium protein comprising an amino acid sequence having at least 85% identity with SEQ ID NO: 14; (57) a targeting sequence comprising amino acids 2-33 of SEQ ID NO: 13; (58) a targeting sequence comprising amino acids 5-33 of SEQ ID NO: 13; (59) a targeting sequence comprising amino acids 8-33 of SEQ ID NO: 13; (60) a targeting sequence comprising amino acids 10-33 of SEQ ID NO: 13; (61) a targeting sequence comprising amino acids 15-33 of SEQ ID NO: 13; (62) a targeting sequence comprising amino acids 1-43 of SEQ ID NO: 15; (63) a targeting sequence comprising amino acids 28-43 of SEQ ID NO: 15; (64) a targeting sequence comprising SEQ ID NO:15; (65) an exosporium protein comprising an amino acid sequence having at least 85% identity with SEQ ID NO: 16; (66) a targeting sequence comprising amino acids 2-43 of SEQ ID NO: 15; (67) a targeting sequence comprising amino acids 5-43 of SEQ ID NO: 15; (68) a targeting sequence comprising amino acids 8-43 of SEQ ID NO: 15; (69) a targeting sequence comprising amino acids 10-43 of SEQ ID NO: 15; (70) a targeting sequence comprising amino acids 15-43 of SEQ ID NO: 15; (71) a targeting sequence comprising amino acids 20-43 of SEQ ID NO: 15; (72) a targeting sequence comprising amino acids 25-43 of SEQ ID NO: 15; (73) a targeting sequence comprising amino acids 1-27 of SEQ ID NO: 17; (74) a targeting sequence comprising amino acids 12-27 of SEQ ID NO: 17; (75) a targeting sequence comprising SEQ ID NO: 17; (76) an exosporium protein comprising an amino acid sequence having at least 85% identity with SEQ ID NO:18; (77) a targeting sequence comprising amino acids 2-27 of SEQ ID NO: 17; (78) a targeting sequence comprising amino acids 5-27 of SEQ ID NO: 17; (79) a targeting sequence comprising amino acids 8-27 of SEQ ID NO: 17; (80) a targeting sequence comprising amino acids 10-27 of SEQ ID NO: 17; (81) a targeting sequence comprising amino acids 1-33 of SEQ ID NO: 19; (82) a targeting sequence comprising amino acids 18-33 of SEQ ID NO: 19; (83) a targeting sequence comprising SEQ ID NO:19; (84) an exosporium protein comprising an amino acid sequence having at least 85% identity with SEQ ID NO:20; (85) a targeting sequence comprising amino acids 2-33 of SEQ ID NO: 19; (86) a targeting sequence comprising amino acids 5-33 of SEQ ID NO: 19; (87) a targeting sequence comprising amino acids 8-33 of SEQ ID NO: 19; (88) a targeting sequence comprising amino acids 10-33 of SEQ ID NO: 19; (89) a targeting sequence comprising amino acids 15-33 of SEQ ID NO: 19; (90) a targeting sequence comprising amino acids 1-33 of SEQ ID NO: 21; (91) a targeting sequence comprising amino acids 18-33 of SEQ ID NO: 21; (92) a targeting sequence comprising SEQ ID NO:21; (93) an exosporium protein comprising an amino acid sequence having at least 85% identity with SEQ ID NO:22; (94) a targeting sequence comprising amino acids 2-33 of SEQ ID NO: 21; (95) a targeting sequence comprising amino acids 5-33 of SEQ ID NO: 21; (96) a targeting sequence comprising amino acids 8-33 of SEQ ID NO: 21; (97) a targeting sequence comprising amino acids 10-33 of SEQ ID NO: 21; (98) a targeting sequence comprising amino acids 15-33 of SEQ ID NO: 21; (99) a targeting sequence comprising amino acids 1-24 of SEQ ID NO: 23;

(100) a targeting sequence comprising amino acids 9-24 of SEQ ID NO: 23; (101) a targeting sequence comprising SEQ ID NO:23; (102) an exosporium protein comprising an amino acid sequence having at least 85% identity with SEQ ID NO:24; (103) a targeting sequence comprising amino acids 2-24 of SEQ ID NO:23; (104) a targeting sequence comprising amino acids 5-24 of SEQ ID NO: 23; (105) a targeting sequence comprising amino acids 8-24 of SEQ ID NO: 23; (106) a targeting sequence comprising amino acids 1-24 of SEQ ID NO: 25; (107) a targeting sequence comprising amino acids 9-24 of SEQ ID NO: 25; (108) a targeting sequence comprising SEQ ID NO:25; (109) an exosporium protein comprising an amino acid sequence having at least 85% identity with SEQ ID NO:26; (110) a targeting sequence comprising amino acids 2-24 of SEQ ID NO: 25; (111) a targeting sequence comprising amino acids 5-24 of SEQ ID NO: 25; (112) a targeting sequence comprising amino acids 8-24 of SEQ ID NO: 25; (113) a targeting sequence comprising amino acids 1-30 of SEQ ID NO: 27; (114) a targeting sequence comprising amino acids 15-30 of SEQ ID NO: 27; (115) a targeting sequence comprising SEQ ID NO:27; (116) an exosporium protein comprising an amino acid sequence having at least 85% identity with SEQ ID NO:28; (117) a targeting sequence comprising amino acids 2-30 of SEQ ID NO: 27; (118) a targeting sequence comprising amino acids 5-30 of SEQ ID NO: 27; (119) a targeting sequence comprising amino acids 8-30 of SEQ ID NO: 27; (120) a targeting sequence comprising amino acids 10-30 of SEQ ID NO: 27; (121) a targeting sequence comprising amino acids 1-33 of SEQ ID NO: 29; (122) a targeting sequence comprising amino acids 18-33 of SEQ ID NO: 29; (123) a targeting sequence comprising SEQ ID NO:29; (124) an exosporium protein comprising an amino acid sequence having at least 85% identity with SEQ ID NO:30; (125) a targeting sequence comprising amino acids 2-33 of SEQ ID NO: 29; (126) a targeting sequence comprising amino acids 5-33 of SEQ ID NO: 29; (127) a targeting sequence comprising amino acids 8-33 of SEQ ID NO: 29; (128) a targeting sequence comprising amino acids 10-33 of SEQ ID NO: 29; (129) a targeting sequence comprising amino acids 15-33 of SEQ ID NO: 29; (130) a targeting sequence comprising amino acids 1-24 of SEQ ID NO: 31; (131) a targeting sequence comprising amino acids 9-24 of SEQ ID NO: 31; (132) a targeting sequence comprising SEQ ID NO:31; (133) an exosporium protein comprising an amino acid sequence having at least 85% identity with SEQ ID NO:32; (134) a targeting sequence comprising amino acids 2-24 of SEQ ID NO: 31; (135) a targeting sequence comprising amino acids 5-24 of SEQ ID NO: 31; (136) a targeting sequence comprising amino acids 8-24 of SEQ ID NO: 31; (137) a targeting sequence comprising amino acids 1-15 of SEQ ID NO: 33; (138) a targeting sequence comprising SEQ ID NO:33; (139) an exosporium protein comprising an amino acid sequence having at least 85% identity with SEQ ID NO:34; (140) a targeting sequence comprising amino acids 1-16 of SEQ ID NO: 35; (141) a targeting sequence comprising SEQ ID NO:35; (142) an exosporium protein comprising an amino acid sequence having at least 85% identity with SEQ ID NO:36; (143) a targeting sequence comprising amino acids 1-29 of SEQ ID NO:43; (144) a targeting sequence comprising amino acids 14-29 of SEQ ID NO: 43; (145) a targeting sequence comprising SEQ ID NO: 43; (146) an exosporium protein comprising an amino acid sequence having at least 85% identity with SEQ ID NO: 44; (147) a targeting sequence comprising amino acids 2-29 of SEQ ID NO: 43; (148) a targeting sequence comprising amino acids 5-29 of SEQ ID NO: 43; (149) a targeting sequence comprising amino acids 8-29 of SEQ ID NO: 43; (150) a targeting sequence comprising amino acids 10-29 of SEQ ID NO: 43; (151) a targeting sequence comprising amino acids 1-35 of SEQ ID NO: 45; (152) a targeting sequence comprising amino acids 20-35 of SEQ ID NO: 45; (153) a targeting sequence comprising SEQ ID NO: 45; (154) an exosporium protein comprising an amino acid sequence having at least 85% identity with SEQ ID NO: 46; (155) a targeting sequence comprising amino acids 2-35 of SEQ ID NO: 45; (156) a targeting sequence comprising amino acids 5-35 of SEQ ID NO: 45; (157) a targeting sequence comprising amino acids 8-35 of SEQ ID NO: 45; (158) a targeting sequence comprising amino acids 10-35 of SEQ ID NO: 45; (159) a targeting sequence comprising amino acids 15-35 of SEQ ID NO: 45; (160) a targeting sequence comprising amino acids 1-43 of SEQ ID NO: 47; (161) a targeting sequence comprising amino acids 28-43 of SEQ ID NO: 47; (162) a targeting sequence comprising SEQ ID NO: 47; (163) an exosporium protein comprising an amino acid sequence having at least 85% identity with SEQ ID NO: 48; (164) a targeting sequence comprising amino acids 2-43 of SEQ ID NO: 47; (165) a targeting sequence comprising amino acids 5-43 of SEQ ID NO: 47; (166) a targeting sequence comprising amino acids 8-43 of SEQ ID NO: 47; (167) a targeting sequence comprising amino acids 10-43 of SEQ ID NO: 47; (168) a targeting sequence comprising amino acids 15-43 of SEQ ID NO: 47; (169) a targeting sequence comprising amino acids 20-43 of SEQ ID NO: 47; (170) a targeting sequence comprising amino acids 25-43 of SEQ ID NO: 47; (171) a targeting sequence comprising amino acids 1-32 of SEQ ID NO: 49; (172) a targeting sequence comprising amino acids 17-32 of SEQ ID NO: 49; (173) a targeting sequence comprising SEQ ID NO: 49; (174) an exosporium protein comprising an amino acid sequence having at least 85% identity with SEQ ID NO: 50; (175) a targeting sequence comprising amino acids 2-32 of SEQ ID NO: 49; (176) a targeting sequence comprising amino acids 5-32 of SEQ ID NO: 49; (177) a targeting sequence comprising amino acids 8-32 of SEQ ID NO: 49; (178) a targeting sequence comprising amino acids 10-32 of SEQ ID NO: 49; (179) a targeting sequence comprising amino acids 15-32 of SEQ ID NO: 49; (180) a targeting sequence comprising amino acids 1-33 of SEQ ID NO: 51; (181) a targeting sequence comprising amino acids 18-33 of SEQ ID NO: 51; (182) a targeting sequence comprising SEQ ID NO: 51; (183) an exosporium protein comprising an amino acid sequence

having at least 85% identity with SEQ ID NO: 52; (184) a targeting sequence comprising amino acids 2-33 of SEQ ID NO: 51; (185) a targeting sequence comprising amino acids 5-33 of SEQ ID NO: 51; (186) a targeting sequence comprising amino acids 8-33 of SEQ ID NO: 51; (187) a targeting sequence comprising amino acids 10-33 of SEQ ID NO: 51; (188) a targeting sequence comprising amino acids 15-33 of SEQ ID NO: 51; (189) a targeting sequence comprising amino acids 1-33 of SEQ ID NO: 53; (190) a targeting sequence comprising amino acids 18-33 of SEQ ID NO: 53; (191) a targeting sequence comprising SEQ ID NO: 53; (192) an exosporium protein comprising an amino acid sequence having at least 85% identity with SEQ ID NO: 54; (193) a targeting sequence comprising amino acids 2-33 of SEQ ID NO: 53; (194) a targeting sequence comprising amino acids 5-33 of SEQ ID NO: 53; (195) a targeting sequence comprising amino acids 8-33 of SEQ ID NO: 53; (196) a targeting sequence comprising amino acids 10-33 of SEQ ID NO: 53; (197) a targeting sequence comprising amino acids 15-33 of SEQ ID NO: 53; (198) a targeting sequence comprising amino acids 1-30 of SEQ ID NO: 55; (199) a targeting sequence comprising amino acids 15-30 of SEQ ID NO: 55; (200) a targeting sequence comprising SEQ ID NO: 55; (201) an exosporium protein comprising an amino acid sequence having at least 85% identity with SEQ ID NO: 56; (202) a targeting sequence comprising amino acids 2-30 of SEQ ID NO: 55; (203) a targeting sequence comprising amino acids 5-30 of SEQ ID NO: 55; (204) a targeting sequence comprising amino acids 8-30 of SEQ ID NO: 55; (205) a targeting sequence comprising amino acids 10-30 of SEQ ID NO: 55; (206) a targeting sequence comprising amino acids 1-130 of SEQ ID NO: 57; (207) a targeting sequence comprising amino acids 115-130 of SEQ ID NO: 57; (208) a targeting sequence comprising SEQ ID NO: 57; (209) an exosporium protein comprising an amino acid sequence having at least 85% identity with SEQ ID NO: 58; (210) a targeting sequence comprising amino acids 2-130 of SEQ ID NO: 57; (211) a targeting sequence comprising amino acids 5-130 of SEQ ID NO: 57; (212) a targeting sequence comprising amino acids 10-130 of SEQ ID NO: 57; (213) a targeting sequence comprising amino acids 20-130 of SEQ ID NO: 57; (214) a targeting sequence comprising amino acids 30-130 of SEQ ID NO: 57; (215) a targeting sequence comprising amino acids 40-130 of SEQ ID NO: 57; (216) a targeting sequence comprising amino acids 50-130 of SEQ ID NO: 57; (217) a targeting sequence comprising amino acids 60-130 of SEQ ID NO: 57; (218) a targeting sequence comprising amino acids 70-130 of SEQ ID NO: 57; (219) a targeting sequence comprising amino acids 80-130 of SEQ ID NO: 57; (220) a targeting sequence comprising amino acids 90-130 of SEQ ID NO: 57; (221) a targeting sequence comprising amino acids 100-130 of SEQ ID NO: 57; (222) a targeting sequence comprising amino acids 110-130 of SEQ ID NO: 57; (223) an exosporium protein fragment comprising an amino acid sequence having at least 85% identity with SEQ ID NO: 95; (224) a targeting sequence comprising SEQ ID NO: 96; (225) a targeting sequence comprising SEQ ID NO: 97; (226) a targeting sequence comprising SEQ ID NO: 98; (227) a targeting sequence comprising SEQ ID NO: 99; (228) a targeting sequence comprising SEQ ID NO: 100; (229) a targeting sequence comprising SEQ ID NO: 101; (230) a targeting sequence comprising SEQ ID NO: 102; (231) a targeting sequence comprising SEQ ID NO: 103; (232) a targeting sequence comprising SEQ ID NO: 104; (233) a targeting sequence comprising SEQ ID NO: 105; (234) a targeting sequence comprising SEQ ID NO: 106; (235) an exosporium protein comprising an amino acid sequence having at least 85% identity with SEQ ID NO: 108; (236) an exosporium protein comprising an amino acid sequence having at least 85% identity with SEQ ID NO: 109; (237) an exosporium protein comprising an amino acid sequence having at least 85% identity with SEQ ID NO: 110; (238) an exosporium protein comprising an amino acid sequence having at least 85% identity with SEQ ID NO: 111; (239) an exosporium protein comprising an amino acid sequence having at least 85% identity with SEQ ID NO: 112; (240) an exosporium protein comprising an amino acid sequence having at least 85% identity with SEQ ID NO: 113; (241) an exosporium protein comprising an amino acid sequence having at least 85% identity with SEQ ID NO: 114; (242) an exosporium protein comprising an amino acid sequence having at least 85% identity with SEQ ID NO: 115; (243) an exosporium protein comprising an amino acid sequence having at least 85% identity with SEQ ID NO: 116; (244) an exosporium protein comprising an amino acid sequence having at least 85% identity with SEQ ID NO: 117; (245) an exosporium protein comprising an amino acid sequence having at least 85% identity with SEQ ID NO: 118; (246) an exosporium protein comprising an amino acid sequence having at least 85% identity with SEQ ID NO: 119; (247) an exosporium protein comprising an amino acid sequence having at least 85% identity with SEQ ID NO: 120; (248) an exosporium protein comprising an amino acid sequence having at least 85% identity with SEQ ID NO: 121; (249) a targeting sequence comprising amino acids 22-31 of SEQ ID NO: 1; (250) a targeting sequence comprising amino acids 22-33 of SEQ ID NO: 1; (251) a targeting sequence comprising amino acids 20-31 of SEQ ID NO: 1; (252) a targeting sequence comprising amino acids 14-23 of SEQ ID NO: 3; (253) a targeting sequence comprising amino acids 14-25 of SEQ ID NO: 3; (254) a targeting sequence comprising amino acids 12-23 of SEQ ID NO: 3; (255) a targeting sequence comprising amino acids 1-30 of SEQ ID NO: 59; (256) a targeting sequence comprising SEQ ID NO: 59; (257) an exosporium protein comprising an amino acid sequence having at least 85% identity with SEQ ID NO: 60; (258) a targeting sequence comprising amino acids 2-30 of SEQ ID NO: 59; (259) a targeting sequence comprising amino acids 4-30 of SEQ ID NO: 59; (260) a targeting sequence comprising amino acids 6-30 of SEQ ID NO: 59; (261) a targeting sequence comprising amino acids 1-33 of SEQ ID NO: 61; (262) a

targeting sequence comprising amino acids 18-33 of SEQ ID NO: 61; (263) a targeting sequence comprising SEQ ID NO: 61; (264) an exosporium protein comprising an amino acid sequence having at least 85% sequence identity with SEQ ID NO: 62; (265) a targeting sequence comprising amino acids 2-33 of SEQ ID NO: 61; (266) a targeting sequence comprising amino acids 5-33 of SEQ ID NO: 61; (267) a targeting sequence comprising amino acids 10-33 of SEQ ID NO: 61; (268) a targeting sequence comprising amino acids 15-33 of SEQ ID NO: 61; (269) a targeting sequence comprising amino acids 1-35 of SEQ ID NO: 63; (270) a targeting sequence comprising SEQ ID NO: 63; (271) an exosporium protein comprising an amino acid sequence having at least 85% identity with SEQ ID NO: 64; (272) a targeting sequence comprising amino acids 2-35 of SEQ ID NO: 63; (273) a targeting sequence comprising amino acids 5-35 of SEQ ID NO: 63; (274) a targeting sequence comprising amino acids 8-35 of SEQ ID NO: 63; (275) a targeting sequence comprising amino acids 10-35 of SEQ ID NO: 63; (276) a targeting sequence comprising amino acids 15-35 of SEQ ID NO: 63; (277) a targeting sequence comprising amino acids 1-24 of SEQ ID NO: 65; (278) a targeting sequence comprising amino acids 9-24 of SEQ ID NO: 65; (279) a targeting sequence comprising SEQ ID NO: 65; (280) an exosporium protein comprising an amino acid sequence having at least 85% identity with SEQ ID NO: 66; (281) a targeting sequence comprising SEQ ID NO: 107; (282) a targeting sequence comprising amino acids 2-24 of SEQ ID NO: 65; (283) a targeting sequence comprising amino acids 5-24 of SEQ ID NO: 65; (284) a targeting sequence comprising amino acids 1-27 of SEQ ID NO: 67; (285) a targeting sequence comprising amino acids 12-27 of SEQ ID NO: 67; (286) a targeting sequence comprising SEQ ID NO: 67; (287) an exosporium protein comprising an amino acid sequence having at least 85% identity with SEQ ID NO: 68; (288) an targeting sequence comprising amino acids 2-27 of SEQ ID NO: 67; (289) a targeting sequence comprising amino acids 5-27 of SEQ ID NO: 67; (290) a targeting sequence comprising amino acids 10-27 of SEQ ID NO: 67; (291) a targeting sequence comprising amino acids 1-38 of SEQ ID NO: 69; (292) a targeting sequence comprising amino acids 23-38 of SEQ ID NO: 69; (293) a targeting sequence comprising SEQ ID NO: 69; (294) an exosporium protein comprising an amino acid sequence having at least 85% identity with SEQ ID NO: 70; (295) a targeting sequence comprising amino acids 2-38 of SEQ ID NO: 69; (296) a targeting sequence comprising amino acids 5-38 of SEQ ID NO: 69; (297) a targeting sequence comprising amino acids 10-38 of SEQ ID NO: 69; (298) a targeting sequence comprising amino acids 15-38 of SEQ ID NO: 69; (299) an exosporium protein comprising SEQ ID NO: 72; (300) a targeting sequence comprising SEQ ID NO: 73; (301) an exosporium protein comprising an amino acid sequence having at least 95% identity with SEQ ID NO: 74; (302) a targeting sequence comprising amino acids 1-42 of SEQ ID NO: 75; (303) a targeting sequence comprising amino acids 27-42 of SEQ ID NO: 75; (304) a targeting sequence comprising SEQ ID NO: 75; (305) an exosporium protein comprising an amino acid sequence having at least 85% identity with SEQ ID NO: 76; (306) a targeting sequence comprising amino acids 2-42 of SEQ ID NO: 75; (307) a targeting sequence comprising amino acids 5-42 of SEQ ID NO: 75; (308) a targeting sequence comprising amino acids 10-42 of SEQ ID NO: 75; (309) a targeting sequence comprising amino acids 15-42 of SEQ ID NO: 75; (310) a targeting sequence comprising amino acids 20-42 of SEQ ID NO: 75; (311) a targeting sequence comprising amino acids 25-42 of SEQ ID NO: 75; (312) a targeting sequence comprising amino acids 1-24 of SEQ ID NO: 77; (313) a targeting sequence comprising amino acids 9-24 of SEQ ID NO: 77; (314) a targeting sequence comprising SEQ ID NO: 77; (315) an exosporium protein comprising an amino acid sequence having at least 85% identity with SEQ ID NO: 78; (316) a targeting sequence comprising amino acids 2-24 of SEQ ID NO: 77; (317) a targeting sequence comprising amino acids 5-24 of SEQ ID NO: 77; (318) an exosporium protein comprising an amino acid sequence having at least 85% identity with SEQ ID NO: 80; (319) a targeting sequence comprising amino acids 1-38 of SEQ ID NO: 81; (320) a targeting sequence comprising amino acids 23-38 of SEQ ID NO: 81; (321) a targeting sequence comprising SEQ ID NO: 81; (322) an exosporium protein comprising an amino acid sequence having at least 85% identity with SEQ ID NO: 82; (323) a targeting sequence comprising amino acids 2-38 of SEQ ID NO: 81; (324) a targeting sequence comprising amino acids 5-38 of SEQ ID NO: 81; (325) a targeting sequence comprising amino acids 10-38 of SEQ ID NO: 81; (326) a targeting sequence comprising amino acids 15-38 of SEQ ID NO: 81; (327) a targeting sequence comprising amino acids 20-38 of SEQ ID NO: 81; (328) a targeting sequence comprising amino acids 1-34 of SEQ ID NO: 83; (329) a targeting sequence comprising SEQ ID NO: 83; (330) an exosporium protein comprising an amino acid sequence having at least 85% identity with SEQ ID NO: 84; (331) an exosporium protein comprising an amino acid sequence having at least 85% identity with SEQ ID NO: 86; (332) a targeting sequence comprising amino acids 1-28 of SEQ ID NO: 87; (333) a targeting sequence comprising amino acids 13-28 of SEQ ID NO: 87; (334) a targeting sequence comprising SEQ ID NO: 87; (335) an exosporium protein comprising an amino acid sequence having at least 85% identity with SEQ ID NO: 88; (336) a targeting sequence comprising amino acids 2-28 of SEQ ID NO: 87; (337) a targeting sequence comprising amino acids 5-28 of SEQ ID NO: 87; (338) a targeting sequence comprising amino acids 10-28 of SEQ ID NO: 87; (339) a targeting sequence comprising amino acids 1-28 of SEQ ID NO: 89; (340) a targeting sequence comprising SEQ ID NO: 89; (341) an exosporium protein comprising an amino acid sequence having at least 85% identity with SEQ ID NO: 90; (342) a targeting sequence comprising amino acids 2-28 of SEQ ID NO: 89; (343) a targeting sequence comprising amino acids 5-28 of SEQ ID NO: 89; (344) a targeting sequence comprising amino acids 10-28 of SEQ

ID NO: 89; (345) a targeting sequence comprising amino acids 1-93 of SEQ ID NO: 91; (346) a targeting sequence comprising SEQ ID NO: 91; (347) an exosporium protein comprising an amino acid sequence having at least 85% identity with SEQ ID NO: 92; (348) a targeting sequence comprising amino acids 2-93 of SEQ ID NO: 91; (349) a targeting sequence comprising amino acids 10-93 of SEQ ID NO: 91; (350) a targeting sequence comprising amino acids 20-93 of SEQ ID NO: 91; (351) a targeting sequence comprising amino acids 30-93 of SEQ ID NO: 91; (352) a targeting sequence comprising amino acids 40-93 of SEQ ID NO: 91; (353) a targeting sequence comprising amino acids 50-93 of SEQ ID NO: 91; (354) a targeting sequence comprising amino acids 60-93 of SEQ ID NO: 91; (355) a targeting sequence comprising amino acids 1-130 of SEQ ID NO: 93; (356) a targeting sequence comprising SEQ ID NO: 93; (357) an exosporium protein comprising an amino acid sequence having at least 85% identity with SEQ ID NO: 94; (358) a targeting sequence comprising amino acids 2-130 of SEQ ID NO: 93; (359) a targeting sequence comprising amino acids 10-130 of SEQ ID NO: 93; (360) a targeting sequence comprising amino acids 20-130 of SEQ ID NO: 93; (361) a targeting sequence comprising amino acids 30-130 of SEQ ID NO: 93; (362) an exosporium protein comprising an amino acid sequence having at least 85% identity with SEQ ID NO: 122; (363) a targeting sequence consisting of amino acids 20-33 of SEQ ID NO: 1; (364) a targeting sequence consisting of amino acids 21-33 of SEQ ID NO: 1; (365) a targeting sequence consisting of amino acids 23-31 of SEQ ID NO: 1; (366) a targeting sequence consisting of amino acids 1-15 of SEQ ID NO: 96; (367) a targeting sequence consisting of amino acids 1-13 of SEQ ID NO: 96; (368) a targeting sequence consisting of amino acids 12-25 of SEQ ID NO: 3; (369) a targeting sequence consisting of amino acids 13-25 of SEQ ID NO: 3; (370) a targeting sequence consisting of amino acids 15-23 of SEQ ID NO: 3; (371) a targeting sequence consisting of amino acids 1-15 of SEQ ID NO: 97; (372) a targeting sequence consisting of amino acids 1-13 of SEQ ID NO: 98; (373) a targeting sequence consisting of amino acids 23-36 of SEQ ID NO: 5; (374) a targeting sequence consisting of amino acids 23-34 of SEQ ID NO: 5; (375) a targeting sequence consisting of amino acids 24-36 of SEQ ID NO: 5; (376) a targeting sequence consisting of amino acids 26-34 of SEQ ID NO: 5; (377) a targeting sequence consisting of amino acids 13-26 of SEQ ID NO: 7; (378) a targeting sequence consisting of amino acids 13-24 of SEQ ID NO: 7; (379) a targeting sequence consisting of amino acids 14-26 of SEQ ID NO: 7; (380) a targeting sequence consisting of amino acids 16-24 of SEQ ID NO: 7; (381) a targeting sequence consisting of amino acids 9-22 of SEQ ID NO: 9; (382) a targeting sequence consisting of amino acids 9-20 of SEQ ID NO: 9; (383) a targeting sequence consisting of amino acids 10-22 of SEQ ID NO: 9; (384) a targeting sequence consisting of amino acids 12-20 of SEQ ID NO: 9; (385) a targeting sequence consisting of amino acids 1-15 of SEQ ID NO: 105; (386) a targeting sequence consisting of amino acids 1-13 of SEQ ID NO: 105; (387) a targeting sequence consisting of amino acids 18-31 of SEQ ID NO: 11; (388) a targeting sequence consisting of amino acids 18-29 of SEQ ID NO: 11; (389) a targeting sequence consisting of amino acids 19-31 of SEQ ID NO: 11; (390) a targeting sequence consisting of amino acids 1-15 of SEQ ID NO: 98; (391) a targeting sequence consisting of amino acids 1-13 of SEQ ID NO: 98; (392) a targeting sequence consisting of amino acids 18-31 of SEQ ID NO: 13; (393) a targeting sequence consisting of amino acids 18-29 of SEQ ID NO: 13; (394) a targeting sequence consisting of amino acids 19-31 of SEQ ID NO: 13; (395) a targeting sequence consisting of amino acids 21-29 of SEQ ID NO: 13; (396) a targeting sequence consisting of amino acids 1-15 of SEQ ID NO: 99; (397) a targeting sequence consisting of amino acids 1-13 of SEQ ID NO: 99; (398) a targeting sequence consisting of amino acids 28-41 of SEQ ID NO: 15; (399) a targeting sequence consisting of amino acids 28-39 of SEQ ID NO: 15; (400) a targeting sequence consisting of amino acids 29-41 of SEQ ID NO: 15; (401) a targeting sequence consisting of amino acids 31-39 of SEQ ID NO: 15; (402) a targeting sequence consisting of amino acids 12-25 of SEQ ID NO: 17; (403) a targeting sequence consisting of amino acids 13-25 of SEQ ID NO: 17; (404) a targeting sequence consisting of amino acids 1-15 of SEQ ID NO: 100; (405) a targeting sequence consisting of amino acids 18-31 of SEQ ID NO: 19; (406) a targeting sequence consisting of amino acids 18-29 of SEQ ID NO: 19; (407) a targeting sequence consisting of amino acids 19-31 of SEQ ID NO: 19; (408) a targeting sequence consisting of amino acids 21-29 of SEQ ID NO: 19; (409) a targeting sequence consisting of amino acids 18-31 of SEQ ID NO: 21; (410) a targeting sequence consisting of amino acids 18-29 of SEQ ID NO: 21; (411) a targeting sequence consisting of amino acids 19-31 of SEQ ID NO: 21; (412) a targeting sequence consisting of amino acids 21-29 of SEQ ID NO: 21; (413) a targeting sequence consisting of amino acids 1-15 of SEQ ID NO: 101; (414) a targeting sequence consisting of amino acids 1-13 of SEQ ID NO: 101; (415) a targeting sequence consisting of amino acids 9-22 of SEQ ID NO: 23; (416) a targeting sequence consisting of amino acids 9-20 of SEQ ID NO: 23; (417) a targeting sequence consisting of amino acids 10-22 of SEQ ID NO: 23; (418) a targeting sequence consisting of amino acids 12-20 of SEQ ID NO: 23; (419) a targeting sequence consisting of amino acids 1-15 of SEQ ID NO: 102; (420) a targeting sequence consisting of amino acids 1-13 of SEQ ID NO: 102; (421) a targeting sequence consisting of amino acids 9-22 of SEQ ID NO: 25; (422) a targeting sequence consisting of amino acids 9-20 of SEQ ID NO: 25; (423) a targeting sequence consisting of amino acids 10-22 of SEQ ID NO: 25; (424) a targeting sequence consisting of amino acids 12-20 of SEQ ID NO: 25; (425) a targeting sequence consisting of amino acids 1-15 of SEQ ID NO: 103; (426) a targeting sequence consisting of amino acids 1-13 of SEQ ID NO: 103; (427) a targeting sequence consisting of

amino acids 15-28 of SEQ ID NO: 27; (428) a targeting sequence consisting of amino acids 15-26 of SEQ ID NO: 27; (429) a targeting sequence consisting of amino acids 16-28 of SEQ ID NO: 27; (430) a targeting sequence consisting of amino acids 18-26 of SEQ ID NO: 27; (431) a targeting sequence consisting of amino acids 1-15 of SEQ ID NO: 104; (432) a targeting sequence consisting of amino acids 1-13 of SEQ ID NO: 104; (433) a targeting sequence consisting of amino acids 1-13 of SEQ ID NO: 33; (434) a targeting sequence consisting of amino acids 1-11 of SEQ ID NO: 33; (435) a targeting sequence consisting of amino acids 3-11 of SEQ ID NO: 33; (436) a targeting sequence consisting of amino acids 1-14 of SEQ ID NO: 35; (437) a targeting sequence consisting of amino acids 1-12 of SEQ ID NO: 35; (438) a targeting sequence consisting of amino acids 2-14 of SEQ ID NO: 35; (439) a targeting sequence consisting of amino acids 14-27 of SEQ ID NO: 43; (440) a targeting sequence consisting of amino acids 14-25 of SEQ ID NO: 43; (441) a targeting sequence consisting of amino acids 15-27 of SEQ ID NO: 43; (442) a targeting sequence consisting of amino acids 20-33 of SEQ ID NO: 45; (443) a targeting sequence consisting of amino acids 20-31 of SEQ ID NO: 45; (444) a targeting sequence consisting of amino acids 21-33 of SEQ ID NO: 45; (445) a targeting sequence consisting of amino acids 1-15 of SEQ ID NO: 106; (446) a targeting sequence consisting of amino acids 1-13 of SEQ ID NO: 106; (447) a targeting sequence consisting of amino acids 28-41 of SEQ ID NO: 47; (448) a targeting sequence consisting of amino acids 28-39 of SEQ ID NO: 47; (449) a targeting sequence consisting of amino acids 18-31 of SEQ ID NO: 53; (450) a targeting sequence consisting of amino acids 18-29 of SEQ ID NO: 53; (451) a targeting sequence consisting of amino acids 19-31 of SEQ ID NO: 53; (452) a targeting sequence comprising amino acids 18-31 of SEQ ID NO: 61; (453) a targeting sequence comprising amino acids 18-29 of SEQ ID NO: 61; (454) a targeting sequence comprising amino acids 19-31 of SEQ ID NO: 61; (455) a targeting sequence comprising amino acids 9-22 of SEQ ID NO: 65; (456) a targeting sequence comprising amino acids 9-20 of SEQ ID NO: 65; (457) a targeting sequence comprising amino acids 10-22 of SEQ ID NO: 65; (458) a targeting sequence comprising amino acids 1-15 of SEQ ID NO: 107; (459) a targeting sequence comprising amino acids 1-13 of SEQ ID NO: 107; (460) a targeting sequence comprising amino acids 12-25 of SEQ ID NO: 67; (461) a targeting sequence comprising amino acids 12-23 of SEQ ID NO: 67; (462) a targeting sequence comprising amino acids 13-25 of SEQ ID NO: 67; (463) a targeting sequence comprising amino acids 15-23 of SEQ ID NO: 67; (464) a targeting sequence comprising amino acids 23-36 of SEQ ID NO: 69; (465) a targeting sequence comprising amino acids 23-34 of SEQ ID NO: 69; (466) a targeting sequence comprising amino acids 24-36 of SEQ ID NO: 69; (467) a targeting sequence comprising amino acids 26-34 of SEQ ID NO: 69; (468) a targeting sequence comprising amino acids 27-40 of SEQ ID NO: 75; (469) a targeting sequence comprising amino acids 27-38 of SEQ ID NO: 75; (470) a targeting sequence comprising amino acids 9-22 of SEQ ID NO: 77; (471) a targeting sequence comprising amino acids 9-20 of SEQ ID NO: 77; (472) a targeting sequence comprising amino acids 10-22 of SEQ ID NO: 77; (473) a targeting sequence comprising amino acids 12-20 of SEQ ID NO: 77; (474) a targeting sequence comprising amino acids 23-36 of SEQ ID NO: 81; (475) a targeting sequence comprising amino acids 23-34 of SEQ ID NO: 81; (476) a targeting sequence comprising amino acids 24-36 of SEQ ID NO: 81; (477) a targeting sequence comprising amino acids 26-34 of SEQ ID NO: 81; (478) a targeting sequence comprising amino acids 13-26 of SEQ ID NO: 87; (479) a targeting sequence comprising amino acids 13-24 of SEQ ID NO: 87; or (480) a targeting sequence comprising amino acids 14-26 of SEQ ID NO: 87.

(391) For example, the targeting sequence can comprise an amino acid sequence having at least about 50% identity with amino acids 20-35 of SEQ ID NO: 1, wherein the identity with amino acids 25-35 is at least about 63%.

(392) For example, the targeting sequence can comprise an amino acid sequence having at least about 56% identity with amino acids 20-35 of SEQ ID NO: 1, wherein the identity with amino acids 25-35 is at least about 63%.

(393) For example, the targeting sequence can comprise an amino acid sequence having at least about 50% identity with amino acids 20-35 of SEQ ID NO: 1, wherein the identity with amino acids 25-35 is at least about 72%.

(394) For example, the targeting sequence can comprise an amino acid sequence having at least about 62% identity with amino acids 20-35 of SEQ ID NO: 1, wherein the identity with amino acids 25-35 is at least about 72%.

(395) For example, the targeting sequence can comprise an amino acid sequence having at least about 75% identity with amino acids 20-35 of SEQ ID NO: 1, wherein the identity with amino acids 25-35 is at least about 72%.

(396) For example, the targeting sequence can comprise an amino acid sequence having at least about 68% identity with amino acids 20-35 of SEQ ID NO: 1, wherein the identity with amino acids 25-35 is at least about 81%.

(397) For example, the targeting sequence can comprise an amino acid sequence having at least about 75% identity with amino acids 20-35 of SEQ ID NO: 1, wherein the identity with amino acids 25-35 is at least about 81%.

(398) For example, the targeting sequence can comprise an amino acid sequence having at least about 81% identity with amino acids 20-35 of SEQ ID NO: 1, wherein the identity with amino acids 25-35 is at least about 81%.

(399) For example, the targeting sequence can comprise an amino acid sequence having at least about 81% identity with amino acids 20-35 of SEQ ID NO: 1, wherein the identity with amino acids 25-35 is at least about 90%.

(400) For example, the targeting sequence can consist of: (a) an amino acid sequence consisting of 16 amino acids and having at least about 43% identity with amino acids 20-35 of SEQ ID NO: 1, wherein the identity with amino acids 25-35 is at least about 54%; (b) amino acids 1-35 of SEQ ID NO: 1; (c) amino acids 20-35 of SEQ ID NO: 1;

(d) SEQ ID NO: 1; (e) SEQ ID NO: 96; or (f) SEQ ID NO: 120.
(401) The targeting sequence can consist of the amino acid sequence as described in these examples.
(402) The fusion protein can comprise an exosporium protein or an exosporium protein fragment comprising an amino acid sequence having at least 90% identity with SEQ ID NO: 2, 4, 6, 8, 10, 12, 14, 16, 18, 20, 22, 24, 26, 28, 30, 32, 34, 36, 44, 46, 48, 50, 52, 54, 56, 58, 60, 62, 64, 66, 68, 70, 72, 74, 76, 78, 80, 82, 84, 86, 88, 90, 92, 94, 95, 108, 109, 110, 111, 112, 113, 114, 115, 116, 117, 118, 119, 120, 121, and 122.
(403) The fusion protein can comprise an exosporium protein or an exosporium protein fragment comprising an amino acid sequence having at least 95% identity with SEQ ID NO: 2, 4, 6, 8, 10, 12, 14, 16, 18, 20, 22, 24, 26, 28, 30, 32, 34, 36, 44, 46, 48, 50, 52, 54, 56, 58, 60, 62, 64, 66, 68, 70, 72, 74, 76, 78, 80, 82, 84, 86, 88, 90, 92, 94, 95, 108, 109, 110, 111, 112, 113, 114, 115, 116, 117, 118, 119, 120, 121, and 122.
(404) The fusion protein can comprise an exosporium protein or an exosporium protein fragment comprising an amino acid sequence having at least 98% identity with SEQ ID NO: 2, 4, 6, 8, 10, 12, 14, 16, 18, 20, 22, 24, 26, 28, 30, 32, 34, 36, 44, 46, 48, 50, 52, 54, 56, 58, 60, 62, 64, 66, 68, 70, 72, 74, 76, 78, 80, 82, 84, 86, 88, 90, 92, 94, 95, 108, 109, 110, 111, 112, 113, 114, 115, 116, 117, 118, 119, 120, 121, and 122.
(405) The fusion protein can comprise an exosporium protein or an exosporium protein fragment comprising an amino acid sequence having at least 99% identity with SEQ ID NO: 2, 4, 6, 8, 10, 12, 14, 16, 18, 20, 22, 24, 26, 28, 30, 32, 34, 36, 44, 46, 48, 50, 52, 54, 56, 58, 60, 62, 64, 66, 68, 70, 72, 74, 76, 78, 80, 82, 84, 86, 88, 90, 92, 94, 95, 108, 109, 110, 111, 112, 113, 114, 115, 116, 117, 118, 119, 120, 121, and 122.
(406) The fusion protein can comprise an exosporium protein or an exosporium protein fragment comprising an amino acid sequence having 100% identity with SEQ ID NO: 2, 4, 6, 8, 10, 12, 14, 16, 18, 20, 22, 24, 26, 28, 30, 32, 34, 36, 44, 46, 48, 50, 52, 54, 56, 58, 60, 62, 64, 66, 68, 70, 72, 74, 76, 78, 80, 82, 84, 86, 88, 90, 92, 94, 95, 108, 109, 110, 111, 112, 113, 114, 115, 116, 117, 118, 119, 120, 121, and 122.
(407) The fusion protein can comprise an exosporium protein comprising an amino acid sequence having at least 90% identity with SEQ ID NO: 60, 62, 64, 66, 68, 70, 76, 78, 80, 82, 84, 86, 88, 90, 92, 94 or 122.
(408) The fusion protein can comprise an exosporium protein comprising an amino acid sequence having at least 95% identity with SEQ ID NO: 60, 62, 64, 66, 68, 70, 76, 78, 80, 82, 84, 86, 88, 90, 92, 94 or 122.
(409) The fusion protein can comprise an exosporium protein comprising an amino acid sequence having at least 98% identity with SEQ ID NO: 60, 62, 64, 66, 68, 70, 76, 78, 80, 82, 84, 86, 88, 90, 92, 94 or 122.
(410) The fusion protein can comprise an exosporium protein comprising an amino acid sequence having at least 99% identity with SEQ ID NO: 60, 62, 64, 66, 68, 70, 76, 78, 80, 82, 84, 86, 88, 90, 92, 94 or 122.
(411) The fusion protein can comprise an exosporium protein comprising an amino acid sequence having 100% identity with SEQ ID NO: 60, 62, 64, 66, 68, 70, 76, 78, 80, 82, 84, 86, 88, 90, 92, 94 or 122.
(412) The targeting sequence, exosporium protein, or exosporium protein fragment of the fusion protein can comprise the amino acid sequence GXT at its carboxy terminus, wherein X is any amino acid.
(413) The targeting sequence, exosporium protein, or exosporium protein fragment can comprise an alanine residue at the position of the targeting sequence that corresponds to amino acid 20 of SEQ ID NO: 1.
(414) The targeting sequence, exosporium protein, or exosporium protein fragment can further comprise a methionine, serine, or threonine residue at the amino acid position immediately preceding the first amino acid of the targeting sequence, exosporium protein, or exosporium protein fragment or at the position of the targeting sequence that corresponds to amino acid 20 of SEQ ID NO: 1.
IX. Fusion Proteins Comprising a Protein or Peptide of Interest and a Spore Coat Protein, Recombinant Spore-Coat Forming Bacteria, and Seeds Coated with Recombinant Spore-Coat Forming Bacteria
(415) A. Spore Coat Proteins that can be Used to Target a Fusion Protein Comprising the Spore Coat Protein to a Surface of a Spore of a Recombinant Spore-Forming Bacterium
(416) A number of spore coat proteins can be used to display proteins or peptides of interest on a surface of a spore of a recombinant spore-forming bacterium. Such bacteria include any spore-forming bacteria, and in particular include spore-forming bacteria of the genuses *Bacillus, Lysinibacillus, Virginibacillus, Clostridia,* and *Paenibacillus*. Spore-forming bacteria of the genus *Bacillus* include *Bacillus cereus* family members as well as other *Bacillus* species that are not *Bacillus cereus* family members (e.g., *Bacillus* species bacteria that lack an exosporium). These spore coat proteins include CotB, CotC, CgeA, CotB/H, CotG, spore coat protein X, and CotY. For case of reference, the descriptions of the amino acid sequences for exemplary spore coat proteins that can be used for targeting of proteins or peptides of interest to a spore surface of a recombinant spore-forming bacterium are provided in Table 7 below, together with their SEQ ID NOs.
(417) TABLE-US-00007 TABLE 7 Spore coat protein sequences used for targeting of proteins and peptides of interest to a spore surface of a recombinant spore-forming bacterium Spore coat protein SEQ ID NO. CotB (*Bacillus subtilis*) 252 CotC (*Bacillus subtilis*) 253 CgeA (*Bacillus amyloliquefaciens*) 254 CotB/H (*Bacillus amyloliquefaciens*) 255 CotG (*Bacillus subtilis*) 256 Spore Coat Protein X (*Bacillus megaterium*) 257 CotY (*Bacillus amyloliquefaciens*) 258 CotY (*Bacillus licheniformis*) 259

B. Fusion Proteins Comprising a Protein or Peptide of Interest and a Spore Coat Protein

(418) The present invention also relates to fusion proteins comprising at least one protein or peptide of interest and a spore coat protein, wherein the spore coat protein comprises a CotB/H protein, a spore protein X protein, or a CotY protein, wherein the CotY protein comprises an amino acid sequence having at least 80% identity with SEQ ID NO: 258 or 259.

(419) For example, the spore coat protein can comprise a CotB/H protein.

(420) For example, the spore coat protein can comprise a spore protein X protein.

(421) For example, the spore coat protein can comprise a CotY protein, wherein the CotY protein comprises an amino acid sequence having at least 80% identity with SEQ ID NO: 258 or 259.

(422) The spore coat protein can comprises an amino acid sequence having at least 85% identity with SEQ ID NO: 255, 257, 258, or 259.

(423) The spore coat protein can comprises an amino acid sequence having at least 90% identity with SEQ ID NO: 255, 257, 258, or 259.

(424) The spore coat protein can comprises an amino acid sequence having at least 95% identity with SEQ ID NO: 255, 257, 258, or 259.

(425) The spore coat protein can comprises an amino acid sequence having at least 98% identity with SEQ ID NO: 255, 257, 258, or 259.

(426) The spore coat protein can comprises an amino acid sequence having at least 99% identity with SEQ ID NO: 255, 257, 258, or 259.

(427) The spore coat protein can comprises an amino acid sequence having at least 100% identity with SEQ ID NO: 255, 257, 258, or 259.

(428) C. Recombinant Spore-Coat Forming Bacteria that Express Fusion Proteins and Plant Seeds Coated with Recombinant Spore-Coat Forming Bacteria

(429) Recombinant spore-forming bacteria that expresses any of the fusion proteins described in Section IX.B are provided. The recombinant spore-forming bacteria can comprise an endophytic strain of bacteria, a plant growth-promoting strain of bacteria, or a strain of bacteria that is both endophytic and plant growth-promoting.

(430) The present invention further relates to a recombinant spore-forming bacterium that expresses a fusion protein comprising at least one protein or peptide of interest and a spore coat protein that targets the fusion protein to the surface of a spore of the bacterium, wherein the spore coat protein comprises a CotB protein, a CotC protein, a CgeA protein, a CotB/H protein, a CotG protein, a spore coat protein X protein, or a CotY protein; and wherein the recombinant spore-forming bacterium comprises an endophytic strain of bacteria, a plant growth-promoting strain of bacteria, or a strain of bacteria that is both endophytic and plant growth-promoting.

(431) Expression of the fusion protein in an endophytic strain of bacteria allows for delivery of the protein or peptide of interest internally to a plant. The endophytic strains can be delivered to plants using various methods, e.g., the endophytic strains can be delivered via seed treatment, treatment of the plant growth medium (e.g., soil), irrigation, application to the plant itself (e.g., foliar application to the aerial portions of a plant). Once inside the plant, the bacteria multiply and colonize the internal tissues of the plant.

(432) The present invention also relates to plant seeds coated with a recombinant spore-forming bacterium, wherein the recombinant spore-forming bacterium expresses a fusion protein comprising at least one protein or peptide of interest and a spore coat protein that targets the fusion protein to the surface of a spore of the bacterium, wherein the spore coat protein comprises a cotB protein, a CotC protein, a CgeA protein, a CotB/H protein, a Cot G protein, a spore protein X protein, or a cotY protein.

(433) The recombinant spore-coat forming bacterium can comprise a bacterium of the genus *Bacillus* or *Lysinibacillus.*

(434) The present invention further relates to a recombinant bacterium of the genus *Bacillus*, wherein the recombinant bacterium comprises a recombinant spore-forming bacterium and wherein the recombinant spore-forming bacterium expresses a fusion protein comprising at least one protein or peptide of interest and a spore coat protein that targets the fusion protein to the surface of a spore of the bacterium, wherein the spore coat protein comprises a CotB protein, a CotC protein, a CgeA protein, a CotB/H protein, a Cot G protein, a spore coat protein X protein, or a CotY protein. The recombinant spore-coat forming bacterium expresses a protease or a nuclease, wherein the expression of the protease or nuclease is increased as compared to the expression of the protease or the nuclease in a wild-type bacterium of the genus *Bacillus* under the same conditions, and wherein the increased expression of the protease or the nuclease partially or completely inactivates spores of the recombinant bacterium of the genus *Bacillus* or renders spores of the recombinant bacterium of the genus *Bacillus* more susceptible to physical or chemical inactivation. The protease or nuclease can be any of the proteases or nucleases described above in Section V.A, and can be expressed under the control of any of the promoters described above in Section V.A. The invention further relates to plant seeds coated with such spore-forming bacteria. The recombinant bacterium can comprise an endophytic strain of bacteria, a plant growth-promoting strain of bacteria, or a strain of bacteria that is

both endophytic and plant growth-promoting.
(435) In any of the plant seeds described in this Section, the recombinant spore-forming bacterium can comprise an endophytic strain of bacteria, a plant growth-promoting strain of bacteria, or a strain of bacteria that is both endophytic and plant growth-promoting.
(436) In any of the recombinant spore-forming bacteria or seeds, the endophytic strain of bacteria, the plant growth-promoting strain of bacteria, or the strain of bacteria that is both endophytic and plant growth-promoting can comprise *Bacillus megaterium* EE385, *Bacillus* sp. EE387, *Bacillus circulans* EE388, *Bacillus subtilis* EE405, *Lysinibacillus fusiformis* EE442, or *Lysinibacillus sphaericus* EE443, *Bacillus pumilus* EE-B00143, *Bacillus subtilis* EE148, *Bacillus subtilis* EE218, or *Bacillus megaterium* EE281. For example, the endophytic strain of bacteria can comprise *Bacillus subtilis* EE405 or *Bacillus megaterium* EE385.
(437) Alternatively, the endophytic strain, the plant growth-promoting strain of bacteria, or the strain of bacteria that is both endophytic and plant growth-promoting of bacteria can comprise *Bacillus cereus* family member EE349, *Bacillus cereus* family member EE439, *Bacillus thuringiensis* EE417, *Bacillus cereus* EE444, or *Bacillus thuringiensis* EE319, *Bacillus thuringiensis* EE-B00184, *Bacillus cereus* family member EE-B00377, *Bacillus pseudomycoides* EE-B00366, *Bacillus mycoides* EE-B00363, *Bacillus mycoides* BT155, *Bacillus mycoides* EE118, *Bacillus mycoides* EE141, *Bacillus mycoides* BT46-3, *Bacillus cereus* family member EE128, *Bacillus thuringiensis* BT013A, or *Bacillus cereus* family member EE349.
(438) In any of the recombinant spore-forming bacteria or seeds, the spore coat protein can comprise an amino acid sequence having at least 85% identity with any of SEQ ID NOs: 252-259.
(439) The spore coat protein can comprise an amino acid sequence having at least 90% identity with any of SEQ ID NOs: 252-259.
(440) The spore coat protein can comprise an amino acid sequence having at least 95% identity with any of SEQ ID NOs: 252-259.
(441) The spore coat protein can comprise an amino acid sequence having at least 98% identity with any of SEQ ID NOs: 252-259.
(442) The spore coat protein can comprise an amino acid sequence having at least 99% identity with any of SEQ ID NOs: 252-259.
(443) The spore coat protein can comprise an amino acid sequence having 100% identity with any of SEQ ID NOs: 252-259.
(444) A recombinant spore-forming bacterium that expresses a fusion protein comprising at least one protein or peptide of interest and a protein that targets the fusion protein to the surface of a spore of the bacterium is also provided. The recombinant spore-forming bacterium is not a recombinant *Bacillus cereus* family member. The protein that targets the fusion protein to the surface of a spore of the bacterium comprises amino acids 20-35 of SEQ ID NO: 1, SEQ ID NO: 96, or an amino acid sequence having at least 85% identity with SEQ ID NO: 108, SEQ ID NO: 111, SEQ ID NO: 114, SEQ ID NO: 120, or SEQ ID NO: 121.
(445) The protein that targets the fusion protein of the surface of a spore of the bacterium can comprise an amino acid sequence having at least 90% identity with SEQ ID NO: 108, SEQ ID NO: 111, SEQ ID NO: 114, SEQ ID NO: 120, or SEQ ID NO: 121.
(446) The protein that targets the fusion protein of the surface of a spore of the bacterium can comprise an amino acid sequence having at least 95% identity with SEQ ID NO: 108, SEQ ID NO: 111, SEQ ID NO: 114, SEQ ID NO: 120, or SEQ ID NO: 121.
(447) The protein that targets the fusion protein of the surface of a spore of the bacterium can comprise an amino acid sequence having at least 98% identity with SEQ ID NO: 108, SEQ ID NO: 111, SEQ ID NO: 114, SEQ ID NO: 120, or SEQ ID NO: 121.
(448) The protein that targets the fusion protein of the surface of a spore of the bacterium can comprise an amino acid sequence having at least 99% identity with SEQ ID NO: 108, SEQ ID NO: 111, SEQ ID NO: 114, SEQ ID NO: 120, or SEQ ID NO: 121.
(449) The protein that targets the fusion protein of the surface of a spore of the bacterium can comprise an amino acid sequence having at least 100% identity with SEQ ID NO: 108, SEQ ID NO: 111, SEQ ID NO: 114, SEQ ID NO: 120, or SEQ ID NO: 121.
(450) For example, the protein that targets the fusion protein to a surface of a spore of the bacterium can comprise amino acids 20-35 of SEQ ID NO: 1, SEQ ID NO: 96, SEQ ID NO: 108, SEQ ID NO: 120, or SEQ ID NO: 121.
(451) The recombinant-spore forming bacterium comprises an endophytic strain of bacteria, a plant growth-promoting strain of bacteria, or a strain of bacteria that is both endophytic and plant growth-promoting. For example, the endophytic strain of bacteria, the plant growth-promoting strain of bacteria, or the strain of bacteria that is both endophytic and plant growth-promoting comprises *Bacillus megaterium* EE385, *Bacillus* sp. EE387, *Bacillus circulans* EE388, *Bacillus subtilis* EE405, *Lysinibacillus fusiformis* EE442, *Lysinibacillus sphaericus* EE443, *Bacillus pumilus* EE-B00143, *Bacillus subtilis* EE148, *Bacillus subtilis* EE218, or *Bacillus megaterium*

EE281. The endophytic strain of bacteria preferably comprises *Bacillus* sp. EE387.

X. Methods for Making the Fusion Proteins

(452) Any of the fusion proteins described herein can be made using standard cloning and molecular biology methods known in the art. For example, a gene encoding a protein or peptide of interest (e.g., a gene encoding a plant growth stimulating protein or peptide) can be amplified by polymerase chain reaction (PCR) and ligated to DNA coding for any of the above-described targeting sequences, exosporium proteins, exosporium protein fragments, or spore coat proteins, to form a DNA molecule that encodes the fusion protein. The DNA molecule encoding the fusion protein can be cloned into any suitable vector, for example a plasmid vector. The vector suitably comprises a multiple cloning site into which the DNA molecule encoding the fusion protein can be easily inserted. The vector also suitably contains a selectable marker, such as an antibiotic resistance gene, such that bacteria transformed, transfected, or mated with the vector can be readily identified and isolated. Where the vector is a plasmid, the plasmid suitably also comprises an origin of replication. Alternatively, DNA coding for the fusion protein can be integrated into the chromosomal DNA of the *B. cereus* family member or spore-forming bacterium host.

XI. Tags, Markers, and Linkers that can be Included in the Fusion Proteins

(453) Any of the fusion proteins described herein can also comprise additional polypeptide sequences that are not part of the targeting sequence, exosporium protein, exosporium protein fragment, or the plant growth stimulating protein or peptide, the protein or peptide that protects a plant from a pathogen, the protein or peptide that enhances stress resistance in a plant, or the plant binding protein or peptide. For example, the fusion protein can include tags or markers to facilitate purification or visualization of the fusion protein (e.g., a polyhistidine tag or a fluorescent protein such as GFP or YFP) or visualization of recombinant *Bacillus cereus* family member spores expressing the fusion protein.

(454) Expression of fusion proteins on the exosporium of a *Bacillus cereus* family member or on a surface of a spore of a spore-forming bacterium using the targeting sequences, exosporium proteins, exosporium protein fragments, and spore coat proteins described herein is enhanced due to a lack of secondary structure in the amino-termini of these sequences, which allows for native folding of the fused proteins and retention of activity. Proper folding can be further enhanced by the inclusion of a short amino acid linker between the targeting sequence, exosporium protein, exosporium protein fragment, spore coat protein, and the protein or peptide of interest.

(455) Thus, any of the fusion proteins described herein can comprise an amino acid linker between the targeting sequence, the exosporium protein, the exosporium protein fragment, or the spore coat protein and the protein or peptide of interest.

(456) The linker can comprise a polyalanine linker or a polyglycine linker. A linker comprising a mixture of both alanine and glycine residues can also be used.

(457) For example, in a fusion protein where the targeting sequence comprises SEQ ID NO: 1, a fusion protein can have one of the following structures: No linker: SEQ ID NO: 1-POI Alanine Linker: SEQ ID NO: 1-A.sub.n-POI *Glycine* Linker: SEQ ID NO: 1-G.sub.n-POI Mixed Alanine and *Glycine* Linker: SEQ ID NO: 1-(A/G).sub.n-POI where A.sub.n, G.sub.n, and (A/G).sub.n are any number of alanines, any number of glycines, or any number of a mixture of alanines and glycines, respectively. For example, n can be 1 to 25, and is preferably 6 to 10. Where the linker comprises a mixture of alanine and glycine residues, any combination of glycine and alanine residues can be used. In the above structures, “POI” represents the protein or peptide of interest.

(458) Alternatively or in addition, the linker can comprise a protease recognition site. Inclusion of a protease recognition site allows for targeted removal, upon exposure to a protease that recognizes the protease recognition site, of the protein or peptide of interest.

XII. Proteins and Peptides of Interest

(459) The protein or peptide of interest can comprise any protein or peptide.

(460) The protein or peptide of interest in the fusion proteins described herein can comprise, for example: (a) a plant growth stimulating protein or peptide; (b) a protein or peptide that protects a plant from a pathogen; (c) a protein or peptide that enhances stress resistance of a plant; (d) a plant binding protein or peptide; (e) an enzyme that catalyzes the production of nitric oxide; (f) a nucleic acid binding protein or peptide; or (g) a plant signaling molecule or a protein or peptide that alters the composition of a plant; (h) an antigen; (i) a remediation enzyme; (j) an enzyme suitable for breaking an emulsion or gel in a hydraulic fracturing fluid; or (k) an antibacterial protein or peptide.

(461) A. Plant Growth Stimulating Proteins or Peptides

(462) The protein or peptide of interest can comprise a plant growth stimulating protein or peptide.

(463) The plant growth stimulating protein or peptide can comprise a peptide hormone, a non-hormone peptide, an enzyme involved in the production or activation of a plant growth stimulating compound, or an enzyme that degrades or modifies a bacterial, fungal, or plant nutrient source.

(464) For example, the plant growth stimulating protein or peptide can comprise a peptide hormone.

(465) The peptide hormone can comprise a phytosulfokine (e.g., phytosulfokine-α), clavata 3 (CLV3), systemin,

ZmlGF, or a SCR/SP11.
(466) The plant growth stimulating protein or peptide can comprise a non-hormone peptide.
(467) The non-hormone peptide can comprise a RKN 16D10, Hg-Syv46, an eNOD40 peptide, melittin, mastoparan, Mas7, RHPP, POLARIS, or kunitz trypsin inhibitor (KTI).
(468) The plant growth stimulating protein or peptide can comprise an enzyme involved in the production or activation of a plant growth stimulating compound. The enzyme involved in the production or activation of a plant growth stimulating compound can be any enzyme that catalyzes any step in a biological synthesis pathway for a compound that stimulates plant growth or alters plant structure, or any enzyme that catalyzes the conversion of an inactive or less active derivative of a compound that stimulates plant growth or alters plant structure into an active or more active form of the compound.
(469) The plant growth stimulating compound can comprise a compound produced by bacteria or fungi in the rhizosphere, e.g., 2,3-butanediol.
(470) Alternatively, the plant growth stimulating compound can comprise a plant growth hormone.
(471) The plant growth hormone can comprise a cytokinin or a cytokinin derivative, ethylene, an auxin or an auxin derivative, a gibberellic acid or a gibberellic acid derivative, abscisic acid or an abscisic acid derivative, or a jasmonic acid or a jasmonic acid derivative.
(472) Where the plant growth stimulating compound comprises a cytokinin or a cytokinin derivative, the cytokinin or the cytokinin derivative can comprise kinetin, cis-zeatin, trans-zeatin, 6-benzylaminopurine, dihydroxyzeatin, N6-(D2-isopentenyl) adenine, ribosylzeatin, N6-(D2-isopentenyl) adenosine, 2-methylthio-cis-ribosylzeatin, cis-ribosylzeatin, trans-ribosylzeatin, 2-methylthio-trans-ribosylzeatin, ribosylzeatin-5-monosphosphate, N6-methylaminopurine, N6-dimethylaminopurine, 2′-deoxyzeatin riboside, 4-hydroxy-3-methyl-trans-2-butenylaminopurine, ortho-topolin, meta-topolin, benzyladenine, ortho-methyltopolin, meta-methyltopolin, or a combination thereof.
(473) Where the plant growth stimulating compound comprises an auxin or an auxin derivative, the auxin or the auxin derivative can comprise an active auxin, an inactive auxin, a conjugated auxin, a naturally occurring auxin, or a synthetic auxin, or a combination thereof. For example, the auxin or auxin derivative can comprise indole-3-acetic acid, indole-3-pyruvic acid, indole-3-acetaldoxime, indole-3-acetamide, indole-3-acetonitrile, indole-3-ethanol, indole-3-pyruvate, indole-3-acetaldoxime, indole-3-butyric acid, a phenylacetic acid, 4-chloroindole-3-acetic acid, a glucose-conjugated auxin, or a combination thereof.
(474) The enzyme involved in the production or activation of a plant growth stimulating compound can comprise an acetoin reductase, an indole-3-acetamide hydrolase, a tryptophan monooxygenase, an acetolactate synthetase, an α-acetolactate decarboxylase, a pyruvate decarboxylase, a diacetyl reductase, a butanediol dehydrogenase, an aminotransferase (e.g., tryptophan aminotransferase), a tryptophan decarboxylase, an amine oxidase, an indole-3-pyruvate decarboxylase, an indole-3-acetaldehyde dehydrogenase, a tryptophan side chain oxidase, a nitrile hydrolase, a nitrilase, a peptidase, a protease, an adenosine phosphate isopentenyltransferase, a phosphatase, an adenosine kinase, an adenine phosphoribosyltransferase, CYP735A, a 5′ribonucleotide phosphohydrolase, an adenosine nucleosidase, a zeatin cis-trans isomerase, a zeatin O-glucosyltransferase, a β-glucosidase, a cis-hydroxylase, a CK cis-hydroxylase, a CK N-glucosyltransferase, a 2,5-ribonucleotide phosphohydrolase, an adenosine nucleosidase, a purine nucleoside phosphorylase, a zeatin reductase, a hydroxylamine reductase, a 2-oxoglutarate dioxygenase, a gibberellic 2B/3B hydrolase, a gibberellin 3-oxidase, a gibberellin 20-oxidase, a chitosanase, a chitinase, a β-1,3-glucanase, a β-1,4-glucanase, a β-1,6-glucanase, an aminocyclopropane-1-carboxylic acid deaminase, or an enzyme involved in producing a nod factor (e.g., nodA, nodB, or nodI).
(475) Where the enzyme comprises a protease or peptidase, the protease or peptidase can be a protease or peptidase that cleaves proteins, peptides, proproteins, or preproproteins to create a bioactive peptide. The bioactive peptide can be any peptide that exerts a biological activity.
(476) Examples of bioactive peptides include RKN 16D10 and RHPP.
(477) The protease or peptidase that cleaves proteins, peptides, proproteins, or preproproteins to create a bioactive peptide can comprise subtilisin, an acid protease, an alkaline protease, a proteinase, an endopeptidase, an exopeptidase, thermolysin, papain, pepsin, trypsin, pronase, a carboxylase, a serine protease, a glutamic protease, an aspartate protease, a cysteine protease, a threonine protease, or a metalloprotease.
(478) The protease or peptidase can cleave proteins in a protein-rich meal (e.g., soybean meal or yeast extract).
(479) Where the enzyme comprises a chitosanase, the chitosanase can comprise an amino acid sequence having at least 85% identity with SEQ ID NO: 313.
(480) The chitosanase can comprise an amino acid sequence having at least 90% identity with SEQ ID NO: 313.
(481) The chitosanase can comprise an amino acid sequence having at least 95% identity with SEQ ID NO: 313.
(482) The chitosanase can comprise an amino acid sequence having at least 98% identity with SEQ ID NO: 313.
(483) The chitosanase can comprise an amino acid sequence having at least 99% identity with SEQ ID NO: 313.
(484) The chitosanase can comprise an amino acid sequence having at least 100% identity with SEQ ID NO: 313.

(485) For example, the fusion protein can comprise amino acids 20-35 of BclA (amino acids 20-35 of SEQ ID NO: 1) as the targeting sequence and an amino acid sequence comprising SEQ ID NO: 313 as the enzyme that is specific for a cellular component of a bacterium or fungus. The fusion protein can further comprise a linker (e.g., a polyalanine linker) between the targeting sequence and the enzyme.
(486) The plant growth stimulating protein or peptide can comprise an enzyme that degrades or modifies a bacterial, fungal, or plant nutrient source.
(487) The enzyme that degrades or modifies a bacterial, fungal, or plant nutrient source can comprise a cellulase, a lipase, a lignin oxidase, a protease, a glycoside hydrolase, a phosphatase, a nitrogenase, a nuclease, an amidase, a nitrate reductase, a nitrite reductase, an amylase, an ammonia oxidase, a ligninase, a glucosidase, a phospholipase, a phytase, a pectinase, a glucanase, a sulfatase, a urease, a xylanase, or a siderophore.
(488) When introduced into a plant growth medium or applied to a plant, seed, or an area surrounding a plant or a plant seed, fusion proteins comprising enzymes that degrade or modify a bacterial, fungal, or plant nutrient source can aid in the processing of nutrients in the vicinity of the plant and result in enhanced uptake of nutrients by the plant or by beneficial bacteria or fungi in the vicinity of the plant.
(489) The enzyme that degrades or modifies a bacterial, fungal, or plant nutrient source can comprise a cellulase.
(490) The cellulase can comprise an endocellulase (e.g., an endoglucanase such as a *Bacillus subtilis* endoglucanase, a *Bacillus thuringiensis* endoglucanase, a *Bacillus cereus* endoglucanase, or a *Bacillus clausii* endoglucanase), an exocellulase (e.g., a *Trichoderma reesei* exocellulase), or a β-glucosidase (e.g., a *Bacillus subtilis* β-glucosidase, a *Bacillus thuringiensis* β-glucosidase, a *Bacillus cereus* β-glucosidase, or a *Bacillus clausii* β-glucosidase). The cellulase preferably comprises a *Bacillus subtilis* endoglucanase.
(491) The endoglucanase can comprise an amino acid sequence having at least 85% identity with SEQ ID NO: 311.
(492) The endoglucanase can comprise an amino acid sequence having at least 90% identity with SEQ ID NO: 311.
(493) The endoglucanase can comprise an amino acid sequence having at least 95% identity with SEQ ID NO: 311.
(494) The endoglucanase can comprise an amino acid sequence having at least 98% identity with SEQ ID NO: 311.
(495) The endoglucanase can comprise an amino acid sequence having at least 99% identity with SEQ ID NO: 311.
(496) The endoglucanase can comprise an amino acid sequence having 100% identity with SEQ ID NO: 311.
(497) For example, the fusion protein can comprise amino acids 20-35 of BclA (amino acids 20-35 of SEQ ID NO: 1) as the targeting sequence and an amino acid sequence comprising SEQ ID NO: 311 as the enzyme that degrades or modifies a bacterial, fungal, or plant nutrient source. The fusion protein can further comprise a linker (e.g., a polyalanine linker) between the targeting sequence and the enzyme.
(498) The enzyme that degrades or modifies a bacterial, fungal, or plant nutrient source can comprise a lipase (e.g., a *Bacillus subtilis* lipase, a *Bacillus thuringiensis* lipase, a *Bacillus cereus* lipase, or a *Bacillus clausii* lipase).
(499) The enzyme that degrades or modifies a bacterial, fungal, or plant nutrient source can comprise a lignin oxidase. For example, the lignin oxidase can comprise a lignin peroxidase, a laccase, a glyoxal oxidase, a ligninase, or a manganese peroxidase.
(500) The enzyme that degrades or modifies a bacterial, fungal, or plant nutrient source can comprise a protease. For example, the protease can comprise a subtilisin, an acid protease, an alkaline protease, a proteinase, a peptidase, an endopeptidase, an exopeptidase, a thermolysin, a papain, a pepsin, a trypsin, a pronase, a carboxylase, a serine protease, a glutamic protease, an aspartate protease, a cysteine protease, a threonine protease, or a metalloprotease.
(501) The enzyme that degrades or modifies a bacterial, fungal, or plant nutrient source can comprise a phosphatase. For example, the phosphatase can comprise a phosphoric monoester hydrolase, a phosphomonoesterase (e.g., PhoA4), a phosphoric diester hydrolase, a phosphodiesterase, a triphosphoric monoester hydrolase, a phosphoryl anhydride hydrolase, a pyrophosphatase, a phytase (e.g., a *Bacillus subtilis* EE148 phytase or a *Bacillus thuringiensis* BT013A phytase), a trimetaphosphatase, or a triphosphatase.
(502) The enzyme that degrades or modifies a bacterial, fungal, or plant nutrient source can comprise a nitrogenase. For example the nitrogenase can comprise a Nif family nitrogenase (e.g., *Paenibacillus massiliensis* NifBDEHKNXV).
(503) The enzyme that degrades or modifies a bacterial, fungal, or plant nutrient source can comprise a phospholipase. For example, the phospholipase can comprise a phospholipase A1, a phospholipase A2, a phospholipase C, a phospholipase D, or a lysophospholipase. The phospholipase preferably comprises a phospholipsae C.
(504) The phospholipase C can comprise an amino acid sequence having at least 85% identity with SEQ ID NO: 312.
(505) The phospholipase C can comprise an amino acid sequence having at least 90% identity with SEQ ID NO: 312.
(506) The phospholipase C can comprise an amino acid sequence having at least 95% identity with SEQ ID NO: 312.
(507) The phospholipase C can comprise an amino acid sequence having at least 98% identity with SEQ ID NO:

312.
(508) The phospholipase C can comprise an amino acid sequence having at least 99% identity with SEQ ID NO: 312.
(509) The phospholipase C can comprise an amino acid sequence having 100% identity with SEQ ID NO: 312.
(510) For example, the fusion protein can comprise amino acids 20-35 of BclA (amino acids 20-35 of SEQ ID NO: 1) as the targeting sequence and an amino acid sequence comprising SEQ ID NO: 312 as the enzyme that degrades or modifies a bacterial, fungal, or plant nutrient source. The fusion protein can further comprise a linker (e.g., a polyalanine linker) between the targeting sequence and the enzyme.
(511) B. Proteins or Peptides that Protect Plants from Pathogens
(512) The protein or peptide of interest can comprise a protein or peptide that protects a plant from a pathogen.
(513) The protein or peptide that protects a plant from a pathogen can comprise a plant immune system enhancer protein or peptide.
(514) For example, the plant immune system enhancer protein or peptide can comprise a harpin, a harpin-like protein, an α-elastin, a β-elastin, a systemin, a phenylalanine ammonia-lyase, an elicitin, a defensin, a cryptogein, a flagellin protein, or a flagellin peptide (e.g., flg22).
(515) The protein or peptide that protects a plant from a pathogen can be a protein or peptide that has antibacterial activity, antifungal activity, or both antibacterial and antifungal activity. Examples of such proteins and peptides include bacteriocins, lysozymes, lysozyme peptides (e.g., LysM), siderophores, avidins, streptavidins, non-ribosomal active peptides, conalbumins, albumins, lactoferrins, lactoferrin peptides (e.g., LfcinB), and TasA.
(516) The protein or peptide that protects a plant from a pathogen can be a protein or a peptide that has insecticidal activity, helminthicidal activity, suppresses insect or worm predation, or a combination thereof. For example, the protein or peptide that protects a plant from a pathogen can comprise an insecticidal bacterial toxin (e.g., a VIP insecticidal protein), an endotoxin, a Cry toxin (e.g., a Cry toxin from *Bacillus thuringiensis*), a protease inhibitor protein or peptide (e.g., a trypsin inhibitor or an arrowhead protease inhibitor), a cysteine protease, or a chitinase. Where the Cry toxin comprises a Cry toxin from *Bacillus thuringiensis*, the Cry toxin can be a Cry5B protein or a Cry21A protein. Cry5B and Cry21A have both insecticidal and nematocidal activity.
(517) The protein that protects a plant from a pathogen can comprise an enzyme. For example, the enzyme can comprise a protease or a lactonase. The proteases and lactonases can be specific for a bacterial signaling molecule (e.g., a bacterial lactone homoserine signaling molecule).
(518) Where the enzyme comprises a lactonase, the lactonase can comprise 1,4-lactonase, 2-pyrone-4,6-dicarboxylate lactonase, 3-oxoadipate enol-lactonase, actinomycin lactonase, deoxylimonate A-ring-lactonase, gluconolactonase L-rhamnono-1,4-lactonase, limonin-D-ring-lactonase, steroid-lactonase, triacetate-lactonase, or xylono-1,4-lactonase.
(519) The enzyme can comprise an enzyme that is specific for a cellular component of a bacterium or fungus. For example, the enzyme can comprise a β-1,3-glucanase, a β-1,4-glucanase, a β-1,6-glucanase, a chitosanase, a chitinase, a chitosanase-like enzyme, a lyticase, a peptidase, a proteinase, a protease (e.g., an alkaline protease, an acid protease, or a neutral protease), a mutanolysin, a stapholysin, or a lysozyme.
(520) Where the enzyme comprises a chitosanase, the chitosanase can comprise an amino acid sequence having at least 85% identity with SEQ ID NO: 313.
(521) The chitosanase can comprise an amino acid sequence having at least 90% identity with SEQ ID NO: 313.
(522) The chitosanase can comprise an amino acid sequence having at least 95% identity with SEQ ID NO: 313.
(523) The chitosanase can comprise an amino acid sequence having at least 98% identity with SEQ ID NO: 313.
(524) The chitosanase can comprise an amino acid sequence having at least 99% identity with SEQ ID NO: 313.
(525) The chitosanase can comprise an amino acid sequence having at least 100% identity with SEQ ID NO: 313.
(526) For example, the fusion protein can comprise amino acids 20-35 of BclA (amino acids 20-35 of SEQ ID NO: 1) as the targeting sequence and an amino acid sequence comprising SEQ ID NO: 313 as the enzyme that is specific for a cellular component of a bacterium or fungus. The fusion protein can further comprise a linker (e.g., a polyalanine linker) between the targeting sequence and the enzyme.
(527) For any of the above proteins or peptides that protect a plant from a pathogen, the pathogen can comprise a protein or a peptide of interest that protects a plant from a bacterial pathogen, a fungal pathogen, a worm pathogen, or an insect pathogen.
(528) For example, the bacterial pathogen can comprise an α-class Proteobacterium, a β-class Proteobacterium, a γ-class Proteobacterium, or a combination thereof; or wherein the bacterial pathogen comprises *Agrobacterium tumefaciens, Pantoea stewartii, Erwinia carotovora, Ralstonia solanacearum, Pseudomonas syringae, Pseudomonas aeruginosa, Xanthomonas campestris*, or a combination thereof.
(529) The protein or peptide that protects a plant from a pathogen can comprise a protein or peptide protects the plant from predation by a worm or an insect pathogen.
(530) The worm or insect pathogen can comprise an army worm, a black cutworm, a European corn borer, a fall

armyworm, a cutworm, a Japanese beetle, a lesser cornstalk borer, a maize billbug, a seed corn maggot, a webworm, a southern cornstalk borer, a southern corn rootworm, a southern potato wireworm, a stalk borer, a sugarcane beetle, a white grub, a cabbage looper, a boll weevil, a yellow striped armyworm, a cereal leaf beetle, a chinch bug, an aphid, a beet armyworm, a Mexican bean beetle, a soybean looper, soybean stem borer, or a combination thereof.
(531) C. Proteins or Peptides that Enhance Stress-Resistance in Plants
(532) The protein or peptide of interest can comprise a protein or peptide that enhances stress resistance in a plant.
(533) For example, the protein or peptide that enhances stress resistance in a plant can comprise an enzyme that degrades a stress-related compound. Stress-related compounds include, but are not limited to, aminocyclopropane-1-carboxylic acid (ACC), reactive oxygen species, nitric oxide, oxylipins, and phenolics. Specific reactive oxygen species include hydroxyl, hydrogen peroxide, oxygen, and superoxide.
(534) The enzyme that degrades a stress-related compound can comprise a superoxide dismutase, an oxidase, a catalase, an aminocyclopropane-1-carboxylic acid deaminase, a peroxidase, an antioxidant enzyme, or an antioxidant peptide.
(535) When the enzyme that degrades a stress-related compound comprises a superoxide dismutase, the superoxide dismutase can comprise superoxide dismutase 1 (SODA1) or superoxide dismutase 2 (SODA2).
(536) The superoxide dismutase can comprise an amino acid sequence having at least 85% identity with SEQ ID NO: 155 or 156.
(537) The superoxide dismutase can comprise an amino acid sequence having at least 90% identity with SEQ ID NO: 155 or 156.
(538) The superoxide dismutase can comprise an amino acid sequence having at least 95% identity with SEQ ID NO: 155 or 156.
(539) The superoxide dismutase can comprise an amino acid sequence having at least 98% identity with SEQ ID NO: 155 or 156.
(540) The superoxide dismutase can comprise an amino acid sequence having at least 99% identity with SEQ ID NO: 155 or 156.
(541) The superoxide dismutase can comprise an amino acid sequence having 100% identity with SEQ ID NO: 155 or 156.
(542) The protein or peptide that enhances stress resistance in a plant can comprise a protein or peptide that protects a plant from an environmental stress. The environmental stress can comprise, for example, drought, flood, heat, freezing, salt, heavy metals, low pH, high pH, or a combination thereof. For instance, the protein or peptide that protects a plant from an environmental stress can comprises an ice nucleation protein, a prolinase, a phenylalanine ammonia lyase, an isochorismate synthase, an isochorismate pyruvate lyase, or a choline dehydrogenase.
(543) D. Plant Binding Proteins or Peptides
(544) The protein or peptide of interest can comprise a plant binding protein or peptide. The plant binding protein or peptide can be any protein or peptide that is capable of specifically or non-specifically binding to any part of a plant (e.g., a plant root or an aerial portion of a plant such as a leaf, stem, flower, or fruit) or to plant matter. Thus, for example, the plant binding protein or peptide can be a root binding protein or peptide, or a leaf binding protein or peptide.
(545) Suitable plant binding proteins and peptides include adhesins (e.g., rhicadhesin), flagellins, omptins, lectins, expansins, biofilm structural proteins (e.g., TasA or YuaB) pilus proteins, curlus proteins, intimins, invasins, agglutinins, and afimbrial proteins.
(546) E. Enzymes that Catalyze the Production of Nitric Oxide
(547) Many plant species do not inherently have a high germination rate. For such plants, it would be desirable to increase the germination rate. Nitric oxide is a powerful germinant that when present in proximity to a plant seed, increases germination.
(548) The present invention relates to fusion proteins comprising any of the targeting sequences, exosporium proteins, exosporium protein fragments, or spore coat proteins described herein and an enzyme that catalyzes the production of nitric oxide synthase. Thus, the protein or peptide of interest can comprise an enzyme that catalyzes the production of nitric oxide. Fusion proteins comprising an enzyme that catalyzes the production of nitric oxide can be expressed in recombinant *Bacillus cereus* family members or recombinant spore-forming bacteria for the purpose of delivering the enzyme that catalyzes the production of nitric oxide to a plant seed, a plant, a plant growth medium, or an area surrounding a plant or a plant seed, and thereby stimulating germination.
(549) For example, the enzyme that catalyzes the production of nitric oxide can comprise a nitric oxide synthase (e.g., a *Bacillus thuringiensis* nitric oxide synthase or a *Bacillus subtilis* nitric oxide synthase, for example a nitric oxide synthase from *Bacillus thuringiensis* BT013A or *Bacillus subtilis* 168) or an arginase.
(550) For example, the nitric oxide synthase can comprise one of the amino acid sequences described below in Table 8.
(551) TABLE-US-00008 TABLE 8 Exemplary nitric oxide synthase sequences Nitric oxide synthase SEQ ID NO.

Bacillus subtilis Nitric Oxide Synthatase 260 *Bacillus thuringiensis* Nitric Oxide Synthatase 261

(552) The nitric oxide synthase can also comprise a sequence having a high degree of sequence identity with the nitric oxide synthase sequences shown in Table 8 above. For example, the nitric oxide synthase can comprise an amino acid sequence having at least 85% sequence identity with SEQ ID NO: 260 or 261.

(553) The nitric oxide synthase can comprise an amino acid sequence having at least 90% sequence identity with SEQ ID NO: 260 or 261.

(554) the nitric oxide synthase can comprise an amino acid sequence having at least 95% sequence identity with SEQ ID NO: 260 or 261.

(555) the nitric oxide synthase can comprise an amino acid sequence having at least 98% sequence identity with SEQ ID NO: 260 or 261.

(556) the nitric oxide synthase can comprise an amino acid sequence having at least 99% sequence identity with SEQ ID NO: 260 or 261.

(557) the nitric oxide synthase can comprise an amino acid sequence having at least 100% sequence identity with SEQ ID NO: 260 or 261.

(558) When the protein or peptide of interest comprises a nitric oxide synthase, the fusion protein can comprise one of the amino acid sequences shown in Table 9 below. In the sequences shown in Table 9 below, the targeting sequence is shown in boldface text, a six amino acid alanine linker is indicated by underlining, and the sequence of the nitric oxide synthase is shown in plain text. Thus, the fusion protein can comprise SEQ ID NO: 262 or 263.

(559) TABLE-US-00009 TABLE 9 Exemplary fusion proteins comprising a nitric oxide synthase

Fusion protein (SEQ ID NO)	Amino Acid Sequence
Bacillus subtilis Nitric Oxide Synthatase (SEQ ID NO: 262)	Met + Amino acids **MAFDPNLVGPTLPPIPP**<u>AAAAAA</u>MEEKEI 20-35 of BclA, alanine linker, and KSEIDLTGSYVHTKEELEHGAKMAWRNSN RCIGRLFWNSLNVIDRRDVRTKEEVRDAL FHHIETATNNGKIRPTITIFPPEEKGEKQ VEIWNHQLIRYAGYESDGERIGDPASCSL TAACEELGWRGERTDFDLLPLIFRMKGDE QPVWYELPRSLVIEVPITHPDIEAFSDLE LKWYGVPIISDMKLEVGGIHYNAAPFNGW YMGTEIGARNLADEKRYDKLKKVASVIGI AADYNTDLWKDQALVELNKAVLHSYKKQG VSIVDHHTAASQFKRFEEQEEEAGRKLTG DWTWLIPPISPAATHIFFIRSYDNSIVKP NYFYQDKPYE
Bacillus thuringiensis Nitric Oxide Synthatase (SEQ ID NO: 263)	Met + Amino acids **MAFDPNLVGPTLPPIPP**<u>AAAAAA</u>MSKTKQ 20-35 of BclA, alanine linker, and QAEIEKTGTYEHTFEELVHGSRMAWRNSN RCIGRLFWSKMHILDAREVNDEEGVYHAL IHHIKYATNDGKVKPTITIFKQYQGEENN IRIYNHQLIRYAGYKTEMGVTGDSHSTAF TDFCQELGWQGEGTNFDVLPLVFSIDGKA PIYKEIPKEEVKEVPIEHPEYPISSLGAK WYGVPMISDMRLEIGGISYTAAPFNGWYM GTEIGARNLADHDRYNLLPAVAEMMDLDT SRNGTLWKDKALIELNVAVLHSFKKQGVS IVDHHTAAQQFQQFEKQEAACGRVVTGNW VWLIPPLSPATTHIYHKPYPNEILKPNFF H

(560) Nitric oxide synthases from a number species, including *Bacillus thuringiensis, Bacillus cereus, Bacillus subtilis* and *Bacillus mycoides* can be used as the protein or peptide of interest in the fusion proteins.

(561) F. Nucleic Acid Binding Proteins and Peptides

(562) The delivery of nucleic acids to plants in the field would be desirable, but has been hampered by the instability of nucleic acids, which degrade rapidly when introduced the environment (e.g., into a plant growth medium such as soil).

(563) The present invention relates to fusion proteins comprising any of the targeting sequences, exosporium proteins, exosporium protein fragments, or spore coat proteins described herein and a nucleic acid binding protein or peptide. Such fusion proteins stabilize nucleic acids and can be used to deliver nucleic acids to soil and/or to plants.

(564) Thus, the protein or peptide of interest can comprise a nucleic acid binding protein or peptide. For example, the nucleic acid binding protein or peptide can comprise an RNA binding protein or peptide or a DNA binding protein or peptide.

(565) The RNA binding protein or peptide can comprise a non-specific RNA binding protein or peptide or a specific RNA binding protein or peptide.

(566) For example, the RNA binding peptide can comprise an Hfq protein (e.g., a *Bacillus thuringiensis* Hqf protein).

(567) The DNA binding protein or peptide can comprise a small acid-soluble spore protein (SASP). For example, the SASP can comprise a SASP encoded by an SspA gene, an SspB gene, an SspC gene, an SspD gene, an SspE gene, an SspF gene, an SspG gene, an SspH gene, an SspI gene, an SspJ gene, an SspK gene, an SspL gene, an SspM gene, an SspN gene, an SspO gene, or an SspP gene. For example, the SASP can comprise a SASPα, a SASPβ, or a SASPγ. The SASP can comprise a *Bacillus thuringiensis* SASP.

(568) The nucleic acid binding protein can comprise one of the amino acid sequences described below in Table 10.
(569) TABLE-US-00010 TABLE 10 Exemplary SASP and Hfq sequences Fusion protein SEQ ID NO. SASPα 264 SASPγ 265 Hfq 266
(570) The nucleic acid binding protein can also comprise a sequence having a high degree of sequence identity with any of the sequences shown above in Table 10. For example, the nucleic acid binding protein can comprise a nucleic acid sequence having at least 85% identity with any of SEQ ID NOs: 264-266.
(571) The nucleic acid binding protein can comprise a nucleic acid sequence having at least 90% identity with any of SEQ ID NOs: 264-266.
(572) The nucleic acid binding protein can comprise a nucleic acid sequence having at least 95% identity with any of SEQ ID NOs: 264-266.
(573) The nucleic acid binding protein can comprise a nucleic acid sequence having at least 98% identity with any of SEQ ID NOs: 264-266.
(574) The nucleic acid binding protein can comprise a nucleic acid sequence having at least 99% identity with any of SEQ ID NOs: 264-266.
(575) The nucleic acid binding protein can comprise a nucleic acid sequence having at least 100% identity with any of SEQ ID NOs: 264-266.
(576) For example, when the protein or peptide of interest comprises a nucleic acid binding protein or peptide, the fusion protein can comprise one of the amino acid sequences shown in Table 11 below. In the sequences shown in Table 11 below, the targeting sequence is shown in boldface text, a six amino acid alanine linker is indicated by underlining, and the sequence of the nucleic acid binding protein or peptide (SASPα, SASPβ, or Hfq) is shown in plain text. Thus, for example, the fusion protein can comprise SEQ ID NO: 267, 268, or 269.
(577) TABLE-US-00011 TABLE 11 Exemplary fusion proteins comprising a nucleic acid binding protein Fusion protein (SEQ ID NO) Amino Acid Sequence Met + Amino acids **MAFDPNLVGPTLPPIPP**<u>AAAAAAAA</u>MAQQSRSR 20-35 of BclA, SNNNNDLLIPQAASAIEQMKLEIASEFGVQLGA alanine linker, ETTSRANGSVGGEITKRLVRLAQQNMGGQFH and SASPα (SEQ ID NO: 267) Met + Amino acids **MAFDPNLVGPTLPPIPP**<u>AAAAAAAA</u>MANNNSGN 20-35 of BclA, SNNLLVPGAAQAIDQMKLEIASEFGVNLGADTT alanine linker, SRANGSVGGEITKRLVSFAQQNMGGGQF and SASPγ (SEQ ID NO: 268) Met + Amino acids **MAFDPNLVGPTLPPIPP**<u>AAAAAAAA</u>MKPINIQD 20-35 of BclA, QFLNQIRKENTYVTVFLLNGFQLRGQVKGFDNF alanine linker, TVLLESEGKQQLIYKHAISTFAPQKNVQLELE and Hfq (SEQ ID NO: 269)
(578) Nucleases can also be used to both bind to and cleave nucleic acid molecules. Nucleases have high affinity for RNA and DNA molecules, and exert their enzymatic activity by cleaving RNA and/or DNA molecules into smaller RNA and/or DNA fragments. Nucleases can be specific, recognizing and cleaving specific DNA or RNA sequences, or non-specific, cleaving any DNA and/or RNA that they come in contact with. Nucleases can be categorized into exonucleases (nucleases that cleave nucleotides off of the ends of RNA and/or DNA molecules), or endonucleases (nucleases that cleave a phosphodiester bond within a polynucleotide chain). Each nuclease enzyme has an active site that comprises particular amino acids that act to catalyze the cleavage of the nucleic acid molecule. Mutation of these active sites can inactivate the active site and allow for high affinity binding of the nuclease to its nucleic acid substrate, without cleavage of the substrate. Thus, such mutants can bind to and stabilize the nucleic acid molecule without cleaving the nucleic acid molecule.
(579) Thus, the nucleic acid binding protein can comprise a nuclease (e.g., a nuclease having an inactivated active site).
(580) When the protein or peptide of interest comprises a nucleic acid binding protein or peptide, a nucleic acid molecule can be bound to the nucleic acid binding protein or peptide. The nucleic acid can comprise, for example, a modulating RNA molecule; an RNAi molecule; a microRNA; an aptamer; or a DNA molecule that encodes a modulating RNA molecule, an RNAi molecule, a microRNA, or an aptamer.
XIII. Recombinant *Bacillus cereus* Family Member Hosts
(581) As described above, a *Bacillus cereus* family member can serve as a host for expression of fusion proteins comprising a targeting sequence, an exosporium protein, or an exosporium protein fragment that targets the fusion protein to the exosporium of the *Bacillus cereus* family member; serve as a host for expression of modulator proteins that modulate the expression of a fusion protein; can serve as a host for overexpression of an exosporium enzyme; can be genetically inactivated; or can comprise a mutation or other genetic alteration that allows for collection of free exosporium.
(582) The recombinant *Bacillus cereus* family member can coexpress two or more of any of the fusion proteins discussed above. For example, the recombinant *Bacillus cereus* family member can coexpress at least one fusion protein that comprises a plant binding protein or peptide, together with a fusion protein comprising a plant growth

stimulating protein or peptide, a fusion protein comprising a protein or peptide that protects a plant from a pathogen, a fusion protein comprising protein or peptide that enhances stress resistance in a plant, a fusion protein comprising an enzyme that catalyzes the production of nitric oxide, or a fusion protein comprising a nucleic acid binding protein or peptide.

(583) The recombinant *Bacillus cereus* family member can comprise any *Bacillus* species that is capable of producing an exosporium. For example, the recombinant *Bacillus cereus* family member can comprise *Bacillus anthracis, Bacillus cereus, Bacillus thuringiensis, Bacillus mycoides, Bacillus pseudomycoides, Bacillus samanii, Bacillus gaemokensis, Bacillus weihenstephensis, Bacillus toyoiensis,* or a combination thereof. In particular, the recombinant *Bacillus cereus* family member can comprise *Bacillus thuringiensis* or *Bacillus mycoides.*

(584) To generate a recombinant *Bacillus cereus* family member expressing a fusion protein, any *Bacillus cereus* family member can be conjugated, transduced, or transformed with a vector encoding the fusion protein using standard methods known in the art (e.g., by electroporation). The bacteria can then be screened to identify transformants by any method known in the art. For example, where the vector includes an antibiotic resistance gene, the bacteria can be screened for antibiotic resistance. Alternatively, DNA encoding the fusion protein can be integrated into the chromosomal DNA of a *B. cereus* family member host. The recombinant *Bacillus cereus* family member can then exposed to conditions which will induce sporulation. Suitable conditions for inducing sporulation are known in the art. For example, the recombinant *Bacillus cereus* family member can be plated onto agar plates, and incubated at a temperature of about 30° C. for several days (e.g., 3 days).

(585) Inactivated strains, non-toxic strains, or genetically manipulated strains of any of the above species can also suitably be used. For example, a *Bacillus thuringiensis* that lacks the Cry toxin can be used. Alternatively or in addition, once the recombinant *B. cereus* family member spores expressing the fusion protein have been generated, they can be inactivated to prevent further germination once in use. Any method for inactivating bacterial spores that is known in the art can be used. Suitable methods include, without limitation, heat treatment, gamma irradiation, x-ray irradiation, UV-A irradiation, UV-B irradiation, chemical treatment (e.g., treatment with gluteraldehyde, formaldehyde, hydrogen peroxide, acetic acid, bleach, or any combination thereof), or a combination thereof. Alternatively, spores derived from nontoxigenic strains, or genetically or physically inactivated strains, can be used.

(586) Many *Bacillus cereus* family member strains have inherent beneficial attributes. For example, some strains have plant-growth promoting effects. Any of the recombinant *Bacillus cereus* family members described herein can comprise a plant-growth promoting strain of bacteria.

(587) The plant-growth promoting strain of bacteria can comprise a strain of bacteria that produces an insecticidal toxin (e.g., a Cry toxin), produces a fungicidal compound (e.g., a β-1,3-glucanase, a chitosanase, a lyticase, or a combination thereof), produces a nematocidal compound (e.g., a Cry toxin), produces a bacteriocidal compound, is resistant to one or more antibiotics, comprises one or more freely replicating plasmids, binds to plant roots, colonizes plant roots, forms biofilms, solubilizes nutrients, secretes organic acids, or any combination thereof.

(588) For example, where the recombinant *Bacillus cereus* family member comprises a plant-growth promoting strain of bacteria, the plant growth-promoting strain of bacteria can comprise (a) *Bacillus mycoides* BT155 (NRRL No. B-50921), (b) *Bacillus mycoides* EE118 (NRRL No. B-50918), (c) *Bacillus mycoides* EE141 (NRRL No. B-50916), (d) *Bacillus mycoides* BT46-3 (NRRL No. B-50922), (c) *Bacillus cereus* family member EE128 (NRRL No. B-50917), (f) *Bacillus thuringiensis* BT013A (NRRL No. B-50924), (g) *Bacillus cereus* family member EE349 (NRRL No. B-50928), (h) *Bacillus cereus* family member EE-B00377 (NRRL B-67119), (i) *Bacillus pseudomycoides* EE-B00366 (NRRL B-67120), or (j) *Bacillus mycoides* EE-B00363 (NRRL B-67121). Each of the strains (a) through (g) was deposited with the United States Department of Agriculture (USDA) Agricultural Research Service (ARS), having the address 1815 North University Street, Peoria, Illinois 61604 U.S.A., on Mar. 10, 2014, and is identified by the NRRL deposit number provided in parentheses. *Bacillus thuringiensis* BT013A is also known as *Bacillus thuringiensis* 4Q7. Each of the strains (h) through (j) were deposited with the USDA ARS on Aug. 19, 2015, and is identified by the NRRL deposit number provided in parentheses. It is hereby certified that the deposits were made in compliance with the terms of the Budapest Treaty and that: (a) during the pendency of this application, access to the deposited organisms will be afforded to the Commissioner upon request; (b) all restrictions upon availability to the public of the deposited materials will be irrevocably removed upon granting of the patent, subject to 37 C.F.R. Y 1.808(b); (c) the deposit will be maintained for a period of 30 years or 5 years after the last request or for the effective life of the patent, whichever is longer; and (d) the deposit will be replaced if it should ever become non-viable.

(589) These plant-growth promoting strains were isolated from the rhizospheres of various vigorous plants and were identified by their 16S rRNA sequences (listed below in Table 12), and through biochemical assays. The strains were identified at least to their genus designation by means of conventional biochemistry and morphological indicators. Biochemical assays for confirmed Gram-positive strains such as *Bacillus* included growth on PEA medium and nutrient agar, microscopic examination, growth on 5% and 7.5% NaCl medium, growth at pH 5 and pH 9, growth at 42° C. and 50° C., the ability to produce acid upon fermentation with cellobiose, lactose, glycerol,

glucose, sucrose, d-mannitol, and starch; fluorescent pigment production; gelatin hydrolysis; nitrate reduction; catalase production, starch hydrolysis; oxidase reaction, urease production and motility. Identification of these strains and demonstration of their plant-growth promoting effects are described further in the Examples hereinbelow.

(590) TABLE-US-00012 TABLE 12 Partial 16S rRNA sequences for plant-growth promoting *Bacillus cereus* family members SEQ ID NO. for partial 16S Strain ribosomal RNA sequence *Bacillus mycoides* EE118 270 *Bacillus mycoides* EE141 271 *Bacillus mycoides* BT46-3 272 *Bacillus cereus* family member EE128 273 *Bacillus thuringiensis* BT013A 274 *Bacillus cereus* family member EE349 275 *Bacillus mycoides* BT155 276

(591) For example, the recombinant *Bacillus cereus* family member comprising a plant-growth promoting strain of bacteria can comprise *Bacillus mycoides* BT155, *Bacillus mycoides* EE141, or *Bacillus thuringiensis* BT013A.

(592) The recombinant *Bacillus cereus* family member can comprises an endophytic strain of bacteria. For example, the endophytic strain of bacteria can comprise *Bacillus cereus* family member EE349, *Bacillus cereus* family member EE439, *Bacillus thuringiensis* EE417, *Bacillus cereus* EE444, or *Bacillus thuringiensis* EE319, *Bacillus thuringiensis* EE-B00184, *Bacillus cereus* family member EE-B00377; *Bacillus pseudomycoides* EE-B00366; or *Bacillus mycoides* EE-B00363.

(593) *Bacillus cereus* family member EE349 is also a plant growth promoting strain of bacteria and is described above. As discussed further in the Examples below, *Bacillus cereus* family member EE349 has also been found to be endophytic.

(594) *Bacillus cereus* family member EE439, *Bacillus thuringiensis* EE417, *Bacillus cereus* EE444, *Bacillus thuringiensis* EE319, *Bacillus thuringiensis* EE-B00184, *Bacillus cereus* family member EE-B00377; *Bacillus pseudomycoides* EE-B00366; or *Bacillus mycoides* EE-B00363 are described further below in Section XIV.

(595) The endophytic strain of bacteria can comprise *Bacillus cereus* family member EE439, *Bacillus thuringiensis* EE417, *Bacillus cereus* EE444, *Bacillus thuringiensis* EE319, *Bacillus thuringiensis* EE-B00184, *Bacillus cereus* family member EE-B00377; *Bacillus pseudomycoides* EE-B00366; or *Bacillus mycoides* EE-B00363.

(596) The recombinant *Bacillus cereus* family member can comprise a strain of bacteria that is capable of degrading an herbicide or a pesticide. As discussed further below in the Examples, *Bacillus cereus* family member EE349, *Bacillus cereus* family member EE-B00377, *Bacillus pseudomycoides* EE-B00366, and *Bacillus mycoides* EE-B00363 have been found to be capable of degrading herbicides and/or pesticides. Thus, when the recombinant *Bacillus cereus* family member comprises a strain of bacteria that is capable of degrading an herbicide, the strain of bacteria that is capable of degrading an herbicide can comprise *Bacillus cereus* family member EE349, *Bacillus cereus* family member EE-B00377, *Bacillus pseudomycoides* EE-B00366, or *Bacillus mycoides* EE-B00363.

(597) The strain of bacteria that is capable of degrading an herbicide or a pesticide can degrade a sulfonylurea herbicide (e.g., sulfentrazone), an aryl triazine herbicide, dicamba, 2,4-D, a phenoxy herbicide, a pyrethrin, a pyrethroid, or a combination thereof.

(598) The strain of bacteria that is capable of degrading a pesticide can be a strain of bacteria that is capable of degrading a pyrethrin.

(599) The recombinant *Bacillus cereus* family member can comprise a probiotic strain of bacteria. For example, the probiotic strain of bacteria can comprise *Bacillus cereus* family member EE349, *Bacillus cereus* family member EE439, *Bacillus thuringiensis* EE417, or *Bacillus cereus* EE444.

(600) The recombinant *Bacillus cereus* family member can comprise an inactivating mutation in its BclA gene, its CotE gene, or its CotO gene (e.g., a knock-out of the BclA gene, CotE gene, or CotO gene). For example, the recombinant *Bacillus cereus* family member can comprise an inactivating mutation in its BclA gene (e.g., a knock-out of the BclA gene). It has been found that expression of fusion proteins in a recombinant *Bacillus cereus* family member having such a mutation results in increased expression levels of the fusion protein.

XIV. Endophytic Bacterial Strains

(601) The present invention further relates to endophytic bacterial strains. While many bacteria of the rhizosphere have a symbiotic relationship with the plant, only a small subset of these bacteria are capable of being internalized into the plant and growing endophytically. As described further in the Examples hereinbelow, several *Bacillus cereus* family member strains and several non-*Bacillus cereus* family member bacterial strains were isolated from corn seedlings and found to have the ability to grow endophytically in plants.

(602) A. Endophytic *Bacillus cereus* Family Members

(603) The present invention relates to biologically pure bacterial cultures of bacteria that have the ability to grow endophytically. The bacterial strain in each of these bacterial cultures can be: (a) *Bacillus cereus* family member EE439 (NRRL B-50979); (b) *Bacillus thuringiensis* EE417 (NRRL B-50974); (c) *Bacillus cereus* EE444 (NRRL B-50977); (d) *Bacillus thuringiensis* EE319 (NRRL B-50983), (c) *Bacillus thuringiensis* EE-B00184 (NRRL B-67122); (f) *Bacillus cereus* family member EE-B00377 (NRRL B-67119); (g) *Bacillus pseudomycoides* EE-B00366 (NRRL B-67120); or (h) *Bacillus mycoides* EE-B00363 (NRRL B-67121). Each of strains (a) through (c) was deposited with the United States Department of Agriculture (USDA) Agricultural Research Service (ARS), having

the address 1815 North University Street, Peoria, Illinois 61604 U.S.A., on Sep. 10, 2014, and are identified by the NRRL numbers provided in parentheses following the names of each strain. Strain (d) was deposited with the USDA ARS on Sep. 17, 2014 and is identified by the NRRL number provided in parentheses following the name of the strain. Each of strains (c) through (h) was deposited with the USDA ARS on Aug. 19, 2015 and are identified by the NRRL numbers provided in parentheses following the names of each strain. It is hereby certified that the deposits were made in compliance with the terms of the Budapest Treaty and that: (a) during the pendency of this application, access to the deposited organisms will be afforded to the Commissioner upon request; (b) all restrictions upon availability to the public of the deposited materials will be irrevocably removed upon granting of the patent, subject to 37 C.F.R. Y 1.808(b); (c) the deposit will be maintained for a period of 30 years or 5 years after the last request or for the effective life of the patent, whichever is longer; and (d) the deposit will be replaced if it should ever become non-viable.

(604) The novel strains disclosed herein were identified by 16S ribosomal RNA (rRNA) sequencing. Thus, *Bacillus cereus* family member EE439 has a 16S ribosomal RNA sequence having at least 98%, at least 99%, or 100% sequence identity with the sequence of SEQ ID NO: 277. *Bacillus thuringiensis* EE417 has a 16S ribosomal RNA sequence having at least 98%, at least 99%, or 100% sequence identity with the sequence of SEQ ID NO: 278. *Bacillus cereus* EE444 has a 16S ribosomal RNA sequence having at least 98%, at least 99%, or 100% sequence identity with the sequence of SEQ ID NO: 279. *Bacillus thuringiensis* EE319 has a 16S ribosomal RNA sequence having at least 98%, at least 99%, or 100% sequence identity with the sequence of SEQ ID NO: 280. *Bacillus thuringiensis* EE-B00184 has a 16S ribosomal RNA sequence having at least 98%, at least 99%, or 100% sequence identity with the sequence of SEQ ID NO: 301. *Bacillus cereus* family member EE-B00377 has a 16S ribosomal RNA sequence having at least 98%, at least 99%, or 100% sequence identity with the sequence of SEQ ID NO: 304. *Bacillus pseudomycoides* EE-B00366 has a 16S ribosomal RNA sequence having at least 98%, at least 99%, or 100% sequence identity with the sequence of SEQ ID NO: 303. *Bacillus mycoides* EE-B00363 (NRRL B-67121) and the bacteria has a 16S ribosomal RNA sequence having at least 98%, at least 99%, or 100% sequence identity with the sequence of SEQ ID NO: 302. The 16S rRNA sequences are listed below in Table 13.

(605) TABLE-US-00013 TABLE 13 Partial 16S rRNA sequences for *Bacillus cereus* family member endophytic strains SEQ ID NO. for partial Strain 16S rRNA sequence *Bacillus cereus* family member EE439 277 *Bacillus thuringiensis* EE417 278 *Bacillus cereus* EE444 279 *Bacillus thuringiensis* EE319 280 *Bacillus thuringiensis* EE-B00184 301 *Bacillus mycoides* EE-B00363 302 *Bacillus pseudomycoides* EE-B00366 303 *Bacillus cereus* family member EE-B00377 304

(606) The present invention further relates to a biologically pure bacterial culture wherein the bacteria in the bacterial culture are mutants of *Bacillus cereus* family member EE439, *Bacillus thuringiensis* EE417, *Bacillus cereus* EE444, *Bacillus thuringiensis* EE319, *Bacillus thuringiensis* EE-B00184, *Bacillus cereus* family member EE-B00377, *Bacillus pseudomycoides* EE-B00366, or *Bacillus mycoides* EE-B00363 comprising one or more mutations, wherein the bacteria are endophytic.

(607) B. Other Endophytic Bacterial Strains

(608) The present invention also relates to other biologically pure bacterial cultures of bacteria (non-*Bacillus cereus* family members) that have the ability to grow endophytically. These strains were isolated from corn seedlings, as described in detail below in the Examples.

(609) The bacterial strain in each of these bacterial cultures can be (a) *Bacillus megaterium* EE385 (NRRL B-50980), (b) *Bacillus* sp. EE387 (NRRL B-50981), (c) *Bacillus circulans* EE388 (NRRL B-50982), (d) *Bacillus subtilis* EE405 (NRRL B-50978), (c) *Lysinibacillus fusiformis* EE442 (NRRL B-50975), (f) *Lysinibacillus sphaericus* EE443 (NRRL B-50976), or (g) *Bacillus pumilus* EE-B00143 (NRRL B-67123). Each of the strains (a) through (f) was deposited with the United States Department of Agriculture (USDA) Agricultural Research Service (ARS), having the address 1815 North University Street, Peoria, Illinois 61604 U.S.A., on Sep. 10, 2014, and are identified by the NRRL numbers provided in parentheses following the names of each strain. Following deposit, *Bacillus* sp. EE387 was determined to be a *Bacillus pumilus*-like strain. Strain (g) was deposited with the USDA ARS on Aug. 19, 2015 and is identified by the NRRL number provided in parentheses following its name.

(610) The novel strains disclosed herein were identified by 16S ribosomal RNA (rRNA) sequencing. Thus, *Bacillus megaterium* EE385 has a 16S ribosomal RNA sequence having at least 98%, at least 99%, or 100% sequence identity with the sequence of SEQ ID NO: 281. *Bacillus* sp. EE387 has a 16S ribosomal RNA sequence having at least 98%, at least 99%, or 100% sequence identity with the sequence of SEQ ID NO: 282. *Bacillus circulans* EE388 has a 16S ribosomal RNA sequence having at least 98%, at least 99%, or 100% sequence identity with the sequence of SEQ ID NO: 283. *Bacillus subtilis* EE405 has a 16S ribosomal RNA sequence having at least 98%, at least 99%, or 100% sequence identity with the sequence of SEQ ID NO: 284. *Lysinibacillus fusiformis* EE442 has a 16S ribosomal RNA sequence having at least 98%, at least 99%, or 100% sequence identity with the sequence of SEQ ID NO: 285. *Lysinibacillus sphaericus* EE443 has a 16S ribosomal RNA sequence having at least 98%, at least 99%, or 100% sequence identity with the sequence of SEQ ID NO: 286. *Bacillus pumilus* EE-B00143 has a 16S

ribosomal RNA sequence having at least 98%, at least 99%, or 100% sequence identity with the sequence of SEQ ID NO: 305. The 16s rRNA sequences are listed below in Table 14.

(611) TABLE-US-00014 TABLE 14 Partial 16S rRNA sequences for non-*Bacillus cereus* family member endophytic strains SEQ ID NO. for Strain (SEQ ID NO) partial 16S rRNA sequence *Bacillus megaterium* EE385 281 *Bacillus* sp. EE387 282 *Bacillus circulans* EE388 283 *Bacillus subtilis* EE405 284 *Lysinibacillus fusiformis* EE442 285 *Lysinibcaillus sphaericus* EE443 286 *Bacillus pumilus* EE-B00143 305

(612) The present invention further relates to a biologically pure bacterial culture wherein the bacteria in the bacterial culture are mutants of *Bacillus megaterium* EE385, *Bacillus* sp. EE387, *Bacillus circulans* EE388, *Bacillus subtilis* EE405, *Lysinibacillus fusiformis* EE442, or *Lysinibacillus sphaericus* EE443, comprising one or more mutations, wherein the bacteria are endophytic.

(613) The present invention also relates to a biologically pure bacterial culture wherein the bacteria in the bacterial culture are mutants of *Bacillus megaterium* EE385, *Bacillus* sp. EE387, *Bacillus circulans* EE388, *Bacillus subtilis* EE405, *Lysinibacillus fusiformis* EE442, or *Lysinibacillus sphaericus* EE443, comprising one or more mutations, wherein the bacteria are probiotic.

XV. Inoculums

(614) The invention further relates to inoculums of any of the biologically pure bacterial strains described above in the preceding section. The inoculums are for application to plants, plant seeds, a plant growth medium, or an area surrounding a plant or a plant seed, wherein the inoculum comprises an effective amount of any one of the biologically pure bacterial cultures and an agriculturally acceptable carrier.

(615) The inoculum can comprise an effective amount of a mixture comprising at least two of the biologically pure bacterial cultures described above in the immediately preceding section.

(616) The inoculum can further comprise an effective amount of a rhizobacteria. The rhizobacteria can be a biologically pure bacterial culture of a rhizobacteria strain. The rhizobacteria can comprise *Bradyrhizobium* genus bacteria (e.g., *Bradyrhizobium japonicum*), *Rhizobium* genus bacteria (e.g., *Rhizobium phaseoli, Rhizobium leguminosarum*, or a combination thereof), or a combination thereof.

XVI. Plant Seeds Coated with an Enzyme that Catalyzes the Production of Nitric Oxide or with Recombinant Bacteria that Overexpress an Enzyme that Catalyzes the Production of Nitric Oxide

(617) A plant seed is also provided which is coated with: (i) an enzyme that catalyzes the production of nitric oxide; (ii) a superoxide dismutase or (iii) a recombinant microorganism that expresses an enzyme that catalyzes the production of nitric oxide or a superoxide dismutase, wherein the expression of the enzyme that catalyzes the production of nitric oxide or the superoxide dismutase is increased as compared to the expression of the enzyme that catalyzes the production of nitric oxide or the superoxide dismutase in a wild-type microorganism under the same conditions.

(618) The enzyme that catalyzes the production of nitric oxide can comprise a nitric oxide synthase or an arginase.

(619) The enzyme that catalyzes the production of nitric oxide can comprise a nitric oxide synthase, such as a nitric oxide synthase from *Bacillus thuringiensis* BT013A or *Bacillus subtilis* 168.

(620) For example, the nitric oxide synthase can comprise an amino acid sequence having at least 85% sequence identity with SEQ ID NO: 260 or 261.

(621) The nitric oxide synthase can comprise an amino acid sequence having at least 90% sequence identity with SEQ ID NO: 260 or 261.

(622) The nitric oxide synthase can comprise an amino acid sequence having at least 95% sequence identity with SEQ ID NO: 260 or 261.

(623) The nitric oxide synthase can comprise an amino acid sequence having at least 98% sequence identity with SEQ ID NO: 260 or 261.

(624) The nitric oxide synthase can comprise an amino acid sequence having at least 99% sequence identity with SEQ ID NO: 260 or 261.

(625) The nitric oxide synthase can comprise an amino acid sequence having 100% sequence identity with SEQ ID NO: 260 or 261.

(626) The superoxide dismutase can comprise superoxide dismutase 1 (SODA1) or superoxide dismutase 2 (SODA2).

(627) The superoxide dismutase comprises an amino acid sequence having at least 85% identity with SEQ ID NO: 155 or 156.

(628) The superoxide dismutase comprises an amino acid sequence having at least 90% identity with SEQ ID NO: 155 or 156.

(629) The superoxide dismutase comprises an amino acid sequence having at least 95% identity with SEQ ID NO: 155 or 156.

(630) The superoxide dismutase comprises an amino acid sequence having at least 98% identity with SEQ ID NO: 155 or 156.

(631) The superoxide dismutase comprises an amino acid sequence having at least 99% identity with SEQ ID NO: 155 or 156.
(632) The superoxide dismutase comprises an amino acid sequence having at least 100% identity with SEQ ID NO: 155 or 156.
(633) When the plant seed is coated with the recombinant microorganism, the recombinant microorganism can comprise a *Bacillus* species, *Escherichia coli*, an *Aspergillus* species such as *Aspergillus niger*, or a *Saccharomyces* species such as *Saccharomyces cerevisiae.*
(634) For example, the recombinant microorganism can comprise a *Bacillus cereus* family member, *Bacillus subtilis*, *Bacillus licheniformis*, or *Bacillus megaterium.*
(635) Amino acid sequences for exemplary nitric oxide synthetase enzymes are provided above in Table 8. Amino acid sequences for exemplary superoxide dismutases are provided above in Table 2.
XVII. Formulations
(636) Formulations are provided which comprise a recombinant *Bacillus cereus* family member as described herein, exosporium fragments derived from spores of a recombinant *Bacillus cereus* family member as described herein or a recombinant spore-forming bacterium as described herein, and an agriculturally acceptable carrier.
(637) The agriculturally acceptable carrier can comprise an additive, such as an oil, a gum, a resin, a clay, a polyoxyethylene glycol, a terpene, a viscid organic, a fatty acid ester, a sulfated alcohol, an alkyl sulfonate, a petroleum sulfonate, an alcohol sulfate, a sodium alkyl butane diamate, a polyester of sodium thiobutane dioate, a benzene acetonitrile derivative, a proteinaceous material, or a combination thereof.
(638) The agriculturally acceptable carrier can comprise a thickener, such as a long chain alkylsulfonate of polyethylene glycol, a polyoxyethylene oleate, or a combination thereof; a surfactant such as a heavy petroleum oil, a heavy petroleum distillate, a polyol fatty acid ester, a polyethoxylated fatty acid ester, an aryl alkyl polyoxyethylene glycol, an alkyl amine acetate, an alkyl aryl sulfonate, a polyhydric alcohol, an alkyl phosphate, or a combination thereof; or an anti-caking agent such as a sodium salt (e.g., a sodium salt of monomethyl naphthalene sulfonate, a sodium salt of dimethyl naphthalene sulfonate, a sodium sulfite, a sodium sulfate, or a combination thereof), a calcium carbonate, diatomaceous earth, or a combination thereof.
(639) The additive can comprise a proteinaceous material such as a milk product, wheat flour, soybean meal, blood, albumin, gelatin, alfalfa meal, yeast extract, or a combination thereof;
(640) The agriculturally acceptable carrier can comprise vermiculite, charcoal, sugar factory carbonation press mud, rice husk, carboxymethyl cellulose, peat, perlite, fine sand, calcium carbonate, flour, alum, a starch, talc, polyvinyl pyrrolidone, or a combination thereof.
(641) The formulation can comprise a seed coating formulation, a liquid formulation for application to plants or to a plant growth medium, or a solid formulation for application to plants or to a plant growth medium. The seed coating formulation can comprise an aqueous or oil-based solution for application to seeds or a powder or granular formulation for application to seeds. The liquid formulation for application to plants or to a plant growth medium can comprise a concentrated formulation or a ready-to-use formulation. The solid formulation for application to plants or to a plant growth medium can comprise a granular formulation or a powder agent.
(642) The formulation further can comprise a fertilizer, a micronutrient fertilizer material, an insecticide, an herbicide, a plant growth amendment, a fungicide, an insecticide, a molluscicide, an algicide, a bacterial inoculant, a fungal inoculant, or a combination thereof.
(643) The bacterial inoculant can comprise a bacterial inoculant of the genus *Rhizobium*, a bacterial inoculant of the genus *Bradyrhizobium*, a bacterial inoculant of the genus *Mesorhizobium*, a bacterial inoculant of the genus *Azorhizobium*, a bacterial inoculant of the genus *Allorhizobium*, a bacterial inoculant of the genus *Sinorhizobium*, a bacterial inoculant of the genus *Kluyvera*, a bacterial inoculant of the genus *Azotobacter*, a bacterial inoculant of the genus *Pseudomonas*, a bacterial inoculant of the genus *Azospirillium*, a bacterial inoculant of the genus *Bacillus*, a bacterial inoculant of the genus *Streptomyces*, a bacterial inoculant of the genus *Paenibacillus*, a bacterial inoculant of the genus *Paracoccus*, a bacterial inoculant of the genus *Enterobacter*, a bacterial inoculant of the genus *Alcaligenes*, a bacterial inoculant of the genus *Mycobacterium*, a bacterial inoculant of the genus *Trichoderma*, a bacterial inoculant of the genus *Gliocladium*, a bacterial inoculant of the genus *Glomus*, a bacterial inoculant of the genus *Klebsiella*, or a combination thereof.
(644) The bacterial inoculant can comprise a plant-growth promoting strain of bacteria. The plant-growth promoting strain of bacteria can produce an insecticidal toxin, produce a fungicidal compound, produce a nematocidal compound, produce a bacteriocidal compound, can be resistant to one or more antibiotics, can comprise one or more freely replicating plasmids, bind to plant roots, colonize plant roots, form biofilms, solubilize nutrients, secrete organic acids, or combinations thereof.
(645) For example, the bacterial inoculant can comprise *Bacillus aryabhattai* CAP53 (NRRL No. B-50819), *Bacillus aryabhattai* CAP56 (NRRL No. B-50817), *Bacillus flexus* BT054 (NRRL No. B-50816), *Paracoccus kondratievae* NC35 (NRRL No. B-50820), *Bacillus mycoides* BT155 (NRRL No. B-50921), *Enterobacter cloacae*

CAP12 (NRRL No. B-50822), *Bacillus nealsonii* BOBA57 (NRRL No. NRRL B-50821), *Bacillus mycoides* EE118 (NRRL No. B-50918), *Bacillus subtilis* EE148 (NRRL No. B-50927), *Alcaligenes faecalis* EE107 (NRRL No. B-50920), *Bacillus mycoides* EE141 (NRRL NO. B-50916), *Bacillus mycoides* BT46-3 (NRRL No. B-50922), *Bacillus cereus* family member EE128 (NRRL No. B-50917), *Bacillus thuringiensis* BT013A (NRRL No. B-50924), *Paenibacillus massiliensis* BT23 (NRRL No. B-50923), *Bacillus cereus* family member EE349 (NRRL No. B-50928), *Bacillus subtilis* EE218 (NRRL No. B-50926), *Bacillus megaterium* EE281 (NRRL No. B-50925), *Bacillus cereus* family member EE-B00377 (NRRL B-67119); *Bacillus pseudomycoides* EE-B00366 (NRRL B-67120), *Bacillus mycoides* EE-B00363 (NRRL B-67121), *Bacillus pumilus* EE-B00143 (NRRL B-67123), or *Bacillus thuringiensis* EE-B00184 (NRRL B-67122) or a combination thereof. Each of these strains was deposited with the United States Department of Agriculture (USDA) Agricultural Research Service (ARS), having the address 1815 North University Street, Peoria, Illinois 61604 U.S.A., on Mar. 7, 2013 (*Bacillus aryabhattai* CAP53, *Bacillus aryabhattai* CAP56, *Bacillus flexus* BT054, *Paracoccus kondratievae* NC35, *Enterobacter cloacae* CAP12, and *Bacillus nealsonii* BOBA57), on Mar. 10, 2014 (*Bacillus mycoides* BT155, *Bacillus mycoides* EE118, *Bacillus subtilis* EE148, *Alcaligenes faecalis* EE107, *Bacillus mycoides* EE141, *Bacillus mycoides* BT46-3, *Bacillus cereus* family member EE128, *Bacillus thuringiensis* BT013A, *Paenibacillus massiliensis* BT23, *Bacillus cereus* family member EE349, *Bacillus subtilis* EE218, and *Bacillus megaterium* EE281), or on Aug. 19, 2015 (*Bacillus cereus* family member EE-B00377; *Bacillus pseudomycoides* EE-B00366, *Bacillus mycoides* EE-B00363, *Bacillus pumilus* EE-B00143, or *Bacillus thuringiensis* EE-B00184) and is identified by the NRRL numbers provided in parentheses.

(646) These plant-growth promoting strains were isolated from the rhizospheres of various vigorous plants and were identified by their 16S rRNA sequences, and through biochemical assays. The strains were identified at least to their genus designation by means of conventional biochemistry and morphological indicators. Biochemical assays for confirmed Gram-negative strains such as *Paracoccus kondratievae*, *Alcaligenes faecalis*, and *Enterobacter cloacae* included growth on MacConkey medium and nutrient agar, microscopic examination, growth on 5% and 7.5% NaCl medium, growth at pH 5 and pH 9, growth at 42° C. and 50° C., the ability to produce acid upon fermentation with cellobiose, lactose, glycerol, glucose, sucrose, d-mannitol, and starch; fluorescent pigment production; gelatin hydrolysis; nitrate reduction; starch hydrolysis; oxidase reaction, catalase production, urease production and motility. Similarly, the biochemical assays for confirmed Gram-positive strains such as *Bacillus* and *Paenibacillus* included growth on PEA medium and nutrient agar, microscopic examination, growth on 5% and 7.5% NaCl medium, growth at pH 5 and pH 9, growth at 42° C. and 50° C., the ability to produce acid upon fermentation with cellobiose, lactose, glycerol, glucose, sucrose, d-mannitol, and starch; fluorescent pigment production; gelatin hydrolysis; nitrate reduction; catalase production, starch hydrolysis; oxidase reaction, urease production and motility. Identification of these strains and demonstration of their plant-growth promoting effects are described further in the Examples hereinbelow. Partial 16S rRNA sequences for the strains *Bacillus mycoides* BT155, *Bacillus mycoides* EE118, *Bacillus mycoides* EE141, *Bacillus mycoides* BT46-3, *Bacillus cereus* family member EE128, *Bacillus thuringiensis* BT013A, and *Bacillus cereus* family member EE349 are provided in Table 12 above. Partial 16S rRNA sequences for the strains *Bacillus aryabhattai* CAP53, *Bacillus aryabhattai* CAP56, *Bacillus flexus* BT054, *Paracoccus kondratievae* NC35, *Enterobacter cloacae* CAP12, *Bacillus nealsonii* BOBA57, *Bacillus subtilis* EE148, *Alcaligenes faecalis* EE107, *Paenibacillus massiliensis* BT23, *Bacillus subtilis* EE218, and *Bacillus megaterium* EE281 are listed in Table 15 below.

(647) TABLE-US-00015 TABLE 15 Partial 16S rRNA sequences for additional plant-growth promoting strains of bacteria SEQ ID NO. for partial 16S Strain ribosomal RNA sequence *Bacillus aryabhattai* CAP53 287 *Bacillus aryabhattai* CAP56 288 *Bacillus flexus* BT054 289 *Paracoccus kondratievae* NC35 290 *Enterobacter cloacae* CAP12 291 *Bacillus nealsonii* BOBA57 292 *Bacillus subtilis* EE148 293 *Alcaligenes faecalis* EE107 294 *Paenibacillus massiliensis* BT23 295 *Bacillus subtilis* EE218 296 *Bacillus megaterium* EE281 297

(648) For example, the formulation can comprise a plant-growth promoting strain of bacteria comprising *Paracoccus kondratievae* NC35, *Bacillus aryabhattai* CAP53, or *Bacillus megaterium* EE281, wherein the formulation further comprises any of the recombinant *Bacillus cereus* family members described herein, including any of the recombinant plant-growth promoting *Bacillus cereus* family member strains herein (e.g., recombinant *Bacillus mycoides* BT155, *Bacillus mycoides* EE141, or *Bacillus thuringiensis* BT013A).

(649) The fungal inoculant can comprise a fungal inoculant of the family Glomeraceae, a fungal inoculant of the family Claroidoglomeraceae, a fungal inoculant of the family Gigasporaceae, a fungal inoculant of the family Acaulosporaceae, a fungal inoculant of the family Sacculosporaceae, a fungal inoculant of the family Entrophosporaceae, a fungal inoculant of the family Pacidsporaceae, a fungal inoculant of the family Diversisporaceae, a fungal inoculant of the family Paraglomeraceae, a fungal inoculant of the family Archaeosporaceae, a fungal inoculant of the family Geosiphonaceae, a fungal inoculant of the family Ambisporaceae, a fungal inoculant of the family Scutellosporaceae, a fungal inoculant of the family Dentiscultataceae, a fungal inoculant of the family Racocetraceae, a fungal inoculant of the phylum Basidiomycota,

a fungal inoculant of the phylum Ascomycota, a fungal inoculant of the phylum Zygomycota, or a combination thereof.

(650) The spore-forming bacterium, alone or in combination with the insecticide, can further comprise an effective amount of at least one fungicide.

(651) Typical fungicidal ingredients also include Captan (N-trichloromethyl)thio-4-cyclohexane-1,2-dicarboximide), Fludioxoni 1 (4-(2,2-difluoro-1,3-benzodioxol-4-yl)-1-H-pyrrol-3-carbonitril; carbendazim iprodione (commercially available under the tradename Rovral®), tebuconazole, thiabendazole, azoxystrobin, prochloraz, and Oxadixyl (N-(2,6-dimethylphenyl)-2-methoxy-N-(2-oxo-3-oxazolidinyl) acetamide).

(652) If a formulation, plant seed, or inoculum comprises a fungicide, the fungicide can comprise aldimorph, ampropylfos, ampropylfos potassium, andoprim, anilazine, azaconazole, azoxystrobin, benalaxyl, benodanil, benomyl, benzamacril, benzamacryl-isobutyl, bialaphos, binapacryl, biphenyl, bitertanol, blasticidin-S, boscalid, bromuconazole, bupirimate, buthiobate, calcium polysulphide, capsimycin, captafol, captan, carbendazim, carvon, quinomethionate, chlobenthiazone, chlorfenazole, chloroneb, chloropicrin, chlorothalonil, chlozolinate, clozylacon, cufraneb, cymoxanil, cyproconazole, cyprodinil, cyprofuram, debacarb, dichlorophen, diclobutrazole, diclofluanid, diclomezine, dicloran, diethofencarb, dimethirimol, dimethomorph, dimoxystrobin, diniconazole, diniconazole-M, dinocap, diphenylamine, dipyrithione, ditalimfos, dithianon, dodemorph, dodine, drazoxolon, edifenphos, epoxiconazole, etaconazole, ethirimol, etridiazole, famoxadon, fenapanil, fenarimol, fenbuconazole, fenfuram, fenitropan, fenpiclonil, fenpropidin, fenpropimorph, fentin acetate, fentin hydroxide, ferbam, ferimzone, fluazinam, flumetover, fluoromide, fluquinconazole, flurprimidol, flusilazole, flusulfamide, flutolanil, flutriafol, folpet, foscetyl-aluminium, fosetyl-sodium, fthalide, fuberidazole, furalaxyl, furametpyr, furcarbonil, furconazole, furconazole-cis, furmecyclox, guazatine, hexachlorobenzene, hexaconazole, hymexazole, imazalil, imibenconazole, iminoctadine, iminoctadine albesilate, iminoctadine triacetate, iodocarb, iprobenfos (IBP), iprodione, irumamycin, isoprothiolane, isovaledione, kasugamycin, kresoxim-methyl, copper preparations, such as: copper hydroxide, copper naphthenate, copper oxychloride, copper sulphate, copper oxide, oxine-copper and Bordeaux mixture, mancopper, mancozeb, maneb, meferimzone, mepanipyrim, mepronil, metalaxyl, metconazole, methasulfocarb, methfuroxam, metiram, metomeclam, metsulfovax, mildiomycin, myclobutanil, myclozolin, nickel dimethyldithiocarbamate, nitrothal-isopropyl, nuarimol, ofurace, oxadixyl, oxamocarb, oxolinic acid, oxycarboxim, oxyfenthiin, paclobutrazole, pefurazoate, penconazole, pencycuron, phosdiphen, pimaricin, piperalin, polyoxin, polyoxorim, probenazole, prochloraz, procymidonc, propamocarb, propanosine-sodium, propiconazole, propineb, prothiocinazole, pyrazophos, pyrifenox, pyrimethanil, pyroquilon, pyroxyfur, quinconazole, quintozene (PCNB), sulphur and sulphur preparations, tebuconazole, tecloftalam, tecnazene, tetcyclasis, tetraconazole, thiabendazole, thicyofen, thifluzamide, thiophanate-methyl, tioxymid, tolclofos-methyl, tolylfluanid, triadimefon, triadimenol, triazbutil, triazoxide, trichlamide, tricyclazole, tridemorph, trifloxystrobin, triflumizole, triforine, uniconazole, validamycin A, vinclozolin, viniconazole, zarilamide, zineb, ziram and also Dagger G, OK-8705, OK-8801, a-(1,1-dimethylethyl)-(3-(2-phenoxyethyl)-1H-1,2,4-triazole-1-ethanol, a-(2,4-dichlorophenyl)-[3-fluoro-3-propyl-1H-1,2,4-triazole-1-ethanol, a-(2,4-dichlorophenyl)-[3-methoxy-a-methyl-1H-1,2,4-triazole-1-ethanol, a-(5-methyl-1,3-dioxan-5-yl)-[3-[[4-(trifluoromethyl)-phenyl]-methylene]-1H-1,2,4-triazole-1-ethanol, (5RS,6RS)-6-hydroxy-2,2,7,7-tetramethyl-5-(1H-1,2,4-triazol-1-yl)-3-octanone, (E)-a-(methoxyimino)-N-methyl-2-phenoxy-phenylacetamide, 1-isopropyl{2-methyl-1-[[[1-(4-methylphenyl)-ethyl]-amino]-carbonyl]-propyl}carbamate, 1-(2,4-dichlorophenyl)-2-(1H-1,2,4-triazol-1-yl)-ethanone-O-(phenyl methyl)-oxime, 1-(2-methyl-1-naphthalenyl)-1H-pyrrole-2,5-dione, 1-(3,5-dichlorophenyl)-3-(2-propenyl)-2,5-pyrrolidindione, 1-[(diiodomethyl)-sulphonyl]-4-methyl-benzene, 1-[[2-(2,4-dichlorophenyl)-1,3-dioxolan-2-yl]-methyl]-1H-imidazole, 1-[[2-(4-chlorophenyl)-3-phenyloxiranyl]-methyl]-1H-1,2,4-triazole, 1-[1-[2-[(2,4-dichlorophenyl)-methoxy]-phenyl]-ethenyl]-1H-imidazole, 1-methyl-5-nonyl-2-(phenylmethyl)-3-pyrrolidinole, 2′,6′-dibromo-2-methyl-4′-trifluoromethoxy-4′-trifluoro-methyl-1,3-thiazole-carboxanilide, 2,2-dichloro-N-[1-(4-chlorophenyl)-ethyl]-1-ethyl-3-methyl-cyclopropanecarboxamide, 2,6-dichloro-5-(methylthio)-4-pyrimidinyl-thiocyanate, 2,6-dichloro-N-(4-trifluoromethylbenzyl)-benzamide, 2,6-dichloro-N-[[4-(trifluoromethyl)-phenyl]-methyl]-benzamide, 2-(2,3,3-triiodo-2-propenyl)-2H-tetrazole, 2-[(1-methylethyl)-sulphonyl]-5-(trichloromethyl)-1,3,4-thiadiazole, 2-[[6-deoxy-4-O-(4-O-methyl-(3-D-glycopyranosyl)-a-D-glucopyranosyl]-amino]-4-methoxy-1H-pyrrolo[2,3-d]pyrimidine-5-carbonitrile, 2-aminobutane, 2-bromo-2-(bromomethyl)-pentanedinitrile, 2-chloro-N-(2,3-dihydro-1,1,3-trimethyl-1H-inden-4-yl)-3-pyridinecarboxamide, 2-chloro-N-(2,6-dimethylphenyl)-N-(isothiocyanatomethyl)-acetamide, 2-phenylphenol (OPP), 3,4-dichloro-1-[4-(difluoromethoxy)-phenyl]-pyrrole-2,5-dione, 3,5-dichloro-N-[cyano[(1-methyl-2-propynyl)-oxy]-methyl]-benzamide, 3-(1,1-dimethylpropyl-1-oxo-1H-indene-2-carbonitrile, 3-[2-(4-chlorophenyl)-5-ethoxy-3-isoxazolidinyl]-pyridine, 4-chloro-2-cyano-N,N-dimethyl-5-(4-methylphenyl)-1H-imidazole-1-sulphonamide, 4-methyl-tetrazolo[1,5-a]quinazolin-5(4H)-one, 8-(1,1-dimethylethyl)-N-ethyl-N-propyl-1,4-dioxaspiro[4,5]decane-2-methanamine, 8-hydroxyquinoline sulphate, 9H-xanthene-2-[(phenylamino)-carbonyl]-9-carboxylic hydrazide, bis-(1-methylethyl)-3-methyl-4-[(3-methylbenzoyl)-oxy]-2,5-thiophenedicarboxylate, cis-1-(4-chlorophenyl)-2-(1H-1,2,4-triazol-1-yl)-cycloheptanol, cis-4-[3-[4-(1,1-

dimethylpropyl)-phenyl-2-methylpropyl]-2,6-dimethyl-morpholine hydrochloride, ethyl [(4-chlorophenyl)-azo]-cyanoacetate, potassium bicarbonate, methanetetrathiol-sodium salt, methyl 1-(2,3-dihydro-2,2-dimethyl-inden-1-yl)-1H-imidazole-5-carboxylate, methyl N-(2,6-dimethylphenyl)-N-(5-isoxazolylcarbonyl)-DL-alaninate, methyl N-(chloroacetyl)-N-(2,6-dimethylphenyl)-DL-alaninate, N-(2,3-dichloro-4-hydroxyphenyl)-1-methyl-cyclohexanecarboxamide, N-(2,6-dimethyl phenyl)-2-methoxy-N-(tetra hydro-2-oxo-3-furanyl)-acetamide, N-(2,6-dimethyl phenyl)-2-methoxy-N-(tetrahydro-2-oxo-3-thienyl)-acetamide, N-(2-chloro-4-nitrophenyl)-4-methyl-3-nitro-benzenesulphonamide, N-(4-cyclohexylphenyl)-1,4,5,6-tetrahydro-2-pyrimidinamine, N-(4-hexylphenyl)-1,4,5,6-tetrahydro-2-pyrimidinamine, N-(5-chloro-2-methylphenyl)-2-methoxy-N-(2-oxo-3-oxazolidinyl)-acetamide, N-(6-methoxy)-3-pyridinyl)-cyclopropanecarboxamide, N-[2,2,2-trichloro-1-[(chloroacetyl)-amino]-ethyl]-benzamide, N-[3-chloro-4,5-bis(2-propinyloxy)-phenyl]-N′-methoxy-methanimidamide, N-formyl-N-hydroxy-DL-alanine-sodium salt, 0,0-diethyl [2-(dipropylamino)-2-oxoethyl]-ethylphosphoramidothioate, O-methyl S-phenyl phenylpropylphosphoramidothioate, S-methyl 1,2,3-benzothiadiazole-7-carbothioate, and spiro[2H]-1-benzopyrane-2,1′(3′H)-isobenzofuran]-3′-one, N-trichloromethyl)thio-4-cyclohexane-1,2-dicarboximide, tetramethylthioperoxydicarbonic diamide, methyl N-(2,6-dimethylphenyl)-N-(methoxyacetyl)-DL-alaninate, 4-(2,2-difluoro-1,3-benzodioxol-4-yl)-1-H-pyrrol-3-carbonitril or a combination thereof.

(653) Additionally, suitable fungicides include the following: (1) a compound capable to inhibit the nucleic acid synthesis like benalaxyl, benalaxyl-M, bupirimate, chiralaxyl, clozylacon, dimethirimol, ethirimol, furalaxyl, hymexazol, metalaxyl, metalaxyl-M, ofurace, oxadixyl, oxolinic acid; (2) a compound capable to inhibit the mitosis and cell division like benomyl, carbendazim, diethofencarb, ethaboxam, fuberidazole, pencycuron, thiabendazole thiophanate-methyl, zoxamide; (3) a compound capable to inhibit the respiration for example as CI-respiration inhibitor like diflumetorim; as CII-respiration inhibitor like boscalid, fenfuram, flutolanil, furametpyr, furmecyclox, mepronil, oxycarboxine, penthiopyrad, thifluzamide; as CIII-respiration inhibitor like amisulbrom, azoxystrobin, cyazofamid, dimoxystrobin, enestrobin, famoxadone, fenamidone, fluoxastrobin, kresoxim-methyl, metominostrobin, orysastrobin, picoxystrobin, trifloxystrobin; (4) a compound capable of to act as an uncoupler like dinocap, fluazinam, meptyldinocap; (5) a compound capable to inhibit ATP production like fentin acetate, fentin chloride, fentin hydroxide; (6) a compound capable to inhibit AA and protein biosynthesis like andoprim, blasticidin-S, cyprodinil, kasugamycin, kasugamycin hydrochloride hydrate, mepanipyrim, pyrimethanil; (7) a compound capable to inhibit the signal transduction like fenpiclonil, quinoxyfen; (8) a compound capable to inhibit lipid and membrane synthesis like biphenyl, chlozolinate, edifenphos, etridiazole, iodocarb, iprobenfos, iprodione, isoprothiolane, procymidonc, propamocarb, propamocarb hydrochloride, pyrazophos, tolclofos-methyl, vinclozolin; (9) a compound capable to inhibit ergosterol biosynthesis like aldimorph, azaconazole, bitertanol, bromuconazole, cyproconazole, diclobutrazole, diniconazole, diniconazole-M, dodemorph, dodemorph acetate, epoxiconazole, etaconazole, fenarimol, fenbuconazole, fenhexamid, fenpropidin, fenpropimorph, fluquinconazole, flurprimidol, flusilazole, flutriafol, furconazole, furconazole-cis, hexaconazole, imazalil, imazalil sulfate, imibenconazole, metconazole, myclobutanil, naftifine, nuarimol, oxpoconazole, paclobutrazol, pefurazoate, penconazole, prochloraz, propiconazole, prothioconazole, pyributicarb, pyrifenox, simeconazole, spiroxamine, tebuconazole, terbinafine, tetraconazole, triadimefon, triadimenol, tridemorph, triflumizole, triforine, uniconazole, viniconazole, voriconazole; (10) a compound capable to inhibit cell wall synthesis like benthiavalicarb, bialaphos, dimethomorph, flumorph, iprovalicarb, mandipropamid, polyoxins, polyoxorim, validamycin A; (11) a compound capable to inhibit melanine biosynthesis like carpropamid, diclocymet, fenoxanil, phthalide, pyroquilon, tricyclazole; (12) a compound capable to induce a host defense like acibenzolar-S-methyl, probenazole, tiadinil; (13) a compound capable to have a multisite action like Bordeaux mixture, captafol, captan, chlorothalonil, copper naphthenate, copper oxide, copper oxychloride, copper preparations such as copper hydroxide, copper sulphate, dichlofluanid, dithianon, dodine, dodine free base, ferbam, fluorofolpet, folpet, guazatine, guazatine acetate, iminoctadine, iminoctadine albesilate, iminoctadine triacetate, mancopper, mancozeb, maneb, metiram, metiram zinc, oxine-copper, propineb, sulphur and sulphur preparations including calcium polysulphide, tolylfluanid, zineb, ziram; (14) a compound selected in the following list: (2E)-2-(2-{[6-(3-chloro-2-methylphenoxy)-5-fluoropyrimidin-4-yl]oxy}pheny-1)-2-(methoxyimino)-N-methylacetamide, (2E)-2-{2-[({[(1E)-1-(3-{[(E)-1-fluoro-2-phenylvinyl]oxy}phenyl)ethyliden-e]amino}oxy)methyl]phenyl}-2-(methoxyimino)-N-methylacetamide, 1-(4-chlorophenyl)-2-(1H-1,2,4-triazol-1-yl)cycloheptanol, 1-[(4-methoxyphenoxy)methyl]-2,2-dimethylpropyl-1H-imidazole-1-carboxylat-c, 2,3,5,6-tetrachloro-4-(methylsulfonyl)pyridine, 2-butoxy-6-iodo-3-propyl-4H-chromen-4-one, 2-chloro-N-(1,1,3-trimethyl-2,3-dihydro-1H-inden-4-yl)nicotinamide, 2-phenylphenol and salts, 3,4,5-trichloropyridine-2,6-dicarbonitrile, 3,4-dichloro-N-(2-cyanophenyl)isothiazole-5-carboxamide, 3-[5-(4-chlorophenyl)-2,3-dimethylisoxazolidin-3-yl]pyridine, 5-chloro-6-(2,4,6-trifluorophenyl)-N-[(1R)-1,2,2-trimethylpropyl][1,2,4]t-riazolo[1,5-a]pyrimidin-7-amine, 5-chloro-7-(4-methylpiperidin-1-yl)-6-(2,4,6-trifluorophenyl) [1,2,4]triazolo[1,5-a]pyrimidine, 5-chloro-N-[(1R)-1,2-dimethylpropyl]-6-(2,4,6-trifluorophenyl) [1,2,4]triazolo[1,5-a]pyrimidin-7-amine, 8-hydroxyquinoline sulfate, benthiazole, bethoxazin, capsimycin, carvone, chinomethionat, cufraneb, cyflufenamid, cymoxanil,

dazomet, debacarb, dichlorophen, diclomezine, dicloran, difenzoquat, difenzoquat methylsulphate, diphenylamine, ferimzone, flumetover, fluopicolide, fluoroimide, flusulfamide, fosetyl-aluminium, fosetyl-calcium, fosetyl-sodium, hexachlorobenzene, irumamycin, isotianil, methasulfocarb, methyl (2E)-2-{2-[({cyclopropyl[(4-methoxyphenyl)imino]methyl}thio)methyl]phenyl-}-3-methoxyacrylate, methyl 1-(2,2-dimethyl-2,3-dihydro-1H-inden-1-yl)-1H-imidazole-5-carboxylate, methyl isothiocyanate, metrafenone, mildiomycin, N-[2-(1,3-dimethylbutyl)phenyl]-5-fluoro-1,3-dimethyl-1H-pyrazole-4-carbo-xamide, N-(3′,4′-dichloro-5-fluorobiphenyl-2-yl)-3-(difluoromethyl)-1-meth-yl-1H-pyrazole-4-carboxamide, N-(3-ethyl-3,5,5-trimethylcyclohexyl)-3-(formylamino)-2-hydroxybenzamide, N-(4-chloro-2-nitrophenyl)-N-ethyl-4-methylbenzenesulfonamide, N-(4-chlorobenzyl)-3-[3-methoxy-4-(prop-2-yn-1-yloxy)phenyl]propanamide, N-[(4-chlorophenyl)(cyano)methyl]-3-[3-methoxy-4-(prop-2-yn-1-yloxy)pheny-1]propanamide, N-[(5-bromo-3-chloropyridin-2-yl)methyl]-2,4-dichloronicotinamide, N-[1-(5-bromo-3-chloropyridin-2-yl)ethyl]-2,4-dichloronicotinamide, N-[1-(5-bromo-3-chloropyridin-2-yl)ethyl]-2-fluoro-4-iodonicotinamide, N-[2-(4-{[3-(4-chlorophenyl)prop-2-yn-1-yl]oxy}-3-methoxyphenyl)ethyl]-N&-lt;-(methylsulfonyl)valinamide, N-{(Z)-[(cyclopropylmethoxy)imino][6-(difluoromethoxy)-2,3-difluorophenyl-]methyl}-2-phenylacetamide, N-{2-[1,1′-bi(cyclopropyl)-2-yl]phenyl}-3-(difluoromethyl)-, 1-methyl-1H-pyrazole-4-carboxamide, N-{2-[3-chloro-5-(trifluoromethyl)pyridin-2-yl]ethyl}-2-(trifluoromethyl)-benzamide, natamycin, N-ethyl-N-methyl-N′-{2-methyl-5-(trifluoromethyl)-4-[3-(trimethylsilyl)pr-poxy]phenyl}imidoformamide, N-ethyl-N-methyl-N′-{2-methyl-5-(difluoromethyl)-4-[3-(trimethylsilyl)propoxy]phenyl}imidoformamide, nickel dimethyldithiocarbamate, nitrothal-isopropyl, O-{1-[(4-methoxyphenoxy)methyl]-2,2-dimethylpropyl}1H-imidazole-1-carbothioate, octhilinone, oxamocarb, oxyfenthiin, pentachlorophenol and salts, phosphorous acid and its salts, piperalin, propamocarb fosetylate, propanosine-sodium, proquinazid, pyribencarb, pyrrolnitrine, quintozene, tecloftalam, tecnazene, triazoxide, trichlamide, valiphenal, zarilamid.

(654) The fungicide can comprise a substituted benzene, a thiocarbamate, an ethylene bis dithiocarbamate, a thiophthalidamide, a copper compound, an organomercury compound, an organotin compound, a cadmium compound, anilazine, benomyl, cyclohexamide, dodine, etridiazole, iprodione, metlaxyl, thiamimefon, triforine, or a combination thereof.

(655) If a formulation, plant seed, or inoculum comprises a fungicide, the fungicide can be a foliar fungicide. Foliar fungicides include copper, mancozeb, penthiopyrad, triazoles, cyproconazole, metconazole, propiconazole, prothioconazole, tebuconazole, azoxystrobin, pyraclastobin, fluoxastrobin, picoxystrobin, trifloxystrobin, sulfur, boscalid, thiophanate methyl, chlorothanonil, penthiopyrad, difenconazole, flutriafol, cyprodinil, fluzinam, iprodione, penflufen, cyazofamid, flutolanil, cymoxanil, dimethomorph, pyrimethanil, zoxamide, mandipropamid, metrinam, propamocarb, fenamidone, tetraconazole, chloronab, hymexazol, tolclofos, and fenbuconazole.

(656) If a formulation, plant seed, or inoculum comprises a bacterial inoculant of the genus *Bacillus*, the bacterial inoculant can comprise *Bacillus argri, Bacillus aizawai, Bacillus albolactis, Bacillus amyloliquefaciens, Bacillus cereus, Bacillus coagulans, Bacillus endoparasiticus, Bacillus endorhythmos, Bacillus kurstaki, Bacillus lacticola, Bacillus lactimorbus, Bacillus lactis, Bacillus laterosporus, Bacillus lentimorbus, Bacillus licheniformis, Bacillus megaterium, Bacillus medusa, Bacillus metiens, Bacillus natto, Bacillus nigrificans, Bacillus popillae, Bacillus pumilus, Bacillus siamensis, Bacillus sphearicus, Bacillus* spp., *Bacillus subtilis, Bacillus thuringiensis, Bacillus unifagellatu*, or a combination thereof plus those listed in the category of *Bacillus* Genus in Bergey's Manual of Systematic Bacteriology, First Ed. (1986), hereby incorporated in full by reference.

(657) If a formulation, plant seed, or inoculum comprises an insecticide, the insecticide can be a nematicide. Suitable nematicides include antibiotic nematicides such as abamectin; carbamate nematicides such as acetoprole, *Bacillus chitonosporus*, chloropicrin, benclothiaz, benomyl, *Burholderia cepacia*, carbofuran, carbosulfan, and cleothocard; dazomet, DBCP, DCIP, alanycarb, aldicarb, aldoxycarb, oxamyl, diamidafos, fenamiphos, fosthietan, phosphamidon, cadusafos, chlorpyrifos, diclofenthion, dimethoate, ethoprophos, fensulfothion, fostiazate, harpins, heterophos, imicyafos, isamidofos, isazofos, methomyl, mecarphon, *Myrothecium verrucaria, Paecilomyces lilacinus*, phorate, phosphocarb, terbufos, thionazin, triazophos, dazomet, 1,2-dicloropropane, 1,3-dichloropropene, furfural, iodomethane, metam, methyl bromide, methyl isothiocyanate, and xylenols.

(658) For example and without limitation, the nematicide and insecticide can be provided in the form of the commercial product Avicta Duo, which is a mixture of abamectin and thiamethoxam commercially available from Syngenta.

(659) If a formulation, plant seed, or inoculum comprises a bactericide, it may include streptomycin, penicillins, tetracyclines, ampicillin, and oxolinic acid.

(660) The fertilizer can comprise a liquid fertilizer. The micronutrient fertilizer material can comprise boric acid, a borate, a boron frit, copper sulfate, a copper frit, a copper chelate, a sodium tetraborate decahydrate, an iron sulfate, an iron oxide, iron ammonium sulfate, an iron frit, an iron chelate, a manganese sulfate, a manganese oxide, a manganese chelate, a manganese chloride, a manganese frit, a sodium molybdate, molybdic acid, a zinc sulfate, a zinc oxide, a zinc carbonate, a zinc frit, zinc phosphate, a zinc chelate, or a combination thereof.

(661) The fertilizer can comprise ammonium sulfate, ammonium nitrate, ammonium sulfate nitrate, ammonium chloride, ammonium bisulfate, ammonium polysulfide, ammonium thiosulfate, aqueous ammonia, anhydrous ammonia, ammonium polyphosphate, aluminum sulfate, calcium nitrate, calcium ammonium nitrate, calcium sulfate, calcined magnesite, calcitic limestone, calcium oxide, calcium nitrate, dolomitic limestone, hydrated lime, calcium carbonate, diammonium phosphate, monoammonium phosphate, magnesium nitrate, magnesium sulfate, potassium nitrate, potassium chloride, potassium magnesium sulfate, potassium sulfate, sodium nitrates, magnesian limestone, magnesia, urea, urea-formaldehydes, urea ammonium nitrate, sulfur-coated urea, polymer-coated urea, isobutylidene diurea, K.sub.2SO.sub.4-2MgSO.sub.4, kainite, sylvinite, kieserite, Epsom salts, elemental sulfur, marl, ground oyster shells, fish meal, oil cakes, fish manure, blood meal, rock phosphate, super phosphates, slag, bone meal, wood ash, manure, bat guano, peat moss, compost, green sand, cottonseed meal, feather meal, crab meal, fish emulsion, humic acid, or a combination thereof.
(662) A formulation, plant seed, or inoculum can also include at least one biological control agent selected from (1) bacteria, in particular spore-forming bacteria, (2) fungi or yeasts, and (3) isoflavones. Preference is given to combinations comprising as biological control agent a bacterium, in particular a spore-forming, root-colonizing bacterium, or a bacterium useful as biofungicide, selected from the group consisting of [Group (1)]: (1.1) *Bacillus agri*, (1.2) *Bacillus aizawai*, (1.3) *Bacillus albolactis*, (1.4) *Bacillus amyloliquefaciens*, (1.5) *Bacillus cereus*, (1.6) *Bacillus coagulans*, (1.7) *Bacillus endoparasiticus*, (1.8) *Bacillus endorhythmos*, (1.9), (1.10) *Bacillus kurstaki*, (1.11) *Bacillus lacticola*, (1.12) *Bacillus lactimorbus*, (1.13) *Bacillus lactis*, (1.14) *Bacillus laterosporus*, (1.15) *Bacillus lentimorbus*, (1.16) *Bacillus licheniformis*, (1.17) *Bacillus medusa*, (1.18) *Bacillus megaterium*, (1.19) *Bacillus metiens*, (1.20) *Bacillus natto*, (1.21) *Bacillus nigrificans*, (1.22) *Bacillus popillac*, (1.23) *Bacillus pumilus*, (1.24) *Bacillus siamensis*, (1.25) *Bacillus sphaericus* (products known as VectoLex.sup.S), (1.26) *Bacillus subtilis*, or *B. subtilis* var. *amyloliquefaciens*, (1.27) *Bacillus thuringiensis*, in particular *B. thuringiensis* var. *israelensis* (products known as VectoBac®) or *B. thuringiensis* subsp. *aizawai* strain ABTS-1857 (products known as XenTari), or *B. thuringiensis* subsp. *kurstaki* strain HD-1 (products known as Dipel ES), (1.28) *Bacillus uniflagellatus*, (1.29) *Delftia acidovorans*, in particular strain RAY209 (products known as BioBoost), (1.30) *Lysobacter antibioticus*, in particular strain 13-1 (Biological Control 2008, 45, 288-296), (1.31) *Lysobacter enzymogenes*, in particular strain 3.1T8, (1.32) *Pseudomonas chlororaphis*, in particular strain MA 342 (products known as Cedomon), (1.33) *Pseudomonas* proradix (products known as Proradix®), (1.34) *Streptomyces galbus*, in particular strain K61 (products known as Mycostop®, cf. Crop Protection 2006, 25, 468-475), (1.35) *Streptomyces griseoviridis* (products known as Mycostop®).
(663) Preference is further given to combinations comprising as biological control agent a fungus or a yeast selected from the group consisting of [Group (2)]: (2.1) *Ampelomyces quisqualis*, in particular strain AQ 10 (product known as AQ 10®), (2.2) *Aureobasidium pullulans*, in particular blastospores of strain DSM14940 or blastospores of strain DSM 14941 or mixtures thereof (product known as Blossom Protect®), (2.3) *Beauveria bassiana*, in particular strain ATCC 74040 (products known as Naturalis®), (2.4) *Candida oleophila*, in particular strain O (products known as Nexy), (2.5) *Cladosporium cladosporioides* H39 (cf. Eur. J. Plant Pathol. 2009, 123, 401-414), (2.6), (2.7) *Dilophosphora alopecuri* (products known as Twist Fungus), (2.8) *Gliocladium catenulatum*, in particular strain J1446 (products known as Prestop), (2.9) *Lecanicillium lecanii* (formerly known as *Verticillium lecanii*), in particular conidia of strain KV01 (products known as Mycotal®, Vertalec®), (2.10) *Metarhizium anisopliea* (products known as BIO 1020), (2.11) *Metschnikovia fructicola*, in particular the strain NRRL Y-30752 (products known as Shemer™), (2.12) *Microsphaeropsis ochracea* (products known as Microx), (2.13), (2.14) *Nomuraea rileyi*, (2.15), (2.16) *Penicillium bilaii*, in particular strain ATCC22348 (products known as JumpStart®, PB-50, Provide), (2.17) *Pichia anomala*, in particular strain WRL-076, (2.18) *Pseudozyma flocculosa*, in particular strain PF-A22 UL (products known as Sporodex L), (2.19) *Pythium oligandrum* DV74 (products known as Polyversum), (2.20) *Trichoderma asperellum*, in particular strain ICC 012 (products known as Bioten), (2.21) *Trichoderma harzianum*, in particular *T. harzianum* T39 (products known e.g. as Trichodex).
(664) Preference is further given to combinations comprising as biological control agent an isoflavone selected from the group consisting of [Group (3)]: (3.1) genistein, (3.2) biochanin A10, (3.3) formononetin, (3.4) daidzein. (3.5) glycitein, (3.6) hesperetin, (3.7) naringenin, (3.8) chalcone, (3.9) coumarin, (3.10) Ambiol (2-methyl-4-dimethylaminomethyl-5-hydroxybenzimidazol dihydrochloride) (3.11) ascorbate and (3.12) pratensein and the salts and esters thereof.
(665) If a formulation, plant seed, or inoculum comprises an insecticide, the insecticide can include pyrethroids, organophosphates, caramoyloximes, pyrazoles, amidines, halogenated hydrocarbons, neonicotinoids, and carbamates and derivatives thereof. Particularly suitable classes of insecticides include organophosphates, phenylpyrazoles and pyrethoids. Preferred insecticides are those known as terbufos, chlorpyrifos, chlorethoxyfos, tefluthrin, carbofuran, and tebupirimfos. Commercially available insecticides include thiomethoxam (commercially available from Syngenta under the tradename Cruiser.
(666) The insecticide can comprise an organophosphate, a carbamate, a pyrethroid, an acaricide, an alkyl phthalate,

boric acid, a borate, a fluoride, sulfur, a haloaromatic substituted urea, a hydrocarbon ester, a biologically-based insecticide, or a combination thereof.

(667) Suitable insecticides for use herein also include the following: (1) acetylcholine receptor agonists/antagonists such as chloronicotinyls/nconicotinoids, nicotine, bensultap or cartap. Suitable examples of chloronicotinyls/neonicotinoids include acetamiprid, dinotefuran, nitenpyram, nithiazine, thiacloprid, thiamethoxam, imidaclothiz and (2E)-1-[(2-chloro-1,3-thiazol-5-yl)methyl]-3,5-dimethyl-N-nitro-1,3,5-tri-azinan-2-imine; (2) acetylcholinesterase (ACNE) inhibitors such as carbamates and organophosphates. Suitable examples of carbamates include alanycarb, aldicarb, aldoxycarb, allyxycarb, aminocarb, bendiocarb, benfuracarb, bufencarb, butacarb, butocarboxim, butoxycarboxim, carbaryl, carbofuran, carbosulfan, chlocthocarb, dimetilan, ethiofencarb, fenobucarb, fenothiocarb, formetanate, furathiocarb, isoprocarb, metam-sodium, methomyl, metolcarb, oxamyl, phosphocarb, pirimicarb, promecarb, propoxur, thiofanox, triazamate, trimethacarb, XMC and xylylcarb. Suitable examples of organophosphates include acephate, azamethiphos, azinphos (-methyl, -ethyl), bromophos-ethyl, bromfenvinfos (-methyl), butathiofos, cadusafos, carbophenothion, chlorethoxyfos, chlorfenvinphos, chlormephos, chlorpyrifos (-methyl/-ethyl), coumaphos, cyanofenphos, cyanophos, demeton-S-methyl, demeton-S-methylsulphon, dialifos, diazinon, dichlofenthion, dichlorvos/DDVP, dicrotophos, dimethoate, dimethylvinphos, dioxabenzofos, disulfoton, EPN, cthion, cthoprophos, ctrimfos, famphur, fenamiphos, fenitrothion, fensulfothion, fenthion, flupyrazofos, fonofos, formothion, fosmethilan, fosthiazate, heptenophos, iodofenphos, iprobenfos, isazofos, isofenphos, isopropyl O-salicylate, isoxathion, malathion, mecarbam, methacrifos, methamidophos, methidathion, mevinphos, monocrotophos, naled, omethoate, oxydemeton-methyl, parathion (-methyl/-ethyl), phenthoate, phorate, phosalone, phosmet, phosphamidon, phosphocarb, phoxim, pirimiphos (-methyl/-ethyl), profenofos, propaphos, propetamphos, prothiofos, prothoate, pyraclofos, pyridaphenthion, pyridathion, quinalphos, sebufos, sulfotep, sulprofos, tebupirimfos, temephos, terbufos, tetrachlorvinphos, thiometon, triazophos, triclorfon and vamidothion; (3) sodium channel modulators/voltage-gated sodium channel blockers such as pyrethroids and oxadiazines. Suitable examples of pyrethroids include acrinathrin, allethrin (d-cis-trans, d-trans), beta-cyfluthrin, bifenthrin, bioallethrin, bioallethrin-S-cyclopentyl-isomer, bioethanomethrin, biopermethrin, bioresmethrin, chlovaporthrin, cis-resmethrin, cis-permethrin, clocythrin, cycloprothrin, cyfluthrin, cyhalothrin, cyphenothrin, DDT, deltamethrin, empenthrin (1R-isomer), esfenvalerate, etofenprox, fenfluthrin, fenpropathrin, fenpyrithrin, fenvalerate, flubrocythrinate, flucythrinate, flufenprox, flumethrin, fluvalinate, fubfenprox, gamma-cyhalothrin, imiprothrin, kadethrin, lambda-cyhalothrin, metofluthrin, permethrin (cis-, trans-), phenothrin (1R-trans isomer), prallethrin, profluthrin, protrifenbute, pyresmethrin, resmethrin, RU 15525, silafluofen, tau-fluvalinate, tefluthrin, terallethrin, tetramethrin (1R-isomer), tralocythrin, tralomethrin, transfluthrin, ZXI 8901 and pyrethrins (pyrethrum). Suitable example of oxadiazines includes indoxacarb; (4) acetylcholine receptor modulators such as spinosyns. Suitable example of spinosyns includes spinosad; (5) GABA-gated chloride channel antagonists such as cyclodiene organochlorines and fiproles. Suitable examples of cyclodiene organochlorines include camphechlor, chlordane, endosulfan, gamma-HCH, HCH, heptachlor, lindane and methoxychlor. Suitable examples of fiproles include acetoprole, and vaniliprole; (6) chloride channel activators such as mectins. Suitable examples of mectins include abamectin, avermectin, emamectin, emamectin-benzoate, ivermectin, lepimectin, milbemectin and milbemycin; (7) juvenile hormone mimetics such as diofenolan, epofenonane, fenoxycarb, hydroprene, kinoprene, methoprene, pyriproxifen, triprene; (8) ecdysone agonists/disruptors such as diacylhydrazines. Suitable examples of diacylhydrazines include chromafenozide, halofenozide, methoxyfenozide and tebufenozide; (9) inhibitors of chitinbiosynthesis such as benzoylureas, buprofezin and cyromazine. Suitable examples of benzoylureas include bistrifluron, chlofluazuron, diflubenzuron, fluazuron, flucycloxuron, flufenoxuron, hexaflumuron, lufenuron, novaluron, noviflumuron, penfluron, teflubenzuron and triflumuron; (10) inhibitors of oxidative phosphorylation, ATP disruptors such as organotins and diafenthiuron. Suitable examples of organotins include azocyclotin, cyhexatin and fenbutatin oxide; (11) decouplers of oxidative phosphorylation by disruption of the H proton gradient such as pyrroles and dinitrophenols. Suitable example of pyrroles includes chlorfenapyr. Suitable examples of dinitrophenols include binapacyrl, dinobuton, dinocap and DNOC; (12) site I electron transport inhibitors such as METIs, hydramethylnone and dicofol. Suitable examples of METIs include fenazaquin, fenpyroximate, pyrimidifen, pyridaben, tebufenpyrad, tolfenpyrad; (13) site II electron transport inhibitors such as rotenone; (14) site III electron transport inhibitors such as acequinocyl and fluacrypyrim; (15) microbial disrupters of the intestinal membrane of insects such as *Bacillus thuringiensis* strains; (16) inhibitors of lipid synthesis such as tetronic acids and tetramic acids. Suitable examples of tetronic acids include spirodiclofen, spiromesifen and spirotetramat. Suitable example of tetramic acids includes cis-3-(2,5-dimethylphenyl)-8-methoxy-2-oxo-1-azaspiro[4.5]dec-3-en-4-yl ethyl carbonate (alias: carbonic acid, 3-(2,5-dimethylphenyl)-8-methoxy-2-oxo-1-azaspiro[4.5]dec-3-en-4-yl ethyl ester (CAS Reg. No.: 382608-10-8); (17) carboxamides such as flonicamid; (18) octopaminergic agonists such as amitraz; (19) inhibitors of the magnesium-stimulated ATPase such as propargite; (20) ryanodin receptor agonists such as phthalamides or rynaxapyr. Suitable example of phthalamides includes N.sup.2-[1,1-dimethyl-2-(methylsulphonyl)ethyl]-3-iodo-N.sup.1-[2-methyl-4-[1,2,2,2-tetrafluoro-1-(trifluoromethyl)ethyl]phenyl]-1,2-

benzenedicarbo-xamide (i.e. flubendiamide, CAS reg. No.: 272451-65-7); (21) nercistoxin analogues such as thiocyclam hydrogen oxalate and thiosultap-sodium; (22) biologics, hormones or pheromones such as azadirachtin, *Bacillus* spec., *Beauveria* spec., codlemone, *Metarrhizium* spec., *Paecilomyces* spec., *thuringiensis* and *Verticillium* spec; (23) active compounds having unknown or non-specified mechanisms of action such as fumigants, selective feeding inhibitors, mite growth inhibitors, amidoflumet; benclothiaz, benzoximate, bifenazate, bromopropylate, buprofezin, chinomethioat, chlordimeform, chlorobenzilate, chloropicrin, clothiazoben, cycloprene, cyflumetofen, dicyclanil, fenoxacrim, fentrifanil, flubenzimine, flufenerim, flutenzin, gossyplure, hydramethylnone, japonilure, metoxadiazone, petroleum, piperonyl butoxide, potassium oleate, pyrafluprole, pyridalyl, pyriprole, sulfluramid, tetradifon, tetrasul, triarathene, verbutin, furthermore the compound 3-methylphenyl propylcarbamate (Tsumacide Z), the compound 3-(5-chloro-3-pyridinyl)-8-(2,2,2-trifluoroethyl)-8-azabicyclo[3.2.1]octa-ne-3-carbonitrile (CAS reg. No. 185982-80-3) and the corresponding 3-endo isomer (CAS reg. No. 185984-60-5) (cf. WO 96/37494, WO 98/25923), and also preparations comprising insecticidal effective plant extracts, nematodes, fungi or viruses. Suitable examples of fumigants include aluminium phosphide, methyl bromide and sulphuryl fluoride. Suitable examples of selective feeding inhibitors include cryolite, flonicamid and pymetrozine. Suitable examples of mite growth inhibitors include clofentezine, etoxazole and hexythiazox.

(668) Commercially available nematicidal ingredients include abamectin (commercially available from Syngenta under the tradename Avicta).

(669) If a formulation, plant seed, or inoculum comprises an herbicide, the herbicide can comprise 2,4-D, 2,4-DB, acetochlor, acifluorfen, alachlor, ametryn, atrazine, aminopyralid, benefin, bensulfuron, bensulide, bentazon, bromacil, bromoxynil, butylate, carfentrazone, chlorimuron, chlorsulfuron, clethodim, clomazone, clopyralid, cloransulam, cycloate, DCPA, desmedipham, dicamba, dichlobenil, diclofop, diclosulam, diflufenzopyr, dimethenamid, diquat, diuron, DSMA, endothall, EPTC, ethalfluralin, ethofumesate, fenoxaprop, fluazifop-P, flucarbazone, flufenacet, flumetsulam, flumiclorac, flumioxazin, fluometuron, fluroxypyr, fomesafen, foramsulfuron, glufosinate, glyphosate, halosulfuron, hexazinone, imazamethabenz, imazamox, imazapic, imazaquin, imazethapyr, isoxaben, isoxaflutole, lactofen, linuron, MCPA, MCPB, mesotrione, metolachlor-s, metribuzin, metsulfuron, molinate, MSMA, napropamide, naptalam, nicosulfuron, norflurazon, oryzalin, oxadiazon, oxyfluorfen, paraquat, pelargonic acid, pendimethalin, phenmedipham, picloram, primisulfuron, prodiamine, prometryn, pronamide, propanil, prosulfuron, pyrazon, pyrithiobac, quinclorac, quizalofop, rimsulfuron, sethoxydim, siduron, simazine, sulfentrazone, sulfometuron, sulfosulfuron, tebuthiuron, terbacil, thiazopyr, thifensulfuron, thiobencarb, tralkoxydim, triallate, triasulfuron, tribenuron, triclopyr, trifluralin, triflusulfuron, or a combination thereof.

(670) The herbicide can comprise a chlorophenoxy compound, a nitrophenolic compound, a nitrocresolic compound, a dipyridyl compound, an acetamide, an aliphatic acid, an anilide, a benzamide, a benzoic acid, a benzoic acid derivative, anisic acid, an anisic acid derivative, a benzonitrile, benzothiadiazinone dioxide, a thiocarbamate, a carbamate, a carbanilate, chloropyridinyl, a cyclohexenone derivative, a dinitroaminobenzene derivative, a fluorodinitrotoluidine compound, isoxazolidinone, nicotinic acid, isopropylamine, an isopropylamine derivative, oxadiazolinone, a phosphate, a phthalate, a picolinic acid compound, a triazine, a triazole, a uracil, a urea derivative, endothall, sodium chlorate, a sulfonylurea, an aryl triazine, or a combination thereof.

(671) The formulation can comprise an herbicide and a strain of bacteria that is capable of degrading the herbicide.

(672) The strain of bacteria that is capable of degrading an herbicide can comprise *Bacillus cereus* family member EE349 (NRRL No. B-50928), *Bacillus cereus* family member EE-B00377 (NRRL B-67119), *Bacillus pseudomycoides* EE-B00366 (NRRL B-67120), or *Bacillus mycoides* EE-B00363 (NRRL B-67121), or a combination thereof.

(673) The herbicide to be degraded can comprise a sulfonylurea such as sulfentrazone, an aryl triazine, dicamba, a phenoxy herbicide, 2,4-D, a pyrethrin, a pyrethroid, or a combination thereof

(674) Binders can be included in the formulations, such as carboxymethylcellulose and natural and synthetic polymers in the form of powders, granules, or latexes, such as gum Arabic, chitin, polyvinyl alcohol and polyvinyl acetate, as well as natural phospholipids, such as cephalins and lecithins, and synthetic phospholipids. Binders include those composed preferably of an adhesive polymer that can be natural or synthetic without phytotoxic effect on the seed to be coated. Additional binders that can be included, either alone or in combination, include, for example, polyesters, polyether esters, polyanhydrides, polyester urethanes, polyester amides; polyvinyl acetates; polyvinyl acetate copolymers; polyvinyl alcohols and tylose; polyvinyl alcohol copolymers; polyvinylpyrolidones: polysaccharides, including starches, modified starches and starch derivatives, dextrins, maltodextrins, alginates, chitosanes and celluloses, cellulose esters, cellulose ethers and cellulose ether esters including ethylcelluloses, methylcelluloses, hydroxymethylcelluloses, hydroxypropylcelluloses and carboxymethylcellulose; fats; oils; proteins, including casein, gelatin and zeins; gum arabics; shellacs; vinylidene chloride and vinylidene chloride copolymers; lignosulfonates, in particular calcium lignosulfonates; polyacrylates, polymethacrylates and acrylic copolymers; polyvinylacrylates; polyethylene oxide; polybutenes, polyisobutenes, polystyrene, polybutadiene,

polyethyleneamines, polyethylenamides; acrylamide polymers and copolymers; polyhydroxyethyl acrylate, methylacrylamide monomers; and polychloroprene.
(675) A variety of colorants may be employed, including organic chromophores classified as nitroso, nitro, azo, including monoazo, bisazo, and polyazo, diphenylmethane, triarylmethane, xanthene, methane, acridine, thiazole, thiazine, indamine, indophenol, azine, oxazine, anthraquinone, and phthalocyanine.
(676) Other additives that can be added include trace nutrients such as salts of iron, manganese, boron, copper, cobalt, molybdenum, and zinc.
(677) One or more preservatives (e.g., antimicrobial agents or other biocidal agents) may also be included for preservation and stabilization of the formulation. Examples of suitable bactericides include those based on dichlorophene and benzylalcohol hemi formal (Proxel® from ICI or Acticide® RS from Thor Chemie and Kathon® MK from Dow Chemical) and isothiazolinone derivatives such as alkylisothiazolinones and benzisothiazolinones (Acticide®) MBS from Thor Chemie). As further examples, suitable preservatives include MIT (2-methyl-4-isothiazolin-3-one), BIT (1,2-benzisothiazolin-3-one, which can be obtained from Avecia, Inc. as Proxel GXL as a solution in sodium hydroxide and dipropylene glycol), 5-chloro-2-(4-chlorobenzyl)-3(2H)-isothiazolone, 5-chloro-2-methyl-2H-isothiazol-3-one, 5-chloro-2-methyl-2H-isothiazol-3-one, 5-chloro-2-methyl-2H-isothiazol-3-one-hydrochloride, 4,5-dichloro-2-cyclohexyl-4-isothiazolin-3-one, 4,5-dichloro-2-octyl-2H-isothiazol-3-one, 2-methyl-2H-isothiazol-3-one, 2-methyl-2H-isothiazol-3-one-calcium chloride complex, 2-octyl-2H-isothiazol-3-one and benzyl alcohol hemiformal.
(678) Examples of suitable thickeners for the formulations include polysaccharides, organic clays, or a water-soluble polymer that exhibits pseudoplastic properties in an aqueous medium, such as, for example, gum arabic, gum karaya, gum tragacanth, guar gum, locust bean gum, xanthan gum, carrageenan, alginate salt, casein, dextran, pectin, agar, 2-hydroxyethyl starch, 2-aminoethyl starch, 2-hydroxy ethyl cellulose, methyl cellulose, carboxymethyl cellulose salt, cellulose sulfate salt, polyacrylamide, alkali metal salts of the maleic anhydride copolymers, alkali metal salts of poly(meth)acrylate.
(679) Suitable antifreeze ingredients for the formulation include, for example and without limitation, ethylene glycol, 1,2-propylene glycol, 1,3-propylene glycol, 1,2-butanediol, 1,3-butanediol, 1,4-butanediol, 1,4-pentanediol, 3-methyl-1,5-pentanediol, 2,3-dimethyl-2,3-butanediol, trimethylol propane, mannitol, sorbitol, glycerol, pentaerythritol, 1,4-cyclohexanedimethanol, xylenol, bisphenols such as bisphenol A or the like. In addition, ether alcohols such as diethylene glycol, triethylene glycol, tetraethylene glycol, polyoxyethylene or polyoxypropylene glycols of molecular weight up to about 4000, diethylene glycol monomethylether, diethylene glycol monoethylether, triethylene glycol monomethylether, butoxyethanol, butylene glycol monobutylether, dipentaerythritol, tripentaerythritol, tetrapentaerythritol, diglycerol, triglycerol, tetraglycerol, pentaglycerol, hexaglycerol, heptaglycerol, octaglycerol and combinations thereof.
XVIII. Plant Seeds
(680) The present invention further relates to plant seeds coated with any of the recombinant *Bacillus cereus* family members described herein, with any of the recombinant spore-forming bacteria described herein, with any of the biologically pure bacterial cultures described herein, with any of the inoculums described herein, with any enzyme that catalyzes the production of nitric oxide, with any recombinant microorganism that expresses an enzyme that catalyzes the production of nitric oxide, or with any of the formulations other than vaccines as described herein.
XIX. Methods Relating to Plants and Plant Seeds, Methods for Delaying Germination of a Spore of a Recombinant *Bacillus cereus* Family Member, and Methods for Making and Using Exosporium Fragments
(681) The present invention further relates to methods for stimulating plant growth, methods for protecting a plant from a pathogen or enhancing stress resistance in a plant, methods for immobilizing recombinant *Bacillus cereus* family member spores or recombinant spore forming bacteria on a plant, methods for stimulating germination of a plant seed, methods for delivering nucleic acids to plants, methods for delaying germination of a spore of a recombinant *Bacillus cereus* family member, methods for making and using exosporium fragments, and methods for delivering beneficial bacteria to animals.
(682) A. Methods for Stimulating Plant Growth
(683) The present invention relates to methods for stimulating plant growth.
(684) One method for stimulating plant growth of the present invention comprises introducing into a plant growth medium any of the recombinant *Bacillus cereus* family members described above or any of the formulations comprising a recombinant *Bacillus cereus* family member described above. Alternatively, any of the recombinant *Bacillus cereus* family members described above or any of the formulations comprising a recombinant *Bacillus cereus* family member described above can be applied to a plant, a plant seed, or to an area surrounding a plant or a plant seed. In such methods, the recombinant *Bacillus cereus* family member expresses a fusion protein comprising a plant growth stimulating protein or peptide. The plant growth stimulating protein or peptide can be physically attached to the exosporium of the recombinant *Bacillus cereus* family member.
(685) Another method for stimulating plant growth comprises introducing into a plant growth medium any of the

recombinant spore-forming bacteria described above or any of the formulations comprising a recombinant spore-forming bacterium described above. Alternatively, any of the recombinant spore-forming bacteria described above or any of the formulations comprising a recombinant spore-forming bacterium described above can be applied to a plant, a plant seed, or to an area surrounding a plant or a plant seed. In such methods, the recombinant spore-forming bacterium expresses a fusion protein comprising a plant growth stimulating protein or peptide. The plant growth stimulating protein or peptide can be physically attached to the spore coat of the recombinant spore-forming bacterium.

(686) Yet another method for stimulating plant growth comprises introducing into a plant growth medium a recombinant *Bacillus cereus* family member or a formulation comprising a recombinant *Bacillus cereus* family member. Alternatively, the recombinant *Bacillus cereus* family member or the formulation can be applied to a plant, a plant seed, or to an area surrounding a plant or a plant seed. The recombinant *Bacillus cereus* family member expresses an enzyme involved in nutrient solubilization, a protease, a BclA protein, a BclB protein, a CotE protein a CotO protein, an ExsY protein, an ExsFA/BxpB protein, a CotY protein, an ExsFB protein, an ExsJ protein, an ExsH protein, a YjcA protein, a YjcB protein, a BclC protein, a BxpA protein, a BclE protein, a BetA/BAS3290 protein, an ExsA protein, an ExsK protein, an ExsB protein, a YabG protein, or a Tgl protein, wherein the expression of the enzyme involved in nutrient solubilization, the protease, a BclA protein, a BclB protein, a CotE protein a CotO protein, an ExsY protein, an ExsFA/BxpB protein, a CotY protein, an ExsFB protein, an ExsJ protein, an ExsH protein, a YjcA protein, a YjcB protein, a BclC protein, a BxpA protein, a BclE protein, a BetA/BAS3290 protein, an ExsA protein, an ExsK protein, an ExsB protein, a YabG protein, or a Tgl protein is increased as compared to the expression of the enzyme involved in nutrient solubilization, the protease, a BclA protein, a BclB protein, a CotE protein a CotO protein, an ExsY protein, an ExsFA/BxpB protein, a CotY protein, an ExsFB protein, an ExsJ protein, an ExsH protein, a YjcA protein, a YjcB protein, a BclC protein, a BxpA protein, a BclE protein, a BetA/BAS3290 protein, an ExsA protein, an ExsK protein, an ExsB protein, a YabG protein, or a Tgl protein in a wild-type *Bacillus cereus* family member under the same conditions.

(687) Additional methods for stimulating plant growth, involving the use of exosporium fragments derived from a recombinant *Bacillus cereus* family member, are described below.

(688) B. Methods for protecting a plant from a pathogen or enhancing stress resistance in a plant

(689) The present invention also relates to methods for protecting a plant from a pathogen or enhancing stress resistance in a plant.

(690) One method for protecting a plant from a pathogen or enhancing stress resistance in a plant comprises introducing into a plant growth medium any of the recombinant *Bacillus cereus* family members described above or any of the formulations comprising a recombinant *Bacillus cereus* family member described above. Alternatively, any of the recombinant *Bacillus cereus* family members described above or any of the formulations comprising a recombinant *Bacillus cereus* family member described above can be applied to a plant, a plant seed, or to an area surrounding a plant or a plant seed. In such methods, the recombinant *Bacillus cereus* family member expresses a fusion protein comprising a protein or peptide that protects a plant from a pathogen or a protein or peptide that enhances stress resistance in a plant. The protein or peptide that protects a plant from a pathogen or the protein or peptide that enhances stress resistance in a plant can be physically attached to the exosporium of the recombinant *Bacillus cereus* family member.

(691) Another method for protecting a plant from a pathogen or enhancing stress resistance in a plant comprises introducing into a plant growth medium any of the recombinant spore-forming bacteria described above or any of the formulations comprising a recombinant spore-forming bacterium described above. Alternatively, any of the recombinant spore-forming bacteria described above or any of the formulations comprising a recombinant spore-forming bacterium described above can be applied to a plant, a plant seed, or to an area surrounding a plant or a plant seed. In such methods, the recombinant spore-forming bacterium expresses a fusion protein comprising a protein or peptide that protects a plant from a pathogen or a protein or peptide that enhances stress resistance in a plant. The protein or peptide that protects a plant from a pathogen or the protein or peptide that enhances stress resistance in a plant can be physically attached to the spore coat of the recombinant spore-forming bacterium.

(692) In any of the methods for protecting a plant from a pathogen, plants grown in the plant growth medium comprising the recombinant *Bacillus cereus* family member or the recombinant spore-forming bacterium are preferably less susceptible to infection with the pathogen as compared to plants grown under the same conditions in the identical plant growth medium that does not contain the recombinant *Bacillus cereus* family member or the recombinant spore-forming bacterium.

(693) In any of the methods for enhancing stress resistance in a plant plants grown in the plant growth medium comprising the recombinant *Bacillus cereus* family member or the recombinant spore-forming bacterium are preferably less susceptible to stress as compared to plants grown under the same conditions in the identical plant growth medium that does not contain the recombinant *Bacillus cereus* family member or the recombinant spore-forming bacterium.

(694) Another method for enhancing stress resistance in a plant comprises introducing into a plant growth medium a recombinant *Bacillus cereus* family member or a formulation comprising the recombinant *Bacillus cereus* family member. Alternatively, the recombinant *Bacillus cereus* or the formulation can be applied to a plant, a plant seed, or to an area surrounding a plant or a plant seed. The recombinant *Bacillus cereus* family member expresses a superoxide dismutase or an arginase, wherein the expression of the superoxide dismutase or the arginase is increased as compared to the expression of the superoxide dismutase or the arginase in a wild-type *Bacillus cereus* family member under the same conditions.
(695) Another method for protecting a plant from a pathogen comprises introducing into a plant growth medium a recombinant *Bacillus cereus* family member or a formulation comprising the recombinant *Bacillus cereus* family member. Alternatively, the recombinant *Bacillus cereus* or the formulation can be applied to a plant, a plant seed, or to an area surrounding a plant or a plant seed. The recombinant *Bacillus cereus* family member expresses a protease, wherein the expression of the protease is increased as compared to the expression of the protease in a wild-type *Bacillus cereus* family member under the same conditions.
(696) Additional methods for protecting a plant from a pathogen or enhancing stress resistance in a plant, involving the use of exosporium fragments derived from a recombinant *Bacillus cereus* family member, are described below.
(697) C. Methods for Immobilizing Recombinant *Bacillus cereus* Family Member Spores or Recombinant Spore Forming Bacteria on a Plant
(698) The present invention further relates to methods for immobilizing recombinant *Bacillus cereus* family member spores or recombinant spore forming bacteria on a plant.
(699) One method for immobilizing a recombinant *Bacillus cereus* family member spore on a plant comprises introducing into a plant growth medium any of the recombinant *Bacillus cereus* family members described above or any of the formulations comprising a recombinant *Bacillus cereus* family member described above. Alternatively, any of the recombinant *Bacillus cereus* family members described above or any of the formulations comprising a recombinant *Bacillus cereus* family member described above can be applied to a plant, a plant seed, or to an area surrounding a plant or a plant seed. In such methods, the recombinant *Bacillus cereus* family member expresses a fusion protein comprising a plant binding protein or peptide. The plant binding protein or peptide can be physically attached to the exosporium of the recombinant *Bacillus cereus* family member.
(700) Another method for immobilizing a spore of a recombinant spore-forming bacterium on a plant comprises introducing into a plant growth medium any of the recombinant spore-forming bacteria described above or any of the formulations comprising a recombinant spore-forming bacterium described above. Alternatively, any of the recombinant spore-forming bacteria described above or any of the formulations comprising a recombinant spore-forming bacterium described above can be applied to a plant, a plant seed, or to an area surrounding a plant or a plant seed. In such methods, the recombinant spore-forming bacterium expresses a fusion protein comprising a plant binding peptide and the plant binding protein or peptide can be physically attached to the spore coat of the recombinant spore-forming bacterium.
(701) The plant binding protein or peptide preferably selectively targets and maintains the recombinant *Bacillus cereus* family member or the recombinant spore-forming bacterium on a plant. For example, the plant binding protein or peptide can selectively target and maintain the recombinant *Bacillus cereus* family member on at plant roots, substructures of roots, an aerial portion of a plant, or a substructure of an aerial portion of a plant.
(702) D. Methods for Stimulating Germination of a Plant Seed
(703) 1. Methods for Stimulating Germination Involving the Use of a Recombinant *Bacillus cereus* Family Member of a Recombinant Spore-Forming Bacterium
(704) The present invention also provides methods for stimulating germination of a plant seed.
(705) One method for stimulating germination of a plant seed comprises introducing into a plant growth medium any of the recombinant *Bacillus cereus* family members described above or any of the formulations comprising a recombinant *Bacillus cereus* family member described above. Alternatively, any of the recombinant *Bacillus cereus* family members described above or any of the formulations comprising a recombinant *Bacillus cereus* family member described above can be applied to a plant, a plant seed, or to an area surrounding a plant or a plant seed. In such methods, the recombinant *Bacillus cereus* family member expresses a fusion protein comprising an enzyme that catalyzes the production of nitric oxide. The enzyme that catalyzes the production of nitric oxide can be physically attached to the exosporium of the recombinant *Bacillus cereus* family member.
(706) Another method for stimulating germination of a plant seed comprises introducing into a plant growth medium any of the recombinant spore-forming bacteria described above or any of the formulations comprising a recombinant spore-forming bacterium described above. Alternatively, any of the recombinant spore-forming bacteria described above or any of the formulations comprising a recombinant spore-forming bacterium described above can be applied to a plant, a plant seed, or to an area surrounding a plant or a plant seed. In such methods, the recombinant spore-forming bacterium expresses a fusion protein comprising an enzyme that catalyzes the production of nitric oxide, and the enzyme that catalyzes the production of nitric oxide can be physically attached to

the spore coat of the recombinant spore-forming bacterium.
(707) The above methods for stimulating germination of a plant seed preferably comprise applying the recombinant *Bacillus cereus* family member, the recombinant spore-forming bacterium, or the formulation to a plant seed.
(708) Any of the above methods for stimulating germination of a plant seed can further comprise applying a substrate for the enzyme that catalyzes production of nitric oxide to the plant growth medium, the plant seed, the plant, or the area surrounding the plant or the plant seed. For example, the method suitably further comprises adding L-arginine to the plant growth medium, the plant seed, the plant, or the area surrounding the plant or the plant seed. For example, the L-arginine can be applied to an aerial portion of the plant. The L-arginine is preferably applied to the plant seed.
(709) The presence of L-arginine enhances the reaction and leads to a more pronounced output of NO by the nitric oxide synthase. Furthermore, L-arginine on a plant seed, a plant growth medium, or an area surrounding a plant can serve as a substrate for the production of nitric oxide by native bacterial enzymes.
(710) In any of the above methods for stimulating germination of a plant seed, seeds in the plant growth medium comprising the recombinant *Bacillus cereus* family member or the recombinant spore-forming bacterium or seeds to which the recombinant *Bacillus cereus* family member or the recombinant spore-forming bacterium has been applied preferably have an increased germination rate as compared to seeds grown under the same conditions in the identical plant growth medium that does not contain the recombinant *Bacillus cereus* family member or the recombinant spore-forming bacterium or seeds to which the recombinant *Bacillus cereus* family member or the recombinant spore-forming bacterium has not been applied, grown under the same conditions.
(711) In any of the above methods for stimulating germination of a plant seed, seeds in the plant growth medium comprising the recombinant *Bacillus cereus* family member or the recombinant spore-forming bacterium or seeds to which the recombinant *Bacillus cereus* family member or the recombinant spore-forming bacterium has been applied preferably have a longer taproot after germination as compared to seeds grown under the same conditions in the identical plant growth medium that does not contain the recombinant *Bacillus cereus* family member or the recombinant spore-forming bacterium or seeds to which the recombinant *Bacillus cereus* family member or the recombinant spore-forming bacterium has not been applied under the same conditions.
(712) Additional methods for stimulating germination of a plant seed, involving the use of exosporium fragments derived from a recombinant *Bacillus cereus* family member, are described below.
(713) 2. Methods for Stimulating Germination by Delivering to Plants Enzymes that Catalyze the Production of Nitric Oxide or Recombinant Microorganisms that Overexpress Such Enzymes
(714) Yet another method for stimulating germination of a plant seed comprises introducing into a plant growth medium, or applying to a plant, a plant seed, or an area surrounding a plant or a plant seed: (i) an enzyme that catalyzes the production of nitric oxide; (ii) a superoxide dismutase; or (iii) a recombinant microorganism that expresses an enzyme that catalyzes the production of nitric oxide or a superoxide dismutase, wherein the expression of the enzyme that catalyzes the production of nitric oxide or the superoxide dismutase is increased as compared to the expression of the enzyme that catalyzes the production of nitric oxide or the superoxide dismutase in a wild type microorganism under the same conditions.
(715) The method preferably comprises applying the enzyme or the microorganism to a plant seed.
(716) The method can further comprise applying a substrate for the enzyme that catalyzes production of nitric oxide to the plant growth medium, the plant seed, the plant, or the area surrounding the plant or the plant seed. For example, the method suitably further comprises adding L-arginine to the plant growth medium, the plant seed, the plant, or the area surrounding the plant or the plant seed. For example, the L-arginine can be applied to an aerial portion of the plant. The L-arginine is preferably applied to the plant seed.
(717) Seeds in the plant growth medium comprising the enzyme or the microorganism or seeds to which the enzyme or the microorganism has been applied preferably have an increased germination rate as compared to seeds grown under the same conditions in the identical plant growth medium that does not contain enzyme or the microorganism or seeds to which the enzyme or the microorganism has not been applied, grown under the same conditions.
(718) Seeds in the plant growth medium comprising the enzyme or the microorganism or seeds to which the enzyme or the microorganism has been applied preferably have a longer taproot after germination as compared to seeds grown under the same conditions in the identical plant growth medium that does not contain the enzyme or the microorganism or seeds to which the enzyme or the microorganism has not been applied under the same conditions.
(719) The enzyme that catalyzes the production of nitric oxide synthase can comprise a nitric oxide synthase or an arginase. Where the enzyme that catalyzes the production of nitric oxide comprises a nitric oxide synthase, the nitric oxide synthase can comprise, for example, a nitric oxide synthase from *Bacillus thuringiensis* BT013A or *Bacillus subtilis* 168. For example, the nitric oxide synthase can have at least 85% sequence identity with SEQ ID NO: 260 or 261.
(720) The nitric oxide synthase can have at least 90% sequence identity with SEQ ID NO: 260 or 261.
(721) The nitric oxide synthase can have at least 95% sequence identity with SEQ ID NO: 260 or 261.

(722) The nitric oxide synthase can have at least 98% sequence identity with SEQ ID NO: 260 or 261.
(723) The nitric oxide synthase can have at least 99% sequence identity with SEQ ID NO: 260 or 261.
(724) The nitric oxide synthase can have 100% sequence identity with SEQ ID NO: 260 or 261.
(725) The superoxide dismutase can comprise a superoxide dismutase 1 (SODA1) or a superoxide dismutase 2 (SODA2). The superoxide dismutase can comprise an amino acid sequence having at least 85% identity with SEQ ID NO: 155 or 156.
(726) The superoxide dismutase can comprise an amino acid sequence having at least 90% identity with SEQ ID NO: 155 or 156.
(727) The superoxide dismutase can comprise an amino acid sequence having at least 95% identity with SEQ ID NO: 155 or 156.
(728) The superoxide dismutase can comprise an amino acid sequence having at least 98% identity with SEQ ID NO: 155 or 156.
(729) The superoxide dismutase can comprise an amino acid sequence having at least 99% identity with SEQ ID NO: 155 or 156.
(730) The superoxide dismutase can comprise an amino acid sequence having at least 100% identity with SEQ ID NO: 155 or 156.
(731) The recombinant microorganism that expresses an enzyme that catalyzes the production of nitric oxide can comprise a *Bacillus* species (e.g., a *Bacillus cereus* family member, *Bacillus subtilis, Bacillus licheniformis*, or *Bacillus megaterium*), *Escherichia coli*, an *Aspergillus* species (e.g., *Aspergillus niger*), or a *Saccharomyces* species (e.g., *Saccharomyces cerevisiae*).
(732) In any of the above methods, the enzyme or the recombinant microorganism can be introduced into the plant growth medium, or applied to a plant, a plant seed, or an area surrounding a plant or a plant seed in a formulation comprising the enzyme or the recombinant microorganism and an agriculturally acceptable carrier. The formulation can comprise any of the agriculturally acceptable carriers and other components discussed herein.
(733) The enzyme that catalyzes the production of nitric oxide can be delivered purified or unpurified, and can be delivered alone or in combination with other beneficial proteins, inoculants, or chemicals to the plant seed, the plant growth medium, or an area surrounding the plant or the plant seed.
(734) E. Methods for Delivering Nucleic Acids to Plants
(735) Methods for delivering nucleic acids to plants are also provided by the present invention.
(736) One method for delivering nucleic acids to a plant comprises introducing into a plant growth medium any of the recombinant *Bacillus cereus* family members described above or any of the formulations comprising a recombinant *Bacillus cereus* family member described above. Alternatively, any of the recombinant *Bacillus cereus* family members described above or any of the formulations comprising a recombinant *Bacillus cereus* family member described above can be applied to a plant, a plant seed, or to an area surrounding a plant or a plant seed. In such methods, the recombinant *Bacillus cereus* family member expresses a fusion protein comprising a nucleic acid binding protein. The nucleic acid binding protein or peptide is bound to a nucleic acid molecule. The nucleic acid binding protein or peptide can be physically attached to the exosporium of the recombinant *Bacillus cereus* family member.
(737) In such methods, the recombinant *Bacillus cereus* family member can comprise an endophytic strain of bacteria. The endophytic strain of bacteria can comprise *Bacillus cereus* family member EE349, *Bacillus cereus* family member EE439, *Bacillus thuringiensis* EE417, *Bacillus cereus* EE444, *Bacillus thuringiensis* EE319, *Bacillus thuringiensis* EE-B00184, *Bacillus cereus* family member EE-B00377, *Bacillus pseudomycoides* EE-B00366, or *Bacillus mycoides* EE-B00363. For example, the endophytic strain of bacteria can comprise *Bacillus cereus* family member EE439, *Bacillus thuringiensis* EE417, *Bacillus cereus* EE444, *Bacillus thuringiensis* EE319, *Bacillus thuringiensis* EE-B00184, *Bacillus cereus* family member EE-B00377, *Bacillus pseudomycoides* EE-B00366, or *Bacillus mycoides* EE-B00363.
(738) Another method for delivering nucleic acids to a plant comprises introducing into a plant growth medium any of the recombinant spore-forming bacteria described above or any of the formulations comprising a recombinant spore-forming bacterium described above. Alternatively, any of the recombinant spore-forming bacteria described above or any of the formulations comprising a recombinant spore-forming bacterium described above can be applied to a plant, a plant seed, or to an area surrounding a plant or a plant seed. In such methods, the recombinant spore-forming bacterium expresses a fusion protein comprising a nucleic acid binding protein. The nucleic acid binding protein or peptide is bound to a nucleic acid molecule. The nucleic acid binding protein or peptide can be physically attached to the spore coat of the recombinant spore-forming bacterium.
(739) The recombinant spore-forming bacterium can comprise an endophytic strain of bacteria. For example, the endophytic strain of bacteria can comprise *Bacillus megaterium* EE385, *Bacillus* sp. EE387, *Bacillus circulans* EE388, *Bacillus subtilis* EE405, *Lysinibacillus fusiformis* EE442, *Lysinibacillus sphericus* EE443, or *Bacillus pumilus* EE-B00143.

(740) In any of the above methods for delivering nucleic acids to a plant, the nucleic acid molecule can comprise a modulating RNA molecule; an RNAi molecule; a microRNA; an aptamer; or a DNA molecule that encodes a modulating RNA molecule, an RNAi molecule, a microRNA, or an aptamer.
(741) The nucleic acid molecules to be delivered to the plant can be produced by any means known the art (e.g., chemical synthesis, recombinant production by a microorganism, etc.). The nucleic acid molecules can then be bound to the nucleic acid binding protein or peptide portion of the fusion proteins described herein in preparation for delivery of such nucleic acids to a plant or plants. The nucleic acid binding proteins and peptides immobilize and stabilize the nucleic acids and allow them to be delivered to the plant intact. The nucleic acid molecules to be delivered to the plant can be in an active form, or in an inactive form that can be processed into an active form by the plant.
(742) To accomplish the binding of the nucleic acid molecules to the nucleic acid binding protein or peptide, the nucleic acids molecules can be incubated with the any of the recombinant *Bacillus cereus* members or recombinant spore-forming bacteria described herein that express a fusion protein comprising a nucleic acid binding protein or peptide.
(743) Additional methods for delivering nucleic acids to a plant, involving the use of exosporium fragments derived from a recombinant *Bacillus cereus* family member, are described below.
(744) F. Methods for Delaying Germination of a Spore of a Recombinant *Bacillus cereus* Family Member
(745) The present invention further relates to a method for delaying germination of a spore of a *Bacillus cereus* family member. The method comprises modifying the *Bacillus cereus* family member to express an inosine-uridine hydrolase or an alanine racemase, wherein the expression of the inosine-uridine hydrolase or the alanine racemase is increased as compared to the expression of the inosine-uridine hydrolase or the alanine racemase in a wild-type *Bacillus cereus* family member under the same conditions.
(746) G. Inactivation of the *Bacillus cereus* Family Member or Recombinant Spore-Forming Bacterium Prior to Use
(747) In any of the above methods that use a recombinant *Bacillus cereus* family member or a recombinant spore forming bacterium, the method can further comprise inactivating the recombinant *Bacillus cereus* family member or the recombinant spore-forming bacterium prior to introduction into the plant growth medium or application to a plant, a plant seed, or an area surrounding a plant or a plant seed.
(748) For example, the inactivating can comprise subjecting the recombinant *Bacillus cereus* family member or the recombinant spore-forming bacterium to heat treatment; gamma irradiation; x-ray irradiation; UV-A irradiation; UV-B irradiation; treatment with gluteraldehyde, formaldehyde, hydrogen peroxide, acetic acid, bleach, chloroform, or phenol, or a combination thereof.
(749) Alternatively or in addition, the inactivating can comprise modifying the recombinant *Bacillus cereus* family member recombinant or spore-forming bacterium to express a germination spore protease or a non-specific endonuclease, wherein the expression of the germination spore protease or the non-specific endonuclease is increased as compared to the expression of the germination spore protease or the non-specific endonuclease in a wild-type *Bacillus cereus* family member under the same conditions, and wherein the recombinant spore-forming bacterium comprises a recombinant bacterium of the genus *Bacillus.*
(750) H. Methods for Making and Using Exosporium Fragments
(751) The present invention further relates to methods for making and using exosporium fragments. These methods relate to the recombinant *Bacillus cereus* family members described in Section IV hereinabove, i.e., recombinant *Bacillus cereus* family members that comprise a mutation or another genetic alteration that allows for the collection of free exosporium.
(752) Thus, the present invention relates to a method for removing exosporium from spores of a recombinant *Bacillus cereus* family member. The method comprises subjecting a suspension comprising any of the recombinant *Bacillus cereus* family members described in Section IV hereinabove to centrifugation or filtration to produce fragments of exosporium that are separated from the spores. The exosporium fragments comprise the fusion protein.
(753) The method for removing exosporium from spores of a recombinant *Bacillus cereus* family member can comprise subjecting the suspension comprising the spores to centrifugation and collecting the supernatant, wherein the supernatant comprises the fragments of the exosporium and is substantially free of spores.
(754) Alternatively, the method for removing exosporium from spores of a recombinant *Bacillus cereus* family member can comprise subjecting the suspension comprising the spores to filtration and collecting the filtrate, wherein the filtrate comprises the fragments of the exosporium and is substantially free of spores.
(755) The suspension of spores can be agitated or mechanically disrupted prior to centrifugation or filtration.
(756) The exosporium fragments can also be separated from the spores by gradient centrifugation, affinity purification, or by allowing the spores to settle out of the suspension.
(757) The present invention further relates to methods for using the exosporium fragments.
(758) A method for stimulating plant growth is provided. The method comprises introducing exosporium fragments or a formulation of comprising the exosporium fragments and an agriculturally acceptable carrier into a plant

growth medium. Alternatively, the exosporium fragments or the formulation can be applied to a plant, a plant seed, or an area surrounding a plant or a plant seed. The exosporium fragments are derived from spores of a recombinant *Bacillus cereus* family member described in Section IV hereinabove and comprise the fusion protein. The fusion protein comprises a plant growth stimulating protein or peptide.

(759) A method for protecting a plant from a pathogen or enhancing stress resistance in a plant is also provided. The method comprises introducing exosporium fragments or a formulation of comprising the exosporium fragments and an agriculturally acceptable carrier into a plant growth medium. Alternatively, the exosporium fragments or the formulation can be applied to a plant, a plant seed, or an area surrounding a plant or a plant seed. The exosporium fragments are derived from spores of a recombinant *Bacillus cereus* family member described in Section IV hereinabove and comprise the fusion protein. The fusion protein comprises a protein or peptide that protects a plant from a pathogen or a protein or peptide that enhances stress resistance in a plant.

(760) When the method is a method for protecting a plant from a pathogen, the fusion protein comprises protein or peptide that protects a plant from a pathogen.

(761) In the methods for protecting a plant from a pathogen, plants grown in the plant growth medium comprising the exosporium fragments are preferably less susceptible to infection with the pathogen as compared to plants grown under the same conditions in the identical plant growth medium that does not contain the exosporium fragments.

(762) When the method is a method for enhancing stress resistance in a plant, the fusion protein comprises a protein or peptide that enhances stress resistance in a plant.

(763) In the methods for enhancing stress resistance in a plant of, plants grown in the plant growth medium comprising the exosporium fragments are preferably less susceptible to stress as compared to plants grown under the same conditions in the identical plant growth medium that does not contain the exosporium fragments.

(764) A method for immobilizing exosporium fragments on a plant is also provided. The method comprises introducing exosporium fragments or a formulation of comprising the exosporium fragments and an agriculturally acceptable carrier into a plant growth medium. Alternatively, the exosporium fragments or the formulation can be applied to a plant, a plant seed, or an area surrounding a plant or a plant seed. The exosporium fragments are derived from spores of a recombinant *Bacillus cereus* family member described in Section IV hereinabove and comprise the fusion protein. The fusion protein comprises a plant binding protein or peptide.

(765) The plant binding protein or peptide preferably selectively targets and maintains the exosporium fragments on a plant. For example, the plant binding protein or peptide can selectively target and maintain the exosporium fragments on at plant roots, substructures of roots, an aerial portion of a plant, or a substructure of an aerial portion of a plant.

(766) A method for stimulating germination of a plant seed is also provided. The method comprises introducing exosporium fragments or a formulation of comprising the exosporium fragments and an agriculturally acceptable carrier into a plant growth medium. Alternatively, the exosporium fragments or the formulation can be applied to a plant, a plant seed, or an area surrounding a plant or a plant seed. The exosporium fragments are derived from spores of a recombinant *Bacillus cereus* family member described in Section IV hereinabove and comprise the fusion protein. The fusion protein comprises a superoxide dismutase or an enzyme that catalyzes the production of nitric oxide.

(767) In the methods for stimulating germination, the method preferably comprises applying the exosporium fragments to a plant seed.

(768) The methods for stimulating germination can further comprise applying a substrate for the enzyme that catalyzes production of nitric oxide to the plant growth medium, the plant seed, the plant, or the area surrounding the plant or the plant seed. For example, the method suitably further comprises adding L-arginine to the plant growth medium, the plant seed, the plant, or the area surrounding the plant or the plant seed. For example, the L-arginine can be applied to an aerial portion of the plant. The L-arginine is preferably applied to the plant seed.

(769) The presence of L-arginine enhances the reaction and leads to a more pronounced output of NO by the nitric oxide synthase. Furthermore, L-arginine on a plant seed, a plant growth medium, or an area surrounding a plant can serve as a substrate for the production of nitric oxide by native bacterial enzymes.

(770) In the methods for stimulating germination of a plant seed, seeds in the plant growth medium comprising the exosporium fragments or seeds to which the exosporium fragments have been applied preferably have an increased germination rate as compared to the same seeds grown under the same conditions in the identical plant growth medium that does not contain the exosporium fragments or the same seeds grown under the same conditions to which the exosporium fragments have not been applied.

(771) In the methods for stimulating germination of a plant seed, seeds in the plant growth medium comprising the exosporium fragments or seeds to which the exosporium fragments have been applied preferably have a longer taproot after germination as compared to the same seeds grown under the same conditions in the identical plant growth medium that does not contain the exosporium fragments or the same seeds grown under the same conditions

to which the exosporium fragments have not been applied.
(772) A method for delivering nucleic acids to a plant is also provided. The method comprises introducing exosporium fragments or a formulation of comprising the exosporium fragments and an agriculturally acceptable carrier into a plant growth medium. Alternatively, the exosporium fragments or the formulation can be applied to a plant, a plant seed, or an area surrounding a plant or a plant seed. The exosporium fragments are derived from spores of a recombinant *Bacillus cereus* family member described in Section IV hereinabove and comprise the fusion protein. The fusion protein comprises a nucleic acid binding protein or peptide. The nucleic acid binding protein or peptide is bound to a nucleic acid molecule.
(773) In the method for delivering nucleic acids to a plant, the nucleic acid molecule can comprise a modulating RNA molecule; an RNAi molecule; a microRNA; an aptamer; or a DNA molecule that encodes a modulating RNA molecule, an RNAi molecule, a microRNA, or an aptamer.
(774) The nucleic acid molecules to be delivered to the plant can be produced by any means known the art (e.g., chemical synthesis, recombinant production by a microorganism, etc.). The nucleic acid molecules can then be bound to the nucleic acid binding protein or peptide portion of the fusion proteins described herein in preparation for delivery of such nucleic acids to a plant or plants. The nucleic acid binding proteins and peptides immobilize and stabilize the nucleic acids and allow them to be delivered to the plant intact. The nucleic acid molecules to be delivered to the plant can be in an active form, or in an inactive form that can be processed into an active form by the plant.
(775) To accomplish the binding of the nucleic acid molecules to the nucleic acid binding protein or peptide, the nucleic acids molecules can be incubated with the exosporium fragments containing a fusion protein comprising a nucleic acid binding protein or peptide.
(776) I. Plant Growth Medium
(777) In any of the methods described herein involving the use of a plant growth medium, the plant growth medium can comprise soil, water, an aqueous solution, sand, gravel, a polysaccharide, mulch, compost, peat moss, straw, logs, clay, soybean meal, yeast extract, or a combination thereof.
(778) Furthermore, the plant growth medium can be supplemented with a substrate or a cofactor for an enzyme. For example, the substrate or the cofactor can comprise tryptophan, an adenosine monophosphate, an adenosine diphosphate, an adenosine triphosphate (e.g., adenosine-3-triphosphate), indole, a trimetaphosphate, ferrodoxin, acetoin, diacetyl, pyruvate, acetolactate, pectin, cellulose, methylcellulose, starch, chitin, pectin, a protein meal, a cellulose derivative, a phosphate, acetoin, chitosan, an inactive derivative of indole-3-acetic acid, an inactive derivative of gibberellic acid, a xylan, an arabinoxylan, a fat, a wax, an oil, a phytic acid, a lignin, a humic acid, choline, a choline derivative, proline, a polyproline, a proline-rich protein, a proline-rich meal, phenylalanine, chorismate, L-arginine, NADH, NADPH, ATP, GTP, cytochrome C, cytochrome p450, or a combination thereof.
(779) J. Methods of Application
(780) The methods described herein can comprise coating seeds with the recombinant *Bacillus cereus* family member, the recombinant spore-forming bacterium, or the exosporium fragments or a formulation containing the recombinant *Bacillus cereus* family member, the recombinant spore-forming bacterium, or the or exosporium fragments prior to planting.
(781) The methods described herein can comprise applying the recombinant *Bacillus cereus* family member, the recombinant spore-forming bacterium, or the exosporium fragments, or a formulation containing the recombinant *Bacillus cereus* family member, the recombinant spore-forming bacterium, or the exosporium fragments to an aerial portion of a plant.
(782) In the methods described herein, introducing the recombinant *Bacillus cereus* family member, the recombinant spore-forming bacterium, or the exosporium fragments into the plant growth medium can comprise applying a liquid or solid formulation containing the recombinant *Bacillus cereus* family member, the recombinant spore-forming bacterium, or the exosporium fragments to the medium. The plant growth medium can comprise soil (e.g., potting soil), compost, peat moss, sand, seed starter mix, or a combination thereof. The method can comprise applying the formulation to the plant growth medium prior to, concurrently with, or after planting of seeds, seedlings, cuttings, bulbs, or plants in the plant growth medium.
(783) K. Agrochemicals
(784) In the methods described herein, the method can further comprise introducing at least one agrochemical into the plant growth medium or applying at least one agrochemical to plants or seeds.
(785) The agrochemical can comprise a fertilizer (e.g., a liquid fertilizer), a micronutrient fertilizer material (e.g., boric acid, a borate, a boron frit, copper sulfate, a copper frit, a copper chelate, a sodium tetraborate decahydrate, an iron sulfate, an iron oxide, iron ammonium sulfate, an iron frit, an iron chelate, a manganese sulfate, a manganese oxide, a manganese chelate, a manganese chloride, a manganese frit, a sodium molybdate, molybdic acid, a zinc sulfate, a zinc oxide, a zinc carbonate, a zinc frit, zinc phosphate, a zinc chelate, or a combination thereof), an insecticide (e.g., an organophosphate, a carbamate, a pyrethroid, an acaricide, an alkyl phthalate, boric acid, a

borate, a fluoride, sulfur, a haloaromatic substituted urea, a hydrocarbon ester, a biologically-based insecticide, or a combination thereof), an herbicide (e.g., a chlorophenoxy compound, a nitrophenolic compound, a nitrocresolic compound, a dipyridyl compound, an acetamide, an aliphatic acid, an anilide, a benzamide, a benzoic acid, a benzoic acid derivative, anisic acid, an anisic acid derivative, a benzonitrile, benzothiadiazinone dioxide, a thiocarbamate, a carbamate, a carbanilate, chloropyridinyl, a cyclohexenone derivative, a dinitroaminobenzene derivative, a fluorodinitrotoluidine compound, isoxazolidinone, nicotinic acid, isopropylamine, an isopropylamine derivatives, oxadiazolinone, a phosphate, a phthalate, a picolinic acid compound, a triazine, a triazole, a uracil, a urea derivative, endothall, sodium chlorate, or a combination thereof), a fungicide (e.g., a substituted benzene, a thiocarbamate, an ethylene bis dithiocarbamate, a thiophthalidamide, a copper compound, an organomercury compound, an organotin compound, a cadmium compound, anilazine, benomyl, cyclohexamide, dodine, etridiazole, iprodione, metlaxyl, thiamimefon, triforine, or a combination thereof), a molluscicide, an algicide, a plant growth amendment, a bacterial inoculant (e.g., a bacterial inoculant of the genus *Rhizobium*, a bacterial inoculant of the genus *Bradyrhizobium*, a bacterial inoculant of the genus *Mesorhizobium*, a bacterial inoculant of the genus *Azorhizobium*, a bacterial inoculant of the genus *Allorhizobium*, a bacterial inoculant of the genus *Sinorhizobium*, a bacterial inoculant of the genus *Kluyvera*, a bacterial inoculant of the genus *Azotobacter*, a bacterial inoculant of the genus *Pseudomonas*, a bacterial inoculant of the genus *Azospirillium*, a bacterial inoculant of the genus *Bacillus*, a bacterial inoculant of the genus *Streptomyces*, a bacterial inoculant of the genus *Paenibacillus*, a bacterial inoculant of the genus *Paracoccus*, a bacterial inoculant of the genus *Enterobacter*, a bacterial inoculant of the genus *Alcaligenes*, a bacterial inoculant of the genus *Mycobacterium*, a bacterial inoculant of the genus *Trichoderma*, a bacterial inoculant of the genus *Gliocladium*, a bacterial inoculant of the genus *Glomus*, a bacterial inoculant of the genus *Klebsiella*, or a combination thereof), a fungal inoculant (e.g., a fungal inoculant of the family Glomeraceae, a fungal inoculant of the family Claroidoglomeraceae, a fungal inoculant of the family Gigasporaceae, a fungal inoculant of the family Acaulosporaceae, a fungal inoculant of the family Sacculosporaceae, a fungal inoculant of the family Entrophosporaceae, a fungal inoculant of the family Pacidsporaceae, a fungal inoculant of the family Diversisporaceae, a fungal inoculant of the family Paraglomeraceae, a fungal inoculant of the family Archacosporaceae, a fungal inoculant of the family Geosiphonaceae, a fungal inoculant of the family Ambisporaceae, a fungal inoculant of the family Scutellosporaceae, a fungal inoculant of the family Dentiscultataceae, a fungal inoculant of the family Racocetraceac, a fungal inoculant of the phylum Basidiomycota, a fungal inoculant of the phylum Ascomycota, a fungal inoculant of the phylum Zygomycota, or a combination thereof), or a combination thereof.

(786) The fertilizer can comprise ammonium sulfate, ammonium nitrate, ammonium sulfate nitrate, ammonium chloride, ammonium bisulfate, ammonium polysulfide, ammonium thiosulfate, aqueous ammonia, anhydrous ammonia, ammonium polyphosphate, aluminum sulfate, calcium nitrate, calcium ammonium nitrate, calcium sulfate, calcined magnesite, calcitic limestone, calcium oxide, calcium nitrate, dolomitic limestone, hydrated lime, calcium carbonate, diammonium phosphate, monoammonium phosphate, magnesium nitrate, magnesium sulfate, potassium nitrate, potassium chloride, potassium magnesium sulfate, potassium sulfate, sodium nitrates, magnesian limestone, magnesia, urea, urea-formaldehydes, urea ammonium nitrate, sulfur-coated urea, polymer-coated urea, isobutylidene diurea, K.sub.2SO.sub.4-2MgSO.sub.4, kainite, sylvinite, kieserite, Epsom salts, elemental sulfur, marl, ground oyster shells, fish meal, oil cakes, fish manure, blood meal, rock phosphate, super phosphates, slag, bone meal, wood ash, manure, bat guano, peat moss, compost, green sand, cottonseed meal, feather meal, crab meal, fish emulsion, humic acid, or a combination thereof.

(787) The agrochemical can comprise any of the fungicides, bacterial inoculants, or herbicides, described above in section XVII.

(788) L. Plants and Seeds

(789) In any of the above methods relating to plants, the plant can be a dicotyledon, a monocotyledon, or a gymnosperm.

(790) For example, where the plant is a dicotyledon, the dicotyledon can be selected from the group consisting of bean, pea, tomato, pepper, squash, alfalfa, almond, aniseseed, apple, apricot, arracha, artichoke, avocado, bambara groundnut, beet, bergamot, black pepper, black wattle, blackberry, blueberry, bitter orange, bok-choi, Brazil nut, breadfruit, broccoli, broad bean, Brussels sprouts, buckwheat, cabbage, camelina, Chinese cabbage, cacao, cantaloupe, caraway seeds, cardoon, carob, carrot, cashew nuts, cassava, castor bean, cauliflower, celeriac, celery, cherry, chestnut, chickpea, chicory, chili pepper, chrysanthemum, cinnamon, citron, clementine, clove, clover, coffee, cola nut, colza, corn, cotton, cottonseed, cowpea, crambe, cranberry, cress, cucumber, currant, custard apple, drumstick tree, carth pea, eggplant, endive, fennel, fenugreek, fig, filbert, flax, geranium, gooseberry, gourd, grape, grapefruit, guava, hemp, hempseed, henna, hop, horse bean, horseradish, indigo, jasmine, Jerusalem artichoke, jute, kale, kapok, kenaf, kohlrabi, kumquat, lavender, lemon, lentil, lespedeza, lettuce, lime, liquorice, litchi, loquat, lupine, macadamia nut, mace, mandarin, mangel, mango, medlar, melon, mint, mulberry, mustard, nectarine, niger seed, nutmeg, okra, olive, opium, orange, papaya, parsnip, pea, peach, peanut, pear, pecan nut, persimmon, pigcon

pea, pistachio nut, plantain, plum, pomegranate, pomelo, poppy seed, potato, sweet potato, prune, pumpkin, quebracho, quince, trees of the genus *Cinchona*, *quinoa*, radish, ramie, rapeseed, raspberry, rhea, rhubarb, rose, rubber, rutabaga, safflower, sainfoin, salsify, sapodilla, Satsuma, scorzonera, sesame, shea tree, soybean, spinach, squash, strawberry, sugar beet, sugarcane, sunflower, swede, sweet pepper, tangerine, tea, teff, tobacco, tomato, trefoil, tung tree, turnip, urena, vetch, walnut, watermelon, yerba mate, wintercress, shepherd's purse, garden cress, peppercress, watercress, pennycress, star anise, laurel, bay laurel, cassia, jamun, dill, tamarind, peppermint, oregano, rosemary, sage, soursop, pennywort, calophyllum, balsam pear, kukui nut, Tahitian chestnut, basil, huckleberry, hibiscus, passionfruit, star apple, sassafras, cactus, St. John's wort, loosestrife, hawthorn, cilantro, curry plant, kiwi, thyme, zucchini, ulluco, jicama, waterleaf, spiny monkey orange, yellow mombin, starfruit, amaranth, wasabi, Japanese pepper, yellow plum, mashua, Chinese toon, New Zealand spinach, bower spinach, ugu, tansy, chickweed, jocote, Malay apple, paracress, sowthistle, Chinese potato, horse parsley, hedge mustard, campion, agate, cassod tree, thistle, burnet, star gooseberry, saltwort, glasswort, sorrel, silver lace fern, collard greens, primrose, cowslip, purslane, knotgrass, terebinth, tree lettuce, wild betel, West African pepper, yerba santa, tarragon, parsley, chervil, land cress, burnet saxifrage, honeyherb, butterbur, shiso, water pepper, perilla, bitter bean, oca, kampong, Chinese celery, lemon basil, Thai basil, water mimosa, cicely, cabbage-tree, moringa, mauka, ostrich fern, rice paddy herb, yellow sawah lettuce, lovage, pepper grass, maca, bottle gourd, hyacinth bean, water spinach, catsear, fishwort, Okinawan spinach, lotus sweetjuice, gallant soldier, culantro, arugula, cardoon, caigua, mitsuba, chipilin, samphire, mampat, ebolo, ivy gourd, cabbage thistle, sea kale, chaya, huauzontle, Ethiopian mustard, magenta spreen, good king henry, epazole, lamb's quarters, centella plumed cockscomb, caper, rapini, napa cabbage, mizuna, Chinese savoy, kai-lan, mustard greens, Malabar spinach, chard, marshmallow, climbing wattle, China jute, paprika, annatto seed, spearmint, savory, marjoram, cumin, chamomile, lemon balm, allspice, bilberry, cherimoya, cloudberry, damson, pitaya, durian, elderberry, feijoa, jackfruit, jambul, jujube, physalis, purple mangosteen, rambutan, redcurrant, blackcurrant, salal berry, satsuma, ugli fruit, azuki bean, black bean, black-eyed pea, borlotti bean, common bean, green bean, kidney bean, lima bean, mung bean, navy bean, pinto bean, runner bean, mangetout, snap pea, broccoflower, calabrese, nettle, bell pepper, raddichio, daikon, white radish, skirret, tat soi, broccolini, black radish, burdock root, fava bean, broccoli raab, lablab, lupin, sterculia, velvet beans, winged beans, yam beans, mulga, ironweed, umbrella bush, tjuntjula, wakalpulka, witchetty bush, wiry wattle, chia, beech nut, candlenut, colocynth, mamoncillo, Maya nut, mongongo, ogbono nut, paradise nut, and cempedak.

(791) Where the plant is a monocotyledon, the monocotyledon can be selected from the group consisting of corn, wheat, oat, rice, barley, millet, banana, onion, garlic, asparagus, ryegrass, millet, fonio, raishan, nipa grass, turmeric, saffron, galangal, chive, cardamom, date palm, pineapple, shallot, leck, scallion, water chestnut, ramp, Job's tears, bamboo, ragi, spotless watermeal, arrowleaf elephant car, Tahitian spinach, abaca, areca, bajra, betel nut, broom millet, broom sorghum, citronella, coconut, cocoyam, maize, dasheen, durra, durum wheat, edo, fique, formio, ginger, orchard grass, esparto grass, Sudan grass, guinea corn, Manila hemp, henequen, hybrid maize, jowar, lemon grass, maguey, bulrush millet, finger millet, foxtail millet, Japanese millet, proso millet, New Zealand flax, oats, oil palm, palm palmyra, sago palm, redtop, sisal, sorghum, spelt wheat, sweet corn, sweet sorghum, taro, teff, timothy grass, triticale, vanilla, wheat, and yam.

(792) Where the plant is a gymnosperm, the gymnosperm can be from a family selected from the group consisting of Araucariaceae, Boweniaceae, Cephalotaxaceae, Cupressaceae, Cycadaceae, Ephedraceae, Ginkgoaceae, Gnetaceae, Pinaceae, Podocarpaceae, Taxaceae, Taxodiaceae, Welwitschiaceae, and Zamiaceae.

(793) The plants and plant seeds described herein may include transgenic plants or plant seeds, such as transgenic cereals (wheat, rice), maize, soybean, potato, cotton, tobacco, oilseed rape and fruit plants (fruit of apples, pears, citrus fruits and grapes. Preferred transgenic plants include corn, soybeans, potatoes, cotton, tobacco and oilseed rape.

(794) Suitable transgenic plants and seeds can be characterized by the plant's formation of toxins, especially from the *Bacillus thuringiensis* genetic material (e.g., by gene CryIA (a), CryIA (b), CryIA (c), CryIIA, CryIIIA, CrylIIB2, Cry9c, Cry2Ab, Cry3Bb, CryIF or a combination thereof). The formation of toxins in plants increases the plant's resistance to insects, arachnids, nematodes and slugs and snails (hereinafter referred to as “Bt plants”). Bt plants, for example, are commercially available under the tradename YIELD GARD® (for example maize, cotton, soybeans), KnockOut® (for example maize), StarLink® (for example maize), Bollgard® (cotton), Nucotn® (cotton) and NewLeaf® (potato) maize varieties, cotton varieties, soybean varieties and potato varieties. Herbicide tolerance plants include plants under the trade names Roundup Ready® (a glyphosate tolerance, such as corn, cotton, soybeans), Clearfield® (for example maize), Liberty Link® (tolerance with glufosinate, for example oilseed rape), IMI® (with imidazolinone tolerance) and STS® (tolerance to a sulfonylurea, such as maize).

(795) Plant seeds as described herein can be genetically modified (e.g., any seed that results in a genetically modified plant or plant part that expresses herbicide tolerance, tolerance to environmental factors such as water stress, drought, viruses, and nitrogen production, or resistance to bacterial, fungi or insect toxins). Suitable genetically modified seeds include those of cole crops, vegetables, fruits, trees, fiber crops, oil crops, tuber crops,

coffee, flowers, legume, cereals, as well as other plants of the monocotyledonous and dicotyledonous species. Preferably, the genetically modified seeds include peanut, tobacco, grasses, wheat, barley, rye, sorghum, rice, rapeseed, sugarbeet, sunflower, tomato, pepper, bean, lettuce, potato, and carrot. Most preferably, the genetically modified seeds include cotton, soybean, and corn (sweet, field, seed, or popcorn).

(796) Particularly useful transgenic plants which may be treated according to the invention are plants containing transformation events, or a combination of transformation events, that are listed for example in the databases from various national or regional regulatory agencies (see for example gmoinfo.jrc.it/gmp_browse.aspx and www.agbios.com/dbase.php).

XX. Methods for Delivering Beneficial Bacteria and Proteins or Peptides to Animals

(797) The present invention further relates to methods for delivering beneficial bacteria and/or proteins or peptides to animals.

(798) The administration of bacterial strains that are both probiotic and are also endophytic to a plant allows for entry of the bacteria into the plant where they divide and multiply. The endophytic and probiotic strains can be delivered to plants using various methods, e.g., the endophytic and probiotic strains can be delivered via seed treatment, treatment of the plant growth medium (e.g., soil), irrigation, application to the plant itself (e.g., foliar application to the aerial portions of a plant). Once inside the plant, the bacteria multiply and colonize the internal tissues of the plant. The plant can then be fed to an animal, which allows for delivery of the probiotic bacteria to the animal. Costs are decreased as to traditional methods for delivering probiotic bacteria to animals, since the endophytic nature of the bacteria allows them to divide and multiply within the plant. By initially delivering a small amount of a probiotic and endophytic strain of bacteria to a plant and allowing the bacteria to increase in number inside the plant, the dose increases. In addition, the probiotic and endophytic strain can spread across a target crop prior to harvest and digestion.

(799) Bacterial strains that are capable of colonizing the phylloplane of a plant and are also probiotic can also be used for these purposes. Strains that are capable of colonizing the phylloplane of a plant can be initially delivered to plants in small doses, and will then divide and colonize the external surfaces of the plants.

(800) Suitable bacterial strains that are both endophytic or phylloplane-colonizing and probiotic include those strains that can both replicate in the field in or on a plant and that provide benefits to animals upon ingestion. Benefits of probiotic bacteria in animals include but are not limited to regulation of the microbiome of the digestive tract of the animal, secretion of enzymes that aid in digestion of plant material, and stimulation of the animals immune system. Examples of digestion-enhancing enzymes that would provide benefit include, but are not limited to cellulases, endoglucanases, exoglucanases, β-glucosidases, amylases, proteases, pectinases, xylanases, xylosidases, lipases, phospholipases, and lignases.

(801) The *Bacillus* and *Lysinibacillus* genera are unique in that they contain a large number of species that are both endophytic and thus colonize plants, but that can also act as probiotics in vertebrates. Thus, *Bacillus* and *Lysinibacillus* species are highly suitable for delivery of probiotics to animals through passaging and growth in plants. Common *Bacillus* species that can be both endophytic and probiotic include *Bacillus subtilis, Bacillus firmus, Bacillus amyloliquefaciens, Bacillus cereus, Bacillus toyocerin, Bacillus megaterium, Bacillus pumilus,* and *Bacillus licheniformis. Lysinibacillus* species that are both endophytic and probiotic can also be used.

(802) A method for delivering beneficial bacteria to an animal is provided. The method comprises feeding to an animal a plant modified to comprise a level of an endophytic and probiotic strain of bacteria that is greater than the level of the endophytic and probiotic strain of bacteria in the same plant that has not been modified grown under the same conditions.

(803) The plant fed to the animal can comprise a plant grown in a plant growth medium containing the endophytic and probiotic strain of bacteria or a formulation comprising the endophytic and probiotic strain of bacteria, a plant to which the endophytic and probiotic strain of bacteria was applied, a plant grown from a plant seed to which the endophytic and probiotic strain of bacteria was applied, a plant grown in an area to which the endophytic and probiotic strain of bacteria was applied, or a seed grown in the area to which the endophytic and probiotic strain of bacteria was applied.

(804) The endophytic and probiotic strain of bacteria can comprise a *Bacillus* or *Lysinibacillus* species. For example, the *Bacillus* species can comprise *Bacillus subtilis, Bacillus firmus, Bacillus amyloliquefaciens, Bacillus cereus, Bacillus toyocerin, Bacillus megaterium, Bacillus pumilus, Bacillus licheniformis*, or a combination thereof.

(805) The endophytic and probiotic strain of bacteria can comprise *Bacillus cereus* family member EE349, *Bacillus cereus* family member EE439, *Bacillus thuringiensis* EE417, *Bacillus cereus* EE444, *Bacillus megaterium* EE385, *Bacillus* sp. EE387, *Bacillus circulans EE*388, *Bacillus subtilis* EE405, *Lysinibacillus fusiformis* EE442, *Lysinibacillus sphericus* EE443, *Bacillus pumilus* EE-B00143, or a combination thereof.

(806) In addition, proteins or peptides (e.g., enzymes) can be delivered to animals by feeding recombinant *Bacillus cereus* family members expressing a fusion protein containing the protein or peptide, exosporium fragments comprising such fusion proteins, or recombinant spore-forming bacteria expressing such fusion proteins to the

animals. The recombinant *Bacillus cereus* family member or the recombinant spore-forming bacteria can be an endophytic strain of bacteria or a strain of bacteria that is capable of colonizing the phylloplane of a plant, which allows for delivery of the protein or peptide to the animal via ingestion of a plant that has been colonized by the bacteria. Probiotic recombinant *Bacillus cereus* family member strains or strains of recombinant spore-forming bacteria can also be used so that the animal that ingests the recombinant *Bacillus cereus* family member or the recombinant spore-forming bacteria obtains both the benefits of the probiotic bacteria and the benefits of the protein or peptide. Recombinant *Bacillus cereus* family member strains and strains of recombinant spore-forming bacteria that are both endophytic or phylloplane colonizing and probiotic can also be used to deliver proteins or peptides to animals.

(807) Accordingly, a method for delivering proteins or peptides to an animal is also provided. The method comprises feeding to an animal a recombinant *Bacillus cereus* family member expressing a fusion protein comprising a protein or peptide of interest and a targeting sequence, exosporium protein, or exosporium protein fragment that targets the fusion protein to the exosporium of the recombinant *Bacillus cereus* family member. Alternatively, the method comprises feeding to an animal exosporium fragments derived from a recombinant *Bacillus cereus* family member expressing a fusion protein comprising a protein or peptide of interest and a targeting sequence, exosporium protein, or exosporium protein fragment that targets the fusion protein to the exosporium of the recombinant *Bacillus cereus* family member.

(808) The recombinant *Bacillus cereus* family member can comprise any of the recombinant *Bacillus cereus* family members described herein that express a fusion protein.

(809) The exosporium fragments can comprise exosporium fragments derived from any of the *Bacillus cereus* family members described above in Section IV.

(810) The recombinant *Bacillus cereus* family member can comprise an endophytic strain of bacteria. The endophytic strain of bacteria can comprise *Bacillus cereus* family member EE349, *Bacillus cereus* family member EE439, *Bacillus thuringiensis* EE417, *Bacillus cereus* EE444, *Bacillus thuringiensis* EE319, *Bacillus thuringiensis* EE-B00184, *Bacillus cereus* family member EE-B00377, *Bacillus pseudomycoides* EE-B00366, or *Bacillus mycoides* EE-B00363. For example, the endophytic strain of bacteria comprises *Bacillus cereus* family member EE439, *Bacillus thuringiensis* EE417, *Bacillus cereus* EE444, *Bacillus thuringiensis* EE319, *Bacillus thuringiensis* EE-B00184, *Bacillus cereus* family member EE-B00377, *Bacillus pseudomycoides* EE-B00366, or *Bacillus mycoides* EE-B00363.

(811) The recombinant *Bacillus cereus* family member can comprise a probiotic strain of bacteria. The probiotic strain of bacteria can comprise *Bacillus cereus* family member EE349 (NRRL No. B-50928), *Bacillus cereus* family member EE439 (NRRL B-50979), *Bacillus thuringiensis* EE417 (NRRL B-50979), *Bacillus cereus* EE444 (NRRL B-50977), *Bacillus thuringiensis* BT013A (NRRL No. B-50924), or a combination thereof.

(812) The recombinant *Bacillus cereus* family member can be comprised within a plant that is fed to the animal.

(813) Alternatively, the recombinant *Bacillus cereus* family can comprise a strain of bacteria that is capable of colonizing the phylloplane of a plant. For example, the strain of bacteria that is capable of colonizing the phylloplane of a plant can comprise *Bacillus mycoides* BT155, *Bacillus mycoides* EE118, *Bacillus mycoides* EE141, *Bacillus mycoides* BT46-3, *Bacillus cereus* family member EE218, *Bacillus thuringiensis* BT013A, *Bacillus cereus* family member EE-B00377, *Bacillus pseudomycoides* EE-B00366, or *Bacillus mycoides* EE-B00363.

(814) The recombinant *Bacillus cereus* family member can be present on the phylloplane of a plant that is fed to the animal.

(815) The targeting sequence, exosporium protein, or exosporium protein fragment can comprise: (1) a targeting sequence comprising an amino acid sequence having at least about 43% identity with amino acids 20-35 of SEQ ID NO: 1, wherein the identity with amino acids 25-35 is at least about 54%; (2) a targeting sequence comprising amino acids 1-35 of SEQ ID NO: 1; (3) a targeting sequence comprising amino acids 20-35 of SEQ ID NO: 1; (4) a targeting sequence comprising SEQ ID NO: 1; (5) an exosporium protein comprising an amino acid sequence having at least 85% identity with SEQ ID NO: 2; (6) a targeting sequence comprising amino acids 2-35 of SEQ ID NO: 1; (7) a targeting sequence comprising amino acids 5-35 of SEQ ID NO: 1; (8) a targeting sequence comprising amino acids 8-35 of SEQ ID NO: 1; (9) a targeting sequence comprising amino acids 10-35 of SEQ ID NO: 1; (10) a targeting sequence comprising amino acids 15-35 of SEQ ID NO: 1; (11) a targeting sequence comprising amino acids 1-27 of SEQ ID NO: 3; (12) a targeting sequence comprising amino acids 12-27 of SEQ ID NO: 3; (13) a targeting sequence comprising SEQ ID NO: 3; (14) an exosporium protein comprising an amino acid sequence having at least 85% identity with SEQ ID NO: 4; (15) a targeting sequence comprising amino acids 2-27 of SEQ ID NO: 3; (16) a targeting sequence comprising amino acids 5-27 of SEQ ID NO: 3; (17) a targeting sequence comprising amino acids 8-27 of SEQ ID NO: 3; (18) a targeting sequence comprising amino acids 10-27 of SEQ ID NO: 3; (19) a targeting sequence comprising amino acids 1-38 of SEQ ID NO: 5; (20) a targeting sequence comprising amino acids 23-38 of SEQ ID NO: 5; (21) a targeting sequence comprising SEQ ID NO: 5; (22) an exosporium protein comprising an amino acid sequence having at least 85% identity with SEQ ID NO: 6; (23) a

targeting sequence comprising amino acids 2-38 of SEQ ID NO: 5; (24) a targeting sequence comprising amino acids 5-38 of SEQ ID NO: 5; (25) a targeting sequence comprising amino acids 8-38 of SEQ ID NO: 5; (26) a targeting sequence comprising amino acids 10-38 of SEQ ID NO: 5; (27) a targeting sequence comprising amino acids 15-38 of SEQ ID NO: 5; (28) a targeting sequence comprising amino acids 20-38 of SEQ ID NO: 5; (29) a targeting sequence comprising amino acids 1-28 of SEQ ID NO: 7; (30) a targeting sequence comprising amino acids 13-28 of SEQ ID NO: 7; (31) a targeting sequence comprising SEQ ID NO: 7; (32) an exosporium protein comprising an amino acid sequence having at least 85% identity with SEQ ID NO: 8; (33) a targeting sequence comprising amino acids 2-28 of SEQ ID NO: 7; (34) a targeting sequence comprising amino acids 5-28 of SEQ ID NO: 7; (35) a targeting sequence comprising amino acids 8-28 of SEQ ID NO: 7; (36) a targeting sequence comprising amino acids 10-28 of SEQ ID NO: 7; (37) a targeting sequence comprising amino acids 1-24 of SEQ ID NO: 9; (38) a targeting sequence comprising amino acids 9-24 of SEQ ID NO: 9; (39) a targeting sequence comprising SEQ ID NO: 9; (40) an exosporium protein comprising an amino acid sequence having at least 85% identity with SEQ ID NO: 10; (41) a targeting sequence comprising amino acids 2-24 of SEQ ID NO: 9; (42) a targeting sequence comprising amino acids 5-24 of SEQ ID NO: 9; (43) a targeting sequence comprising amino acids 8-24 of SEQ ID NO: 9; (44) a targeting sequence comprising amino acids 1-33 of SEQ ID NO:11; (45) a targeting sequence comprising amino acids 18-33 of SEQ ID NO: 11; (46) a targeting sequence comprising SEQ ID NO: 11; (47) an exosporium protein comprising an amino acid sequence having at least 85% identity with SEQ ID NO: 12; (48) a targeting sequence comprising amino acids 2-33 of SEQ ID NO: 11; (49) a targeting sequence comprising amino acids 5-33 of SEQ ID NO: 11; (50) a targeting sequence comprising amino acids 8-33 of SEQ ID NO: 11; (51) a targeting sequence comprising amino acids 10-33 of SEQ ID NO: 11; (52) a targeting sequence comprising amino acids 15-33 of SEQ ID NO: 11; (53) a targeting sequence comprising amino acids 1-33 of SEQ ID NO: 13; (54) a targeting sequence comprising amino acids 18-33 of SEQ ID NO: 13; (55) a targeting sequence comprising SEQ ID NO: 13; (56) an exosporium protein comprising an amino acid sequence having at least 85% identity with SEQ ID NO:14; (57) a targeting sequence comprising amino acids 2-33 of SEQ ID NO: 13; (58) a targeting sequence comprising amino acids 5-33 of SEQ ID NO: 13; (59) a targeting sequence comprising amino acids 8-33 of SEQ ID NO: 13; (60) a targeting sequence comprising amino acids 10-33 of SEQ ID NO: 13; (61) a targeting sequence comprising amino acids 15-33 of SEQ ID NO: 13; (62) a targeting sequence comprising amino acids 1-43 of SEQ ID NO: 15; (63) a targeting sequence comprising amino acids 28-43 of SEQ ID NO: 15; (64) a targeting sequence comprising SEQ ID NO:15; (65) an exosporium protein comprising an amino acid sequence having at least 85% identity with SEQ ID NO:16; (66) a targeting sequence comprising amino acids 2-43 of SEQ ID NO: 15; (67) a targeting sequence comprising amino acids 5-43 of SEQ ID NO: 15; (68) a targeting sequence comprising amino acids 8-43 of SEQ ID NO: 15; (69) a targeting sequence comprising amino acids 10-43 of SEQ ID NO: 15; (70) a targeting sequence comprising amino acids 15-43 of SEQ ID NO: 15; (71) a targeting sequence comprising amino acids 20-43 of SEQ ID NO: 15; (72) a targeting sequence comprising amino acids 25-43 of SEQ ID NO: 15; (73) a targeting sequence comprising amino acids 1-27 of SEQ ID NO: 17; (74) a targeting sequence comprising amino acids 12-27 of SEQ ID NO: 17; (75) a targeting sequence comprising SEQ ID NO:17; (76) an exosporium protein comprising an amino acid sequence having at least 85% identity with SEQ ID NO:18; (77) a targeting sequence comprising amino acids 2-27 of SEQ ID NO: 17; (78) a targeting sequence comprising amino acids 5-27 of SEQ ID NO: 17; (79) a targeting sequence comprising amino acids 8-27 of SEQ ID NO: 17; (80) a targeting sequence comprising amino acids 10-27 of SEQ ID NO: 17; (81) a targeting sequence comprising amino acids 1-33 of SEQ ID NO: 19; (82) a targeting sequence comprising amino acids 18-33 of SEQ ID NO: 19; (83) a targeting sequence comprising SEQ ID NO: 19; (84) an exosporium protein comprising an amino acid sequence having at least 85% identity with SEQ ID NO:20; (85) a targeting sequence comprising amino acids 2-33 of SEQ ID NO: 19; (86) a targeting sequence comprising amino acids 5-33 of SEQ ID NO: 19; (87) a targeting sequence comprising amino acids 8-33 of SEQ ID NO: 19; (88) a targeting sequence comprising amino acids 10-33 of SEQ ID NO: 19; (89) a targeting sequence comprising amino acids 15-33 of SEQ ID NO: 19; (90) a targeting sequence comprising amino acids 1-33 of SEQ ID NO: 21; (91) a targeting sequence comprising amino acids 18-33 of SEQ ID NO: 21; (92) a targeting sequence comprising SEQ ID NO:21; (93) an exosporium protein comprising an amino acid sequence having at least 85% identity with SEQ ID NO:22; (94) a targeting sequence comprising amino acids 2-33 of SEQ ID NO: 21; (95) a targeting sequence comprising amino acids 5-33 of SEQ ID NO: 21; (96) a targeting sequence comprising amino acids 8-33 of SEQ ID NO: 21; (97) a targeting sequence comprising amino acids 10-33 of SEQ ID NO: 21; (98) a targeting sequence comprising amino acids 15-33 of SEQ ID NO: 21; (99) a targeting sequence comprising amino acids 1-24 of SEQ ID NO: 23; (100) a targeting sequence comprising amino acids 9-24 of SEQ ID NO: 23; (101) a targeting sequence comprising SEQ ID NO:23; (102) an exosporium protein comprising an amino acid sequence having at least 85% identity with SEQ ID NO:24; (103) a targeting sequence comprising amino acids 2-24 of SEQ ID NO:23; (104) a targeting sequence comprising amino acids 5-24 of SEQ ID NO: 23; (105) a targeting sequence comprising amino acids 8-24 of SEQ ID NO: 23; (106) a targeting sequence comprising amino acids 1-24 of SEQ ID NO: 25; (107) a targeting sequence comprising amino acids 9-24 of SEQ ID NO: 25;

(108) a targeting sequence comprising SEQ ID NO:25; (109) an exosporium protein comprising an amino acid sequence having at least 85% identity with SEQ ID NO:26; (110) a targeting sequence comprising amino acids 2-24 of SEQ ID NO: 25; (111) a targeting sequence comprising amino acids 5-24 of SEQ ID NO: 25; (112) a targeting sequence comprising amino acids 8-24 of SEQ ID NO: 25; (113) a targeting sequence comprising amino acids 1-30 of SEQ ID NO: 27; (114) a targeting sequence comprising amino acids 15-30 of SEQ ID NO: 27; (115) a targeting sequence comprising SEQ ID NO:27; (116) an exosporium protein comprising an amino acid sequence having at least 85% identity with SEQ ID NO:28; (117) a targeting sequence comprising amino acids 2-30 of SEQ ID NO: 27; (118) a targeting sequence comprising amino acids 5-30 of SEQ ID NO: 27; (119) a targeting sequence comprising amino acids 8-30 of SEQ ID NO: 27; (120) a targeting sequence comprising amino acids 10-30 of SEQ ID NO: 27; (121) a targeting sequence comprising amino acids 1-33 of SEQ ID NO: 29; (122) a targeting sequence comprising amino acids 18-33 of SEQ ID NO: 29; (123) a targeting sequence comprising SEQ ID NO:29; (124) an exosporium protein comprising an amino acid sequence having at least 85% identity with SEQ ID NO:30; (125) a targeting sequence comprising amino acids 2-33 of SEQ ID NO: 29; (126) a targeting sequence comprising amino acids 5-33 of SEQ ID NO: 29; (127) a targeting sequence comprising amino acids 8-33 of SEQ ID NO: 29; (128) a targeting sequence comprising amino acids 10-33 of SEQ ID NO: 29; (129) a targeting sequence comprising amino acids 15-33 of SEQ ID NO: 29; (130) a targeting sequence comprising amino acids 1-24 of SEQ ID NO: 31; (131) a targeting sequence comprising amino acids 9-24 of SEQ ID NO: 31; (132) a targeting sequence comprising SEQ ID NO:31; (133) an exosporium protein comprising an amino acid sequence having at least 85% identity with SEQ ID NO:32; (134) a targeting sequence comprising amino acids 2-24 of SEQ ID NO: 31; (135) a targeting sequence comprising amino acids 5-24 of SEQ ID NO: 31; (136) a targeting sequence comprising amino acids 8-24 of SEQ ID NO: 31; (137) a targeting sequence comprising amino acids 1-15 of SEQ ID NO: 33; (138) a targeting sequence comprising SEQ ID NO:33; (139) an exosporium protein comprising an amino acid sequence having at least 85% identity with SEQ ID NO:34; (140) a targeting sequence comprising amino acids 1-16 of SEQ ID NO: 35; (141) a targeting sequence comprising SEQ ID NO:35; (142) an exosporium protein comprising an amino acid sequence having at least 85% identity with SEQ ID NO:36; (143) a targeting sequence comprising amino acids 1-29 of SEQ ID NO:43; (144) a targeting sequence comprising amino acids 14-29 of SEQ ID NO: 43; (145) a targeting sequence comprising SEQ ID NO: 43; (146) an exosporium protein comprising an amino acid sequence having at least 85% identity with SEQ ID NO: 44; (147) a targeting sequence comprising amino acids 2-29 of SEQ ID NO: 43; (148) a targeting sequence comprising amino acids 5-29 of SEQ ID NO: 43; (149) a targeting sequence comprising amino acids 8-29 of SEQ ID NO: 43; (150) a targeting sequence comprising amino acids 10-29 of SEQ ID NO: 43; (151) a targeting sequence comprising amino acids 1-35 of SEQ ID NO: 45; (152) a targeting sequence comprising amino acids 20-35 of SEQ ID NO: 45; (153) a targeting sequence comprising SEQ ID NO: 45; (154) an exosporium protein comprising an amino acid sequence having at least 85% identity with SEQ ID NO: 46; (155) a targeting sequence comprising amino acids 2-35 of SEQ ID NO: 45; (156) a targeting sequence comprising amino acids 5-35 of SEQ ID NO: 45; (157) a targeting sequence comprising amino acids 8-35 of SEQ ID NO: 45; (158) a targeting sequence comprising amino acids 10-35 of SEQ ID NO: 45; (159) a targeting sequence comprising amino acids 15-35 of SEQ ID NO: 45; (160) a targeting sequence comprising amino acids 1-43 of SEQ ID NO: 47; (161) a targeting sequence comprising amino acids 28-43 of SEQ ID NO: 47; (162) a targeting sequence comprising SEQ ID NO: 47; (163) an exosporium protein comprising an amino acid sequence having at least 85% identity with SEQ ID NO: 48; (164) a targeting sequence comprising amino acids 2-43 of SEQ ID NO: 47; (165) a targeting sequence comprising amino acids 5-43 of SEQ ID NO: 47; (166) a targeting sequence comprising amino acids 8-43 of SEQ ID NO: 47; (167) a targeting sequence comprising amino acids 10-43 of SEQ ID NO: 47; (168) a targeting sequence comprising amino acids 15-43 of SEQ ID NO: 47; (169) a targeting sequence comprising amino acids 20-43 of SEQ ID NO: 47; (170) a targeting sequence comprising amino acids 25-43 of SEQ ID NO: 47; (171) a targeting sequence comprising amino acids 1-32 of SEQ ID NO: 49; (172) a targeting sequence comprising amino acids 17-32 of SEQ ID NO: 49; (173) a targeting sequence comprising SEQ ID NO: 49; (174) an exosporium protein comprising an amino acid sequence having at least 85% identity with SEQ ID NO: 50; (175) a targeting sequence comprising amino acids 2-32 of SEQ ID NO: 49; (176) a targeting sequence comprising amino acids 5-32 of SEQ ID NO: 49; (177) a targeting sequence comprising amino acids 8-32 of SEQ ID NO: 49; (178) a targeting sequence comprising amino acids 10-32 of SEQ ID NO: 49; (179) a targeting sequence comprising amino acids 15-32 of SEQ ID NO: 49; (180) a targeting sequence comprising amino acids 1-33 of SEQ ID NO: 51; (181) a targeting sequence comprising amino acids 18-33 of SEQ ID NO: 51; (182) a targeting sequence comprising SEQ ID NO: 51; (183) an exosporium protein comprising an amino acid sequence having at least 85% identity with SEQ ID NO: 52; (184) a targeting sequence comprising amino acids 2-33 of SEQ ID NO: 51; (185) a targeting sequence comprising amino acids 5-33 of SEQ ID NO: 51; (186) a targeting sequence comprising amino acids 8-33 of SEQ ID NO: 51; (187) a targeting sequence comprising amino acids 10-33 of SEQ ID NO: 51; (188) a targeting sequence comprising amino acids 15-33 of SEQ ID NO: 51; (189) a targeting sequence comprising amino acids 1-33 of SEQ ID NO: 53; (190) a targeting sequence comprising amino acids 18-33 of SEQ ID NO: 53; (191) a targeting sequence comprising SEQ

ID NO: 53; (192) an exosporium protein comprising an amino acid sequence having at least 85% identity with SEQ ID NO: 54; (193) a targeting sequence comprising amino acids 2-33 of SEQ ID NO: 53; (194) a targeting sequence comprising amino acids 5-33 of SEQ ID NO: 53; (195) a targeting sequence comprising amino acids 8-33 of SEQ ID NO: 53; (196) a targeting sequence comprising amino acids 10-33 of SEQ ID NO: 53; (197) a targeting sequence comprising amino acids 15-33 of SEQ ID NO: 53; (198) a targeting sequence comprising amino acids 1-30 of SEQ ID NO: 55; (199) a targeting sequence comprising amino acids 15-30 of SEQ ID NO: 55; (200) a targeting sequence comprising SEQ ID NO: 55; (201) an exosporium protein comprising an amino acid sequence having at least 85% identity with SEQ ID NO: 56; (202) a targeting sequence comprising amino acids 2-30 of SEQ ID NO: 55; (203) a targeting sequence comprising amino acids 5-30 of SEQ ID NO: 55; (204) a targeting sequence comprising amino acids 8-30 of SEQ ID NO: 55; (205) a targeting sequence comprising amino acids 10-30 of SEQ ID NO: 55; (206) a targeting sequence comprising amino acids 1-130 of SEQ ID NO: 57; (207) a targeting sequence comprising amino acids 115-130 of SEQ ID NO: 57; (208) a targeting sequence comprising SEQ ID NO: 57; (209) an exosporium protein comprising an amino acid sequence having at least 85% identity with SEQ ID NO: 58; (210) a targeting sequence comprising amino acids 2-130 of SEQ ID NO: 57; (211) a targeting sequence comprising amino acids 5-130 of SEQ ID NO: 57; (212) a targeting sequence comprising amino acids 10-130 of SEQ ID NO: 57; (213) a targeting sequence comprising amino acids 20-130 of SEQ ID NO: 57; (214) a targeting sequence comprising amino acids 30-130 of SEQ ID NO: 57; (215) a targeting sequence comprising amino acids 40-130 of SEQ ID NO: 57; (216) a targeting sequence comprising amino acids 50-130 of SEQ ID NO: 57; (217) a targeting sequence comprising amino acids 60-130 of SEQ ID NO: 57; (218) a targeting sequence comprising amino acids 70-130 of SEQ ID NO: 57; (219) a targeting sequence comprising amino acids 80-130 of SEQ ID NO: 57; (220) a targeting sequence comprising amino acids 90-130 of SEQ ID NO: 57; (221) a targeting sequence comprising amino acids 100-130 of SEQ ID NO: 57; (222) a targeting sequence comprising amino acids 110-130 of SEQ ID NO: 57; (223) an exosporium protein fragment comprising an amino acid sequence having at least 85% identity with SEQ ID NO: 95; (224) a targeting sequence comprising SEQ ID NO: 96; (225) a targeting sequence comprising SEQ ID NO: 97; (226) a targeting sequence comprising SEQ ID NO: 98; (227) a targeting sequence comprising SEQ ID NO: 99; (228) a targeting sequence comprising SEQ ID NO: 100; (229) a targeting sequence comprising SEQ ID NO: 101; (230) a targeting sequence comprising SEQ ID NO: 102; (231) a targeting sequence comprising SEQ ID NO: 103; (232) a targeting sequence comprising SEQ ID NO: 104; (233) a targeting sequence comprising SEQ ID NO: 105; (234) a targeting sequence comprising SEQ ID NO: 106; (235) an exosporium protein comprising an amino acid sequence having at least 85% identity with SEQ ID NO: 108; (236) an exosporium protein comprising an amino acid sequence having at least 85% identity with SEQ ID NO: 109; (237) an exosporium protein comprising an amino acid sequence having at least 85% identity with SEQ ID NO: 110; (238) an exosporium protein comprising an amino acid sequence having at least 85% identity with SEQ ID NO: 111; (239) an exosporium protein comprising an amino acid sequence having at least 85% identity with SEQ ID NO: 112; (240) an exosporium protein comprising an amino acid sequence having at least 85% identity with SEQ ID NO: 113; (241) an exosporium protein comprising an amino acid sequence having at least 85% identity with SEQ ID NO: 114; (242) an exosporium protein comprising an amino acid sequence having at least 85% identity with SEQ ID NO: 115; (243) an exosporium protein comprising an amino acid sequence having at least 85% identity with SEQ ID NO: 116; (244) an exosporium protein comprising an amino acid sequence having at least 85% identity with SEQ ID NO: 117; (245) an exosporium protein comprising an amino acid sequence having at least 85% identity with SEQ ID NO: 118; (246) an exosporium protein comprising an amino acid sequence having at least 85% identity with SEQ ID NO: 119; (247) an exosporium protein comprising an amino acid sequence having at least 85% identity with SEQ ID NO: 120; (248) an exosporium protein comprising an amino acid sequence having at least 85% identity with SEQ ID NO: 121; (249) a targeting sequence comprising amino acids 22-31 of SEQ ID NO: 1; (250) a targeting sequence comprising amino acids 22-33 of SEQ ID NO: 1; (251) a targeting sequence comprising amino acids 20-31 of SEQ ID NO: 1; (252) a targeting sequence comprising amino acids 14-23 of SEQ ID NO: 3; (253) a targeting sequence comprising amino acids 14-25 of SEQ ID NO: 3; (254) a targeting sequence comprising amino acids 12-23 of SEQ ID NO: 3; (255) a targeting sequence comprising amino acids 1-30 of SEQ ID NO: 59; (256) a targeting sequence comprising SEQ ID NO: 59; (257) an exosporium protein comprising an amino acid sequence having at least 85% identity with SEQ ID NO: 60; (258) a targeting sequence comprising amino acids 2-30 of SEQ ID NO: 59; (259) a targeting sequence comprising amino acids 4-30 of SEQ ID NO: 59; (260) a targeting sequence comprising amino acids 6-30 of SEQ ID NO: 59; (261) a targeting sequence comprising amino acids 1-33 of SEQ ID NO: 61; (262) a targeting sequence comprising amino acids 18-33 of SEQ ID NO: 61; (263) a targeting sequence comprising SEQ ID NO: 61; (264) an exosporium protein comprising an amino acid sequence having at least 85% sequence identity with SEQ ID NO: 62; (265) a targeting sequence comprising amino acids 2-33 of SEQ ID NO: 61; (266) a targeting sequence comprising amino acids 5-33 of SEQ ID NO: 61; (267) a targeting sequence comprising amino acids 10-33 of SEQ ID NO: 61; (268) a targeting sequence comprising amino acids 15-33 of SEQ ID NO: 61; (269) a targeting sequence comprising amino acids 1-35 of SEQ ID NO: 63; (270) a targeting sequence

comprising SEQ ID NO: 63; (271) an exosporium protein comprising an amino acid sequence having at least 85% identity with SEQ ID NO: 64; (272) a targeting sequence comprising amino acids 2-35 of SEQ ID NO: 63; (273) a targeting sequence comprising amino acids 5-35 of SEQ ID NO: 63; (274) a targeting sequence comprising amino acids 8-35 of SEQ ID NO: 63; (275) a targeting sequence comprising amino acids 10-35 of SEQ ID NO: 63; (276) a targeting sequence comprising amino acids 15-35 of SEQ ID NO: 63; (277) a targeting sequence comprising amino acids 1-24 of SEQ ID NO: 65; (278) a targeting sequence comprising amino acids 9-24 of SEQ ID NO: 65; (279) a targeting sequence comprising SEQ ID NO: 65; (280) an exosporium protein comprising an amino acid sequence having at least 85% identity with SEQ ID NO: 66; (281) a targeting sequence comprising SEQ ID NO: 107; (282) a targeting sequence comprising amino acids 2-24 of SEQ ID NO: 65; (283) a targeting sequence comprising amino acids 5-24 of SEQ ID NO: 65; (284) a targeting sequence comprising amino acids 1-27 of SEQ ID NO: 67; (285) a targeting sequence comprising amino acids 12-27 of SEQ ID NO: 67; (286) a targeting sequence comprising SEQ ID NO: 67; (287) an exosporium protein comprising an amino acid sequence having at least 85% identity with SEQ ID NO: 68; (288) an targeting sequence comprising amino acids 2-27 of SEQ ID NO: 67; (289) a targeting sequence comprising amino acids 5-27 of SEQ ID NO: 67; (290) a targeting sequence comprising amino acids 10-27 of SEQ ID NO: 67; (291) a targeting sequence comprising amino acids 1-38 of SEQ ID NO: 69; (292) a targeting sequence comprising amino acids 23-38 of SEQ ID NO: 69; (293) a targeting sequence comprising SEQ ID NO: 69; (294) an exosporium protein comprising an amino acid sequence having at least 85% identity with SEQ ID NO: 70; (295) a targeting sequence comprising amino acids 2-38 of SEQ ID NO: 69; (296) a targeting sequence comprising amino acids 5-38 of SEQ ID NO: 69; (297) a targeting sequence comprising amino acids 10-38 of SEQ ID NO: 69; (298) a targeting sequence comprising amino acids 15-38 of SEQ ID NO: 69; (299) an exosporium protein comprising SEQ ID NO: 72; (300) a targeting sequence comprising SEQ ID NO: 73; (301) an exosporium protein comprising an amino acid sequence having at least 95% identity with SEQ ID NO: 74; (302) a targeting sequence comprising amino acids 1-42 of SEQ ID NO: 75; (303) a targeting sequence comprising amino acids 27-42 of SEQ ID NO: 75; (304) a targeting sequence comprising SEQ ID NO: 75; (305) an exosporium protein comprising an amino acid sequence having at least 85% identity with SEQ ID NO: 76; (306) a targeting sequence comprising amino acids 2-42 of SEQ ID NO: 75; (307) a targeting sequence comprising amino acids 5-42 of SEQ ID NO: 75; (308) a targeting sequence comprising amino acids 10-42 of SEQ ID NO: 75; (309) a targeting sequence comprising amino acids 15-42 of SEQ ID NO: 75; (310) a targeting sequence comprising amino acids 20-42 of SEQ ID NO: 75; (311) a targeting sequence comprising amino acids 25-42 of SEQ ID NO: 75; (312) a targeting sequence comprising amino acids 1-24 of SEQ ID NO: 77; (313) a targeting sequence comprising amino acids 9-24 of SEQ ID NO: 77; (314) a targeting sequence comprising SEQ ID NO: 77; (315) an exosporium protein comprising an amino acid sequence having at least 85% identity with SEQ ID NO: 78; (316) a targeting sequence comprising amino acids 2-24 of SEQ ID NO: 77; (317) a targeting sequence comprising amino acids 5-24 of SEQ ID NO: 77; (318) an exosporium protein comprising an amino acid sequence having at least 85% identity with SEQ ID NO: 80; (319) a targeting sequence comprising amino acids 1-38 of SEQ ID NO: 81; (320) a targeting sequence comprising amino acids 23-38 of SEQ ID NO: 81; (321) a targeting sequence comprising SEQ ID NO: 81; (322) an exosporium protein comprising an amino acid sequence having at least 85% identity with SEQ ID NO: 82; (323) a targeting sequence comprising amino acids 2-38 of SEQ ID NO: 81; (324) a targeting sequence comprising amino acids 5-38 of SEQ ID NO: 81; (325) a targeting sequence comprising amino acids 10-38 of SEQ ID NO: 81; (326) a targeting sequence comprising amino acids 15-38 of SEQ ID NO: 81; (327) a targeting sequence comprising amino acids 20-38 of SEQ ID NO: 81; (328) a targeting sequence comprising amino acids 1-34 of SEQ ID NO: 83; (329) a targeting sequence comprising SEQ ID NO: 83; (330) an exosporium protein comprising an amino acid sequence having at least 85% identity with SEQ ID NO: 84; (331) an exosporium protein comprising an amino acid sequence having at least 85% identity with SEQ ID NO: 86; (332) a targeting sequence comprising amino acids 1-28 of SEQ ID NO: 87; (333) a targeting sequence comprising amino acids 13-28 of SEQ ID NO: 87; (334) a targeting sequence comprising SEQ ID NO: 87; (335) an exosporium protein comprising an amino acid sequence having at least 85% identity with SEQ ID NO: 88; (336) a targeting sequence comprising amino acids 2-28 of SEQ ID NO: 87; (337) a targeting sequence comprising amino acids 5-28 of SEQ ID NO: 87; (338) a targeting sequence comprising amino acids 10-28 of SEQ ID NO: 87; (339) a targeting sequence comprising amino acids 1-28 of SEQ ID NO: 89; (340) a targeting sequence comprising SEQ ID NO: 89; (341) an exosporium protein comprising an amino acid sequence having at least 85% identity with SEQ ID NO: 90; (342) a targeting sequence comprising amino acids 2-28 of SEQ ID NO: 89; (343) a targeting sequence comprising amino acids 5-28 of SEQ ID NO: 89; (344) a targeting sequence comprising amino acids 10-28 of SEQ ID NO: 89; (345) a targeting sequence comprising amino acids 1-93 of SEQ ID NO: 91; (346) a targeting sequence comprising SEQ ID NO: 91; (347) an exosporium protein comprising an amino acid sequence having at least 85% identity with SEQ ID NO: 92; (348) a targeting sequence comprising amino acids 2-93 of SEQ ID NO: 91; (349) a targeting sequence comprising amino acids 10-93 of SEQ ID NO: 91; (350) a targeting sequence comprising amino acids 20-93 of SEQ ID NO: 91; (351) a targeting sequence comprising amino acids 30-93 of SEQ ID NO: 91; (352) a targeting sequence comprising amino acids 40-93 of SEQ ID NO:

91; (353) a targeting sequence comprising amino acids 50-93 of SEQ ID NO: 91; (354) a targeting sequence comprising amino acids 60-93 of SEQ ID NO: 91; (355) a targeting sequence comprising amino acids 1-130 of SEQ ID NO: 93; (356) a targeting sequence comprising SEQ ID NO: 93; (357) an exosporium protein comprising an amino acid sequence having at least 85% identity with SEQ ID NO: 94; (358) a targeting sequence comprising amino acids 2-130 of SEQ ID NO: 93; (359) a targeting sequence comprising amino acids 10-130 of SEQ ID NO: 93; (360) a targeting sequence comprising amino acids 20-130 of SEQ ID NO: 93; (361) a targeting sequence comprising amino acids 30-130 of SEQ ID NO: 93; (362) an exosporium protein comprising an amino acid sequence having at least 85% identity with SEQ ID NO: 122; (363) a targeting sequence consisting of amino acids 20-33 of SEQ ID NO: 1; (364) a targeting sequence consisting of amino acids 21-33 of SEQ ID NO: 1; (365) a targeting sequence consisting of amino acids 23-31 of SEQ ID NO: 1; (366) a targeting sequence consisting of amino acids 1-15 of SEQ ID NO: 96; (367) a targeting sequence consisting of amino acids 1-13 of SEQ ID NO: 96; (368) a targeting sequence consisting of amino acids 12-25 of SEQ ID NO: 3; (369) a targeting sequence consisting of amino acids 13-25 of SEQ ID NO: 3; (370) a targeting sequence consisting of amino acids 15-23 of SEQ ID NO: 3; (371) a targeting sequence consisting of amino acids 1-15 of SEQ ID NO: 97; (372) a targeting sequence consisting of amino acids 1-13 of SEQ ID NO: 98; (373) a targeting sequence consisting of amino acids 23-36 of SEQ ID NO: 5; (374) a targeting sequence consisting of amino acids 23-34 of SEQ ID NO: 5; (375) a targeting sequence consisting of amino acids 24-36 of SEQ ID NO: 5; (376) a targeting sequence consisting of amino acids 26-34 of SEQ ID NO: 5; (377) a targeting sequence consisting of amino acids 13-26 of SEQ ID NO: 7; (378) a targeting sequence consisting of amino acids 13-24 of SEQ ID NO: 7; (379) a targeting sequence consisting of amino acids 14-26 of SEQ ID NO: 7; (380) a targeting sequence consisting of amino acids 16-24 of SEQ ID NO: 7; (381) a targeting sequence consisting of amino acids 9-22 of SEQ ID NO: 9; (382) a targeting sequence consisting of amino acids 9-20 of SEQ ID NO: 9; (383) a targeting sequence consisting of amino acids 10-22 of SEQ ID NO: 9; (384) a targeting sequence consisting of amino acids 12-20 of SEQ ID NO: 9; (385) a targeting sequence consisting of amino acids 1-15 of SEQ ID NO: 105; (386) a targeting sequence consisting of amino acids 1-13 of SEQ ID NO: 105; (387) a targeting sequence consisting of amino acids 18-31 of SEQ ID NO: 11; (388) a targeting sequence consisting of amino acids 18-29 of SEQ ID NO: 11; (389) a targeting sequence consisting of amino acids 19-31 of SEQ ID NO: 11; (390) a targeting sequence consisting of amino acids 1-15 of SEQ ID NO: 98; (391) a targeting sequence consisting of amino acids 1-13 of SEQ ID NO: 98; (392) a targeting sequence consisting of amino acids 18-31 of SEQ ID NO: 13; (393) a targeting sequence consisting of amino acids 18-29 of SEQ ID NO: 13; (394) a targeting sequence consisting of amino acids 19-31 of SEQ ID NO: 13; (395) a targeting sequence consisting of amino acids 21-29 of SEQ ID NO: 13; (396) a targeting sequence consisting of amino acids 1-15 of SEQ ID NO: 99; (397) a targeting sequence consisting of amino acids 1-13 of SEQ ID NO: 99; (398) a targeting sequence consisting of amino acids 28-41 of SEQ ID NO: 15; (399) a targeting sequence consisting of amino acids 28-39 of SEQ ID NO: 15; (400) a targeting sequence consisting of amino acids 29-41 of SEQ ID NO: 15; (401) a targeting sequence consisting of amino acids 31-39 of SEQ ID NO: 15; (402) a targeting sequence consisting of amino acids 12-25 of SEQ ID NO: 17; (403) a targeting sequence consisting of amino acids 13-25 of SEQ ID NO: 17; (404) a targeting sequence consisting of amino acids 1-15 of SEQ ID NO: 100; (405) a targeting sequence consisting of amino acids 18-31 of SEQ ID NO: 19; (406) a targeting sequence consisting of amino acids 18-29 of SEQ ID NO: 19; (407) a targeting sequence consisting of amino acids 19-31 of SEQ ID NO: 19; (408) a targeting sequence consisting of amino acids 21-29 of SEQ ID NO: 19; (409) a targeting sequence consisting of amino acids 18-31 of SEQ ID NO: 21; (410) a targeting sequence consisting of amino acids 18-29 of SEQ ID NO: 21; (411) a targeting sequence consisting of amino acids 19-31 of SEQ ID NO: 21; (412) a targeting sequence consisting of amino acids 21-29 of SEQ ID NO: 21; (413) a targeting sequence consisting of amino acids 1-15 of SEQ ID NO: 101; (414) a targeting sequence consisting of amino acids 1-13 of SEQ ID NO: 101; (415) a targeting sequence consisting of amino acids 9-22 of SEQ ID NO: 23; (416) a targeting sequence consisting of amino acids 9-20 of SEQ ID NO: 23; (417) a targeting sequence consisting of amino acids 10-22 of SEQ ID NO: 23; (418) a targeting sequence consisting of amino acids 12-20 of SEQ ID NO: 23; (419) a targeting sequence consisting of amino acids 1-15 of SEQ ID NO: 102; (420) a targeting sequence consisting of amino acids 1-13 of SEQ ID NO: 102; (421) a targeting sequence consisting of amino acids 9-22 of SEQ ID NO: 25; (422) a targeting sequence consisting of amino acids 9-20 of SEQ ID NO: 25; (423) a targeting sequence consisting of amino acids 10-22 of SEQ ID NO: 25; (424) a targeting sequence consisting of amino acids 12-20 of SEQ ID NO: 25; (425) a targeting sequence consisting of amino acids 1-15 of SEQ ID NO: 103; (426) a targeting sequence consisting of amino acids 1-13 of SEQ ID NO: 103; (427) a targeting sequence consisting of amino acids 15-28 of SEQ ID NO: 27; (428) a targeting sequence consisting of amino acids 15-26 of SEQ ID NO: 27; (429) a targeting sequence consisting of amino acids 16-28 of SEQ ID NO: 27; (430) a targeting sequence consisting of amino acids 18-26 of SEQ ID NO: 27; (431) a targeting sequence consisting of amino acids 1-15 of SEQ ID NO: 104; (432) a targeting sequence consisting of amino acids 1-13 of SEQ ID NO: 104; (433) a targeting sequence consisting of amino acids 1-13 of SEQ ID NO: 33; (434) a targeting sequence consisting of amino acids 1-11 of SEQ ID NO: 33; (435) a targeting sequence

consisting of amino acids 3-11 of SEQ ID NO: 33; (436) a targeting sequence consisting of amino acids 1-14 of SEQ ID NO: 35; (437) a targeting sequence consisting of amino acids 1-12 of SEQ ID NO: 35; (438) a targeting sequence consisting of amino acids 2-14 of SEQ ID NO: 35; (439) a targeting sequence consisting of amino acids 14-27 of SEQ ID NO: 43; (440) a targeting sequence consisting of amino acids 14-25 of SEQ ID NO: 43; (441) a targeting sequence consisting of amino acids 15-27 of SEQ ID NO: 43; (442) a targeting sequence consisting of amino acids 20-33 of SEQ ID NO: 45; (443) a targeting sequence consisting of amino acids 20-31 of SEQ ID NO: 45; (444) a targeting sequence consisting of amino acids 21-33 of SEQ ID NO: 45; (445) a targeting sequence consisting of amino acids 1-15 of SEQ ID NO: 106; (446) a targeting sequence consisting of amino acids 1-13 of SEQ ID NO: 106; (447) a targeting sequence consisting of amino acids 28-41 of SEQ ID NO: 47; (448) a targeting sequence consisting of amino acids 28-39 of SEQ ID NO: 47; (449) a targeting sequence consisting of amino acids 18-31 of SEQ ID NO: 53; (450) a targeting sequence consisting of amino acids 18-29 of SEQ ID NO: 53; (451) a targeting sequence consisting of amino acids 19-31 of SEQ ID NO: 53; (452) a targeting sequence comprising amino acids 18-31 of SEQ ID NO: 61; (453) a targeting sequence comprising amino acids 18-29 of SEQ ID NO: 61; (454) a targeting sequence comprising amino acids 19-31 of SEQ ID NO: 61; (455) a targeting sequence comprising amino acids 9-22 of SEQ ID NO: 65; (456) a targeting sequence comprising amino acids 9-20 of SEQ ID NO: 65; (457) a targeting sequence comprising amino acids 10-22 of SEQ ID NO: 65; (458) a targeting sequence comprising amino acids 1-15 of SEQ ID NO: 107; (459) a targeting sequence comprising amino acids 1-13 of SEQ ID NO: 107; (460) a targeting sequence comprising amino acids 12-25 of SEQ ID NO: 67; (461) a targeting sequence comprising amino acids 12-23 of SEQ ID NO: 67; (462) a targeting sequence comprising amino acids 13-25 of SEQ ID NO: 67; (463) a targeting sequence comprising amino acids 15-23 of SEQ ID NO: 67; (464) a targeting sequence comprising amino acids 23-36 of SEQ ID NO: 69; (465) a targeting sequence comprising amino acids 23-34 of SEQ ID NO: 69; (466) a targeting sequence comprising amino acids 24-36 of SEQ ID NO: 69; (467) a targeting sequence comprising amino acids 26-34 of SEQ ID NO: 69; (468) a targeting sequence comprising amino acids 27-40 of SEQ ID NO: 75; (469) a targeting sequence comprising amino acids 27-38 of SEQ ID NO: 75; (470) a targeting sequence comprising amino acids 9-22 of SEQ ID NO: 77; (471) a targeting sequence comprising amino acids 9-20 of SEQ ID NO: 77; (472) a targeting sequence comprising amino acids 10-22 of SEQ ID NO: 77; (473) a targeting sequence comprising amino acids 12-20 of SEQ ID NO: 77; (474) a targeting sequence comprising amino acids 23-36 of SEQ ID NO: 81; (475) a targeting sequence comprising amino acids 23-34 of SEQ ID NO: 81; (476) a targeting sequence comprising amino acids 24-36 of SEQ ID NO: 81; (477) a targeting sequence comprising amino acids 26-34 of SEQ ID NO: 81; (478) a targeting sequence comprising amino acids 13-26 of SEQ ID NO: 87; (479) a targeting sequence comprising amino acids 13-24 of SEQ ID NO: 87; or (480) a targeting sequence comprising amino acids 14-26 of SEQ ID NO: 87.

(816) For example, the targeting sequence can comprise or consist of an amino acid sequence having at least about 50% identity with amino acids 20-35 of SEQ ID NO: 1, wherein the identity with amino acids 25-35 is at least about 63%.

(817) The targeting sequence can comprise or consist of an amino acid sequence having at least about 50% identity with amino acids 20-35 of SEQ ID NO: 1, wherein the identity with amino acids 25-35 is at least about 72%.

(818) The targeting sequence can comprise or consist of an amino acid sequence having at least about 56% identity with amino acids 20-35 of SEQ ID NO: 1, wherein the identity with amino acids 25-35 is at least about 63%.

(819) The targeting sequence can comprise or consist of an amino sequence having at least about 62% identity with amino acids 20-35 of SEQ ID NO: 1, wherein the identity with amino acids 25-35 is at least about 72%.

(820) The targeting sequence can comprise or consist of an amino acid sequence having at least about 68% identity with amino acids 20-35 of SEQ ID NO: 1, wherein the identity with amino acids 25-35 is at least about 81%.

(821) The targeting sequence can comprise or consist of an amino sequence having at least about 75% identity with amino acids 20-35 of SEQ ID NO: 1, wherein the identity with amino acids 25-35 is at least about 72%.

(822) The targeting sequence can comprise or consist of an amino sequence having at least about 75% identity with amino acids 20-35 of SEQ ID NO: 1, wherein the identity with amino acids 25-35 is at least about 81%.

(823) The targeting sequence can comprise or consist of an amino acid sequence having at least about 81% identity with amino acids 20-35 of SEQ ID NO:1, wherein the identity with amino acids 25-35 is at least about 81%.

(824) The targeting sequence can comprise or consist of an amino acid sequence having at least about 81% identity with amino acids 20-35 of SEQ ID NO: 1, wherein the identity with amino acids 25-35 is at least about 90%.

(825) The targeting sequence can consist of: (a) an amino acid sequence consisting of 16 amino acids and having at least about 43% identity with amino acids 20-35 of SEQ ID NO: 1, wherein the identity with amino acids 25-35 is at least about 54%; (b) amino acids 1-35 of SEQ ID NO: 1; (c) amino acids 20-35 of SEQ ID NO: 1; (d) SEQ ID NO: 1; (e) SEQ ID NO: 96; or (f) SEQ ID NO: 120.

(826) The exosporium protein or exosporium protein fragment can comprise an amino acid sequence having at least 90% identity with SEQ ID NO: 2, 4, 6, 8, 10, 12, 14, 16, 18, 20, 22, 24, 26, 28, 30, 32, 34, 36, 44, 46, 48, 50, 52, 54, 56, 58, 60, 62, 64, 66, 68, 70, 76, 78, 80, 82, 84, 86, 88, 90, 92, 94, 95, 108, 109, 110, 111, 112, 113, 114, 115, 116,

117, 118, 119, 120, 121, and 122.
(827) The exosporium protein or exosporium protein fragment can comprise an amino acid sequence having at least 95% identity with SEQ ID NO: 2, 4, 6, 8, 10, 12, 14, 16, 18, 20, 22, 24, 26, 28, 30, 32, 34, 36, 44, 46, 48, 50, 52, 54, 56, 58, 60, 62, 64, 66, 68, 70, 76, 78, 80, 82, 84, 86, 88, 90, 92, 94, 95, 108, 109, 110, 111, 112, 113, 114, 115, 116, 117, 118, 119, 120, 121, and 122.
(828) The exosporium protein or exosporium protein fragment can comprise an amino acid sequence having at least 98% identity with SEQ ID NO: 2, 4, 6, 8, 10, 12, 14, 16, 18, 20, 22, 24, 26, 28, 30, 32, 34, 36, 44, 46, 48, 50, 52, 54, 56, 58, 60, 62, 64, 66, 68, 70, 76, 78, 80, 82, 84, 86, 88, 90, 92, 94, 95, 108, 109, 110, 111, 112, 113, 114, 115, 116, 117, 118, 119, 120, 121, and 122.
(829) The exosporium protein or exosporium protein fragment can comprise an amino acid sequence having at least 99% identity with SEQ ID NO: 2, 4, 6, 8, 10, 12, 14, 16, 18, 20, 22, 24, 26, 28, 30, 32, 34, 36, 44, 46, 48, 50, 52, 54, 56, 58, 60, 62, 64, 66, 68, 70, 76, 78, 80, 82, 84, 86, 88, 90, 92, 94, 95, 108, 109, 110, 111, 112, 113, 114, 115, 116, 117, 118, 119, 120, 121, and 122.
(830) The exosporium protein or exosporium protein fragment can comprise an amino acid sequence having at least 100% identity with SEQ ID NO: 2, 4, 6, 8, 10, 12, 14, 16, 18, 20, 22, 24, 26, 28, 30, 32, 34, 36, 44, 46, 48, 50, 52, 54, 56, 58, 60, 62, 64, 66, 68, 70, 76, 78, 80, 82, 84, 86, 88, 90, 92, 94, 95, 108, 109, 110, 111, 112, 113, 114, 115, 116, 117, 118, 119, 120, 121, and 122.
(831) The targeting sequence, exosporium protein, or exosporium protein fragment can comprise the amino acid sequence GXT at its carboxy terminus, wherein X is any amino acid.
(832) The targeting sequence, exosporium protein, or exosporium protein fragment can comprise an alanine residue at the position of the targeting sequence that corresponds to amino acid 20 of SEQ ID NO: 1.
(833) The targeting sequence, exosporium protein, or exosporium protein fragment can further comprise a methionine, serine, or threonine residue at the amino acid position immediately preceding the first amino acid of the targeting sequence, exosporium protein, or exosporium protein fragment or at the position of the targeting sequence that corresponds to amino acid 20 of SEQ ID NO: 1.
(834) The fusion protein can further comprise an amino acid linker between the targeting sequence, the exosporium protein, or the exosporium protein fragment and the protein or peptide of interest. The linker can be any of the linkers described above in Section XI.
(835) The fusion protein can be expressed under the control of a sporulation promoter native to the targeting sequence, exosporium protein, or exosporium protein fragment of the fusion protein or a portion thereof and/or under the control of a high-expression sporulation promoter. The promoter can be any of the promoters described above in Section III.
(836) A further method for delivering proteins or peptides to an animal is also provided. The method comprises feeding to an animal a recombinant spore-forming bacterium. The recombinant spore-forming bacterium can be any of the recombinant spore-forming bacteria described above in Section IX.
(837) The recombinant spore-forming bacterium can be comprised within a plant that is fed to the animal.
(838) The recombinant spore-forming bacterium can comprise an endophytic and probiotic strain of bacteria. For example, the endophytic and probiotic strain of bacteria can comprise *Bacillus megaterium* EE385 (NRRL B-50980), *Bacillus* sp. EE387 (NRRL B-50981), *Bacillus circulans* EE388 (NRRL B-50982), *Bacillus subtilis* EE405 (NRRL B-50978), *Lysinibacillus fusiformis* EE442 (NRRL B-50975), or *Lysinibacillus sphaericus* EE443 (NRRL B-50976), *Bacillus pumilus* EE-B00143 (NRRL B-67123), or a combination thereof.
(839) In any of the above methods, the plant can be processed prior to feeding to the animal.
(840) In any of the above methods involving feeding a plant to an animal, the method can further comprise introducing the endophytic strain of bacteria or a formulation comprising the endophytic strain of bacteria into a plant growth medium. Alternatively, the method can comprise applying the endophytic strain of bacteria or a formulation comprising the endophytic strain of bacteria to a plant, a plant seed, or an area surrounding a plant or a plant seed. The plant fed to the animal comprises a plant grown in a plant growth medium containing the endophytic and probiotic strain of bacteria or a formulation comprising the endophytic and probiotic strain of bacteria, a plant to which the endophytic and probiotic strain of bacteria was applied, a plant grown from a plant seed to which the endophytic and probiotic strain of bacteria was applied, a plant grown in an area to which the endophytic and probiotic strain of bacteria was applied, or a seed grown in the area to which the endophytic and probiotic strain of bacteria was applied.
(841) In any of the above methods for delivering proteins or peptides to an animal, the protein or peptide of interest comprises an enzyme. For example, the enzyme can comprise a xylanase, a xylosidase, a phytase, a phosphatase, a protease, a cellulase, an endoglucanase, an exogluconase, a glucanase, an amylase (e.g., α-amylase or a β-amylase), a lipase, a phospholipase, a glycosylase, a galactanase, an α-galactosidase, a β-glucosidase, an amylase, a pectinase, a biotinase, a polygalacturonase, a ligninase, or a combination thereof. The lipase can comprise a phospholipase A1, a phospholipase A2, a phospholipase C, a phospholipase D, a lysophospholipase, or a combination thereof. The

enzyme preferably comprises a xylanase or a phytase.
(842) In any of the methods comprising feeding a plant to an animal, the plant can be processed prior to feeding to the animal.
(843) In any of the above methods comprising delivery of bacteria, proteins, or peptides to an animal, the animal can be a mammal (e.g., a sheep, goat, cow, pig, deer, alpaca, bison, camel, donkey, horse, mule, llama, rabbit, dog, or cat), a bird (e.g., a chicken, turkey, duck, goose, quail, or pheasant), a fish (e.g., salmon, trout, tilapia, tuna, catfish, or a carp), or a crustacean (e.g., a shrimp, prawn, lobster, crab, or crayfish).
XXI. Methods for Delivering Beneficial Nucleic Acids to Animals, Insects, Worms, Fungi, and Protozoans
(844) The invention further relates to methods for delivering a nucleic acid molecule to an animal, insect, worm, fungus, or protozoan.
(845) The method can comprise feeding to an animal, an insect, or worm a plant modified to comprise a level of the nucleic acid molecule that is greater than the level of the nucleic acid molecule in the same plant that has not been modified, grown under the same conditions.
(846) A further method for delivering a nucleic acid molecule to an animal, insect, or worm is provided. The method can comprise feeding to an animal, insect, or worm a recombinant *Bacillus cereus* family member expressing a fusion protein comprising a protein or peptide of interest and a targeting sequence, exosporium protein, or exosporium protein fragment that targets the fusion protein to the exosporium of the recombinant *Bacillus cereus* family member. Alternatively, the method can comprise feeding to an animal, insect, or worm a recombinant spore-forming bacterium that expresses a fusion protein comprising at least one protein or peptide of interest and a spore coat protein that targets the fusion protein to the surface of a spore of the bacterium. The protein or peptide of interest comprises a nucleic acid binding protein or peptide and the nucleic acid molecule is bound to the DNA or RNA binding protein or peptide. The nucleic acid binding protein or peptide can be physically attached to the exosporium of the recombinant *Bacillus cereus* family member or to the spore coat of the recombinant spore-forming bacterium.
(847) Another method for delivering a nucleic acid molecule to an animal, insect, or worm is provided. The method comprises feeding to an animal, insect, or worm exosporium fragments derived from a recombinant *Bacillus cereus* family member. The exosporium fragments are derived from spores of a recombinant *Bacillus cereus* family member described in Section IV hereinabove and comprise the fusion protein. The fusion protein comprises a nucleic acid binding protein or peptide, and wherein the nucleic acid binding protein or peptide is bound to a nucleic acid molecule.
(848) The worm is preferably a nematode.
(849) A method for delivering a nucleic acid molecule to a fungus or a protozoan is provided. The method comprises contacting a fungus or a protozoan with a recombinant *Bacillus cereus* family member expressing a fusion protein comprising a protein or peptide of interest and a targeting sequence, exosporium protein, or exosporium protein fragment that targets the fusion protein to the exosporium of the recombinant *Bacillus cereus* family member. Alternatively, the method comprises contacting a fungus or a protozoan with a recombinant spore-forming bacterium that expresses a fusion protein comprising at least one protein or peptide of interest and a spore coat protein that targets the fusion protein to the surface of a spore of the bacterium. The protein or peptide of interest comprises a nucleic acid binding protein or peptide and the nucleic acid molecule is bound to the nucleic acid binding protein or peptide.
(850) A further method for delivering a nucleic acid molecule to a fungus or a protozoan is provided. The method comprises contacting a fungus or a protozoan with exosporium fragments. The exosporium fragments are derived from spores of a recombinant *Bacillus cereus* family member described in Section IV hereinabove and comprise the fusion protein. The fusion protein comprises a nucleic acid binding protein or peptide, and wherein the nucleic acid binding protein or peptide is bound to a nucleic acid molecule.
(851) The nucleic acid molecule can comprise a modulating RNA molecule; an RNAi molecule; a microRNA; an aptamer; or a DNA molecule that encodes a modulating RNA molecule, an RNAi molecule, a microRNA, or an aptamer.
(852) The recombinant *Bacillus cereus* family member can comprise any of the recombinant *Bacillus cereus* family members that express a fusion protein.
(853) The fusion protein can comprise any of the fusion proteins described herein that include a nucleic acid binding protein.
(854) The spore coat protein comprises a CotB protein, a CotC protein, a CgeA protein, a CotB/H protein, a CotG protein, a spore coat protein X protein, or a CotY protein.
(855) The spore coat protein can comprise an amino acid sequence having at least 85% identity with any of SEQ ID NOs: 252-259.
(856) The spore coat protein can comprise an amino acid sequence having at least 90% identity with any of SEQ ID NOs: 252-259.

(857) The spore coat protein can comprise an amino acid sequence having at least 95% identity with any of SEQ ID NOs: 252-259.
(858) The spore coat protein can comprise an amino acid sequence having at least 98% identity with any of SEQ ID NOs: 252-259.
(859) The spore coat protein can comprise an amino acid sequence having at least 99% identity with any of SEQ ID NOs: 252-259.
(860) The spore coat protein can comprise an amino acid sequence having 100% identity with any of SEQ ID NOs: 252-259.
(861) The above-described methods can be used for numerous purposes. For example, these methods can be used to deliver RNA or DNA to animals for the purpose of decreasing susceptibility of the animal to a disease or treating a disease in the animal (e.g., organic disease such as stroke, diabetes, heart disease, and degenerative diseases). RNAs and DNAs have also been demonstrated to be effective for eliminating or treating disease caused by animal pathogens, such as bacteria, viruses, worms (e.g., nematodes), and fungi. The RNAs and DNAs can act directly on the pathogen, or can work with the animal's immune system to activate or increase the immune response.
(862) In addition, the above methods can be used for eliminating pests, including insects, worms (e.g., nematodes), fungi, and protozoans. Delivery of specific RNAs or DNAs to the pest can lead to decreased ability to of the pest to infect a host (e.g., a plant host), decreased feeding on target hosts or plants, direct killing through blocking of key genes, or various other effects.
XXII. Vaccines and a Method of Producing an Immunogenic Response
(863) A vaccine is provided which comprises a pharmaceutically acceptable carrier and recombinant *Bacillus cereus* family member spores that express a fusion protein as described herein above in Section I wherein the protein or peptide of interest is an antigen or an immunogen.
(864) A further vaccine is provided which comprises a pharmaceutically acceptable carrier and exosporium fragments. The exosporium fragments are derived from spores of a recombinant *Bacillus cereus* family member described in Section IV hereinabove and comprise the fusion protein. The fusion protein comprises an antigen.
(865) Yet another vaccine is provided which comprises a pharmaceutically acceptable carrier and a recombinant *Bacillus cereus* family member. The recombinant *Bacillus cereus* family member is a recombinant *Bacillus cereus* family member as described above in Section II.
(866) In the vaccines that comprise exosporium fragments or a recombinant *Bacillus cereus* family member as described above in Section II, the targeting sequence, exosporium protein, or exosporium protein fragment can comprise any of the targeting sequences, exosporium proteins, or exosporium protein fragments described herein. In particular, the targeting sequence, exosporium protein, or exosporium protein fragment can comprise any of the targeting sequences, exosporium protein, or exosporium protein fragments described herein above.
(867) The fusion protein can be expressed under the control of a sporulation promoter native to the targeting sequence, exosporium protein, or exosporium protein fragment of the fusion protein or a portion thereof and/or under the control of a high-expression sporulation promoter. The promoter can be any of the promoters described above in Section III.
(868) When the protein or peptide of interest is an antigen, display of the antigen on the outside of the spore or on an exosporium fragment provides an immune system response to achieve vaccination against various pathogens or diseases. Suitable antigens or small molecules are those that are known or expected to illicit a desired immune response that is sufficient to yield a therapeutic or protective effect when expressed on the exterior of a *Bacillus* spore or displayed on an exosporium fragment. Suitability in large part will be determined by the folding in the three-dimensional structure once the recombinant antigen is incorporated into the exosporium, i.e. the antigenic portion(s) of the recombinant molecule must be available for detection by the immune system.
(869) The pathogens or diseases from which the antigen can be derived include, but are not limited to, Acintobacter infections, caused by *Acinetobacter baumannii*; Actinomycosis, caused by *Actinomyces israelii, Actinomyces gerencseriae,* and *Propionibacterium propionicus*; African sleeping sickness, caused by *Trypanosoma brucei*; Acquired immune deficiency syndrome (AIDS), caused by Human immunodeficiency virus; Amebiasis, caused by *Entamoeba histolytica*; Anaplasmosis, caused by *Anaplasma* genus, Anthrax, caused by *Bacillus anthracis; Arcanobacterium haemolyticum* infection, caused by *Arcanobacterium haemolyticum*; Argentine hemorrhagic fever, caused by Junin virus; Ascariasis, caused by *Ascaris lumbricoides*, Astrovirus infection, caused by Astroviradae family; Babesiosis, *Babesia* genus; *Bacillus cereus* infection, caused by *Bacillus cereus;* Bacterial pneumonia; Bacterial vaginosis; *Bacteroides* infection, caused by *Bacteroides* genus; Balantidiasis, caused by *Balantidium coli; Baylisascaris* infection, caused by *Baylisascaris* genus; BK virus infection, caused by BK virus; Black piedra, caused by *Piedraia hortae; Blastocystis hominis* infection, caused by *Blastocystis hominis*; Blastomycosis, caused by *Blastomyces dermatitidis*; Bolivian hemorrhagic fever, caused by Machupo virus; *Borrelia* infection, caused by *Borrelia* genus; Botulism (and Infant botulism), caused by the intake of *Clostridium botulinum* toxin; Brazilian hemorrhagic fever, caused by Sabia; Brucellosis, caused by *Brucella* genus; *Burkholderia* infection, caused by

usually *Burkholderia cepacia* and other *Burkholderia* species; Buruli ulcer, caused by *Mycobacterium ulcerans*; Calicivirus infection (Norovirus and Sapovirus), caused by Caliciviridae family; Campylobacteriosis, caused by *Campylobacter* genus; Candidiasis (Moniliasis; Thrush) usually caused by *Candida albicans* and other *Candida* species; Cat-scratch disease, caused by *Bartonella henselae*; Cellulitis, caused by usually Group A *Streptococcus* and *Staphylococcus*; Chagas Disease (American trypanosomiasis), caused by *Trypanosoma cruzi*; Chancroid, caused by *Haemophilus ducreyi*; Chickenpox, caused by Varicella zoster virus (VZV); *Chlamydia*, caused by *Chlamydia trachomatis; Chlamydophila pneumoniae* infection, caused by *Chlamydophila pneumoniae*; Cholera, caused by *Vibrio cholerae*; Chromoblastomycosis, caused by usually *Fonsecaea pedrosoi*; Clonorchiasis, caused by *Clonorchis sinensis; Clostridium difficile* infection, caused by *Clostridium difficile*; Coccidioidomycosis, caused by *Coccidioides immitis* and *Coccidioides posadasii*; Colorado tick fever (CTF), caused by Colorado tick fever virus (CTFV); Common cold (Acute viral rhinopharyngitis; Acute coryza), caused by usually rhinoviruses and coronaviruses; Creutzfeldt-Jakob disease (CJD), caused by CJD prion; Crimean-Congo hemorrhagic fever (CCHF), caused by Crimean-Congo hemorrhagic fever virus; Cryptococcosis, caused by *Cryptococcus neoformans*; Cryptosporidiosis, caused by *Cryptosporidium* genus; Cutaneous larva migrans (CLM), caused by usually *Ancylostoma braziliense* and multiple other parasites; Cyclosporiasis, caused by *Cyclospora cayetanensis*; Cysticercosis, caused by *Taenia solium*; Cytomegalovirus infection, caused by Cytomegalovirus; Dengue fever, caused by Dengue viruses (DEN-1, DEN-2, DEN-3 and DEN-4)-Flaviviruses; Dientamoebiasis, caused by *Dientamoeba fragilis*; Diphtheria, caused by *Corynebacterium diphtheriae*; Diphyllobothriasis, caused by *Diphyllobothrium*; Dracunculiasis, caused by *Dracunculus medinensis*; Ebola hemorrhagic fever, caused by Ebolavirus (EBOV); Echinococcosis, caused by *Echinococcus* genus; Ehrlichiosis, caused by *Ehrlichia* genus; Enterobiasis (Pinworm infection), caused by *Enterobius vermicularis; Enterococcus* infection, caused by *Enterococcus* genus; Enterovirus infection, caused by Enterovirus genus; Epidemic typhus, caused by *Rickettsia prowazekii*; Erythema infectiosum (Fifth disease), caused by Parvovirus B19; Exanthem subitum, caused by Human herpesvirus 6 (HHV-6) and Human herpesvirus 7 (HHV-7); Fasciolopsiasis, caused by *Fasciolopsis buski*; Fasciolosis, caused by *Fasciola hepatica* and *Fasciola gigantica*; Fatal familial insomnia (FFI), caused by FFI prion; Filariasis, caused by Filarioidea superfamily; Food poisoning caused by *Clostridium perfringens*; Free-living amebic infection; *Fusobacterium* infection, caused by *Fusobacterium* genus; Gas gangrene (Clostridial myonecrosis), caused by usually *Clostridium perfringens* or other *Clostridium* species; Geotrichosis, caused by *Geotrichum candidum*; Gerstmann-Straussler-Scheinker syndrome (GSS), caused by GSS prion; Giardiasis, caused by Giardia intestinalis; Glanders, caused by *Burkholderia mallei*; Gnathostomiasis, caused by *Gnathostoma spinigerum* and *Gnathostoma hispidum*; Gonorrhea, caused by *Neisseria gonorrhoeae*; Granuloma inguinale (Donovanosis), caused by *Klebsiella granulomatis*; Group A streptococcal infection, caused by *Streptococcus pyogenes*; Group B streptococcal infection, caused by *Streptococcus agalactiae; Haemophilus influenzae* infection, caused by *Haemophilus influenzae*; Hand, foot and mouth disease (HFMD), caused by Enteroviruses, mainly Coxsackie A virus and Enterovirus 71 (EV71); Hantavirus Pulmonary Syndrome (HPS), caused by Sin Nombre virus; *Helicobacter pylori* infection, caused by *Helicobacter pylori*; Hemolytic-uremic syndrome (HUS), caused by *Escherichia coli* O157:H7; Hemorrhagic fever with renal syndrome (HFRS), caused by Bunyaviridae family; Hepatitis A, caused by Hepatitis A Virus; Hepatitis B, caused by Hepatitis B Virus; Hepatitis C, caused by Hepatitis C Virus; Hepatitis D caused by Hepatitis D Virus; Hepatitis E, caused by Hepatitis E Virus; Herpes simplex, caused by Herpes simplex virus 1 and 2 (HSV-1 and HSV-2); Histoplasmosis, caused by *Histoplasma capsulatum*; Hookworm infection, caused by *Ancylostoma duodenale* and *Necator americanus*; Human bocavirus infection, caused by Human bocavirus (HBOV); Human ewingii chrlichiosis, caused by *Ehrlichia ewingii*; Human granulocytic anaplasmosis (HGA), caused by *Anaplasma phagocytophilum*; Human metapneumovirus infection, caused by Human metapneumovirus (hMPV); Human monocytic chrlichiosis, caused by *Ehrlichia chaffeensis*; Human papillomavirus (HPV) infection, caused by Human papillomavirus (HPV); Human parainfluenza virus infection, caused by Human parainfluenza viruses (HPIV); Hymenolepiasis, caused by *Hymenolepis nana* and *Hymenolepis diminuta*; Epstein-Barr Virus Infectious Mononucleosis (Mono), caused by Epstein-Ban Virus (EBV); Influenza (flu), caused by Orthomyxoviridae family; Isosporiasis, caused by *Isospora Belli*; Kawasaki disease (cause unknown but evidence supports that it is infectious); Keratitis; *Kingella kingae* infection, caused by *Kingella kingae*; Kuru, caused by Kuru prion; Lassa fever, caused by Lassa virus; Legionellosis (Legionnaires' disease), caused by *Legionella pneumophila*; Legionellosis (Pontiac fever), caused by *Legionella pneumophila*; Leishmaniasis, caused by *Leishmania* genus; Leprosy, caused by *Mycobacterium leprae* and *Mycobacterium lepromatosis*; Leptospirosis, caused by Leptospira genus; Listeriosis, caused by *Listeria monocytogenes*; Lyme disease (Lyme borreliosis), caused by usually *Borrelia burgdorferi* and other *Borrelia* species; Lymphatic filariasis (Elephantiasis), caused by *Wuchereria bancrofti* and *Brugia malayi*; Lymphocytic choriomeningitis, caused by Lymphocytic choriomeningitis virus (LCMV); Malaria, caused by *Plasmodium* genus; Marburg hemorrhagic fever (MHF), caused by Marburg virus; Measles, caused by Measles virus; Melioidosis (Whitmore's disease), caused by *Burkholderia pseudomallei*; Meningitis; Meningococcal disease, caused by *Neisseria meningitidis*; Metagonimiasis,

caused by usually *Metagonimus yokagawai*; Microsporidiosis, caused by *Microsporidia* phylum; Molluscum contagiosum (MC), caused by Molluscum contagiosum virus (MCV); Mumps, caused by Mumps virus; Murine typhus (Endemic typhus), caused by *Rickettsia typhi; Mycoplasma* pneumonia, caused by *Mycoplasma pneumoniae*; Mycetoma, caused by numerous species of bacteria (Actinomycetoma) and fungi (Eumycetoma); Myiasis, caused by parasitic dipterous fly larvae; Neonatal conjunctivitis (Ophthalmia neonatorum), caused by most commonly *Chlamydia trachomatis* and *Neisseria gonorrhoeae*; (New) Variant Creutzfeldt-Jakob disease (vCJD, nvCJD), caused by vCJD prion; Nocardiosis, caused by usually *Nocardia asteroides* and other *Nocardia* species; Onchocerciasis (River blindness), caused by *Onchocerca volvulus*; Paracoccidioidomycosis (South American blastomycosis), caused by *Paracoccidioides brasiliensis*; Paragonimiasis, caused by usually *Paragonimus westermani* and other *Paragonimus* species; Pasteurellosis, caused by *Pasteurella* genus; Pediculosis capitis (Head lice), caused by *Pediculus humanus* capitis; *Pediculosis corporis* (Body lice), caused by *Pediculus humanus corporis; Pediculosis pubis* (Pubic lice, Crab lice), caused by *Phthirus pubis*; Pelvic inflammatory disease (PID); Pertussis (Whooping cough), caused by *Bordetella pertussis*; Plague, caused by *Yersinia pestis*; Pneumococcal infection, caused by *Streptococcus pneumoniae; Pneumocystis* pneumonia (PCP), caused by *Pneumocystis jirovecii*; Pneumonia; Poliomyelitis, caused by Poliovirus; *Prevotella* infection, caused by *Prevotella* genus; Primary amoebic meningoencephalitis (PAM), caused by usually *Naegleria fowleri*; Progressive multifocal leukoencephalopathy, caused by JC virus; Psittacosis, caused by *Chlamydophila psittaci*; Q fever, caused by *Coxiella burnetii*; Rabies, caused by Rabies virus; Rat-bite fever, caused by *Streptobacillus moniliformis* and Spirillum minus; Respiratory syncytial virus infection, caused by Respiratory syncytial virus (RSV); Rhinosporidiosis, caused by *Rhinosporidium seeberi*; Rhinovirus infection, caused by Rhinovirus; Rickettsial infection, caused by *Rickettsia* genus; Rickettsialpox, caused by *Rickettsia akari*; Rift Valley fever (RVF), caused by Rift Valley fever virus; Rocky mountain spotted fever (RMSF), caused by *Rickettsia rickettsii*; Rotavirus infection, caused by Rotavirus; Rubella, caused by Rubella virus; *Salmonellosis*, caused by *Salmonella* genus; SARS (Severe Acute Respiratory Syndrome), caused by SARS coronavirus; Scabies, caused by *Sarcoptes scabiei*; Schistosomiasis, caused by *Schistosoma* genus; Sepsis; Shigellosis (Bacillary dysentery), caused by *Shigella* genus; Shingles (Herpes zoster), caused by Varicella zoster virus (VZV); Smallpox (Variola), caused by Variola major or Variola minor; Sporotrichosis, caused by *Sporothrix schenckii*; Staphylococcal food poisoning, caused by *Staphylococcus* genus; Staphylococcal infection, caused by *Staphylococcus* genus; Strongyloidiasis, caused by *Strongyloides stercoralis*; Syphilis, caused by *Treponema pallidum*; Taeniasis, caused by *Taenia* genus; Tetanus (Lockjaw), caused by *Clostridium tetani; Tinea barbae* (Barber's itch), caused by usually *Trichophyton* genus; *Tinea* capitis (Ringworm of the Scalp), caused by usually *Trichophyton tonsurans; Tinea corporis* (Ringworm of the Body), caused by usually *Trichophyton* genus; *Tinea cruris* (Jock itch), caused by usually *Epidermophyton floccosum, Trichophyton rubrum*, and *Trichophyton mentagrophytes; Tinea manuum* (Ringworm of the Hand), caused by *Trichophyton rubrum; Tinea nigra*, caused by usually *Hortaea werneckii; Tinea pedis* (Athlete's foot), caused by usually *Trichophyton* genus; *Tinea unguium* (Onychomycosis), caused by usually *Trichophyton* genus; *Tinea versicolor* (*Pityriasis versicolor*), caused by *Malassezia* genus; Toxocariasis (Ocular Larva Migrans (OLM)), caused by *Toxocara canis* or *Toxocara cati*; Toxocariasis (Visceral Larva Migrans (VLM)), caused by *Toxocara canis* or *Toxocara cati*; Toxoplasmosis, caused by *Toxoplasma gondii*; Trichinellosis, caused by *Trichinella spiralis*; Trichomoniasis, caused by *Trichomonas vaginalis*; Trichuriasis (Whipworm infection), caused by *Trichuris trichiura*; Tuberculosis, caused by usually *Mycobacterium tuberculosis*; Tularemia, caused by *Francisella tularensis; Ureaplasma urealyticum* infection, caused by *Ureaplasma urealyticum*; Venezuelan equine encephalitis, caused by Venezuelan equine encephalitis virus; Venezuelan hemorrhagic fever, caused by Guanarito virus; Viral pneumonia; West Nile Fever, caused by West Nile virus; White piedra (*Tinea blanca*), caused by *Trichosporon beigelii; Yersinia pseudotuberculosis* infection, caused by *Yersinia pseudotuberculosis*; Yersiniosis, caused by *Yersinia enterocolitica*; Yellow fever, caused by Yellow fever virus; Zygomycosis, caused by Mucorales order (Mucormycosis) and Entomophthorales order (Entomophthoramycosis).

(870) When the protein or peptide of interest is an antigen, any *Bacillus cereus* family member can be used to express the fusion protein. *Bacillus thuringiensis* or *Bacillus mycoides* are preferred.

(871) To prepare a vaccine, the antigen of interest is incorporated into the fusion protein by known methods such as PCR splicing by overlapping extension, restriction endonuclease digestion and ligation, or de novo gene synthesis. The fusion protein gene is then introduced into a recombinant *Bacillus cereus* family member by transfection, transformation, conjugation, electroporation or other known methods. The recombinant *Bacillus cereus* family member is then grown in culture media (e.g., minimal liquid media) and allowed to sporulate. Preferably, sporulation continues to completion before the spores are collected and stored. Spores can be collected by either centrifugation or swabbing of spores off of growth plates and introduction into liquid media (e.g., PBS or water) followed by centrifugation and washing of the resulting spore pellet in liquid media. Prior to use, the spore pellet can be resuspended in liquid media to a desired concentration for use or injection. Where the vaccine is to comprise exosporium fragments, the exosporium fragments can be prepared using any of the methods described in section

XIX.H above.
(872) The desired concentration of recombinant *Bacillus cereus* family member spores or exosporium fragments in a vaccine is based on the size of the subject, the amount of active antigen on the surface of the spores, and the presence and concentration of adjuvants in the vaccine formulation. A vaccine of the invention can contain conventional adjuvants including pharmaceutically acceptable carriers.
(873) A method of producing an immunogenic response in a subject is provided. The method comprises administering a vaccine containing recombinant *Bacillus cereus* family member spores expressing fusion proteins or exosporium fragments comprising fusion proteins as described herein to the subject.
(874) The vaccine as described herein is suitable for intravenous, intrarterial, intraperitoneal, intramuscular, subcutaneous, intrapleural, topical, oral, intranasal, intradermal, transepithelial administration or by inhalation.
(875) The vaccine can be administered to a subject which is human, murine, avian, porcine, bovine, ovine, feline, canine, equine, caprine, reptilian or a non-human primate. The subject is preferably mammalian and most preferably human.
XXIII. Remediation
(876) When the protein or peptide of interest is a remediation protein or peptide, a toxic substance is catalytically converted by the remediation protein or peptide to a non-toxic or less toxic substance.
(877) When the remediation protein or peptide comprises an enzyme, the enzyme is displayed and stabilized on the outside of the spore and can be delivered into contaminated soil or contaminated water in a form which is active against a target pollutant or target chemical.
(878) Suitable enzymes depend upon the pollutant or chemical being targeted for remediation.
(879) To prepare a remediation composition, the enzyme of interest is incorporated into the fusion protein by known methods such as PCR splicing by overlapping extension, restriction endonuclease digestion and ligation, or de novo gene synthesis. The fusion protein gene is then introduced into a recombinant *Bacillus cereus* family member by transfection, transformation, conjugation, electroporation or other known methods. The recombinant *Bacillus cereus* family member is then grown in culture media (e.g., minimal liquid media) and allowed to sporulate. Preferably, sporulation continues to completion before the spores are collected and stored. Spores can be collected by either centrifugation or swabbing of spores off of growth plates and introduction into liquid media (e.g., PBS or water) followed by centrifugation and washing of the resulting spore pellet in liquid media. Prior to use, the spore pellet can be resuspended in liquid media to a desired concentration for use. Alternatively, the spore pellet can be formulated into granules at a desired concentration for use and application to the contaminated environment. Where exosporium fragments are to be used for remediation, the exosporium fragments can be prepared using any of the methods described in section XIX.H above.
(880) A method of reducing contaminants in an environment is provided. The method comprises exposing a contaminated environment to a recombinant *Bacillus cereus* family member spore that express the fusion protein as described herein above in Section I wherein the protein or peptide of interest comprises a remediation enzyme.
(881) A further method for reducing contaminants in an environment is provided. The method comprises exposing a contaminated environment to exosporium fragments. The exosporium fragments are derived from a recombinant *Bacillus cereus* family member described in Section IV hereinabove and comprise the fusion protein. The fusion protein comprises a remediation enzyme.
(882) Yet another method for reducing contaminants in an environment is provided. The method comprises exposing a contaminated environment to spores of a recombinant *Bacillus cereus* family member. The recombinant *Bacillus cereus* family member is a recombinant *Bacillus cereus* family member as described above in Section II.
(883) In the methods for reducing contaminants that comprise exposing a contaminated environment to exosporium fragments or to a recombinant *Bacillus cereus* family member as described above in Section II, the targeting sequence, exosporium protein, or exosporium protein fragment can be any of the targeting sequences, exosporium proteins, or exosporium protein fragments described herein. In particular, the targeting sequence, exosporium protein, or exosporium protein fragment can comprise any of the targeting sequences, exosporium protein, or exosporium protein fragments described herein above.
(884) The fusion protein can be expressed under the control of a sporulation promoter native to the targeting sequence, exosporium protein, or exosporium protein fragment of the fusion protein or a portion thereof and/or under the control of a high-expression sporulation promoter. The promoter can be any of the promoters described above in Section III.
(885) When the protein or peptide of interest is a remediation enzyme, any *Bacillus cereus* family member can be used to express the fusion protein. *Bacillus thuringiensis*, *Bacillus cereus*, or *Bacillus mycoides* are preferred.
(886) The recombinant *Bacillus cereus* family member spores can comprise an endophytic strain of bacteria for phytoremediation, such as *Bacillus cereus* family member EE349, *Bacillus cereus* family member EE439, *Bacillus thuringiensis* EE417, *Bacillus cereus* EE444, or *Bacillus thuringiensis* EE319.
(887) The contaminated environment to be treated can be gas, liquid, semi-liquid, gel, film, semi-solid, or solid. The

solid environment can be soil such as surface soil and subsurface soil, compost, crop residue, leaves, mulch, cut trees, a biofilm, a slime layer, mold, sludge, sand, slag, sediment, sewage, waste rock, nuclear waste, munitions and ordnance, hospital waste, junked auto parts, metal cuttings, insulation waste, food waste, asbestos, batteries, industrial scrap, landfill waste, wood waste, textile waste, glass waste, leather waste, rubber waste, plastic waste, electronic component waste, agricultural waste, photographic waste, ceramic waste, pharmaceutical waste, wax, spent catalysts, or a combination thereof. The liquid environment can be drinking water, groundwater, surface water, brine, storage tanks, lagoons, an aquatic system, industrial wastewater, acid mine drainage, spent autofluid, spent plating baths, degreasing solutions, dry cleaning solutions, machine coolants, drilling fluid waste, cutting fluid waste, hydraulic fracturing fluid waste, lubricant waste, paint, greywater, oily wastewater, pulp mill effluent, a water treatment system, a septic system, a sewer system, a precipitation lagoon, a holding pond, a lake, a river, or combinations thereof. The gaseous environment can be air, a flue gas such as emissions from power plants, waste incinerators, crematoria or refineries, a process exhaust stream, landfill gas, natural gas, propane gas, or a combination thereof.

(888) The contaminated environment can be contaminated by various contaminants including, but not limited to, a chemical warfare agent comprising sarin (GB; o-isopropyl methylphosphonofluoridate); soman (GD; o-pinacolyl methylphosphonofluoridate); cyclosarin (GF; o-cyclohexyl methylphosphonofluoridate); VX (O-ethyl S-[2-(diisopropylamino)ethyl]methylphosphonothioate); tabun (GA; N,N-dimethylethyl phosphoroamidocyanidatc), DFP (diisopropyl phophorofluoridate), or a mustard agent; an inorganic compound comprising arsenic, antimony, barium, beryllium, cadmium, chromium, copper, iron, lead, manganese, mercury, nickel, selenium, silver, tin, thallium, uranium, zinc or a combination thereof; an organic compound comprising a polycyclic aromatic hydrocarbon (PAH), a chlorinated aromatic compound, a chlorinated aliphatic compound, a nitroaromatic compound (NAC), a phenolic compound, a cyano compound, dioxin, or a combination thereof; a crude oil, a refined oil, a fuel oil, a diesel oil, a gasoline, a hydraulic oil, and kerosene, or a volatile constituent thereof such as benzene, toluene, ethylbenzene, xylene, or naphthalene; an explosive, a fertilizer, a pesticide, an insecticide, or an herbicide

(889) The concentration of recombinant spores or exosporium fragments needed to treat a contaminated environment is based on factors including the volume or area to be treated, the extent of the target chemical, pollutant or organic matter present, the amount of time available for treatment, and amount of active enzyme on the surface of the spores.

(890) The recombinant *Bacillus cereus* family member spores or exosporium fragments can contact the contaminated environment by incorporating the spores or exosporium fragments into a stream containing the contaminant, contacting a stream containing the contaminant with an immobilization material containing the spores or exosporium fragments (e.g., a filter, membrane, sponge or cassette), incorporating the spores or exosporium fragments into granules to be mixed with the contaminated environment, spraying the spores or exosporium fragments onto or into the contaminated environment, injecting the spores or exosporium fragments into the contaminated environment, or drenching the contaminated environment with the spores or exosporium fragments.

(891) The spores can be combined with bacterial inoculants, chemicals, solvents, and other products that can expedite the decomposition process.

(892) The remediation enzyme includes, but is not limited to, a phosphate binding protein, a protease, a carbohydrate hydrolyase, a lipase, a phospholipase, a nuclease, a nutrient binding protein, a cellulase, an oxidoreductase, a monooxygenase, a diooxygenase, a laccase, a lignin peroxidase, a manganese peroxidase, a peroxidase, a dehalogenase, a catalase, an amylase, a reductase, an oxidase, an amidase, a ligninase, a xylanase, a pectinase, a xylosidase, an endoglucanase, an exoglucanase, a glucosidase, a biofilm inhibitory peptide, an herbicide-degrading enzyme, a pesticide-degrading enzyme (e.g., a pyrethrinase), or a combination thereof.

(893) Where the enzyme comprises an herbicide-degrading enzyme or a pesticide-degrading enzyme, the recombinant *Bacillus cereus* family member suitably comprises a strain of bacteria that is capable of degrading an herbicide or a pesticide. For example, the strain of bacteria that is capable of degrading an herbicide or a pesticide can comprise *Bacillus cereus* family member EE349 (NRRL No. B-50928), *Bacillus cereus* family member EE-B00377 (NRRL B-67119); *Bacillus pseudomycoides* EE-B00366 (NRRL B-67120); or *Bacillus mycoides* EE-B00363 (NRRL B-67121).

(894) A method for phytoremediation of contaminated soil is also provided. The method comprises introducing recombinant *Bacillus cereus* family member spores into contaminated soil; or applying the recombinant *Bacillus cereus* family member spores to a plant planted in contaminated soil, or a plant seed for planting in contaminated soil, or an area of contaminated soil surrounding a plant or a plant seed; wherein the recombinant *Bacillus cereus* family member spores express a fusion protein comprising at least one protein or peptide of interest and a targeting sequence, exosporium protein, or exosporium protein fragment that targets the fusion protein to the exosporium of the recombinant *Bacillus cereus* family member spore, wherein the fusion protein is the fusion protein as described above wherein the protein or peptide of interest comprises a remediation enzyme, and wherein the recombinant

Bacillus cereus family member comprises an endophytic strain of bacteria or a root colonizing strain of bacteria. For example, the recombinant spore-forming bacterium can comprise an endophytic strain of bacteria.

(895) A further method for phytoremediation of contaminated soil is provided. The method comprises expressing a remediation enzyme in a *Bacillus cereus* family member spore, wherein the expression of the remediation enzyme in the recombinant *Bacillus cereus* family member spore is increased as compared to the expression of the remediation enzyme in a wild-type *Bacillus cereus* family member spore.

(896) Another method for phytoremediation of contaminated soil is also provided. The method comprises introducing a recombinant spore-forming bacterium into contaminated soil; or applying the recombinant spore-forming bacterium to a plant planted in contaminated soil, or a plant seed to be planted in contaminated soil, or an area of contaminated soil surrounding a plant or a plant seed. The recombinant spore-forming bacterium expresses a fusion protein comprising at least one protein or peptide of interest and a spore coat protein that targets the fusion protein to the surface of a spore of the bacterium. The spore coat protein comprises a CotB protein, a CotC protein, a CgeA protein, a CotB/H protein, a Cot G protein, a spore coat protein X protein, or a CotY protein. The recombinant spore-forming bacterium comprises an endophytic strain of bacteria or a root colonizing strain of bacteria. The protein or peptide of interest comprises a remediation enzyme.

(897) Another method for phytoremediation of contaminated soil is also provided. The method comprises introducing exosporium fragments into contaminated soil or applying exosporium fragments to a plant planted in contaminated soil, or a plant seed to be planted in contaminated soil, or an area of contaminated soil surrounding a plant or a plant seed. The exosporium fragments are derived from spores of a recombinant *Bacillus cereus* family member described in Section IV herein above and comprise the fusion protein. The fusion protein comprises a remediation enzyme.

(898) Yet another method for phytoremediation of contaminated soil is provided. The method comprises introducing spores of a recombinant *Bacillus cereus* family member into contaminated soil. Alternatively, the method comprises applying spores of a recombinant *Bacillus cereus* family member to a plant planted in contaminated soil, or a plant seed to be planted in contaminated soil, or an area of contaminated soil surrounding a plant or a plant seed. The recombinant *Bacillus cereus* family member is a recombinant *Bacillus cereus* family member as described above in Section II, and the fusion protein comprises a remediation enzyme.

(899) In the methods for phytoremediation of contaminated soil that involve the use of exosporium fragments or a recombinant *Bacillus cereus* family member as described above in Section II, the targeting sequence, exosporium protein, or exosporium protein fragment can comprise any of the targeting sequences, exosporium proteins, or exosporium protein fragments described herein. In particular, the targeting sequence, exosporium protein, or exosporium protein fragment can comprise any of the targeting sequences, exosporium proteins, or exosporium protein fragments described herein above.

(900) The fusion protein can be expressed under the control of a sporulation promoter native to the targeting sequence, exosporium protein, or exosporium protein fragment of the fusion protein or a portion thereof and/or under the control of a high-expression sporulation promoter. The promoter can be any of the promoters described above in Section III.

(901) The remediation enzyme is displayed on the outside of the spores and within the plant so that both the plant and spores can convert the target contaminant. The plant can take up the target contaminant while the spores convert the contaminant into a non-toxic or less toxic form within the plant or its root system.

(902) The recombinant *Bacillus cereus* family member spores can comprise an endophytic strain of bacteria, such as *Bacillus cereus* family member EE349, *Bacillus cereus* family member EE439, *Bacillus thuringiensis* EE417, *Bacillus cereus* EE444, or *Bacillus thuringiensis* EE319, *Bacillus thuringiensis* EE-B00184, *Bacillus cereus* family member EE-B00377, *Bacillus pseudomycoides* EE-B00366, or *Bacillus mycoides* EE-B00363.

(903) The spores or the exosporium fragments can be applied to the plant or the plant seed, and the plant or plant grown from the plant seed is tolerant to a target contaminant to be remediated from the contaminated soil

(904) In the method for phytoremediation, recombinant *Bacillus cereus* family members undergo sporulation within the plant.

(905) The recombinant *Bacillus cereus* family member spores can be introduced into the plant growth medium by various methods such as soil drench at the time of planting. The spores can also be coated onto the plant seed as a seed treatment.

(906) Preferably, the plant to be treated with the remediation enzyme is tolerant to the target contaminant so that the plant is not injured by the target contaminant.

(907) The concentration of recombinant spores needed for the phytoremediation method is based on factors including volume or area to be treated, the ability of the endophytic strains to colonize the plant roots, the extent that the target contaminant is present, and the amount of active enzyme on the surface of the spores.

(908) A further method for reducing contaminants in an environment is provided. The method comprises exposing a contaminated environment to spores of a *Bacillus cereus* family member strain that is capable of degrading an

herbicide or a pesticide. The contaminants in the environment comprise an herbicide, a pesticide, or a combination thereof. The *Bacillus cereus* family member strain that is capable of degrading an herbicide or a pesticide comprises *Bacillus cereus* family member EE349 (NRRL No. B-50928), *Bacillus cereus* family member EE-B00377 (NRRL B-67119); *Bacillus pseudomycoides* EE-B00366 (NRRL B-67120); *Bacillus mycoides* EE-B00363 (NRRL B-67121), or a combination thereof.

(909) The *Bacillus cereus* family member strain that is capable of degrading an herbicide or a pesticide can comprise a recombinant *Bacillus cereus* family member that expresses a fusion protein comprising at least one protein or peptide of interest and a targeting sequence, exosporium protein, or exosporium protein fragment that targets the fusion protein to the exosporium of the recombinant *Bacillus cereus* family member. The protein or peptide of interest preferably comprises an herbicide-degrading enzyme, a pesticide-degrading enzyme, or a combination thereof.

(910) In this way, dual pesticide or herbicide degrading activity can be obtained since both the *Bacillus cereus* family member strains and the herbicide-degrading or pesticide-degrading enzymes in the fusion protein will exert pesticide- and/or herbicide-degrading activity. The herbicides and/or pesticides that are degraded by the *Bacillus cereus* family strain that is capable of degrading an herbicide or a pesticide can be the same as or different from the herbicides and/or pesticides that are degraded by the herbicide-degrading enzyme or the pesticide-degrading enzyme. Thus, where an environment is contaminated with a single type of herbicide or pesticide, dual degrading action against that single herbicide or pesticide can be obtained. Alternatively, where an environment is contaminated with more than one type of herbicide or pesticide, dual degrading action against two or more different herbicides or pesticides can be obtained.

(911) In the methods of reducing contaminants involving the use of one of the *Bacillus cereus* family member strains described herein that is capable of degrading an herbicide or a pesticide, the targeting sequence, exosporium protein, or exosporium protein fragment can comprise any of the targeting sequences, exosporium proteins, or exosporium protein fragments described herein. In particular, the targeting sequence, exosporium protein, or exosporium protein fragment can comprise any of the targeting sequences, exosporium proteins, or exosporium protein fragments described herein above.

XXIV. Breaking Emulsions or Gels in a Hydraulic Fracturing Fluid

(912) A method of treating a hydraulic fracturing fluid to break an emulsion or gel within the fluid is provided. The method comprises adding spores of a recombinant *Bacillus cereus* family member spores to a hydraulic fracturing fluid. The recombinant *Bacillus cereus* family member expresses a fusion protein comprising at least one protein or peptide of interest and a targeting sequence, exosporium protein, or exosporium protein fragment that targets the fusion protein to the exosporium of the recombinant *Bacillus cereus* family member spore. Such a fusion protein is described above wherein the protein or peptide of interest comprises an enzyme suitable for breaking the emulsion or gel.

(913) The recombinant *Bacillus cereus* family member can comprise any of the recombinant *Bacillus cereus* family members described herein that express a fusion protein.

(914) A further method of treating a hydraulic fracturing fluid to break an emulsion or gel within the fluid is provided. The method comprises adding exosporium fragments to a hydraulic fracturing fluid. The exosporium fragments are derived from a recombinant *Bacillus cereus* family member described in Section IV hereinabove and comprise the fusion protein. The fusion protein comprises an enzyme suitable for breaking the emulsion or gel.

(915) The enzyme is selected based upon the target emulsion or gel to be treated and the pH of the hydraulic fracturing fluid. Enzymes include, but are not limited to, a hemicellulase, an amylase, a pectinase, a carbohydrate hydrolyase, a cellulase, an agarase, a polygalacturonase, an endoglucanase, or a combination thereof.

(916) The emulsion or gel contains a polymer or other component which the enzyme can digest. The emulsion or gel can comprise a polymer, *Arabica* gum, agar, xanthan gum, cellulose, carboxymethylcellulose, carboxymethylhydroxyethyl cellulose, hydroxyethyl methylcellulose, guar, a guar derivative, or a combination thereof.

(917) When the protein or peptide of interest is an enzyme for breaking an emulsion or gel, any *Bacillus cereus* family member can be used to express the fusion protein. *Bacillus thuringiensis* or *Bacillus mycoides* are preferred.

(918) The spores or exosporium fragments can be injected into a well that is in contact with a subterranean hydrocarbon-containing formation such as a sandstone reservoir or a carbonate reservoir.

(919) The concentration of spores or exosporium fragments needed is based on factors including the size of the well to be treated, the type of emulsion or gel, the amount of active enzyme on the surface of the spores or exosporium fragments, and the presence and concentration of adjuvants delivered with the enzymes.

(920) The enzymes can digest polymers or other components within the emulsion or gel, or can dissolve such components so that the hydraulic fracturing fluid can be pumped out of the well.

(921) In the methods of treating a hydraulic fracturing fluid to break an emulsion or gel within the fluid, any of the targeting sequences, exosporium proteins, or exosporium protein fragments described herein can be used. In

particular, the targeting sequence, exosporium protein, or exosporium protein fragment can comprise any of the targeting sequences, exosporium protein, or exosporium protein fragments described herein above.
(922) The fusion protein can be expressed under the control of a sporulation promoter native to the targeting sequence, exosporium protein, or exosporium protein fragment of the fusion protein or a portion thereof and/or under the control of a high-expression sporulation promoter. The promoter can be any of the promoters described above in Section III.
XXV. Feedstock Processing
(923) Feedstock is generated from plants that are harvested for their biomass, and processed into feed (bailing, silage, extrusion, pelleting, etc). The plant biomass that constitutes the feedstock is often difficult to digest due to the fibrous nature of the material. The presence of enzymes can greatly assist in the degradation of this fibrous material, leading to a more digestible and easier to process material. Enzymes are traditionally added after the feedstock has been processed and upon delivery to the organism that is ingesting the feedstock. Enzymes delivered in feedstock can improve health and weight gain of target animals, as well as reduce the environmental impact of the waste products of animals fed such enzyme-supplemented feed.
(924) These same systems can be utilized to pretreat feedstock that is destined for biofuel production, including processing into bioethanol, biodiesel, or other biofuels.
(925) Many species of spores have the ability to persist on foliar surfaces, such as leaves, stems, and fruit, for long periods of time. By using spore display technologies as described herein to display the enzymes on these spores, active enzyme is provided to the feedstock that will be present as the feedstock is harvested. These target enzymes can also be delivered to the feedstock plant at planting, either through delivery of recombinant spores on the plant seeds, or delivery of the recombinant spores to the plant growth medium or area around the plant.
(926) A method for delivering enzymes to a plant is provided. The method comprises introducing into a plant growth medium a recombinant *Bacillus cereus* family member that expresses a fusion protein comprising at least one protein or peptide of interest and a targeting sequence, exosporium protein, or exosporium protein fragment that targets the fusion protein to the exosporium of the recombinant *Bacillus cereus* family member or a formulation comprising a recombinant *Bacillus cereus* family member as described herein; or applying to a plant, a plant seed, or an area surrounding a plant or a plant seed the recombinant *Bacillus cereus* family member or the formulation comprising a recombinant *Bacillus cereus* family member. The protein or peptide of interest comprises an enzyme. The enzyme can be physically attached to the exosporium of the recombinant *Bacillus cereus* family member.
(927) Another method for delivering enzymes to a plant is provided. The method comprises introducing into a plant growth medium a recombinant spore-forming bacterium or a formulation comprising the recombinant spore-forming bacterium; or applying to a plant, a plant seed, or an area surrounding a plant or a plant seed the recombinant spore-forming bacterium or a formulation comprising the recombinant spore-forming bacterium. The recombinant spore-forming bacterium expresses a fusion protein comprising at least one protein or peptide of interest and a spore coat protein that targets the fusion protein to the surface of a spore of the bacterium. The spore coat protein comprises a CotB protein, a CotC protein, a CgeA protein, a CotB/H protein, a Cot G protein, a spore coat protein X protein, or a CotY protein. The recombinant spore-forming bacterium comprises an endophytic strain of bacteria. The protein or peptide of interest comprises an enzyme, and the enzyme is physically attached to the spore coat of the recombinant spore-forming bacterium
(928) Yet another method for delivering enzymes to a plant is provided. The method comprises introducing exosporium fragments or a formulation containing the exosporium fragments into a plant growth medium; or applying exosporium fragments or a formulation containing the exosporium fragments to a plant, a plant seed, or an area surrounding a plant or a plant seed. The exosporium fragments are derived from spores of a recombinant *Bacillus cereus* family member described in Section IV hereinabove and comprise the fusion protein. The protein or peptide of interest comprises an enzyme.
(929) Where the method for delivering enzymes to a plant comprises the use of exosporium fragments, the method can further comprise treating the plant with a penetrating agent, a surfactant, a detergent, an oil, or a combination thereof.
(930) Optimal bacteria strains for these methods include, but are not limited to, *Bacillus cereus* family members, including *Bacillus cereus, Bacillus mycoides, Bacillus thuringiensis,* and *Bacillus pseudomycoides,* as well as other *Bacillus* spore formers, including *Bacillus megaterium, Bacillus firmus, Bacillus flexus, Bacillus subtilis* clade members, *Bacillus pumilus, Bacillus licheniformis,* and *Bacillus subtilis.*
(931) Application can be directly onto the plant material, optionally in conjunction with adjuvants, such as nonionic or other surfactants. The recombinant *Bacillus cereus* family member can be applied to foliage of the plant prior to harvest such as by spraying onto the foliage.
(932) Application to the plant seed is generally performed as a seed dip, a slurry, or a polymer-based seed coating. Optionally, the application can be done in conjunction with seed applied inoculants, fungicides, insecticides, or nematocides.

(933) Application to the plant growth medium or area around the plant can be performed prior to planting, at planting, or post planting of seeds, optionally in conjunction with fertilizers, fungicides, herbicides, or insecticides.
(934) The enzyme is suitable for degrading biomass, digesting cellulosic material, aiding digestion in a digestive system of a target animal to which the plant can be fed, or for biofuel production (e.g., for production of bioethanol or biodiesel).
(935) The enzyme includes, but is not limited to, a nonspecific protease, a metalloprotease, a cellulase, a xylanase, a phosphatase, an endoglucanase, an exoglucanase, a β-glucosidase, an amylase, a pectinase, a xylosidase, a lipase, a phospholipase, or a combination thereof.
(936) The selection of enzymes may depend on the feedstock and the intended use of the feedstock. The enzymes are preferably degradative enzymes.
(937) Enzymes of interest in the protease family include nonspecific proteases, such as serine proteases, histidine proteases, aspartate proteases, as well as metalloproteases.
(938) Enzymes of interest in the cellulase family would include exoglucanases, endoglucanases, β-1,3 glucosidases, cellulases, hemicellulases, a-glucosidases.
(939) Enzymes of interest in the xylanases family include xylosidases, endoxylanases, exoxylanases, pectinases, methyl pectinases, polygalacturonase.
(940) Enzymes of interest in the phosphatases include acid phosphatases, alkaline phosphatases, polyphosphatases, phytases, monophosphatases, and diphosphatases.
(941) Many of these enzymes are also beneficial to plant growth.
(942) These enzymes can not only “predigest” some of the feedstock to increase absorption of key nutrients by a target animal to which the feedstock is fed, but can also aid digestion in the digestive system of the target animal.
(943) The “predigestion” of cellulosic material at harvest can liberate free cellulose during processing for bioethanol and biofuel production, as well as preprocessing of oils destined for biofuel production.
(944) The bacterium can be an endophytic bacterium. Selection of endophytic recombinant bacteria will allow for the bacteria to enter into the plant, but also colonize and grow inside the plant tissues. This will establish a growing number of recombinant spore forming organisms inside the plant as it grows from use of a relatively minor amount of recombinant spores on the seed or with the seed at planting. Upon harvest of the plant biomass material, the bacterial will undergo sporulation, creating new enzymes in planta, which are active on the feedstock as it is harvested, transported, and utilized, for example, either as animal feed or for biofuel production. This can significantly reduce the input cost of degradative enzymes as compared to existing techniques. This is a unique method of delivering digestive enzymes to the biomass prior to industrial processing.
(945) While the optimal bacterial strains are as described above, selection of endophytic strains will increase efficacy. Preferably, the endophytic bacteria comprises *Bacillus cereus* family member EE349, *Bacillus cereus* family member 439, *Bacillus thuringiensis* EE417, *Bacillus cereus* EE444, *Bacillus megaterium* EE385, *Bacillus* sp. EE387, *Bacillus circulans* EE388, *Bacillus subtilis* EE405, *Lysinibacillus fusiformis* EE442, *Lysinibacillus sphaericus* EE443, or a combination thereof.
(946) The plant can be a crop selected from corn, alfalfa, wheat, a pasture crop, a forage crop, soybean, switchgrass, jicama, sweet sorghum, sugarcane, or a combination thereof, and other biofuel and bioethanol feedstocks.
(947) For the methods for delivering enzymes to a plant, any of the targeting sequences, exosporium proteins, or exosporium protein fragments described herein can be used.

XXVI. Use of Spores in Altering Properties of Target Plants

(948) The recombinant *Bacillus cereus* family members and recombinant spore-forming bacterium as described herein allow for the interaction of surface displayed signaling molecules impacting biochemical pathways, and a number of other proteins that benefit plant health. The presence of the spore displayed proteins or peptides can lead to alteration in the metabolism of the target plant, leading to changes in the composition of the plant, its fruit, or other properties or characteristics.
(949) The expression of fusion proteins can be directly used to alter the composition of the target plant. Selection of different enzymes leads to varying effects on the target plant.
(950) A method for altering a property of a plant is provided. The method comprises introducing into a plant growth medium a recombinant *Bacillus cereus* family member that expresses a fusion protein comprising at least one protein or peptide of interest and a targeting sequence, exosporium protein, or exosporium protein fragment that targets the fusion protein to the exosporium of the recombinant *Bacillus cereus* family member or a formulation comprising a recombinant *Bacillus cereus* family member as described herein; or applying to a plant, a plant seed, or an area surrounding a plant or a plant seed the recombinant *Bacillus cereus* family member or the formulation comprising a recombinant *Bacillus cereus* family member. The protein or peptide of interest comprises a plant signaling molecule or an enzyme that affects plant composition, and the protein or peptide of interest can be physically attached to the exosporium of the recombinant *Bacillus cereus* family member.
(951) Another method for altering a property of a plant is provided. The method comprises introducing into a plant

growth medium a recombinant spore-forming bacterium or a formulation comprising the recombinant spore-forming bacterium; or applying to a plant, a plant seed, or an area surrounding a plant or a plant seed the recombinant spore-forming bacterium or a formulation comprising the recombinant spore-forming bacterium. The recombinant spore-forming bacterium expresses a fusion protein comprising at least one protein or peptide of interest and a spore coat protein that targets the fusion protein to the surface of a spore of the bacterium. The spore coat protein comprises a CotB protein, a CotC protein, a CgeA protein, a CotB/H protein, a Cot G protein, a spore coat protein X protein, or a CotY protein. The recombinant spore-forming bacterium comprises an endophytic strain of bacteria. The protein or peptide of interest comprises a plant signaling molecule or an enzyme that affects plant composition, and the protein or peptide of interest can be physically attached to the spore coat of the recombinant spore-forming bacterium

(952) Yet another method for altering a property of a plant is provided. The method comprises introducing exosporium fragments or a formulation containing the exosporium fragments into a plant growth medium; or applying exosporium fragments or a formulation containing the exosporium fragments to a plant, a plant seed, or an area surrounding a plant or a plant seed. The exosporium fragments are derived from spores of a recombinant *Bacillus cereus* family member described in Section IV hereinabove and comprise the fusion protein. The protein or peptide of interest comprises a plant signaling molecule or an enzyme that affects plant composition.

(953) Where the method for altering a property of a plant comprises the use of exosporium fragments, the method can further comprise treating the plant with a penetrating agent, a surfactant, a detergent, an oil, or a combination thereof.

(954) The target bacterium preferably survives or thrives in the environment and on the roots of the target plant. Optimal bacteria strains for these methods include, but are not limited to, *Bacillus cereus* family member EE349, *Bacillus cereus* family member 439, *Bacillus thuringiensis* EE417, *Bacillus cereus* EE444, *Bacillus megaterium* EE385, *Bacillus* sp. EE387, *Bacillus circulans* EE388, *Bacillus subtilis* EE405, *Lysinibacillus fusiformis* EE442, or *Lysinibacillus sphaericus* EE443.

(955) The plant signaling molecules or enzymes can also be delivered to the plant at planting, either through delivery of recombinant spores on the plant seeds, or delivery of the recombinant spores to the plant growth medium or area around the plant.

(956) Application can be directly onto the plant material, optionally in conjunction with adjuvants, such as nonionic or other surfactants. The recombinant *Bacillus cereus* family member, the recombinant spore-forming bacterium, or the exosporium fragments can be applied to foliage of the plant prior to harvest such as by spraying onto the foliage.

(957) Application to the plant seed is generally performed as a seed dip, a slurry, or a polymer-based seed coating. Optionally, the application can be done in conjunction with seed applied inoculants, fungicides, insecticides, or nematocides.

(958) Application to the plant growth medium or area around the plant can be performed prior to planting, at planting, or post planting of seeds, optionally in conjunction with fertilizers, fungicides, herbicides, or insecticides.

(959) The enzyme includes, but is not limited to, comprises endoglucanases, proteases, phospholipases, aminocarboxy-1-propanedeaminase, aminocyclopropane-1-carboxylic acid deaminases, lipases, or a combination thereof.

(960) The plant signaling molecules include, but are not limited to, flg22 and flagellin peptides, cryptogein, harpins, harpin-like proteins, enzymes that degrade or modify a bacterial, fungal, or plant nutrient source, or a combination thereof.

(961) The enzymes or plant signaling molecules can cause desired metabolic changes to the host plant, including increasing the macronutrient and micronutrient uptake or content of the plant tissues through enlargement of the root systems, increasing the protein content of plants such as grains, cereals, and fruit through modifications to metabolism and increased nitrogen uptakes, and modifications to oil content in rapeseed, canola, soybeans and sunflower, sugar content (sucrose) in grapes, sugar cane, switchgrass, sweet sorghum and other biofuel feedstock, medicinal compound content, and cannabinoid content in marijuana. These alterations not only increase the value of the plants of interest, but also increase the utility of these plants in various industries such as biofuel formation, sugar production, and feedstock production.

(962) For the methods for altering a property of a plant, any of the targeting sequences, exosporium proteins, or exosporium protein fragments described herein can be used.

XXVII. Disinfection

(963) A method of disinfecting a surface is provided. The method comprises exposing a surface to a recombinant *Bacillus cereus* family member that expresses a fusion protein as described herein above in Section I, wherein the protein or peptide of interest comprises an antibacterial protein or peptide.

(964) A further method of disinfecting a surface is provided. The method comprises exposing a surface to exosporium fragments. The exosporium fragments are derived from a recombinant *Bacillus cereus* family member described in Section IV hereinabove and comprise the fusion protein. The fusion protein comprises an antibacterial

protein or peptide.
(965) Yet another method of disinfecting a surface is provided. The method comprises exposing a surface to a recombinant *Bacillus cereus* family member. The recombinant *Bacillus cereus* family member is a recombinant *Bacillus cereus* family member as described above in Section II.
(966) In the methods for disinfecting a surface that comprise exposing a surface to exosporium fragments or to a recombinant *Bacillus cereus* family member as described above in Section II, the targeting sequence, exosporium protein, or exosporium protein fragment can be any of the targeting sequences, exosporium proteins, or exosporium protein fragments described herein. In particular, the targeting sequence, exosporium protein, or exosporium protein fragment can comprise any of the targeting sequences, exosporium protein, or exosporium protein fragments described herein above.
(967) The fusion protein can be expressed under the control of a sporulation promoter native to the targeting sequence, exosporium protein, or exosporium protein fragment of the fusion protein or a portion thereof and/or under the control of a high-expression sporulation promoter. The promoter can be any of the promoters described above in Section III.
(968) The antibacterial protein or peptide minimizes or prevents viral agents, bacteria, amoebas, pests, or molds from forming on or binding to the surface.
(969) The antibacterial protein or peptide includes, but is not limited to, proteases, nucleases, antimicrobial peptides, LysM, LfcinB, lysostaphin, albumin, defensins, bacteriocins, lipopeptides, innate immune system peptides, lysozyme, lyticase, or a combination thereof.
(970) The recombinant *Bacillus cereus* family member spores can be used in conjunction with other antimicrobial agents, including disinfectants, cleaners, antibiotics, antifungals, and antivirals.
(971) Although any of the *Bacillus cereus* family can be utilized to express the fusion proteins, either *Bacillus thuringiensis* or *Bacillus mycoides* is preferred.
(972) For these methods, any of the targeting sequences, exosporium proteins, or exosporium protein fragments described herein can be used.
XXVIII. Other Uses
(973) The fusion proteins wherein the protein or peptide of interest is an enzyme or recombinant *Bacillus cereus* members wherein the protein or peptide of interest is an enzyme that can be used for grease, oil, or fat treatment or degumming; leather hide processing; biofuel, biodiesel, or bioethanol formation; sugar processing or conversion; starch treatment; paper or linen processing; animal or fungal byproduct treatment or amino acid recovery; targeted digestion of facility wastes; feed or food additives; dietary supplements; animal nutrition; industrial cleaning; grain processing; cosmetic manufacturing; odor control; food or beverage processing; brewing enhancement or additives; detergent additives; or textile or yarn processing.
(974) By displaying an enzyme on the outside of the spore or on exosporium fragments, the enzyme can be stabilized, immobilized, and able to be reused.
(975) Industrial processes generally involve harsh conditions, including high temperatures, presence of solvents, and large amounts of organic matter. These conditions hinder traditional enzymes. Expression of the target enzyme on the surface of the spore or on exosporium fragments allows for resistance to high temperatures and harsh conditions, and allows for the enzymes to be reisolated and reused.
(976) Key enzymes of interest for such uses include: β-lactamases, proteases, lipases, phospholipases, cellulases, endoglucanases, exogluconases, pectinases, ligninases, amylases (e.g., α-amylases, β-amylases, or glucoamylases), polygalacturonases, glucosidases, galactosidases, carbohydrate hydrolyases, cell wall hydrolases, nucleases, hemicellulases, xylanases, mannases, laccases, lactases, esterases (e.g., pectin methyl esterases), phytases, phosphatases, invertases, glucose oxidases, catalases, lyticases, acetolactate decarboxylass, and ureases.
(977) Preferred enzymes for grease, oil, or fat treatment or degumming, or for cosmetic manufacturing include lipases, phospholipases, esterases, and proteases.
(978) Preferred enzymes for leather hide processing include lipases, proteases, peptidases, collagenases, and phospholipases.
(979) Preferred enzymes for biofuel, biodiesel, or bioethanol formation can include, but are not limited to, lipases and esterases.
(980) Preferred enzymes for sugar processing or conversion, for grain processing, and for textile or yarn processing include carbohydrate hydrolases, amylases, mannases, glucoamylases, invertases, cellulases, hemicellulases, pectinases, pectin methyl esterases, xylanases, endoglucanases, exoglucanases, glucosidases, galactosidases, laccases, lactases, catalases, and glucose oxidases.
(981) Preferred enzymes for starch treatment include amylases and glucoamylases.
(982) Preferred enzymes for paper or linen processing include cellulases, hemicellulases, xylanases, endoglucanases, laccases, ligninases, exoglucanases, phytases, catalases, and glucosidases.
(983) Preferred enzymes for animal or fungal byproduct treatment or amino acid recovery include proteases,

peptidases, lipases, lyticases, cell wall hydrolases, phospholipases, endoglucanases, cellulases, glucanases and carbohydrate hydrolases.
(984) Preferred enzymes for targeted digestion of facility wastes, industrial cleaning, detergent additives, and odor control include lipases, phospholipases, proteases, peptidases, amylases, lyticases, cell wall hydrolases, glucoamylases, cellulases, hemicellulases, xylanases, esterases, glucosidases, galactosidases, laccases, lactases, ureases, phytases, phosphatases, and carbohydrate hydrolases.
(985) Preferred enzymes for feed or food additives, dietary supplements, animal nutrition, brewing additives, beverage additives, or wine processing include mannases, laccases, lyticases, proteases, peptidases, carbohydrate hydrolases, pectinases, pectin methyl esterases, esterases, lipases, cellulases, hemicellulases, xylanases, phytases, phosphatases, invertases, glucosidases, galactosidases, lactases, catalases, glucanases, endoglucanases, acetolactate decarboxylase, and glucose oxidases.
(986) Although any of the *Bacillus cereus* family can be utilized to express the fusion proteins for these uses, either *Bacillus thuringiensis* or *Bacillus mycoides* is preferred.
(987) A use of fusion proteins comprising an enzyme as the protein or peptide of interest or a recombinant *Bacillus cereus* family member expressing a fusion protein comprising an enzyme as the protein or peptide of interest is provided. The fusion protein can be any of the fusion proteins described herein above in Section I. The use can be a use for grease, oil, or fat treatment or degumming; leather hide processing; biofuel, biodiesel, or bioethanol formation; sugar processing or conversion; starch treatment; paper or linen processing; animal or fungal byproduct treatment or amino acid recovery; targeted digestion of facility wastes; feed or food additives; dietary supplements; animal nutrition; industrial cleaning; grain processing; cosmetic manufacturing; odor control; food or beverage processing; brewing enhancement or additives; detergent additives; or textile or yarn processing.
(988) A use of exosporium fragments is also provided. The use can be for grease, oil, or fat treatment or degumming; leather hide processing; biofuel, biodiesel, or bioethanol formation; sugar processing or conversion; starch treatment; paper or linen processing; animal or fungal byproduct treatment or amino acid recovery; targeted digestion of facility wastes; feed or food additives; dietary supplements; animal nutrition; industrial cleaning; grain processing; cosmetic manufacturing; odor control; food or beverage processing; brewing enhancement or additives; detergent additives; or textile or yarn processing. The exosporium fragments are derived from a recombinant *Bacillus cereus* family member described in Section IV hereinabove and comprise the fusion protein. The fusion protein comprises an enzyme.
(989) A further use of a recombinant *Bacillus cereus* family member is provided. The recombinant *Bacillus cereus* family member is a recombinant *Bacillus cereus* family member as described above in Section II. The use can be for grease, oil, or fat treatment or degumming; leather hide processing; biofuel, biodiesel, or bioethanol formation; sugar processing or conversion; starch treatment; paper or linen processing; animal or fungal byproduct treatment or amino acid recovery; targeted digestion of facility wastes; feed or food additives; dietary supplements; animal nutrition; industrial cleaning; grain processing; cosmetic manufacturing; odor control; food or beverage processing; brewing enhancement or additives; detergent additives; or textile or yarn processing. The fusion protein comprises an enzyme.
(990) In the uses of exosporium fragments or the recombinant *Bacillus cereus* family members as described above in Section II, the targeting sequence, exosporium protein, or exosporium protein fragment can be any of the targeting sequences, exosporium proteins, or exosporium protein fragments described herein. In particular, the targeting sequence, exosporium protein, or exosporium protein fragment can comprise any of the targeting sequences, exosporium protein, or exosporium protein fragments described herein above.
(991) The fusion protein can be expressed under the control of a sporulation promoter native to the targeting sequence, exosporium protein, or exosporium protein fragment of the fusion protein or a portion thereof and/or under the control of a high-expression sporulation promoter. The promoter can be any of the promoters described above in Section III.
(992) Having described the invention in detail, it will be apparent that modifications and variations are possible without departing from the scope of the invention defined in the appended claims.
EXAMPLES
(993) The following non-limiting examples are provided to further illustrate the present invention.
Example 1. Use of a Recombinant *Bacillus cereus* Family Member Displaying a Lipase or an Endoglucanase to Stimulate Plant Growth in Soybeans
(994) The *Bacillus subtilis* lipase and endoglucanase genes were amplified via polymerase chain reaction (PCR) using the following primers shown below in Table 16:
(995) TABLE-US-00016 TABLE 16 lipase endoglucanase forward ggatccatggctgaacacaatcc ggatccatgaaacgg (SEQ ID NO: 37) tcaatc (SEQ ID NO: 39) reverse ggatccttaattcgtattctggcc ggatccttactaatt (SEQ ID NO: 38) tggttctgt (SEQ ID NO: 40)
(996) To create fusion constructs, genes were fused to the native bclA promoter of *Bacillus thuringiensis* DNA

encoding the first 35 amino acids of BclA (amino acids 1-35 of SEQ ID NO:1) using the splicing by overlapping extension (SOE) technique. Correct amplicons were cloned into the *E. coli/Bacillus* shuttle vector pHP13, and correct clones screened by DNA sequencing. Correct clones were electroporated into *Bacillus thuringiensis* (Cry-, plasmid-) and screened for chloramphenicol resistance. Correct transformants were grown in brain heart infusion broth overnight at 30° C., plated onto nutrient agar plates, and incubated at 30° C. for 3 days. Spores expressing the fusion construct (BEMD spores) were collected off of the plates by washing in phosphate buffered saline (PBS) and purified by centrifugation and additional washes in PBS. Non-transformed control *Bacillus thuringiensis* (B.t.) spores were created identically.

(997) Soybeans (strain Jake 011-28-04) were planted 2.54 cm deep in 10 cm deep pots filled with standard loam topsoil. Spores were diluted to a concentration of 1×10.sup.4/ml in 50 ml of water and applied to each seed at planting. Plants were grown under ideal light using T5 lamps, 54 watts, and exposed to 11 hours of light a day under controlled temperature conditions between 15.5-25.5° C. Plants were watered to saturation every three days over a two week trial. At the end of two weeks, the height of each plant was measured and measurements were normalized to control *Bacillus thuringiensis* spores. Two independent trials were performed.

(998) Results are shown in Table 17, together with the standard error of the mean. In both trials, soybeans grown in the presence of BEMD spores displaying either lipase or endoglucanase grew significantly taller than control B.t. spore treated soybeans (statistical analysis assayed via a t-test).

(999) TABLE-US-00017 TABLE 17 Soybeans Comparison Avg. Height, to Treatment cm Control SEM Trial #1 Control *Bt* 14.034 100.0% .521 Lipase, BEMD 17.93 127.8% .395 Endocellulase, BEMD 16.31 116.2% .411 Trial #2 Control *Bt* 15.39 100.0% .749 Lipase, BEMD 19.15 124.4% .428 Endocellulase, BEMD 17.65 114.7% .313

Example 2. Use of a Recombinant *Bacillus cereus* Family Member Displaying an Endoglucanase to Stimulate Plant Growth in Corn

(1000) BEMD spores expressing endoglucanase were created in an identical fashion as described above in Example 1. Field corn was planted 3.8 cm deep in 10 cm deep pots filled with standard loam topsoil. Spores, control and BEMD expressing endoglucanase, were diluted to a concentration of 1×10.sup.4/ml in 50 ml of water and applied to each plant at planting. A water-only control was also included. Plants were grown under ideal light using T5 lamps, 54 watts, and exposed to 11 hours of light a day under controlled temperature conditions between 15.5-25.5° C. Plants were watered to saturation every three days over the one week trial. At the end of one week, the height of each plant was measured, and measurements were normalized to control *Bacillus thuringiensis* spores.

(1001) Results are shown in Table 18, together with the standard error of the mean. Corn grown in the presence of BEMD spores displaying endoglucanase grew significantly taller than both control B.t. spore treated soybeans and water-only control plants (statistical analysis assayed via a t-test).

(1002) TABLE-US-00018 TABLE 18 Height, cm Comparison SEM H.sub.2O 15.44 100% 0.318 *Bt* 18.92 122.50% 0.645 BEMD Endo 22.71 143.40% 0.616

Example 3. Use of a Recombinant *Bacillus cereus* Family Member Displaying an Endoglucanase or a Protease to Stimulate Plant Growth in Wheat

(1003) BEMD spores expressing endoglucanase were created in an identical fashion as described above in Example 1. BEMD spores expressing *E. coli* protease PtrB were created using similar methods to those described above in Example 1 and the following primers: ggatccatgctaccaaaagcc (forward, SEQ ID NO: 41) and ggatccttagtccgcaggcgtagc (reverse, SEQ ID NO: 42).

(1004) Winter hard wheat was planted 2.54 cm deep in 10 cm deep pots filled with standard loam topsoil. Spores, control and BEMD expressing endoglucanase or protease, were diluted to a concentration of 1×10.sup.4/ml in 50 ml of water and applied to each plant at planting. A water-only control was also included. Plants were grown under ideal light using T5 lamps, 54 watts, and exposed to 11 hours of light a day under controlled temperature conditions between 15.5-25.5° C. Plants were watered to saturation every three days over the one week trial. At the end of one week, the height of each plant was measured, and measurements were normalized to control water only plants.

(1005) Results are shown in Table 19, together with the standard error of the mean. Wheat grown in the presence of BEMD spores displaying endoglucanase or protease grew significantly taller than control B.t. spore treated or water control soybeans (statistical analysis assayed via a t-test).

(1006) TABLE-US-00019 TABLE 19 Height, cm Comparison SEM H.sub.2O 18.11 100% 0.721 *Bt* Control 19.96 110.33% 0.752 BEMD Endo 24.76 136.80% 0.21 BEMD Protease 22.35 123.40% 0.354

Example 4. Use of Recombinant *Bacillus cereus* Family Members Displaying an Endoglucanase to Stimulate Plant Growth in Ryegrass

(1007) BEMD spores expressing endoglucanase were created in an identical fashion as described above in Example 1. Perennial ryegrass was planted 6.4 mm deep in 10 cm deep pots filled with standard loam topsoil. Spores, both control and BEMD expressing endoglucanase, were diluted to a concentration of 1×10.sup.4/ml in 50 ml of water and applied to each plant at planting. A water-only control was also included. Plants were grown under ideal light

using T5 lamps, 54 watts, and exposed to 11 hours of light a day under controlled temperature conditions between 15.5-25.5° C. Plants were watered to saturation every three days over the two week trial. At the end of two weeks, the height of each plant was measured, and measurements were normalized to control water only plants.

(1008) Results are shown in Table 20, together with the standard error of the mean. Ryegrass grown in the presence of BEMD spores displaying endocellulase grew significantly taller than control B.t. spore treated or water control ryegrass (statistical analysis assayed via a t-test).

(1009) TABLE-US-00020 TABLE 20 Height, cm Comparison SEM H.sub.2O 11.43 100.0% 0.137 *Bt* Control 12.29 107.7% 0.128 BEM Endo 12.78 111.9% 0.137

Example 5. Use of Recombinant *Bacillus cereus* Family Members Displaying Enzymes Involved in the Synthesis or Activation of Plant Hormones to Stimulate Plant Growth

(1010) The BEMD system can also be used to display enzymes involved in the synthesis of plant hormones. For example, the plant hormone indole-3-acetic acid is a potent growth stimulator in plants. Indole-3-acetic acid is synthesized in vivo from tryptophan by the enzymes tryptophan monooxygenase and indole-3-acetamide hydrolase. Indole-3-acetic acid and other auxin hormones can also be synthesized in vivo from tryptophan and/or indole by the enzymes nitrilase, tryptophan aminotransferase, indole-3-acetaldehyde dehydrogenase, indole-3-pyruvate decarboxylase, amine oxidase, tryptophan decarboxylase, and tryptophan side chain oxidases.

(1011) The BEMD system can also be used to display enzymes involved in the modification of plant growth hormones into bioactive or inactive forms. For example, nitrilase can be expressed on the BEMD system to catalyze the conversion of indole-3-acetonitrile into the bioactive indole-3-acetic acid. Additionally, inactive forms of plant hormones, such as indole-3-acetonitrile can be added into the plant growth media with the BEMD-expressed nitrilase to provide a gradual release of active hormone into the plant growth media. Many other inactive or less active forms of plant hormones can be modified using their corresponding enzymes.

(1012) Related plant growth hormones (auxins) include indole-3-pyruvic acid, indole-3-acetaldoxime, indole-3-acetamide, indole-3-acetonitrile, indole-3-ethanol, indole-3-pyruvate, indole-3-butyric acid, phenylacetic acids, 4-chloroindole-3-acetic acid, and indole-3-acetaldoxime. These hormones are synthesized from tryptophan and/or indole in vivo via the enzymes tryptophan monooxygenase, indole-3-acetamide hydrolase, nitrilase, nitrile hydrolase, acetolactate synthetase, alpha acetolactate decarboxylase, tryptophan aminotransferase, indole-3-acetaldehyde dehydrogenase, indole-3-pyruvate decarboxylase, amine oxidase, tryptophan decarboxylase, and tryptophan side chain oxidases.

(1013) Growth hormones of the cytokinin family can also be synthesized by enzymes expressed in the BEMD system. Examples of cytokinins include kinetin, zeatin (cis and trans), 6-benzylaminopurine, dihydroxyzeatin, N6-(D2-isopentenyl) adenine, ribosylzeatin, N6-(D2-isopentenyl) adenosine, 2 methylthio-cis-ribosylzeatin, cis ribosylzeatin, ribosylzeatin-5-monosphosphate, N6-methylaminopurine, N6-dimethylaminopurine, 2′-deoxyzeatin riboside, 4-hydroxy-3-methyl-trans-2-butenylaminopurine, ortho-topolin, meta-topolin, benzyladenine, ortho-methyltopolin, and meta-methyltopolin. These plant growth stimulating compounds are synthesized in vivo from mevalonate or adenosine mono/di/triphosphate by enzymes including adenosine phosphate isopentenyltransferases, phosphatases, adenosine kinases, adenine phosphoribosyltransferase, CYP735A, 5′ribonucleotide phosphohydrolase, adenosine nucleosidases, zeatin cis-trans isomerase, zeatin O-glucosyltransferases, β-glucosidases, cis-hydroxylases, CK cis-hydroxylases, CK N-glucosyltransferases, 2,5-ribonucleotide phosphohydrolases, adenosine nucleosidases, purine nucleoside phosphorylases, and zeatin reductases.

(1014) Using methods similar to those described above in Example 1, any of these enzymes can be incorporated into the BEMD system for display on BEMD spores by creating a fusion construct comprising the enzyme and a targeting sequence that targets the expressed enzyme to the exosporium when the fusion construct is expressed in a *Bacillus cereus* family member. A recombinant *Bacillus cereus* family member expressing such a construct can then be added to the soil or other plant growth medium or applied directly to plant foliage using methods similar to those described above in Example 1 for stimulation of plant growth.

(1015) The plant growth medium can be supplemented with precursors or substrates for the enzymes. For example, the plant growth medium can be supplemented with tryptophan, adenosine monophosphates, adenosine diphosphates, adenosine triphosphates, or indole. Suitable concentrations of these substrates are between 100 nM and 100 μM.

Example 6. Use of Recombinant *Bacillus cereus* Family Members Displaying Proteases or Peptidases that Cleave Proteins, Peptides, Proproteins, or Preproproteins into Bioactive Peptides for Stimulation of Plant Growth

(1016) Proteases and peptidases can be expressed in the BEMD system that can enzymatically cleave available proteins in the plant growth media to bioactive peptides that can act on the plant directly or indirectly. Examples include the enzymatic cleavage of soybean meal, yeast extract, or other protein rich meals added to the plant growth medium into active peptides that can directly stimulate plant growth. Bioactive peptides generated by enzymatic cleavage of protein meals include RHPP and RKN 16D10, potent stimulators of plant root development. Additionally, proproteins or preproproteins can be cleaved into active forms by BEMD-expressed proteases and

peptidases to their bioactive forms. Inactive proproteins or preproproteins can be added in the plant growth medium to facilitate their gradual cleavage by BEMD proteases and slow release of bioactive proteins.

(1017) Using methods similar to those described above in Example 1, any of these proteases and peptidases can be incorporated into the BEMD system for display on BEMD spores by creating a fusion construct comprising the protease or peptidase and a targeting sequence that targets the expressed enzyme to the exosporium when the fusion construct is expressed in a *Bacillus cereus* family member. A recombinant *Bacillus cereus* family member expressing such a construct can then be added to soil or other plant growth medium supplemented with soybean meal, yeast extract, or another-protein-rich meal for stimulation of plant growth. The soybean meal, yeast extract, or other protein-rich meal is suitably added to the plant growth medium in the form of a liquid composition comprising about 10 μg/L to about 100 mg/L of the protein meal, yeast extract, or other protein-rich meal.

Example 7. Use of BEMD Spores Expressing the Protease PtrB for Stimulation of Plant Growth

(1018) BEMD spores expressing *E. coli* protease PtrB were created as described above in Example 3. Soybean seeds were planted 2.54 cm deep in 10 cm deep pots filled with standard loam topsoil. Spores, both control and BEMD expressing protease, were diluted to a concentration of 1×10.sup.4/ml in 50 ml of water and applied to each plant at planting. A water-only control was also included. Soybean meal at 25 mg/pot was added in water at planting. Plants were grown under ideal light using T5 lamps, 54 watts, and exposed to 13 hours of light a day under controlled temperature conditions between 15.5-25.5° C. Plants were watered to saturation every three days over the one week trial. At the end of two weeks, the height of each plant was measured, and measurements were normalized to control water only plants.

(1019) Results are shown in Table 21, together with the standard error of the mean as a percentage of water control. Soy grown in the presence of BEMD spores displaying protease grew significantly taller than control B.t. spore treated or water control soybeans (statistical analysis assayed via a t-test). The addition of soybean meal to water control or *B. thuringiensis* control plants had little effect. By contrast, in the presence of the soybean meal and the BEMD protease system, the soybean plants responded significantly over all other treatments.

(1020) TABLE-US-00021 TABLE 21 SEM, as Soybean Height Normalized percentage of Treatment Meal (cm) to water water Water only No 12.10 100% 3.1% Water only 25 mg/pot 12.43 102.7% 7.4% *B. thuringiensis* No 12.52 103.5% 5.2% *B. thuringiensis* 25 mg/pot 11.99 99.1% 5.0% BEMD Protease No 12.97 107.2% 6.1% BEMD Protease 25 mg/pot 14.44 119.3% 4.8%

Example 8. Use of Recombinant *Bacillus cereus* Family Members Displaying Proteins or Peptides Involved in the Stimulation of Plant Growth

(1021) The BEMD system can also be used to display proteins or peptides that are directly involved in the promotion of plant growth. For example, plant peptide hormones or non-hormone peptides that stimulate plant growth can be expressed in the BEMD system. For example, non-hormone peptides that directly bind to and active plant receptors can be expressed in the BEMD system to directly act on receptors in the plant and roots of target plants. Such peptide hormones and non-hormone peptides include phytosulfokine, calcalva 3 (CLV3), systemin, RKN 16D10, Hg-Syv46, eNOD40, NOD family proteins, ZmlGF, SCR/SP11 family proteins and peptides, RHPP, POLARIS, and KTI. These peptides and related peptides can be expressed in the BEMD system and delivered to plant growth medium or directly applied to foliage to stimulate plant growth.

(1022) Using methods similar to those described above in Example 1, any of these proteins or peptides can be incorporated into the BEMD system for display on BEMD spores by creating a fusion construct comprising the enzyme and a targeting sequence that targets the expressed enzyme to the exosporium when the fusion construct is expressed in a *Bacillus cereus* family member. A recombinant *Bacillus cereus* family member expressing such a construct can then be added to the soil or other plant growth medium or applied directly to plant foliage using methods similar to those described above in Example 1 for stimulation of plant growth.

Example 9. Use of BEMD Spores Expressing POLARIS or KTI for Stimulation of Plant Growth

(1023) BEMD spores expressing the plant peptide POLARIS and soy peptide KTI were created by synthesizing genes coding for the POLARIS or KIT peptides linked to the targeting sequence of SEQ ID NO: 96. The genes were then introduced genes into *Bacillus thuringiensis* and spores were made as described in Example 1. Soybean seeds were planted 2.54 cm deep in 10 cm deep pots filled with standard loam topsoil. BEMD spores expressing POLARIS or KTI were diluted to a concentration of 1×10.sup.4/ml in 50 ml of water and applied to each plant at planting. A water-only control was also included. Pure POLARIS and KTI peptides were also tested for their effects on soybeans at 0.05 mg/pot. Plants were grown under ideal light using T5 lamps, 54 watts, and exposed to 13 hours of light a day under controlled temperature conditions between 15.5-25.5° C. Plants were watered to saturation every three days over the two week trial. At the end of two weeks, the height of each plant was measured, the roots measured, and measurements were normalized to control water only plants.

(1024) Results are shown in Table 22, together with the standard error of the mean as a percentage of water control. Soy grown in the presence of BEMD spores displaying POLARIS grew taller and had a slight increase in root development than water control soybeans. The presence of free KTI peptide led to a significant stunting of the

plants, losing between 6-8% of their heights, but adding 15% to the length of the roots. Expression of KTI on the BEMD system led to the root growth benefit, but without the stunting effect on the plant height. Importantly, the presence of the *Bacillus thuringiensis* control spores with the free KTI peptide did not prevent the stunting effect of KTI, while the BEMD with KTI displayed no such stunting.

(1025) TABLE-US-00022 TABLE 22 Roots Height, Normalized Normalized Treatment Peptide to Water SEM to Water SEM Water No 100% 6.8% 100% 4.3% Water KTI, 0.05 115% 8.4% 91.8% 3.1% mg/Pot BEMD No 106.3% 7.9% 107.3% 1.7% POLARIS BEMD KTI No 113.3% 5.8% 99.4% 3.4% *B. thuringiensis* KTI, 0.05 115% 7.7% 93.4% 4.2% mg/pot

Example 10. Use of Recombinant *Bacillus cereus* Family Members Displaying Enzymes that Degrade or Modify a Bacterial, Fungal, or Plant Nutrient Source to Stimulate Plant Growth and/or Process Nutrients

(1026) The BEMD system can also be used to display enzymes that degrade or modify beneficially a bacterial, fungal, or plant nutrient source present in soil or another plant growth medium. Such enzymes degrade products present in the soil or other plant growth medium into forms that can easily be taken up by plants and/or the beneficial bacteria and/or fungi of the rhizosphere. Such enzymes include, for example, glucoside hydrolases to degrade complex carbohydrates, cellulases to degrade cellulose; lipases to degrade lipids, including oil, fats, and waxes; lignin oxidases to degrade lignin and humic acids; proteases to degrade polypeptides; phospholipases to degrade membranes; amidases and nitrogenases to recover nitrogen; amylases to process starches; nucleases to recover nucleotides, pectinases to break down pectin, sulfatases to recover sulfur, and xylanases to break down xylans and arabinoxylans. The resultant products, including simple sugars, amino acids, fatty acids, and other nutrients will be readily available for direct uptake by plants and/or for stimulating beneficial bacteria and/or fungi to grow and thrive in the rhizospheres of the plants.

(1027) In addition, enzymes and other biological molecules can be utilized to release or sequester phosphate, nitrogen, and other key elemental nutrients for plant uptake from their various organic and inorganic forms in soil. For example, phosphatases can be used to degrade phosphates in the environment into usable inorganic phosphates for plant use. The phosphates can be naturally occurring phosphates present in a plant growth medium. Alternatively or in addition, the plant growth medium can be supplemented with phosphates such as trimetaphosphate, a common agricultural amendment. Examples of useful phosphatases include phosphoric monoester hydrolases, phosphomonoesterases, phosphoric diester hydrolases, phosphodiesterases, triphosphoric monoester hydrolases, phosphoryl anhydride hydrolases, pyrophosphatases, phytase, trimetaphosphatases, and triphosphatases. For example, the enzymes trimetaphosphatase, triphosphatase, and pyrophosphatase sequentially break down trimetaphosphate into usable inorganic phosphate.

(1028) The nitrogenase family of enzymes converts atmospheric nitrogen (N.sub.2) into ammonia, thereby converting nitrogen that would otherwise be inaccessible to plants into a usable form. Suitable enzymes belong to the Nif family of nitrogenases.

(1029) Chemical energy can also be directly added into the plant growth medium as adenosine-3-triphosphate, ferrodoxin, or additional enzymes that create such energy into the BEMD system. These are cofactors for the nitrogenases and are limited in soil. Thus, such cofactors can be added to soil to enhance the reactions described above.

(1030) Other supplements that can be added to the plant growth medium include starches, cellulose and cellulose derivatives, pectins, xylans and arabinoxylans, fats, waxes, oils, phytic acids, lignins, humic acids, and other nutrient sources that the above enzyme classes exert activity upon.

(1031) Using methods similar to those described above in Example 1, any of these enzymes can be incorporated into the BEMD system for display on BEMD spores by creating a fusion construct comprising the enzyme and a targeting sequence for targeting the fusion construct to the exosporium of a *Bacillus cereus* family member. The fusion construct can then be expressed in a *Bacillus cereus* family member, and this recombinant *Bacillus cereus* family member can be added to soil or another plant growth medium using methods similar to those described above in Example 1 for stimulation of plant growth.

Example 11. Use of BEMD Spores Expressing a Phosphatase for Stimulation of Plant Growth

(1032) BEMD spores expressing *Bacillus subtilis* Phosphatase A4 (PhoA4) were created by synthesizing a gene coding for PhoA4 linked to the targeting sequence of SEQ ID NO: 96. This gene was then introduced into *Bacillus thuringiensis* and spores were made as in Example 1. Corn was planted 2.54 cm deep in 10 cm deep pots filled with standard loam topsoil. BEMD spores expressing PhoA4, were diluted to a concentration of 1×10.sup.4/ml in 50 ml of water and applied to each plant at planting. A water-only control was also included. Polyphosphate was added to pots in liquid at a rate of 0.5 mg/pot. Plants were grown under ideal light using T5 lamps, 54 watts, and exposed to 13 hours of light a day under controlled temperature conditions between 15.5-25.5° C. Plants were watered to saturation every three days over the two week trial. At the end of two weeks, the height of each plant was measured, and measurements were normalized to control water only plants.

(1033) Results are shown in Table 23. Corn grown in the presence of BEMD spores displaying PhoA4 exhibit

enhanced growth, especially in the presence of added polyphosphate. This effect was greater than the effect of the polyphosphate alone.

(1034) TABLE-US-00023 TABLE 23 Growth, Comparison Treatment Additive to Water Water None 100% Water Polyphosphate 110.8% BEMD PhoA4 None 108.3% BEMD PhoA4 Polyphosphate 114.8%

Example 12. Use of Recombinant *Bacillus cereus* Family Members Displaying Enzymes Involved in the Synthesis of 2,3-Butanediol or the Synthesis or Activation of Gibberellic Acid for Stimulation of Plant Growth

(1035) The BEMD system can also be used display enzymes involved in the synthesis of the plant-growth promoting compound 2,3-butanediol. In vivo, 2,3-butanediol is synthesized by beneficial bacteria and fungi in the rhizosphere from acetoin, diacetyl, acetolactate, or pyruvate by the enzymes acetolactate synthetase, α-acetolactate decarboxylase, pyruvate decarboxylase, diacetyl reductase, butanediol dehydrogenases, and acetoin reductase.

(1036) The BEMD system can also be used to display enzymes involved in the synthesis or activation of the plant-growth promoting compound gibberellic acid. Gibberellic acid can be produced from inactive or less active forms via the action of enzymes, including but not limited to hydroxylamine reductases, 2-oxogluturate dioxygenases, gibberellin 2B/3B hydrolases, gibberellin 3-oxidases, and gibberellin 20-oxidases.

(1037) Any of these enzymes can be incorporated into the BEMD system for display on BEMD spores using methods similar to those described above in Example 1. A fusion construct can be prepared that comprises the enzyme and a targeting sequence that targets the enzyme to the exosporium when the fusion construct is expressed in a *Bacillus cereus* family member. The fusion construct is then expressed in a *Bacillus cereus* family member, and the *Bacillus cereus* family member is added to soil or another plant growth medium for stimulation of plant growth.

(1038) To increase the effect of the enzymes displayed on BEMD, the soil can be supplemented with substrates for the enzymes. For example, the soil or other plant growth medium can be supplemented with acetoin, which is a substrate for acetoin reductase; pyruvate, which is a substrate for pyruvate decarboxylase; diacetyl, which is a substrate for diacetyl reductase; and/or acetolactate, which is a substrate for acetolactate decarboxylase. Alternatively or in addition, the soil or other plant growth medium can be supplemented with less potent or inactive forms of gibberellic acid, which will converted into more active forms by the enzymes described above in the soil or other plant growth medium.

Example 13. Use of Recombinant *Bacillus cereus* Family Members Displaying Proteases for Protecting Plants from Pathogens

(1039) The BEMD system can also be used display proteases that protect plants from one or more pathogens. For example, certain bacterial pathogens can communicate between individual members via secretion of bacterial lactone homoserines or related signaling molecules. Thus, proteases specific for bacterial lactone homoserine signaling molecules can protect plants from such bacterial pathogens by disrupting communication between bacteria, a step essential for the bacteria to secrete toxins and upregulate virulence factors. Suitable proteases specific for bacterial lactone homoserine signaling molecules include endopeptidases and exopeptidases.

(1040) Proteases specific for bacterial lactone homoserine signaling molecules can be incorporated into the BEMD system using methods similar to those described above in Example 1. A fusion construct can be prepared that comprises the protease and a targeting sequence that targets the protease to the exosporium when the fusion construct is expressed in a *Bacillus cereus* family member. The fusion construct is then expressed in a *Bacillus cereus* family member, and the *Bacillus cereus* family member is added to soil or another plant growth medium. The protease can then degrade the bacterial lactone homoserine signaling molecules, blocking a key step in the virulence of these organisms and thereby helping to protect the plant from these pathogens. Other proteases and peptidases work effectively in this capacity on the BEMD system as demonstrated above in Example 6 and 7.

Example 14. Use of Recombinant *Bacillus cereus* Family Members Displaying Antimicrobial Proteins and Peptides for Protecting Plants from Pathogens

(1041) The BEMD system can also be used display enzymes that exhibit antibacterial and/or antifungal activities that can help protect plants from one or more pathogens. For example, antimicrobial proteins and peptides such as bacteriocins, lysozymes (e.g., LysM), siderophores, avidins, streptavidins, conalbumin, albumin, lactoferrins (e.g., LfcinB), or TasA can all be expressed in the BEMD system to exert their effect on bacterial and fungal pathogens of plants. Bacteriocins, albumin, conalbumin, lysozymes, and lactoferrin exert direct antimicrobial action on their targets, whereas siderophores, avidins, and streptavidins bind essential nutrients that pathogens require for virulence. For example, the peptide LfcinB of lactoferrin, when expressed on the surface of the BEMD system would lyse bacteria cells that are susceptible to the lactoferrin peptides in the plant growth medium. These proteins and peptides have specific action on select microbes, and can selectively target a group of pathogens without obstructing all microbes in the plant growth medium.

(1042) Any of these proteins or peptides can be incorporated into the BEMD system for display on BEMD spores using methods similar to those described above in Example 1. A fusion construct can be prepared that comprises the enzyme and a targeting sequence that targets the enzyme to the exosporium when the fusion construct is expressed in a *Bacillus cereus* family member. The fusion construct is then expressed in a *Bacillus cereus* family member, and

the *Bacillus cereus* family member is added to soil or another plant growth medium for protection of plants from one or more pathogens.

Example 15. Use of BEMD Spores Expressing Antimicrobial Peptides for Protecting Plants from Bacteria

(1043) Genes were synthesized that coded for either of two antimicrobial peptides, LfcinB (derived from bovine lactoferrin) and LysM (derived from chicken lysozyme), linked to a BclA targeting sequence (SEQ ID NO: 96), under the control of the BclA promoter (SEQ ID NO: 215). The genes were introduced into *Bacillus thuringiensis* BT013A and spores were made by growing an overnight culture of the transformed *Bacillus* in brain heart infusion broth, plating onto nutrient agar plates at 30° C. and allowing to grow for 3 days. Spores were washed off the plates and rinsed 3× in PBS. *Staphylococcus epidermidis* cultures were grown overnight in TSB broth at 37° C. The overnight culture was then pelleted, washed in PBS, and resuspended in PBS at an Abs595=0.2. 1×10.sup.4 BEMD expressing the LysM or LfcinB peptides was incubated in the PBS with the *S. epidermidis* for 3 hours at 37° C., with shaking. A control sample of *S. epidermidis* was left untreated (no BEMD spores). After the 3 hour incubation, dilution plates of the *S. epidermidis* were made and incubated at 37° C. overnight. *S. epidermidis* cultures were counted the next day, and percent killing quantified. In Table 24 below, a record of the killing activity was recorded. The BEMD expressed peptides killed a significant number of *S. epidermidis* cells. This would directly translate into killing of bacteria on the rhizosphere, seed, or other plant material. The selection of peptides specific to certain classes of bacteria can also skew the population of the microorganisms near the plant in a beneficial way, or can selectively target key pathogens.

(1044) TABLE-US-00024 TABLE 24 Treatment Survival % Killed None 100% 0% BEMD LysM 71% 29% BEMD LfcinB 23% 77%

Example 16. Use of Recombinant *Bacillus cereus* Family Members Displaying Enzymes for Protecting Plants from Pathogens

(1045) The BEMD system can also be used display enzymes that protect plants from one or more pathogens. For example, yeast and mold cell walls are degraded by enzymes such as β-1,3-glucanases, β-1,4-glucanases, β-1,6-glucanases, chitosanases, chitinases, chitosanase-like proteins, and lyticases. Bacteria cell walls are degraded by enzymes selected from proteinases, proteases, mutanolysin, stapholysin, and lysozymes. Each of these cell wall degrading enzymes can be expressed on the BEMD system and added to plant growth medium for selective inhibition of pathogenic microbes in the rhizosphere.

(1046) The BEMD system can also be used to display enzymes or proteins that protect plants from insect or worm pathogens, for example by suppressing insect and/or worm predation of desired plants. Examples of such proteins and enzymes of interest include endotoxins, Cry toxins, other insecticidal protein toxins, protease inhibitors, cysteine proteases, the Cry5B protein, the Cry 21A protein, chitinase, protease inhibitor proteins, protease inhibitor peptides, trypsin inhibitors, and arrowhead protease inhibitors.

(1047) Any of these proteins or peptides can be incorporated into the BEMD system for display on BEMD spores using methods similar to those described above in Example 1. A fusion construct can be prepared that comprises the enzyme and a targeting sequence that targets the enzyme to the exosporium when the fusion construct is expressed in a *Bacillus cereus* family member. The fusion construct is then expressed in a *Bacillus cereus* family member, and the *Bacillus cereus* family member is added to soil or another plant growth medium for protection of plants from pathogens.

Example 17. Use of BEMD Spores Expressing an Antifungal Enzyme for Protecting Plants, and Demonstration of Efficacy Against *Saccharomyces*

(1048) A gene was synthesized that encoded an antifungal enzyme, β-1,3-glucanase from *Bacillus subtilis*, linked to a BclA targeting sequence (SEQ ID NO: 96) under the control of the BclA promoter (SEQ ID NO: 215). The gene was and introduced into *Bacillus thuringiensis* BT013A and pores were made by growing an overnight culture of the transformed *Bacillus* in brain heart infusion broth, plating onto nutrient agar plates at 30° C., and allowing to grow for 3 days. Spores were washed off the plates and rinsed 3× in PBS. *Saccharomyces cerevisiae* cultures were grown overnight in YZ broth at 37° C. The overnight culture was then pelleted, washed in PBS, and resuspended in PBS at an Abs595=0.2. 1×10.sup.4 BEMD expressing β-1,3-glucanase was incubated in the PBS with the *Saccharomyces* for 1 hour at 37° C., with shaking. A control sample of *Saccharomyces* was left untreated (no BEMD spores). After the 3 hour incubation, dilution plates of the *Saccharomyces* were made and incubated at 37° C. overnight. *Saccharomyces* cultures were counted the next day, and percent killing quantified. In Table 25 below shows the killing activity of the BEMD spores expressing β-1,3-glucanase. The BEMD-expressed enzyme killed a significant number of *Saccharomyces* cells. This would directly translate into killing of fungal microorganisms on the rhizosphere, seed, or other plant material. The selection of proteins specific to certain classes of fungi can also skew the population of the microorganisms near the plant in a beneficial way, or can selectively target key fungal pathogens.

(1049) TABLE-US-00025 TABLE 25 Treatment Survival % Killed None 100% 0% BEMD β-1,3-glucanase 83% 17%

Example 18. Use of Recombinant *Bacillus cereus* Family Members Displaying Plant Immune System Stimulatory Peptides or Proteins for Protecting Plants from Pathogens

(1050) The BEMD system can also be used display plant immune system enhancer peptides and proteins. These proteins can be expressed on the outside of the BEMD spore and delivered into the plant growth medium to stimulate the plant immune system to allow the plant to protect itself from plant pathogens. Example proteins and peptides include harpin, α-elastins, β-elastins, systemins, phenylalanine ammonia-lyase, elicitins, defensins, cryptogein, and flagellin proteins and peptides. Exposure of plants to these proteins and peptides will stimulate resistance to many plant pathogens in plants.

(1051) Any of these proteins or peptides can be incorporated into the BEMD system for display on BEMD spores using methods similar to those described above in Example 1. A fusion construct can be prepared that comprises the enzyme and a targeting sequence that targets the enzyme to the exosporium when the fusion construct is expressed in a *Bacillus cereus* family member. The fusion construct is then expressed in a *Bacillus cereus* family member, and the *Bacillus cereus* family member is added to soil or another plant growth medium for protection of plants from pathogens.

Example 19. Use of Recombinant *Bacillus cereus* Family Members Displaying a Root or Leaf Binding Protein or Peptide to Immobilize the Recombinant *Bacillus cereus* Family Member on a Root System of a Plant or on Plant Leaves

(1052) Root and leaf binding proteins and peptides can also be incorporated into the BEMD system to allow the BEMD spores to be immobilized on a root system or on leaves of a plant. Display of such root or leaf binding ligands on the BEMD spores allows for targeting of the spores to the root system of a plant or to substructures of the root system or to the leaves or to substructures of leaves to maintain the BEMD spores at an optimal location for other displayed biological molecules and enzymes to be effective.

(1053) For example, rhicadhesin is a root binding ligand that binds to root hairs. Thus, display of rhicadhesin on the BEMD spores thus targets the spores to root hairs. Additional proteins that could be utilized for selective binding to plant roots or leaves include adhesins, flagellin, omptins, lectins, pili proteins, curlus proteins, intimins, invasins, agglutinin, afimbrial proteins, TasA, or YuaB.

(1054) Such root or leaf binding proteins and peptides can be incorporated into the BEMD system using methods similar to those described above in Example 1. A fusion construct can be prepared that comprises the root or leaf binding protein or peptide and a targeting sequence that targets the protein or peptide to the exosporium when the construct is expressed in a *Bacillus cereus* family member. The fusion construct containing the root or leaf binding ligand is then expressed in a *Bacillus cereus* family member. Such fusion constructs can be coexpressed with one or more additional fusion constructs comprising any of the beneficial enzymes discussed herein (e.g., an enzyme involved in the synthesis of a plant hormone, an enzyme that degrades a nutrient source, or a proteases that protects a plant from a pathogen). The recombinant *Bacillus cereus* family member is added to soil or another plant growth medium, or applied to the leaves of a plant. The root or leaf binding ligand targets the *Bacillus cereus* family member to the root system of the plant or to the leaves of the plant and immobilizes it there, thus allowing the coexpressed fusion construct to exert its effects in close proximity to the root or leaf system.

Example 20. Use of Recombinant *Bacillus cereus* Family Members Displaying Proteins or Enzymes to Enhance Stress Resistance of Plants

(1055) Proteins, peptides, and enzymes that enhance stress resistance in a plant can be incorporated into the BEMD system and delivered to target plants via addition to roots, leaves, or the plant growth medium. During periods of stress, plants release stress-related compounds, including aminocyclopropane-1-carboxlic acid (ACC), reactive oxygen species, and others, resulting in a negative impact on plant growth. The BEMD system can be used to display enzymes that degrade such stress-related compounds, such as aminocyclopropane-1-carboxylic acid deaminase, superoxide dismutases, oxidases, catalases, and other enzymes that act on reactive oxygen species. Such enzymes reduce the amount of these stress-related compounds and allow plants to continue to grow and even thrive under stressed conditions.

(1056) Any of these proteins or peptides can be incorporated into the BEMD system for display on BEMD spores using methods similar to those described above in Example 1. A fusion construct can be prepared that comprises the enzyme and a targeting sequence that targets the enzyme to the exosporium when the fusion construct is expressed in a *Bacillus cereus* family member. The fusion construct is then expressed in a *Bacillus cereus* family member, and the *Bacillus cereus* family member is added to soil or to another plant growth medium or applied to the leaves of a plant for enhancing the stress resistance of a target plant.

Example 21. Preparation of BEMD Spores Expressing the Protective Enzyme Catalase

(1057) A gene was synthesized that encoded the protective enzyme catalase from *Bacillus cereus* linked to a BetA targeting sequence (SEQ ID NO: 97) under the control of the BetA promoter (SEQ ID NO: 197). This gene was and introduced into *Bacillus thuringiensis* BT013A. Spores were made by growing an overnight culture of the transformed *Bacillus* and wildtype strain in brain heart infusion broth, plating onto nutrient agar plates at 30° C.,

and allowing to grow for 3 days. Spores were washed off the plates and rinsed 3× in PBS. 3 drops of hydrogen peroxide was added to each spore pellet. The enzyme catalase converts the hydrogen peroxide into water and O.sub.2 gas. The control spores did not bubble, while the BEMD-catalase spores readily did, demonstrating enzyme activity on the surface of the spores. Other protective enzymes can be displayed in a similar fashion and delivered to the plant to act upon free radicals produced during stress by the plants.

Example 22. Use of Recombinant *Bacillus cereus* Family Members Displaying Proteins or Enzymes that Protect Seeds or Plants from an Environmental Stress

(1058) Proteins, peptides, and enzymes that protect a plant from an environmental stress can be incorporated into the BEMD system and delivered to target plants via addition to roots, leaves, fruit, or the plant growth medium. During periods of freezing, plants can be damaged by the effect of ice. The BEMD system can be used to display peptides, proteins, or enzymes that protect plants from such effects. For example, the BEMD system can be used to display choline dehydrogenases, which act by producing protective products that protect the plant or seed from frost. Substrates for these enzymes (e.g., choline and/or choline derivatives) can also be added to the plant growth medium. Addition of such substrates can enhance the amount of protectant (betaine and related chemistries) produced in the plant environment by the BEMD expressed enzymes. Betaine derivatives are known to protect seeds from cold stress.

(1059) Any of these proteins or peptides can be incorporated into the BEMD system for display on BEMD spores using methods similar to those described above in Example 1. A fusion construct can be prepared that comprises the enzyme and a targeting sequence that targets the enzyme to the exosporium when the fusion construct is expressed in a *Bacillus cereus* family member. The fusion construct is then expressed in a *Bacillus cereus* family member, and the *Bacillus cereus* family member is added to soil or to another plant growth medium or applied to the leaves of a plant for protecting the plant from environmental stresses and factors.

Example 23. Enhanced Expression of Fusion Constructs on the BEMD System by Use of Enhanced or Alternative Promoter Elements

(1060) The BEMD system can display a wide range of proteins, peptides, and enzymes using one or more of the targeting sequences described herein. Some of these targeting sequences have a high affinity for the exosporium which would be beneficial for fusion protein expression, but their low fusion protein expression level limits their use on the BEMD system. For such fusion proteins and sequences, alternative high-expression sporulation promoters can be used instead of the native promoters.

(1061) For example, SEQ ID NO: 13 (amino acids 1-39 of *B. weihenstephensis* KBAB4 gene 3572) provides a very effective N-terminal sequence for the delivery of proteins to the exosporium of *Bacillus cereus* family members, as shown in Table 26 below. All genes were synthesized in their complete form (including promoter regions and regions coding for fusion proteins) as described herein. When the native promoter elements for *B. weihenstephensis* KBAB4 gene 3572 (SEQ ID NO: 217) were used to express a fusion protein comprising the targeting sequence of SEQ ID NO: 13 fused to a β-galactosidase enzyme (from *E. coli*), a low level of fusion protein was expressed, leading to a reduction in enzyme activity on the surface of the spore. Enzyme activity was measure by the conversion of 0.5M o-nitrophenylgalactoside in solution over 10 minutes. Enzyme conversion was measured with a spectrophotometer at ABS540. Replacement of the native promoter elements of the *B. weihenstephensis* KBAB4 gene 3572 with the high-expression promoters of SEQ ID NO: 197 (*B. anthracis* BetA/BAS3290) or SEQ ID NO: 218 (*B. weihenstephensis* KBAB4 YVTN β-propeller protein) led to a dramatic increase in the enzymatic activity of the spores. On the other hand, replacement of the native promoter elements for *B. weihenstephensis* KBAB4 gene 3572 with the promoter native to *B. anthracis* Sterne BAS1882 (SEQ ID NO: 216) led to a decrease in the enzymatic activity of the spores. The expression level of the targeting sequence of SEQ ID NO: 13 fused to β-galactosidase was much lower (0.38×) when driven by the promoter of BAS1882 (SEQ ID NO: 216), and was greatly improved when driven from the BetA promoter (SEQ ID NO: 197) or YVTN protein promoter (SEQ ID NO: 218).

(1062) TABLE-US-00026 TABLE 26 β-galactosidase activity on BEMD Fold Promoter Fusion Protein system, normalized Change SEQ ID NO: 217 SEQ ID NO: 100% 13-β-galactosidase SEQ ID NO: 197 SEQ ID NO: 213.4% 2.13× 13-β-galactosidase SEQ ID NO: 218 SEQ ID NO: 220.7% 2.21× 13-β-galactosidase SEQ NO: ID 216 SEQ ID NO: 38.1% 0.38× 13-β-galactosidase

Example 24. Isolation and Identification of Plant-Growth Promoting Bacterial Strains

(1063) Soil samples from rhizospheres of the healthiest and most resistant potato (*Solanum tuberosum*), yellow summer squash (*Cucurbita pepo*), tomato (*Solanum lycopersicum*), and pole bean (*Phaseolus coccineus*) plants were collected, diluted in sterile water, and spread onto nutrient agar plates. Bacterial isolates that demonstrated high growth rates and were able to be passaged and propagated were selected for further study. The selected strains were grown in minimal media (KH.sub.2PO.sub.4 3 g, Na.sub.2HPO.sub.4 6 g, NH.sub.4Cl 1 g, NaCl 0.50 g, MgSO.sub.4 7H.sub.2O 0.15 g, CaCl.sub.2) 2H.sub.2O 0.013 g, and glucose 1 g, per L dry weight). Overnight cultures (30° C.) of selected strains were spun down, media decanted off, and resuspended in an equal amount of

distilled water. Ten lettuce seeds per treatment were planted at a depth of 1 cm in loam top soil (Columbia, MO) that was sieved to remove large debris. Seeds were inoculated at planting in 4 cm pots with 0.5 μl of resuspended bacteria in water mixed into 10 ml of H.sub.2O. Ten ml of H.sub.2O was sufficient to deliver the bacteria into the 3 in.sup.3 (7.62 cm.sup.3) of soil as well as saturate the soil for proper germination of seeds. Plants were grown at temperatures between 65-75° F. (18-24° C.) with 11 hours of light/day, and 5 ml of watering every 3 days. After one week, plant heights and leaf diameters, as well as overall health of the plants were collected. Initial screening of rhizosphere isolates resulted in obtaining greater than 200 distinct species of bacteria and fungi from the rhizosphere of the four plants. Some of the bacterial species are described in Table 27. Identified strains are indicated by their proper bacterial identifications. Other strains are indicated by their unknown identification number. Inoculants giving results near control (+/−2%) were not included in the table.

(1064) TABLE-US-00027 TABLE 27 Butterhead Lettuce Bacterial Inoculant Avg. Height (cm) Comparison SEM Uninoculated 1.8 Control .07 *Paracoccus kondratiavae* 2 111.1% .05 NC35 *B. aryabhattai* CAP53 3.65 202.8% .45 *B. flexus* BT054 2.45 136.1% .11 *Bacillus mycoides* strain 2.17 120.4% .21 BT155 *B. aryabhattai* CAP56 2.1 116.7% .20 *B. nealsonii* BOBA57 2.8 155.6% .03 *E. cloacae* CAP12 2.4 133.3% .41 Unknown 8 1.77 77.8% .65 Unknown 122 1.9 105.6% .11 Unknown 15 1.4 77.8% .41 Unknown 39 1.8 100.0% .20 Unknown 401 2 111.1% .21 Unknown 402 1.53 85.2% .27 Unknown 41 1.45 80.6% .31 Unknown 42 1.4 77.8% .15 Unknown 44 2.2 133.3% .08 Unknown 51 1.83 102.9% .21

(1065) Bacterial strains that produced the greatest effect on the overall plant health and plant height in the initial lettuce trial were subjected to further identification. Bacterial strains were grown overnight in Luria Bertani broth at 37° C., and overnight cultures were spun down in a centrifuge. Media was decanted and the remaining bacterial pellet was subjected to chromosomal DNA isolation using the Qiagen Bacterial Chromosomal DNA Isolation kit. Chromosomal DNA was subjected to PCR amplification of the 16S rRNA coding regions using the primers E338F 5′-ACT CCT ACG GGA GGC AGC AGT-3′ (SEQ ID NO: 298), E1099R A 5′-GGG TTG CGC TCG TTG C-3′ (SEQ ID NO: 299), and E1099R B 5′-GGG TTG CGC TCG TTA C-3′ (SEQ ID NO: 300). PCR amplicons were purified using a Promega PCR purification kit, and the resultant amplicons were diluted and sent to the University of Missouri DNA Core for DNA sequencing. DNA sequences were compared to the NCBI BLAST database of bacterial isolates, and genus and species were identified by direct comparison to known strains. Top identified species are indicated in Table 27. In many cases, 16S rRNA DNA sequences were only able to delineate the genus of the selected bacterial strain. In cases where a direct identification was not forthcoming, additional biochemistry analyses, using methods standard in the field, were performed to differentiate strains at the species and strain levels, and are listed in Table 28.

(1066) TABLE-US-00028 TABLE 28 *E. cloacae P. kondratiavae B. aryabhattai B. flexus B. mycoides B. aryabhattai B. nealsoni* Test CAP12 NC35 CAP53 BT054 BT155 CAP56 BOBA57 Urease − − − − − − + Catalase + + + + + + + Oxidase − + + + − − − Nitrate + + − + + − + Growth, 5% NaCl + − + + − + + Growth, 7.5% NaCl − − + + − + − Growth, 42° C. + + + + + + + Growth, 50° C. − − + + − + − Growth, pH 5 + − + + − + − Growth, pH 9 + + + + + + + Acid, Cellobiose + − + + + + − Acid, Lactose + − + + + − + Acid, Starch − − − + − + −

Example 25. Isolation and Identification of Additional Plant-Growth Promoting Bacterial Strains

(1067) Soil samples from agricultural fields near Gas, Kansas were collected, diluted in sterile water, and spread onto nutrient agar plates. Bacterial isolates that demonstrated high growth rates and were able to be passaged and propagated were selected for further study. The selected strains were grown in minimal media (KH.sub.2PO.sub.4 3 g, Na.sub.2HPO.sub.4 6 g, NH.sub.4Cl 1 g, NaCl 0.50 g, MgSO.sub.4 7H.sub.2O 0.15 g, CaCl.sub.2) 2H.sub.2O 0.013 g, and glucose 1 g, per L dry weight). Overnight cultures (30° C.) of selected strains were spun down, media decanted off, and resuspended in an equal amount of distilled water. Corn seeds were coated with commercial seed polymer mixed with water alone (1.6 μl per seed total) or commercial seed polymer containing selected bacterial strains (1.6 μl per seed total). Coated seeds were planted in (3 inch) 7.62 cm diameter pots at a depth of 1 inch (2.54 cm) in loam top soil (Columbia, MO) that was sieved to remove large debris. Plants were grown at temperatures between 18-24° C. (65-75° F.) with 11 hours of light/day, and 50 ml of watering at planting and every 3 days. After two weeks, plant heights and leaf diameters, as well as overall health of the plants were collected. For germination assays and determining 3 day root length, seeds were coated as indicated above and evenly dispersed at 10 seeds per paper towel. The paper towels were wetted with 10 mls of water, rolled up, placed in a small plastic bag and incubated at 30° C. or placed on a germination heat mat at 27-30° C. (80-85° F.). Root measurements were recorded after 3 days. Initial screening of rhizosphere isolates resulted in obtaining greater than 100 distinct species of bacteria and fungi from the rhizosphere. Some of the bacterial species are described in Table 29. Identified strains are indicated by their proper bacterial identifications.

(1068) TABLE-US-00029 TABLE 29 Corn Seed Treatments Avg. Height Avg. Root Length (2 weeks) (3 days) normalized to normalized to polymer control polymer control Bacterial Inoculant (%) (%) Polymer control 100 100 *B. mycoides* EE118 111.1 189.1 *B. subtilis* EE148 99.4 172.8 *Alcaligenes faecalis* EE107 111.5 129.2 *B. mycoides* EE141 109.2 143.5 *B. mycoides* BT46-3 105.6 141.3 *B. cereus* family member EE128 105.6 — *B. thuringiensis*

BT013A 101.8 103.8 *Paenibacillus massiliensis* BT23 104.2 139.4 *B. cereus* family member EE349 105.2 — *B. subtilis* EE218 106.6 — *B. megaterium* EE281 107.8 —

(1069) Bacterial strains that produced the greatest effect on plant health are described in Table 29. Bacterial strains were grown overnight in Luria Bertani broth at 37° C., and overnight cultures were spun down in a centrifuge. Media was decanted and the remaining bacterial pellet was subjected to chromosomal DNA isolation using the Qiagen Bacterial Chromosomal DNA Isolation kit. Chromosomal DNA was subjected to PCR amplification of the 16S rRNA coding regions using the primers E338F 5′-ACT CCT ACG GGA GGC AGC AGT-3′ (SEQ ID NO: 298), E1099R A 5′-GGG TTG CGC TCG TTG C-3′ (SEQ ID NO: 299), and E1099R B 5′-GGG TTG CGC TCG TTA C-3′ (SEQ ID NO: 300). PCR amplicons were purified using a Promega PCR purification kit, and the resultant amplicons were diluted and sent to the University of Missouri DNA Core for DNA sequencing. DNA sequences were compared to the NCBI BLAST database of bacterial isolates, and genus and species were identified by direct comparison to known strains. Top identified species are indicated in Table 16. In many cases, 16S rRNA DNA sequences were only able to delineate the genus of the selected bacterial strain. In cases where a direct identification was not forthcoming, additional biochemistry analyses, using methods standard in the field, were performed to differentiate strains at the species and strain levels, and the differentiated strains are listed in Table 30.

(1070) TABLE-US-00030 TABLE 30 *B. thuringiensis B. cereus* family *B. subtilis B. subtilis B. megaterium Paenibacillus* Test BT013A member EE349 EE148 EE218 EE281 *massiliensis* BT23 Motility + + + + + + Rhizoid Colony − − − − − + Catalase + + + + + + Oxidase + − − − − − Nitrate + + wk − − − Growth, 5% NaCl + wk − + + − Growth, 7.5% NaCl Wk − − + + − Growth, 42° C. − + + + + + Growth, 50° C. − − − − − − Growth, pH 5 Wk − + + + − Growth, pH 9 + + − + + − Acid, Cellobiose − − wk + − + Acid, Lactose − + + + + − Acid, Starch − + − + + − *B. mycoides Alcaligenes faecalis B. mycoides B. cereus* family *B. mycoides* Test BT46-3 EE107 EE118 member EE128 EE141 Motility − + − − − Rhizoid Colony + − + − + Catalase + + + + + Oxidase − + − − − Nitrate + + + + + Growth, 5% NaCl + + − + − Growth, 7.5% NaCl − − − − − Growth, 42° C. + + − + − Growth, 50° C. − − − − − Growth, pH 5 wk + − + − Growth, pH 9 wk + + + − Acid, Cellobiose + wk + − wk Acid, Lactose + + − + wk Acid, Starch + wk + + − wk = weak growth or low growth

Example 26. Testing of Plant-Growth Promoting Bacterial Strains on Alfalfa

(1071) The selected strains were grown in minimal media (KH.sub.2PO.sub.4 3 g, Na.sub.2HPO.sub.4 6 g, NH.sub.4Cl 1 g, NaCl 0.50 g, MgSO.sub.4 7H.sub.2O 0.15 g, CaCl.sub.2) 2H.sub.2O 0.013 g, and glucose 1 g, per L dry weight). Overnight cultures (30° C.) of selected strains were spun down, media decanted off, and bacteria resuspended in an equal amount of distilled water. Ten Zeba-coated alfalfa seeds were planted for each treatment at a depth of 0.6 cm in loam top soil (Columbia, MO) that was sieved to remove large debris. Seeds were inoculated at planting with 0.5 μl of resuspended bacteria in water mixed into 10 ml of H.sub.2O. Ten ml of H.sub.2O was sufficient to deliver the bacteria into the 3 in.sup.3 (7.62 cm.sup.3) of soil as well as saturate the soil for proper germination of seeds. Plants were grown at temperatures between 65-75° F. (18-24° C.) with 11 hours of light/day, and 5 ml of watering every 3 days. Alfalfa was allowed to grow for 1 week to analyze emergence and initial outgrowth of plants under described conditions. Identified strains indicated by their proper bacterial identifications and final height data are listed in Table 31.

(1072) TABLE-US-00031 TABLE 31 Alfalfa Avg. Height Bacterial Inoculant (cm) Comparison SEM Uninoculated 4.82 — .008 *B. aryabhattai* CAP56 4.85 101.20% .016 *B. nealsonii* BOBA57 4.86 101.70% .021 *E. cloacae* CAP12 5.6 116.23% .020

Example 27. Testing of Plant-Growth Promoting Bacterial Strains on Cucumbers

(1073) The selected strains were grown in minimal media (KH.sub.2PO.sub.4 3 g, Na.sub.2HPO.sub.4 6 g, NH.sub.4Cl 1 g, NaCl 0.50 g, MgSO.sub.4 7H.sub.2O 0.15 g, CaCl.sub.2) 2H.sub.2O 0.013 g, and glucose 1 g, per L dry weight). Overnight cultures (30° C.) of selected strains were spun down, media decanted off, and resuspended in equal amount of distilled water. Ten cucumber seeds were planted for each treatment at a depth of 1 cm in loam top soil (Columbia, MO) that was sieved to remove large debris. Seeds were inoculated at planting with 0.5 μl of resuspended bacteria in water mixed into 10 ml of H.sub.2O. Ten ml of H.sub.2O was sufficient to deliver the bacteria into the 3 in.sup.3 (7.62 cm.sup.3) of soil as well as saturate the soil for proper germination of seeds. Plants were grown at temperatures between 65-75° F.)(18-24° C. with 11 hours of light/day, and 5 ml of watering every 3 days. Cucumbers were allowed to grow for 2 weeks to analyze emergence and initial outgrowth of plants under described conditions. Identified strains indicated by their proper bacterial identifications and final height data are listed in Table 32.

(1074) TABLE-US-00032 TABLE 32 Cucumbers Avg. Height Bacterial Inoculant (cm) Comparison SEM Uninoculated 11.23 — .067 *B. aryabhattai* CAP53 11.5 102.00% .023 *B. aryabhattai* CAP56 11.35 101.20% .035 *B. nealsonii* BOBA57 11.33 101.10% .014

Example 28. Testing of Plant-Growth Promoting Bacterial Strains on Yellow Squash

(1075) The selected strains were grown in minimal media (KH.sub.2PO.sub.4 3 g, Na.sub.2HPO.sub.4 6 g, NH.sub.4Cl 1 g, NaCl 0.50 g, MgSO.sub.4 7H.sub.2O 0.15 g, CaCl.sub.2) 2H.sub.2O 0.013 g, and glucose 1 g, per

L dry weight). Overnight cultures (30° C.) of selected strains were spun down, media decanted off, and resuspended in an equal amount of distilled water. Ten yellow squash seeds were planted for each treatment at a depth of 1 cm in loam top soil (Columbia, MO) that was sieved to remove large debris. Seeds were inoculated at planting with 0.5 μl of resuspended bacteria in water mixed into 10 ml of H.sub.2O. Ten ml of H.sub.2O was sufficient to deliver the bacteria into the 3 in.sup.3 (7.62 cm.sup.3) of soil as well as saturate the soil for proper germination of seeds. Plants were grown at temperatures between 65-75° F. (18-24° C.) with 11 hours of light/day, and 5 ml of watering every 3 days. Squash was allowed to grow for 2 weeks to analyze emergence and initial outgrowth of plants under described conditions. Identified strains indicated by their proper bacterial identifications, final height data, and final leaf diameter (by span of the two leaves) data are listed in Table 33.

(1076) TABLE-US-00033 TABLE 33 Avg. Yellow Leaf Bacterial Height Squash Diameter Inoculant (cm) Comparison SEM (cm) Comparison Uninoculated 10.16 — .028 5.08 — *B. aryabhattai* 11.75 115.60% .055 7.25 142.60% CAP53 *B. flexus* 11.88 116.90% .017 6.36 125.20% BT054 *Bacillus* 11.92 117.20% .051 6.33 124.60% *mycoides* BT155 *B. aryabhattai* 11.95 117.60% .027 6.33 124.60% CAP56 *B. nealsonii* 11.89 117.00% .118 6.42 126.40% BOBA57 *E. cloacae* 11.42 112.30% .039 6.83 134.40% CAP12

Example 29. Testing of Plant-Growth Promoting Bacterial Strains on Ryegrass

(1077) The selected strains were grown in minimal media (KH.sub.2PO.sub.4 3 g, Na.sub.2HPO.sub.4 6 g, NH.sub.4Cl 1 g, NaCl 0.50 g, MgSO.sub.4 7H.sub.2O 0.15 g, CaCl.sub.2) 2H.sub.2O 0.013 g, and glucose 1 g, per L dry weight). Overnight cultures (30° C.) of selected strains were spun down, media decanted off, and resuspended in an equal amount of distilled water. Thirty ryegrass seeds were planted for each treatment at a depth of 0.3 cm in loam top soil (Columbia, MO) that was sieved to remove large debris. Seeds were inoculated at planting with 0.5 μl of resuspended bacteria in water mixed into 10 ml of H.sub.2O. Ten ml of H.sub.2O was sufficient to deliver the bacteria into the 3 in.sup.3 (7.62 cm.sup.3) of soil as well as saturate the soil for proper germination of seeds. Plants were grown at temperatures between 65-75° F. (18-24° C.) with 11 hours of light/day, and 5 ml of watering every 3 days. Ryegrass was allowed to grow for 1.5 weeks to analyze emergence and initial outgrowth of plants under described conditions. Identified strains indicated by their proper bacterial identifications and height data are listed in Table 34.

(1078) TABLE-US-00034 TABLE 34 Ryegrass Avg. Height Bacterial Inoculant (cm) Comparison SEM Uninoculated 1.61 — .023 *B. aryabhattai* CAP53 2.01 124.70% .012 *B. flexus* BT054 2.21 137.30% .034 Bacillus mycoides BT155 2.29 142.20% .049 *B. aryabhattai* CAP56 2.19 136.00% .009 *B. nealsonii* BOBA57 2.29 142.40% .045 *E. cloacae* CAP12 1.98 122.50% .015

Example 30. Testing of Plant-Growth Promoting Bacterial Strains on Corn

(1079) The selected strains were grown in minimal media (KH.sub.2PO.sub.4 3 g, Na.sub.2HPO.sub.4 6 g, NH.sub.4Cl 1 g, NaCl 0.50 g, MgSO.sub.4 7H.sub.2O 0.15 g, CaCl.sub.2) 2H.sub.2O 0.013 g, and glucose 1 g, per L dry weight). Overnight cultures (30° C.) of selected strains were spun down, media decanted off, and resuspended in an equal amount of distilled water. Ten corn seeds were planted for each treatment at a depth of 2.5 cm in loam top soil (Columbia, MO) that was sieved to remove large debris. Seeds were inoculated at planting with 0.5 μl of resuspended bacteria in water mixed into 10 ml of H.sub.2O. Ten ml of H.sub.2O was sufficient to deliver the bacteria into the 3 in.sup.3 (7.62 cm.sup.3) of soil as well as saturate the soil for proper germination of seeds. Plants were grown at temperatures between 65-75° F. (18-24° C.) with 11 hours of light/day, and 5 ml of watering every 3 days. Corn was allowed to grow for 2 weeks to analyze emergence and initial outgrowth of plants under described conditions. Identified strains indicated by their proper bacterial identifications and final height data are listed in Table 35.

(1080) TABLE-US-00035 TABLE 35 Corn Avg. Height Bacterial Inoculant (cm) Comparison SEM Uninoculated 8.9 — .039 *B. aryabhattai* CAP53 11.01 123.60% .081 *B. flexus* BT054 9.96 112.00% .095 *Bacillus mycoides* strain BT155 9.6 107.90% .041 *B. aryabhattai* CAP56 9.54 107.10% .088 *B. nealsonii* BOBA57 9.23 103.70% .077

Example 31. Testing of Plant-Growth Promoting Bacterial Strains on Soybeans

(1081) The selected strains were grown in minimal media (KH.sub.2PO.sub.4 3 g, Na.sub.2HPO.sub.4 6 g, NH.sub.4Cl 1 g, NaCl 0.50 g, MgSO.sub.4 7H.sub.2O 0.15 g, CaCl.sub.2) 2H.sub.2O 0.013 g, and glucose 1 g, per L dry weight, or for *Bradyrhizobium* or *Rhizobium* on yeast mannitol media). Overnight cultures (30° C.) of selected strains were spun down, media decanted off, and resuspended in equal amount of distilled water. Ten soybean seeds were planted for each treatment at a depth of 2.5 cm in loam top soil (Columbia, MO) that was sieved to remove large debris. Seeds were inoculated at planting with 0.5 μl of resuspended bacteria in water mixed into 10 ml of H.sub.2O. When testing two bacterial strains, 0.5 μl of each resuspended bacteria was mixed into 10 ml of H.sub.2O. Ten ml of H.sub.2O was sufficient to deliver the bacteria into the 3 in.sup.3 (7.62 cm.sup.3) of soil as well as saturate the soil for proper germination of seeds. Plants were grown at temperatures between 65-75° F. (18-24° C.) with 11 hours of light/day, and 5 ml of watering every 3 days. Soybeans were allowed to grow for 2 weeks to analyze emergence and initial outgrowth of plants under described conditions. Identified strains indicated by their proper bacterial identifications and final height data are listed in Table 36. Co-inoculation of bacteria strains

in the present invention with members of the *Bradyrhizobium* sp. or *Rhizobium* sp. lead to an increase in plant growth compared to either inoculant alone.

(1082) TABLE-US-00036 TABLE 36 Soybeans Avg. Height Bacterial Inoculant (cm) Comparison SEM Uninoculated 13.94 — .089 *B. aryabhattai* CAP53 16.32 117.1% .146 *B. flexus* BT054 17.85 128.0% .177 *Bacillus mycoides* strain BT155 18.93 135.8% .117 *B. aryabhattai* CAP56 17.23 123.6% .133 *B. aryabhattai* CAP53 16.32 117.1% .077 *B. aryabhattai* CAP53 and 16.72 119.9% .182 *Bradyrhizobium* sp. *B. aryabhattai* CAP53 and 17.32 124.2% .086 *Rhizobium* sp. *Bradyrhizobium* sp. 14.25 102.2% *Rhizobium* sp. 14.75 105.8%

Example 32. *Bacillus cereus* Family Members with Plant Growth Promoting Attributes

(1083) *Bacillus mycoides* strain BT155, *Bacillus mycoides* strain EE118, *Bacillus mycoides* strain EE141, *Bacillus mycoides* strain BT46-3, *Bacillus cereus* family member strain EE349, *Bacillus thuringiensis* strain BT013A, and *Bacillus megaterium* strain EE281 were grown in Luria Bertani broth at 37° C. and overnight cultures were spun down, media decanted off, and resuspended in equal amount of distilled water. 20 corn seeds were planted for each treatment at a depth of 2.5 cm in loam top soil (Columbia, MO) that was sieved to remove large debris. Seeds were inoculated at planting with 0.5 μl of resuspended bacteria in water mixed into 50 ml of H.sub.2O. Fifty ml of H.sub.2O was sufficient to deliver the bacteria into the 29 in.sup.3 (442.5 cm.sup.3) of soil as well as saturate the soil for proper germination of seeds. Plants were grown at temperatures between 65-72° F. with 13 hours of light/day, and 5 ml of watering every 3 days. Seedlings were allowed to grow for 2 weeks to analyze emergence and initial outgrowth of plants under described conditions. Identified strains indicated by their proper bacterial identifications and final height data are listed in Table 37.

(1084) TABLE-US-00037 TABLE 37 Avg. Height, cm, Bacterial Inoculant Corn Percentage SEM, H.sub.2O Control 11.41 100% .123 *B. mycoides* EE118 12.43 108.9% .207 *B. mycoides* EE141 12.84 112.5% .231 *B. mycoides* BT46-3 11.81 103.5% .089 *Bacillus thuringiensis* 12.05 105.6% .148 BT013A *Bacillus cereus* family 13.12 114.9% .159 member EE128 *Bacillus mycoides* BT155 12.85 112.6% .163 *Bacillus megaterium* EE281 11.99 105.1% .098

(1085) All plant growth promoting bacteria tested had a beneficial effect on corn height at two weeks under the described conditions. The *Bacillus cereus* family member EE128 strain had the greatest effect in this trial, giving a greater than at 14% boost in corn height.

Example 33. Enhanced Selection of *Bacillus cereus* Family Members to Screen for Plant Growth-Promoting and Other Beneficial Activities as BEMD Expression Host

(1086) The BEMD system can be used to display a wide range of proteins, peptides, and enzymes using any of the targeting sequences described herein to provide beneficial agricultural effects. Additional beneficial effects can be obtained by selecting an expression host (a *Bacillus cereus* family member) having inherent beneficial attributes. Many strains of members of the *Bacillus cereus* family have plant-growth promoting benefits. Additionally, many *Bacillus cereus* family member strains provide have protective effects, through direct fungicidal, insecticidal, nematocidal, or other protective activities. By using such strains these as the expression host for the BEMD system, the end spore product would have a combination of positive benefits in agriculture.

(1087) Table 38 provides results for an experiment wherein a fusion protein was expressed in various *Bacillus cereus* family member strains. All strains are expressed a fusion protein comprising amino acids 1-35 of SEQ ID NO: 1 and the phosphatase PhoA4 from *Bacillus subtilis*, a beneficial enzyme for enhanced phosphate uptake in corn. The gene was synthesized, cloned into the pMK4 vector, and introduced into each of the *Bacillus* spp. indicated in Table 38 below. Strains were taken into sporulation by incubation at 30° C. on nutrient agar plates containing chloramphenicol 10 μg/ml for three days. Spores were collected, washed, and applied to corn at planting at a rate of 1×10.sup.5 CFU/ml in 50 ml of water per 7.62 cm diameter pot with 5 mg polyphosphate per pot. Corn was grown in silt loam soil for two weeks. Plants were grown under ideal light using T5 lamps, 54 watts, and exposed to 13 hours of light a day under controlled temperature conditions between 15.5-25.5° C. Plants were watered to saturation every three days over a two week trial. At the end of two weeks, the height of each plant was measured and measurements were normalized to control *Bacillus thuringiensis* spores. Expression of the SEQ ID NO: 1-Phosphatase fusion protein led to an increase in corn height at 2 weeks regardless of the expression host strain selected. As shown in Table 38, use of a plant-growth promoting *Bacillus cereus* family member further increased corn height.

(1088) TABLE-US-00038 TABLE 38 Height at 2 weeks, *Bacillus* Species Strain Fusion Protein Normalized *B. thuringiensis* Strain BT013A None 100% *B. thuringiensis* Strain BT013A SEQ ID NO: 117.4% 1-Phosphatase *B. mycoides* Strain EE141 None 107.3% *B. mycoides* Strain EE141 SEQ ID NO: 123.3% 1-Phosphatase *B. cereus* family Strain EE128 None 124.1% member *B. cereus* family Strain EE128 SEQ ID NO: 131.7% member 1-Phosphatase *B. mycoides* Strain BT155 None 104.8% *B. mycoides* Strain BT155 SEQ ID NO: 121.9% 1-Phosphatase

Example 34. Use of Various Targeting Sequences to Express β-Galactosidase on the Surface of *Bacillus thuringiensis*

(1089) A wide variety of targeting sequences that that have a high degree homology with amino acids 20-35 of BclA (amino acids 20-35 of SEQ ID NO: 1) can be used to display enzymes, proteins, and peptides on the surface of *Bacillus cereus* family members. Several targeting sequences were compared by making fusion proteins containing the targeting sequences linked to *Bacillus subtilis* lipase. Fusion constructs were synthesized using the promoters native to the targeting sequence, cloned into the replicating plasmid pMK4, and introduced into *Bacillus thuringiensis* BT013A. Strains were taken into sporulation by incubation at 30° C. on nutrient agar plates containing chloramphenicol 10 μg/ml for 3 days. Spores were collected, washed, and resuspended in PBS at a rate of 1×10.sup.8/ml. 1×10.sup.5 spores for each fusion construct spores were suspended in 400 μl dH.sub.2O. The reactions were warmed with the reaction components to the desired reaction temperature)(40° C. 200 μl working buffer was added (9:1 Solution A:Solution B). Solution A was 50 mM Tris pH 10 and 13.6 mM deoxycholic acid and Solution B was 3 mg/ml p-nitrophenyl palmitate in isopropanol. The reaction was incubated at 40° C. for 10 minutes and placed on ice, centrifuged to remove spores, and absorbance at 420 nm was recorded. The results are shown in Table 39 below. Activity was normalized to a control fusion protein comprising amino acids 1-35 of SEQ ID NO: 1 fused to *Bacillus subtilis* lipase.

(1090) TABLE-US-00039 TABLE 39 Strain Targeting sequence Enzyme Relative activity *B. thuringiensis* Amino acids 1-35 of Lipase 100% BT013A SEQ ID NO: 1 *B. thuringiensis* Amino acids 1-27 of Lipase 92.5% BT013A SEQ ID NO: 3 *B. thuringiensis* Amino acids 1-28 of Lipase 13.5% BT013A SEQ ID NO: 7 *B. thuringiensis* Amino acid 1-24 of Lipase 24.8% BT013A SEQ ID NO: 9 *B. thuringiensis* Amino acid 1-33 of Lipase 98.5% BT013A SEQ ID NO: 13 *B. thuringiensis* Amino acid 1-33 of Lipase 107.8% BT013A SEQ ID NO: 21 *B. thuringiensis* SEQ ID NO: 96 Lipase 137.1% BT013A *B. thuringiensis* SEQ ID NO: 98 Lipase 146.3% BT013A *B. thuringiensis* SEQ ID NO: 100 Lipase 115.7% BT013A *B. thuringiensis* SEQ ID NO: 104 Lipase 81.5% BT013A

(1091) Several targeting sequences linked to lipase result in higher expression levels and activity of enzyme on the surface of spores. In particular, SEQ ID NOs. 96, 98, and 100, each containing a shorter targeting sequence, resulted in enhanced fusion expression on the surface of the BEMD spores. All the fusion proteins containing targeting sequences tested resulted in surface display of lipase.

Example 35. Use of Various Exosporium Sequences to Express Lipase on the Surface of *Bacillus thuringiensis* and Demonstration of Fusion Protein Localization to the Exosporium Surface

(1092) A wide variety of exosporium proteins can be used to display enzymes, proteins, and peptides on the surface of *Bacillus cereus* family members. Several different exosporium proteins were compared by making fusion proteins containing the exosporium proteins linked to *Bacillus subtilis* lipase as described in Example 34. Fusion constructs were synthesized using the promoter native to the exosporium protein indicated in Table 40 below, cloned into the replicating plasmid pMK4, and introduced into *Bacillus thuringiensis* BT013A. Spores displaying the various exosporium protein-*Bacillus subtilis* 168 lipase fusions were made by growing the transformed bacteria in brain heart infusion broth with selective pressure from 10 μg/ml chloramphenicol, plating onto nutrient agar plates, and incubating at 30° C. for 3 days. After 3 days, the spores were washed off the plates, purified by centrifugation, and resuspended in PBS at 1×10.sup.8 CFU/ml.

(1093) 1×10.sup.5 spores for each fusion construct were resuspended in 400 μl dH.sub.2O.

(1094) The reactions were warmed with the reaction components to the desired reaction temperature (40° C.) 200 μl of working buffer was added (9:1 Solution A:Solution B). Solution A was 50 mM Tris pH 10 and 13.6 mM deoxycholic acid and Solution B was 3 mg/ml p-nitrophenyl palmitate in isopropanol. The reaction was incubated at 40° C. for 10 minutes and placed on ice, centrifuged to remove spores and absorbance at 420 nm was recorded. Results are shown in Table 40 below. Activity was normalized to SEQ ID NO: 109 linked to lipase.

(1095) TABLE-US-00040 TABLE 40 Strain Exosporium protein Enzyme Relative activity *B. thuringiensis* SEQ ID NO: 109 Lipase 100% BT013A *B. thuringiensis* SEQ ID NO: 110 Lipase 134.5% BT013A *B. thuringiensis* SEQ ID NO: 113 Lipase 17.8% BT013A *B. thuringiensis* SEQ ID NO: 117 Lipase 19.8% BT013A *B. thuringiensis* SEQ ID NO: 118 Lipase 8.2% BT013A

(1096) Use of the exosporium proteins of SEQ ID NOs. 109 and 110 resulted in the highest enzyme activity on the spore. All the fusion proteins containing exosporium proteins resulted in surface display of active *Bacillus subtilis* 168 lipase, albeit at different levels.

(1097) Additional exosporium proteins were demonstrated to result in targeting of fusion proteins to the exosporium using the fluorescent reporter mCherry. Fusion constructs were created that contained the exosporium proteins of SEQ ID NOs. 111, 120, and 110 linked to the mCherry reporter. Spores were grown for 1.5 days, collected, and resuspended as described above. 7 μl of fluorescent spores were put under a Nikon E1000 microscope and imaged during late sporulation. Circular localization in a ring is indicative of outer spore layer localization, and the appearance matches that of an exosporium protein. Fluorescent microscopy results are shown in FIG. **2**. Panels A, B, and C of FIG. **2** are fluorescent microscopy images of spores expressing fusion proteins comprising the exosporium proteins of SEQ ID NOs. 111, 120, and 110, respectively, and the mCherry reporter. All three fusions demonstrated high levels of fluorescence and exosporium localization, demonstrating their potential utility for the

expression of foreign proteins on the surface of the exosporium.

Example 36. Use of Various Targeting Sequences and Exosporium Proteins to Express Phosphatase in *Bacillus subtilis* Spores and Effects of the Phosphatase-Expressing Spores in Soybeans

(1098) BEMD spores expressing *Bacillus subtilis* EE148 Phosphatase A4 (PhoA4) were created by gene synthesis of the genes coding for various targeting sequences and exosporium proteins under the control of their native promoters linked to PhoA4. The synthesized genes were cloned into pMK4 and introduced into *Bacillus thuringiensis* BT013A. Spores displaying the various exosporium protein-*Bacillus subtilis* EE148 PhoA4 fusions were made by growing the transformed bacteria in brain heart infusion broth with selective pressure from 10 μg/ml chloramphenicol, plating onto nutrient agar plates, and incubating at 30° C. for three days. After three days, the spores were washed off the plates, purified by centrifugation, and resuspended in PBS at 1×10.sup.8 CFU/ml.

(1099) Soybeans were planted 2.54 cm deep in 10 cm deep pots filled with standard loam topsoil. BEMD spores expressing PhoA4 were diluted to a concentration of 1×10.sup.4/ml in 50 ml of water and applied to each plant at planting. A water-only control was also included. Polyphosphate was added to pots in liquid at a rate of 0.5 mg/pot. Plants were grown under ideal light using T5 lamps, 54 watts, and exposed to 13 hours of light a day under controlled temperature conditions between 15.5-25.5° C. Plants were watered to saturation every three days over the two week trial. At the end of two weeks, the height of each plant was measured, and measurements were normalized to control water-only plants.

(1100) Results are shown in Table 41. Soy grown in the presence of BEMD spores expressing fusion proteins containing PhoA4 linked to various targeting sequences and exosporium proteins with different fusion partners with PhoA4 all exhibited enhanced growth, but the extent of the effect varied depending on the targeting sequence or exosporium protein used.

(1101) TABLE-US-00041 TABLE 41 Targeting sequence or Height at exosporium protein 2 weeks, *Bacillus* species linked to PhoA4 Normalized H2O (No bacteria) N/A 100% *Bacillus thuringiensis* Amino acids 1-35 of 100% Strain BT013A SEQ ID NO: 1 Bacillus thuringiensis Amino acids 1-28 of 117.4% Strain BT013A SEQ ID NO: 3 *Bacillus thuringiensis* Amino acids 1-33 of 107.3% Strain BT013A SEQ ID NO: 21 *Bacillus thuringiensis* SEQ ID NO: 96 123.3% Strain BT013A *Bacillus thuringiensis* SEQ ID NO: 98 124.1% Strain BT013A *Bacillus thuringiensis* SEQ ID NO: 109 131.7% Strain BT013A *Bacillus thuringiensis* SEQ ID NO: 110 104.8% Strain BT013A

Example 37. Co-Application of BEMD Spores and Seed Treatments, Liquid Fertilizers, and Other Additives

(1102) BEMD spores expressing fusion proteins were tested for compatibility with various seed treatments. The BEMD spores expressed fusion proteins comprising the targeting sequence of amino acids 1-35 SEQ ID NO: 1 linked to a phosphatase (PhoA4) from *Bacillus subtilis* EE148 or the POLARIS peptide. The synthesized genes were cloned into pMK4 and introduced into *Bacillus thuringiensis* BT013A. Spores displaying the various exosporium protein-*Bacillus subtilis* EE148 PhoA4 or POLARIS fusions were made by growing the transformed bacteria in brain heart infusion broth with selective pressure from 10 μg/ml chloramphenicol, plating onto nutrient agar plates, and incubating at 30° C. for three days. After three days, the spores were washed off the plates, purified by centrifugation, and resuspended in PBS at 1×10.sup.8 CFU/ml.

(1103) Plants were grown under ideal light using T5 lamps, 54 watts, and exposed to 13 hours of light a day under controlled temperature conditions between 15.5-25.5° C. Plants were watered to saturation every three days over the two week trial. At the end of two weeks, the height of each plant was measured, and measurements were normalized to control water only plants. Results are shown in Table 42 below. Drench=applied to soil at 50 ml per pot. Polymer=ACCELERON seed coating polymer only. BEMD spores were added at 1×10.sup.4 cells/50 ml for drench applications. BEMD spores were added at 1.3×10.sup.4/cells/seed for seed coating applications. 10-34-0 and 6-24-6 are standard commercial starter fertilizer compositions. 10-34-0 is liquid ammonium phosphate. 6-24-6 is low salt liquid phosphate fertilizer with an ortho/poly formulation. Colorant=Becker Underwood red seed coating coloring agent. MACHO, APRON, and CRUISER are commercial fungicides used on seeds. MACHO contains the active ingredient imidacloprid, APRON contains the active ingredient mefenoxam, and CRUISER contains a mixture of the active ingredients thiamethoxam, mefenoxam, and fludioxonil. The spores were found to be compatible with many seed applications and retained their ability to stimulate plant growth in corn.

(1104) TABLE-US-00042 TABLE 42 Corn height at 2 BEMD treatment Chemical weeks, normalized None None (Water Drench) 100% None Polymer Only 101.3% BEMD PhoA4 N/A (Drench) 111.3% BEMD POLARIS N/A (Drench) 106.7% BEMD PhoA4 Polymer 109.3% BEMD POLARIS Polymer 107.3% BEMD PhoA4 Polymer + Colorant 102.3% BEMD PhoA4 Polymer + MACHO 107.9% BEMD PhoA4 Polymer + APRON 112.3% BEMD PhoA4 Polymer + CRUISER 116.8% BEMD PhoA4 Polymer + Colorant + 113.7% MACHO + APRON + CRUISER None 10-34-0 Starter 108.5% (Drench) BEMD PhoA4 10-34-0 Starter 114.7% Fertilizer (Drench) None 6-24-6 Starter 102.6% Fertilizer (Drench) BEMD PhoA4 6-24-6 Starter 112.9% Fertilizer (Drench)

(1105) BEMD spores were found to be compatible with all seed coating amendments tested. There was a slight decrease in activity when BEMD PhA4 spores were combined with colorant and polymer alone, but the spores

regained full activity with colorant in combination with other fungicides. BEMD spores also worked well with liquid fertilizers. Starter fertilizers contributed to plant growth most likely through direct nutrient supplementation. BEMD spores worked with both starter fertilizers, suggesting that phosphatase activity can still lead to increased plant growth in the presence of excess nutrients. Combinations of BEMD spores with fungicides exhibited greater increases in plant growth than BEMD spores alone, likely due to protection given to young corn plants during early growth.

Example 38. The Use of the BEMD Spores as a Foliar Addition for Reducing Stress Inhibition of Growth on Corn

(1106) The BEMD spore display system can be used to deliver enzymes that can alleviate some stress from growing plants in the field or greenhouse. To accomplish this, enzymes were selected that selectively act upon reactive oxygen species in soil. Reactive oxygen species are a key marker of stress in plants.

(1107) BEMD spores expressing fusion proteins comprising the targeting sequence of amino acids 1-35 of SEQ ID NO: 1 linked to chitosanase, superoxide dismutase, catalase, or B1,3 glucanase from *Bacillus thuringiensis* BT013A were generated. The synthesized genes were cloned into pMK4 and introduced into *Bacillus thuringiensis* BT013A. Spores displaying the various protein fusions were made by growing the transformed bacteria in brain heart infusion broth with selective pressure from 10 μg/ml chloramphenicol, plating onto nutrient agar plates, and incubating at 30° C. for three days. After three days, the spores were washed off the plates, purified by centrifugation, and resuspended in PBS at 1×10.sup.8 CFU/ml.

(1108) Three week old corn plants at the V5 stage were grown under ideal light using T5 lamps, 54 watts, and exposed to 13 hours of light a day under controlled temperature conditions between 15.5-25.5° C. Plants were watered to saturation every three days over the course of the trial. As the plants reach V5, BEMD spores or positive control chemicals were sprayed on the leaves at either 1×10.sup.5 BEMD spores/ml or at the recommended rates for the chemicals. A total of 1 ml of spray was applied to each plant individually. Plant heights were taken just prior to the application of the foliar sprays. The corn plants were then stressed by warming to 32.2° C. and decreasing watering to once per week. Plants were kept under stressed conditions for two weeks. At the end of the two weeks, plant heights were again measured, and visual appearance recorded. Under these stressed conditions, plant growth was minimal in control treatments. The ability to continue to grow under stressed conditions was measured by an increase in plant height over the two week span as compared to the water-only control. Results are shown in Table 43 below.

(1109) TABLE-US-00043 TABLE 43 Change in plant Height over 2 week Treatment Rate stress None None 0% *Bacillus thuringiensis* 1 ml/plant −1.6% BT013A spores BEMD Chitosanase 1 ml/plant 0.3% BEMD Chitosanase 1 ml/plant and 5 mM 4.7% and Chitosan BEMD Superoxide 1 ml/plant 8.3% Dismutase BEMD B1,3 1 ml/plant 4.9% Glucanase Salicylic Acid 1 ml/plant 5.8% Benzothiadiazole 1 ml/plant 7.3% (BTH) BEMD Catalase 1 ml/plant −0.5%

(1110) Several destressing enzymes were applied to corn using the BEMD system, as shown in in Table 43 above. Control spores had no significant effect (decrease in plant height of −1.6%. The BEMD chitosanase enzyme had a positive effect when combined with its substrate, chitosan. The two best performing enzymes were BEMD β-1,3-glucanase and BEMD superoxide dismutase. BEMD β-1,3-glucanase has a primarily antifungal activity, but can also have direct effects on plants. Salicylic acid and BTH were positive controls for the foliar assay, and positive responses were seen for both. This foliar delivery method can be used for delivering destressing enzymes to the plants at various times of the season.

Example 39. Expression Levels of Fusion Proteins Using Various Sigma-K Containing Promoters

(1111) As shown in Example 23 above, replacing native promoter of a targeting sequence, exosporium protein, or exosporium protein fragment can greatly affect the level of fusion protein expressed on the exosporium of a *Bacillus cereus* family spore. For example, replacing the native BclA promoter with the BclB promoter greatly reduces the level of fusion protein on the surface of *Bacillus cereus* family member spores. Alternatively, replacement of native BclB promoter with the BclA promoter increases fusion protein levels on the exosporium dramatically.

(1112) Relative promoter expression levels for various exosporium proteins under the control of their native sporulation promoters were obtained from microarray data from Bergman et al., 2008. The relative expression levels were determined during late sporulation timing (300 minutes after the start of the experiment), when sigma K promoters are most active. Sigma K promoters are key promoters for expression of exosporium localized genes and associated proteins. Relative expression is the increase in a gene's expression level when compared to the average of all other genes of the chromosome at all given times. Table 44 below shows the relative expression levels of a variety of sigma K driven genes in *Bacillus cereus* family members.

(1113) TABLE-US-00044 TABLE 44 Relative Expression (Fold increase Protein (Promoter SEQ ID NO.) in mRNA) CotO (SEQ ID NO: 226) 79.21 Rhamnose (SEQ ID NO: 225) 75.69 BclC (SEQ ID NO: 179) 14.44 Sigma K (SEQ ID NO: 227) 64 BclA adjacent US Glycosyl 72.25 transferase promoter 1 (SEQ ID NO: 229) BclA adjacent DS Glycosyl 73.96 transferase promoter 2 (SEQ ID NO: 230) BclA (SEQ ID NO: 215) 77.44 ExsY (SEQ ID NO:

220) 32.49 YjcA (SEQ ID NO: 222) 64 YjcB (SEQ ID NO: 223) 70.56 BxpB/ExsFA (SEQ ID NO: 224) 30.25 InhA (SEQ ID NO: 228) 34.25

Example 40. Preparation and Testing of BEMD Spores Expressing a Fusion Protein Comprising a Nitric Oxide Synthase, and Use of Such Spores for Stimulating Germination of Plant Seeds

(1114) BEMD spores expressing a fusion protein containing amino acids 20-35 of BclA, a 6-alanine linker, and the nitric oxide synthase enzyme from *Bacillus subtilis* 168 were generated. The nitric oxide synthase (NOS) enzyme from *Bacillus subtilis* 168 was gene synthesized in fusion to the BclA promoter, ribosomal binding site (RBS), start codon and amino acids 20-35 of BclA. A six-alanine linker region was included to separate the BclA targeting sequence from the NOS enzymes. The amino acids sequences of these fusion proteins, including the methionine encoded by the BclA start codon, amino acids 20-35 of BclA, the six-amino acid linker, and the NOS enzyme, are provided above in Table 9. These clones were subcloned in the shuttle vector pHP13 via digestion with XhoI and ligation into the SalI site of pHP13. Correct constructs were sequenced and verified, transformed into *E. coli* cells. The resultant plasmids were transformed into *Bacillus thuringiensis* BT013A and *Bacillus mycoides* EE155.

(1115) The recombinant *Bacillus thuringiensis* BT013A and *Bacillus mycoides* EE155 transformed with the plasmids encoding the NOS fusion proteins were then induced to sporulate by swabbing the bacteria onto nutrient agar plates and incubating the plates at 30° C. for 72 hours. After 72 hours, the bacterial spores were collected from the plate by swabbing into sterile phosphate buffered saline (PBS), and were purified by density centrifugation three times.

(1116) The spores were then applied to commercial corn and soy hybrid seeds at rates of 1×10.sup.5 spores/seed. The soybean hybrid variety was BECK 335NR, which contains the cyst nematode protection gene, the ROUNDUP READY glyphosate resistance gene, and the K-gene for *Phytophthora* resistance. The corn hybrid variety was BECK 5540RR, which contains the ROUNDUP READY glyphosate resistance gene. The seeds were then lightly dusted with L-arginine. A control set of seeds was dusted with L-arginine, but with no spores. Seeds were then placed between two paper towels, which were then wetted with 25 ml of H.sub.2O. The paper towels were then rolled, placed into a small sandwich bag, and sealed tightly. These bags were then placed in a 30° C. incubator and allowed to germinate for 24, or 48 hours. The number of seeds germinated at each timepoint was measured, and the results compared to untreated and control seeds. The results of these experiments are shown in Tables 45 and 46 below.

(1117) TABLE-US-00045 TABLE 45 Increase in germination rate in hybrid soybean seeds treated with spores of recombinant *Bacillus cereus* family members expressing a fusion protein containing nitric oxide synthase. Germination Germination Treatment Day 1 (%) Day 2 (%) Naked soybean seed 15.0% 92.3% Soybean seed plus L-Arginine 20.5% 94.9% Soybean seed plus *B.* 28.9% 97.5% *thuringiensis* BT013A expressing *B. subtilis* NOS fusion protein Soybean seed with L-arginine 30.0% 97.5% and *B. mycoides* EE155 expressing *B. subtilis* NOS

(1118) TABLE-US-00046 TABLE 46 Increase in germination rate in hybrid corn seeds treated with spores of recombinant *Bacillus cereus* family members expressing a fusion protein containing nitric oxide synthase. Germination Germination Treatment Day 1 (%) Day 2 (%) Naked corn seed 0.0% 77.5% Corn seed plus L-Arginine 4.1% 80.5% Corn seed plus *B. thuringiensis* 6.5% 82.5% BT013A expressing *B. subtilis* NOS fusion protein Corn seed with L-arginine and *B.* 4.3% 95.0% *mycoides* EE155 expressing *B. subtilis* NOS

(1119) As can be seen from Tables 45 and 46, treatment of seeds with L-arginine and a recombinant *Bacillus cereus* family member expressing a fusion protein comprising a nitric oxide synthase enzyme led to an increase in the number of germinated seeds, in both soybeans and corn.

Example 41. Preparation and Testing of BEMD Spores Expressing a Fusion Protein Comprising Nucleic Acid Binding Proteins

(1120) BEMD spores expressing a fusion protein containing amino acids 20-35 of BclA, an eight-alanine linker, and the non-specific DNA binding protein SASPα from *Bacillus subtilis* 168 or the non-specific DNA binding protein SASPγ from *Bacillus subtilis* 168. DNA encoding SASPα and SASPY was gene synthesized in frame with the BclA promoter, RBS, start codon BclA and amino acids 20-35 of BclA. An eight alanine linker region was included between the BclA targeting sequence and the RNA/DNA binding proteins. The linker allows for greater flexibility and protein folding of the fusion proteins. The amino acid sequences for these fusion proteins, including the methionine encoded by the BclA start codon, amino acids 20-35 of BclA, the eight-amino acid linker, and the SASPα or SASPγ protein are provided above in Table 11. The synthesized genes were digested with XhoI, and ligated into the SalI site of pHP13 to generate the plasmids pHP13-BclA20-35-SASPα and pHP13-BclA20-25-SASPγ. PHP13 is a well characterized 5.5 kbp shuttle vector plasmid having chloramphenicol and erythromycin resistance cassettes. It was constructed by the ligation of plasmids pE194, pC194, and pUC9.

(1121) Correct clones were subjected to DNA sequencing and transformed into the SCS110 strain of *E. coli*. The plasmid DNA was then purified, and transformed into the *Bacillus thuringiensis* BT013A. These bacteria were then induced to sporulate by swabbing onto nutrient agar plates for 72 hours at 30° C. The spores were collected and purified as described above in the immediately preceding example.

(1122) To assess the ability of the recombinant spores to bind nucleic acids, the recombinant *Bacillus cereus* family members transformed with the plasmids encoding the SASPα and SASPγ fusion proteins were then incubated in PBS with random DNA primers that contained a fluorescein tag on the 5′ ends. A control using non-recombinant spores was also included in the experiment. The spores were incubated for ten minutes with 50 mM tagged DNA, and then washed by centrifugation for one minute at 10,000 rpm. The supernatant was removed, and the spores were resuspended in 1 ml of PBS. The spores were again pelleted and the supernatant removed after centrifugation, and then subjected to analysis. The fluorescein-labeled DNA treated spores were examined under an E600 Nikon fluorescent microscope and DNA binding was determined by the change in the total fluorescence overall as compared to the control spores that did not contain the DNA-binding fusion proteins. The results this assays are shown in Table 47 below.

(1123) TABLE-US-00047 TABLE 47 DNA binding to recombinant *Bacillus cereus* family member spores expressing a fusion protein comprising a DNA binding protein DNA Binding Treatment (Normalized) *B. thuringiensis* BT013A 100% spores (non-recombinant) *B. thuringiensis* BT013A 341.2% spores expressing BclA-SASPα fusion protein *B. thuringiensis* BT013A 250.1% spores expressing BclA- SASPγ fusion protein

(1124) In addition, FIG. **3** shows DNA binding to spores as measured by fluorescein-labeled DNA binding. In FIG. **3**, "control" refers to non-recombinant *B. thuringiensis* BT013A spores (non-recombinant), "SASPa" refers to *B. thuringiensis* BT013A spores expressing BclA-SASPα fusion protein, and SASPc refers to *B. thuringiensis* BT013A spores expressing BclA-SASPγ fusion protein.

(1125) As can be seen from the data shown in Table 47 and FIG. **3**, the spores expressing the SASPα or SASPγ fusion proteins bound a significantly greater amount of DNA than the non-recombinant spores, demonstrating a strong affinity of these spores for DNA.

Example 42. Preparation and Testing of BEMD Spores Expressing a Fusion Protein Comprising a Nuclease

(1126) In addition to the non-specific DNA and RNA binding proteins discussed above in the immediately preceding example, nucleases can also be used to both bind to and cleave nucleic acid molecules. BEMD spores expressing a fusion protein containing amino acids 20-35 of BclA and an endonuclease enzyme were generated and assayed for their ability to bind to and cleave DNA.

(1127) The *Bacillus subtilis* endonuclease 1 was PCR amplified and fused in frame to the BclA promoter, RBS, start codon and amino acids 20-35 of BclA. This construct was then cloned into the pHP13 plasmid to create the plasmid pHP13-BclA20-35-endonuclease. This construct was sequenced and transformed into and propagated in *E. coli*. The plasmid DNA was then isolated from the *E. coli* and introduced into *Bacillus thuringiensis* BT013A. Spores were created and purified as described in Example 40 above.

(1128) Endonuclease activity was assayed by incubating recombinant spores expressing the endonuclease fusion protein and non-recombinant control spores in PBS at a concentration of 1×10.sup.8 spores/ml in PBS with 300 ng of salmon sperm DNA and 1 μg/ml DAPI (4′,6-diamidino-2-phenylindole) DNA stain. The reaction was allowed to proceed continue for 10 minutes at 37° C. After 10 minutes, the supernatant was assayed for cleaved DNA using a fluorometer. As DNA is cleaved, the DAPI stain is released from the individual freed nucleotides, and thus cleavage can be determined by loss of DAPI staining over time. The results of this assay are shown in Table 48 below.

(1129) TABLE-US-00048 TABLE 48 Nuclease Activity and DNA binding by BEMD spores expressing an endonuclease fusion protein Loss of Spore-bound DNA DNA signal (fluorescence Treatment Construct (supernatant) on spores) *Bacillus thuringiensis* — 5% 5.3% BT013A Spores *Bacillus thuringiensis* BclA-endonuclease 65% 21.9% BT013A Spores

(1130) The data provided above in Table 48 show that the endonuclease fusion protein was expressed on the exosporium of the *Bacillus thuringiensis* BT013A spores, and was able to cleave the salmon sperm DNA as evidenced by the loss of DAPI signal in the supernatant. Surprisingly, a portion of the endonuclease bound the DNA tightly without cleaving it, retaining the DAPI fluorescence signal on the spores, even after washing the spores to remove excess DNA. This demonstrates that not all the DNA was processed, and that nucleases expressed on the outside of the spore can bind DNA tightly. To increase this effect, a nuclease having an inactivated active site could be used in the fusion protein, which would lead to less cleavage of the DNA and even more binding DNA on the spores.

Example 43. Agricultural Use of Spores Expressing Fusion Proteins Containing Nucleic Acid Binding Proteins or Peptides

(1131) The recombinant *Bacillus cereus* family or recombinant spore-forming bacteria members expressing fusion proteins comprising nucleic acid binding proteins or peptides can be used in agriculture to deliver nucleic acids to a plant growth medium (e.g., soil) and/or to plants. For example, the recombinant *Bacillus cereus* family members or recombinant spore-forming bacteria can be delivered to plants via seed treatment, in furrow/soil drench treatment, or foliar treatment. Furthermore, the fusion proteins comprising nucleic acid binding proteins or peptides can be expressed in any of the endophytic *Bacillus cereus* family members or any of the other endophytic *Bacillus* species described herein, enabling delivery of nucleic acids bound to the nucleic acid binding proteins internally to the

plant, where they would be more effective in reaching their target cells. For example, the fusion proteins comprising nucleic acid binding proteins can be expressed in the endophytic strain *Bacillus cereus* family member EE349. Expression of another fusion protein (comprising endoglucanase as the protein of interest) in this strain is described in Example 51 hereinbelow, demonstrating that the fusion proteins expressed in this endophytic strain are delivered internally to plants. Thus, expression of the fusion proteins comprising SASPα, SASPγ, Hfq, or a nuclease having an inactivated active site in endophytic *Bacillus cereus* family member strains such as *Bacillus cereus* family member EE349 can provide a means to deliver RNA and DNA (e.g., RNAi or rDNA) internally to a plant. Other non-specific binding nucleic acid binding proteins or peptides could also be used in the fusion proteins for this purpose.

Example 44. Preparation of BEMD Spores that Express a Fusion Protein and Also Overexpress a Protein that Modulates Expression of Fusion Proteins

(1132) Overexpression of various exosporium proteins (referred to herein as "modulator proteins") in a recombinant *Bacillus cereus* family member expressing any of the fusion proteins described herein can modulate (increase or decrease) the expression level of the fusion protein. These modulator proteins include ExsY, ExsFA/BxpB, CotY, CotO, ExsFB, InhA1, InhA2, ExsJ, ExsH, YjcA, YjcB, BclC, AcpC, InhA3, alanine racemase 1, alanine racemasc 2, BclA, BclB, BxpB, BclE, BetA/BAS3290, CotE, ExsA, ExsK, ExsB, YabG, Tgl, superoxide dismutase 1 (SODA1), and superoxide dismutase 2 (SODA2).

(1133) The ability to control the expression level of the fusion protein allows for control of the amount of the protein or peptide of interest of the fusion protein that is displayed on the outside of the spore of the recombinant *Bacillus cereus* family member. For example, when the protein or peptide of interest of the fusion protein comprises a plant growth stimulating protein or peptide (e.g., an enzyme that degrades or modifies a bacterial, fungal, or plant nutrient source), the recombinant *Bacillus cereus* family member expressing the fusion protein produces a spore that when applied to a seed, plant, or plant growth medium, has a beneficial effect on the plant due to the action of the plant growth stimulating protein or peptide. Modulation of the expression level of the fusion protein results in modulation of the level of the peptide or protein of interest that is displayed on the outside of the recombinant *Bacillus cereus* family member spore. In some cases, increasing the level of fusion protein expression would be beneficial (e.g., where there is a desire to increase the expression of an enzyme and thereby increase the amount of enzyme per spore that can be delivered to a plant). In other cases, decreasing the level of fusion protein expression would be beneficial (e.g., where there is a desire to decrease the expression of a protein and thereby decrease the amount of protein per spore that is delivered to a plant, for example, where high levels of the protein would have detrimental effects on the plant).

(1134) To generate plasmids for expression of fusion proteins in *Bacillus cereus* family members, PCR fragments were generated that contained the BclA promoter (SEQ ID NO: 85), start codon, and amino acids 20-35 of BclA fused in frame to either *Bacillus subtilis* 168 endoglucanase or the β-galactosidase gene from *E. coli* DH5α. These PCR fragments were digested with XhoI and ligated into the SalI site of the pSUPER plasmid to generate the plasmids pSUPER-BclA 20-35-Endoglucanase and pSUPER-BclA 20-35-βgal, respectively. The pSUPER plasmid was generated through fusion of the pUC57 plasmid (containing an ampicillin resistance cassette) with the pBC16-1 plasmid from *Bacillus* (containing a tetracycline resistance). This 5.5 kbp plasmid can replicate in both *E. coli* and *Bacillus* spp.

(1135) The pSUPER-BclA 20-35-Endoglucanase and pSUPER-BclA 20-35-βgal plasmids were transformed into and propagated in dam methylase negative *E. coli* strains. The sequences of the pSUPER-BclA 20-35-Endoglucanase and pSUPER-BclA 20-35-βgal plasmids were verified by DNA sequencing.

(1136) The pSUPER-BclA 20-35-Endoglucanase and pSUPER-BclA 20-35-βgal plasmids were transformed into the host strains *Bacillus thuringiensis* BT013A (for pSUPER-BclA 20-35-Endo) or *Bacillus mycoides* BT155 (pSUPER-BclA 20-35-βgal). These transformed strains expressed either the β-galactosidase enzyme or the endoglucanase enzyme on the outside of the spore.

(1137) To generate plasmids for overexpression of modulator proteins, PCR fragments containing the native promoter regions for and genes encoding ExsFA/BxpB, CotO, ExsFB, YjcB, BclC, AcpC, BclA, BclB, BxpB, and CotE were generated, digested with SalI, and ligated into the pHP13 plasmid. The nucleotide sequences for the native promoter regions are provided above in Table 3. The pHP13 plasmid is a multicopy plasmid and therefore results in high expression levels of the encoded modulator proteins when the plasmids are transformed into a *Bacillus cereus* family member host cell. The pHP13 plasmids containing the promoter regions and genes encoding ExsFA/BxpB, CotO, ExsFB, YjcB, BclC, AcpC, BclA, BclB, BxpB, BclE, BetA/BAS3290, and CotE are referred to herein as pHP13-ExsFA/BxpB, pHP13-CotO, pHP13-ExsFB, pHP13-YjcB, pHP13-BclC, pHP13-AcpC, pHP13-BclA, pHP13-BclB, pHP13-BxpB, and pHP13-CotE, respectively.

(1138) The pHP13 plasmids containing the promoter regions and genes encoding the modulator proteins were transformed into and propagated in *E. coli* strains. The sequences of these plasmids were verified by DNA sequencing.

(1139) The pHP13 plasmids encoding the modulator proteins were transformed into *Bacillus thuringiensis* BT013A containing pSUPER-BclA 20-35-Endoglucanase or *Bacillus mycoides* BT155 containing pSUPER-BclA 20-35-βgal. The resultant recombinant bacteria were plated onto nutrient agar plates containing 10 μg/ml chloramphenicol to select for the pHP13 plasmids and 10 μg/ml tetracycline to select for the pSUPER plasmids. Bacteria containing both plasmids were then grown in brain heart infusion broth overnight with both tetracycline and chloramphenicol. The overnight cultures were then swabbed onto nutrient agar, and bacteria were allowed to sporulate at 30° C. for 72 hours. After 72 hours, the bacterial spores were collected from the plate by swabbing into sterile PBS, and were purified by density centrifugation three times. The pure spores were then diluted to 1×10.sup.8 CFU/ml, and assayed for enzyme activity using on a population of 1×10.sup.8 colony forming units (CFU).

Example 45. Enhanced or Diminished Expression of Fusion Proteins on the BEMD System by Overexpression of a Protein that Modulates Expression of the Fusion Construct

(1140) The recombinant *Bacillus mycoides* EE155 spores generated as described above in the immediately preceding example were assayed for β-galactosidase activity, and the recombinant *Bacillus thuringiensis* BT013A spores generated as described above in the immediate preceding example were assayed for endoglucanase activity.

(1141) β-galactosidase activity was assayed by measuring hydrolysis of the chromogenic substrate ortho-Nitrophenyl-β-galactoside (ONPG). A commercial source of β-galactosidase was used to prepare standards (0.2 μg, 0.4 μg, and 0.8 μg from a 100 μg/mL stock). 250 μl of spore preparation was pelleted and the spores were resuspended in 50 μL of enzyme dilution buffer (10 mM TRIS, pH 7.6, 0.2 M NaCl.sub.2, 5% glycerol). 600 μl of prewarmed 37° C. substrate mixture (10 mM KCl, 1 mM MgSO.sub.4.Math.7 H.sub.2O, 100 mM NaH.sub.2PO.sub.4, pH 7.5) containing 1.14 mg/mL ONPG was added to each sample and standard. Each reaction was incubated at room temperature for 2 minutes. 250 μl of 1M sodium carbonate was added to stop the reaction. The solution was centrifuged for 5 min at 14,000×g to remove the spores from the absorbance reading. The absorbance was determined at 420 nm using an IMPLEN nanospectrophotometer model P330. Samples were performed in triplicate with a blank for each reaction. The results of this assay are shown below in Table 49.

(1142) TABLE-US-00049 TABLE 49 Effects of overexpression of exosporium proteins on the expression levels of BclA 20-35-βgal. Plasmid encoding Plasmid encoding Enzyme Activity fusion protein modulator protein (Normalized) — — 0% pSUPER-BclA 20-35-βgal — 100% pSUPER-BclA 20-35-βgal pHP13-CotO 112.8% pSUPER-BclA 20-35-βgal pHP13-CotE 135.4% pSUPER-BclA 20-35-βgal pHP13-YjcB 45.4% pSUPER-BclA 20-35-βgal pHP13-BclA 144.7% pSUPER-BclA 20-35-βgal pHP13-BclB 132.6% pSUPER-BclA 20-35-βgal pHP13-AcpC 76.1% pSUPER-BclA 20-35-βgal pHP13-BxpB 103%

(1143) As can be seen from results shown in Table 49, overexpression of CotO, CotE, BclA, BclB, and BxpB increased the expression of the fusion protein containing β-galactosidase, resulting in increased enzyme activity on the spores. By contrast, overexpression of YjcB or AcpC decreased the expression of the fusion protein containing β-galactosidase, resulting in decreased enzyme activity on the spores.

(1144) The assay for endoglucanase activity was performed by determining cellulase activity using a carboxymethylcellulose (CMC) substrate and a dinitrosalicylic acid (DNS reagent). A commercial source of cellulase enzyme was used to prepare standards in 50 mM citrate buffer, pH 4.8. 1% CMC (carboxymethylcellulose sodium salt) was prepared in 50 mM citrate buffer, pH 4.8 to serve as the substrate for the reaction. 250 μl of spore preparation was pelleted and the spores were resuspended in 150 μL of 50 mM citrate buffer, pH 4.8. The reaction was carried out with a reagent composed of 1% DNS, 1% NaOH, 0.05% Na.sub.2SO.sub.4, 0.2% phenol, and 18.2% Rochelle salts. 150 μl of the sample was mixed with 250 μl of the 1% CMC substrate and incubated in a water bath at 50° C. for 15 minutes. 300 μl of DNS reagent was added and the samples boiled at 100° C. for 10 minutes and then cooled on ice. The solution was centrifuged for 5 minutes at 14,000×g to remove the spores from the absorbance reading. The absorbance was determined at 540 nm using an IMPLEN nanospectrophotometer model P330. Samples were performed in triplicate with a blank for each reaction. The results from this assay are shown in Table 50 below.

(1145) TABLE-US-00050 TABLE 50 Effects of overexpression of exosporium proteins on the expression levels of BclA 20-35-Endoglucanase. Plasmid encoding Plasmid encoding Enzyme Activity fusion protein modulator protein (Normalized) — — 0% pSUPER-BclA 20-35- — 100% endoglucanase pSUPER-BclA 20-35- pHP13-CotO 215.7% endoglucanase pSUPER-BclA 20-35- pHP13-CotE 125.5% endoglucanase pSUPER-BclA 20-35- pHP13-YjcB 89.3% endoglucanase pSUPER-BclA 20-35- pHP13-BclB 193.0% endoglucanase pSUPER-BclA 20-35- pHP13-AcpC 33.7% endoglucanase pSUPER-BclA 20-35- pHP13-BxpB 202.3% endoglucanase pSUPER-BclA 20-35- pHP13-BclC 3.2% endoglucanase

(1146) As shown in Table 50, overexpression of CotO, CotE, BclB, and BxpB increased expression of the fusion protein containing endoglucanase, resulting in increased enzyme activity on the spores. Overexpression of YjcB, AcpC, or BclC, on the other hand, decreased expression of the fusion protein, resulting in decreased enzyme activity on the spores.

(1147) In sum, overexpression of CotO, CotE, BclB, or BxpB increased expression of both fusion proteins,

resulting in increased activity of both β-galactosidase and endogloconase on spores expressing the BclA 20-35-βgal or BclA 20-35-endoglucanase fusion proteins, respectively. Overexpression of YjcB or AcpC on the other hand, decreased expression of both fusion proteins, resulting in decreased activity of β-galactosidase and endogloconase on spores expressing the BclA 20-35-βgal or BclA 20-35-endoglucanase fusion proteins, respectively. Overexpression of BclC and BclA20-35 tagged eGFP also decreased expression of the BclA 20-35-endoglucanase fusion protein, while overexpression of BclA increased expression of the BclA 20-35-βgal fusion protein.

Example 46. Effects of BEMD Spores Expressing a Fusion Protein and Overexpressing a Modulator Protein on Corn Growth

(1148) Application of recombinant *Bacillus thuringiensis* BT103A and *Bacillus mycoides* BT155 spores expressing a fusion protein comprising *Bacillus subtilis* 168 endoglucanase to corn results in increased seedling vigor and growth response over the course of two weeks. Alternations in the expression level of the fusion protein comprising endoglucanase induced by overexpression of a modulator protein in such spores as described above in the immediately preceding example results in corresponding alterations in the effects of the BEMD spores on corn growth.

(1149) To demonstrate this, pSUPER-BclA 20-35-Endoglucanase and the pHP13-CotO or pHP13-BclB were coexpressed in *Bacillus thuringiensis* BT013A. Spores were created on nutrient agar as described above in Example 40. The spores were diluted to a concentration of 1×10.sup.4 spores/50 ml water, and the 50 ml of water was added to commercial hybrid corn seed in potting soil at planting. The corn hybrid variety was BECK 5540RR, which contains the ROUNDUP READY glyphosate resistance gene. The corn seeds were coated with a fungicide and a biological inoculant.

(1150) Plants were grown under artificial light for 14 hours a day and plant growth over a ten day period was determined. Plants were watered every three days over the course of the experiment. After ten days, the plants were measured for height and normalized against the height of untreated corn plants. The results of these experiments are shown in Table 51 below.

(1151) TABLE-US-00051 TABLE 51 Effects of BEMD spores expressing a fusion protein comprising an endogloconase and overexpressing a modulator protein on hybrid corn growth Corn Growth Plasmid (Normalized to encoding pSUPER-BclA 20-35 Plasmid encoding modulator Expression Endoglucanase fusion protein protein Strain alone control) pSUPER-BclA 20- None *Bacillus* 100% 35-Endoglucanase *thuringiensis* BT013A pSUPER-BclA 20- pHP13-CotO *Bacillus* 103.8% 35-Endoglucanase *thuringiensis* BT013A pSUPER-BclA 20- pHP13-BclB *Bacillus* 107.6% 35-Endoglucanase *thuringiensis* BT013A

(1152) As shown in Table 51, overexpression of the exosporium proteins CotO and BclB increased the effects of the BclA 20-35-endoglucanase fusion protein on corn seedling growth and vigor at 10 days. These effects correlate with the expression levels of the fusion protein in BEMD spores expressing BclA 20-35-endoglucanase and pHP13-CotO or pHP13-BclB, indicating that the effects on seedling growth and vigor are attributable to the alteration of fusion protein expression levels by the modulator proteins

Example 47. Genetic Inactivation of *Bacillus cereus* Family Members and Use of Such Inactivated *Bacillus cereus* Family Members for Expression of Fusion Proteins

(1153) As described above, overexpression of germination spore protease (GPR) in its active form in the forespore of a *Bacillus cereus* family member during sporulation results in proteolytic cleavage of proteins in the forespore and inactivation of the spore. Similarly, overexpression of a non-specific endonuclease in the forespore during sporulation destroys the DNA in the spore, leading to an inactivated spore particle in a percentage of the spore population.

(1154) A plasmid encoding a non-specific endonuclease under the control of a sigma G promoter was generated. The non-specific endonuclease 1 from *Bacillus subtilis* 168 and a sigma G promoter (SEQ ID NO: 235) were gene synthesized and ligated into the pHP13 plasmid using the SalI site to generate the plasmid pHP13-SigG-nuclease. Correct clones were sequenced and transformed into and propagated in *E. coli* cells. Plasmid DNA was isolated from the *E. coli* cells and transformed into *Bacillus thuringiensis* BT013A. Correct clones were verified by PCR. The amino acid sequence for *Bacillus subtilis* 168 endonuclease 1 is provided above in Table 4.

(1155) *Bacillus thuringiensis* BT013A cells expressing the sigma G endonuclease were created and purified on nutrient agar plates as described above in Example 40. Spores were quantified visually using a hemocytometer, diluted, and dilution plated onto nutrient agar plates. The ratio of live spores to killed spores was calculated by determining the change from visual counting to plate counts. Control spores (untreated) were included in each assay. Additionally, 1×10.sup.8 spores were UV irradiated for 10 minutes using a handheld UV lamp, and the assay repeated. The visual count and plate count were again compared to assess spore killing. The results from these assays are shown in Table 52 below.

(1156) TABLE-US-00052 TABLE 52 Viability of *Bacillus cereus* family member spores expressing a non-specific nuclease under the control of a sigma G promoter Treatment Live Ratio UV Live Ratio *Bacillus thuringiensis* BT013A 100% 61.3% *Bacillus thuringiensis* BT013A 70.4% 24.5% expressing SigG-endonuclease

(1157) As can be seen from Table 52, expression of endonuclease 1 under the control of a sigma G promoter decreased cell viability by about 30% in spores that were not exposed to UV irradiation and by about 75% in spores that were exposed to UV irradiation.
(1158) Co-expression of both a germination spore protease and a nonspecific endonuclease under the control of sigma G promoters would be expected to further decrease spore viability.
Example 48. Preparation of Exosporium Fragments from Recombinant *Bacillus cereus* Family Members Comprising a Knockout of the CotE Gene
(1159) The plasmid pUCpE was constructed that contained the pUC19 backbone, which is able to replicate in *E. coli*, as well as the origin of replication erythromycin resistance cassette from pE194. This construct is able to replicate in both *E. coli* and *Bacillus* spp. A 1 kb DNA region that corresponding to the upstream region of the CotE gene and a 1 kb region corresponding to the downstream region of the gene CotE were PCR amplified from *Bacillus anthracis* ΔSterne. The two 1 kb regions were then spliced together using splicing by overlapping extension via 15 bp homologous overhangs that corresponded to the opposing PCR amplicons. This 2 kb fragment was digested with XhoI (in external primers) and ligated into the SalI site of pUCpE. This plasmid construct was verified by digestion and DNA sequencing. A Gram-positive omega-kanamycin resistance gene was digested with BamHI and placed between the two 1-kb regions. The final construct was again PCR verified and sequenced, and the final plasmid was introduced into *Bacillus anthracis* ΔSterne. Correct clones were screened by looking for both erythromycin resistance and kanamycin resistance.
(1160) Clones were passaged under high temperature (40° C.) in brain heart infusion broth in the presence of kanamycin (25 μg/ml) and were routinely struck for isolation onto LB agar plates containing kanamycin and grown at 30° C. Individual colonies were toothpicked onto LB agar plates containing erythromycin 5 μg/ml and grown at 30° C. Clones that maintained kanamycin resistance but lost erythromycin resistance (signifying loss of the plasmid but recombination and removal of the CotE gene) were grown in brain heart infusion broth plus kanamycin, and chromosomal DNA was isolated using a Qiagen Chromosomal DNA isolation kit. Proper deletion of the CotE gene was determined by PCR amplification of the CotE gene region and loss of CotE, and gain of the kanamycin resistance cassette.
(1161) A construct was generated (pHP13-AcpC-cGFP) that encoded the exosporium protein ApcC (acid phosphatase) fused in frame to the fluorescent reporter protein cGFP (enhanced green fluorescent protein). The pHP13-ApcC-eGFP construct included the native ApcC promoter, ribosomal binding site, and coding sequence for ApcC (from *B. anthracis* ΔSterne), fused in frame to eGFP (from pGFPuv). This construct was generated by PCR amplification of the individual AcpC and eGFP genes with corresponding primers that contained a 15 bp overlapping region corresponding to the alternate amplicons. The two PCR amplicons were then purified, and combined into a second PCR reaction using external primers that contained XhoI sites. The two amplicons prime each other with their compatible ends, and create a fusion PCR amplicons, that were purified and digested with XhoI for 1 hour at 37° C. The spliced PCR product was cloned into the SalI site of pHP13, and correct clones were sequence verified and transformed into SCS110 *E. coli*. The plasmid DNA was subsequently isolated from the *E. coli* and introduced into *B. anthracis* ΔSterne CotE:Kan generated as described above, which was grown in brain heart infusion broth containing 10 μg/ml chloramphenicol overnight at 30° C. One milliliter of this culture was inoculated into nutrient broth (50 ml) in a baffled flask and grown at 30° C. for 3 days. Spores were collected via centrifugation at 10,000×g for 5 minutes, and the supernatant (containing the broken exosporium fragments) was filtered through a 100,000 Da membrane filter to obtain purified exosporium fragments containing the fusion proteins.
(1162) A transmission electron micrograph showing the CotE knockout spores is provided in FIG. **4**. The closed arrows indicate fragments of exosporium that have been separated from the spores, and the open arrow indicates a spore from which the exosporium has been removed.
(1163) The purification of the exosporium fragments was performed as follows: CotE:kan spores were grown in brain heart infusion broth overnight at 30° C. and swabbed onto nutrient agar plates and grown at 30° C. for 3 days. After 3 days, the spores were collected by swabbing the plates with cotton swabs wetted with PBS and resuspended into 1 ml of PBS in a microcentrifuge tube. The spores were separated from the culture by centrifugation, and supernatant containing the exosporium fragments filtered through a 0.22 μM filter to remove any residual spores. The filtrate was then filtered through a 100 kDa filter to collect exosporium fragments but allow free proteins to pass through the filter. The 100 kDa filter was washed, and the collected exosporium fragments boiled in SDS buffer for 5 minutes and separated by SDS-PAGE electrophoresis. FIG. **5** provides a photograph of an SDS-PAGE gel showing the purified exosporium fragments (lane 2) and a protein marker standard (lane 1). The exosporium fragments shown in lane 2 represent the individual proteins that constitute the exosporium fragments. Only a subset of bands that would normally be seen in a whole spore SDS-PAGE preparation are apparent.
(1164) Ten microliters of the exosporium fragment preparation containing the AcpC-eGFP fusion protein was tested for activity in a phosphatase assay against pNPP (p-nitrophenyl polyphosphate). Acid phosphatase activity was

detected by spectrophotometry based on release of p-nitrophenol from phosphate through phosphatase activity. Briefly, 1 ml of 10 mM pNPP in phosphate buffer at pH 6.0 was incubated with exosporium fragments in a 1 ml microcentrifuge tube and allowed to incubate at 37° C. for 10 minutes. After 10 minutes, the tube was centrifuged for 1 minute to remove excess spores, and the supernatant read on a spectrophotometer at 420 nm for free p-nitrophenol. It was found that the purified exosporium fragments were able to effectively release the phosphate groups from pNPP, demonstrating that the ApcC was present in the exosporium fragments. The results of this assay are shown in FIG. **6**. In FIG. **6**, "CotE control spores" refers to CotE knock-out spores alone (not expressing the AcpC-eGFP fusion protein), "CotE Acp-eGFP" refers to the CotE knock-out spores expressing the AcpC-cGFP fusion protein, and "CotE AcpC-eGFP fragments" refers to the exosporium fragments obtained as described above from the CotE knock-out spores expressing the AcpC-cGFP fusion protein.

(1165) These results demonstrate that mutations that disrupt the exosporium, such as a knock-out mutation in the CotE gene, can be used to generate exosporium fragments that are substantially free of spores, and demonstrates that these exosporium fragments contain fusion proteins that are targeted to the exosporium.

Example 49. Expression of Fusion Proteins in Recombinant *Bacillus cereus* Family Members that are Capable of Degrading Herbicides, and Use of Such Recombinant *Bacillus cereus* Family Members for Stimulation of Plant Growth

(1166) Recombinant *Bacillus cereus* family members expressing fusion proteins can have potent effects on plant health and growth, as illustrated, for example, in Examples 1-4, 7, 9, 11, 33, 36, 37, and 38 above. The fusion proteins comprising a targeting sequence, an exosporium protein, or an exosporium protein fragment described herein can be used in a number of different species and strains within the *Bacillus cereus* family, which includes *Bacillus anthracis, Bacillus cereus, Bacillus thuringiensis, Bacillus mycoides, Bacillus pseudomycoides, Bacillus samanii, Bacillus gaemokensis, Bacillus weihenstephensis*, and *Bacillus toyoiensis*. Many members of the *Bacillus cereus* family are potent degraders of organic and inorganic material in the environment, and some *Bacillus cereus* family members have the ability to degrade herbicides. Expression of the fusion proteins in such strains would be advantageous since this would provide herbicide degrading activity, thereby alleviating the stress on plants that can be caused by the use of herbicides, in addition to the ability to stimulate plant growth or confer other benefits to plant health, depending on the peptide or protein of interest selected for inclusion in the fusion protein.

(1167) *Bacillus cereus* family member EE349 was isolated, identified, and characterized as described above in Example 25, and was found to have the ability to stimulate plant growth. This strain has further been found to have the ability to degrade multiple herbicides, including sulfonylureas and aryl triazines.

(1168) To demonstrate the ability of *Bacillus cereus* family member EE349 to degrade herbicides, 1×10.sup.5 *Bacillus cereus* family member EE349 spores were coated onto lentils planted into soil containing various concentrations of sulfentrazone. The seeds were allowed to grow at 24° C. for 3 weeks on a 13 hour day/night cycle, with watering every 3 days. After 3 weeks, the plants were measured for root growth. A control set of seeds without *Bacillus cereus* family member EE349 was planted under identical conditions.

(1169) The results of this experiment can be seen in FIG. **7**. In FIG. **7**, "protected" refers to seeds treated with *Bacillus cereus* family member EE349, and "unprotected" refers to untreated seeds. The y-axis shows the root length normalized against a water-only control. FIG. **7** shows that as the concentration of the herbicide was increased, the inhibition of root growth also increased. However, application of *Bacillus cereus* family member EE349 to seeds alleviated the majority of this inhibition, even at full strength of the herbicide in soil. Thus, as can be seen from FIG. **7**, *Bacillus cereus* family member EE349 can act as a safener.

(1170) Moreover, the ability of *Bacillus cereus* family member EE349 to express fusion proteins is demonstrated in Example 51 below. Thus, *Bacillus cereus* family member EE349 can be used as a dual-purpose safener and host for expression of the fusion proteins comprising a targeting sequence, an exosporium protein, or an exosporium protein fragment that targets the fusion protein to the exosporium.

Example 50. Preparation of Recombinant *Bacillus cereus* Family Members that Overexpress Exosporium Enzymes and Effects of Such Recombinant *Bacillus cereus* Family Members on Plants

(1171) The exosporiums of *Bacillus cereus* family members naturally contain various natural enzymes that can have beneficial effects on plants. For example, the exosporiums of *Bacillus cereus* family members contain enzymes involved in nutrient solubilization (e.g., acid phosphatases such as AcpC), inosine uridine hydrolases, proteases (e.g., metalloproteases such as InhA1, InhA2, and InhA3), enzymes that catalyze the degradation of free radicals (e.g., superoxide dismutases such as SODA1 and SODA2), arginases, and alanine racemases. Overexpression of such enzymes in *Bacillus cereus* family members can provide recombinant *Bacillus cereus* family members that will have beneficial effects when applied to seeds, plants, a plant growth media, or an area surrounding a plant or a plant seed.

(1172) The metalloproteases InhA2 and InhA3, acid phosphatase (AcpC), and superoxide dismutase 1 and 2 were PCR amplified with their native promoters with primers that contained XhoI sites (amino acid sequences for InhA2, InhA3, AcpC, SODA1 and SODA 2 are provided above in Tables 1 and 2, and nucleotide sequences for the native

promoters for these proteins are provided above in Table 3). The PCR products were digested with XhoI, and cloned into the *E. coli/Bacillus* shuttle vector pHP13 via its SalI site. Correct clones were verified by PCR and DNA sequencing. The plasmids were introduced into *Bacillus thuringiensis* BT013A and *Bacillus mycoides* EE155. Correct clones were screened by plating onto LB agar plates containing chloramphenicol. Overnight cultures of correct clones were grown in brain heart infusion broth containing chloramphenicol, and 1 ml of this overnight culture was inoculated into 50 ml of nutrient broth and cultured for 3 days at 30° C. Sporulation was verified via light microscopy. Spores were then subjected to enzymatic assays.

(1173) *Bacillus mycoides* EE155 spores overexpressing AcpC (i.e., spores containing the pHP13-AcpC (acid phosphatase) plasmid) were assayed for phosphatase activity. One milliliter of the sporulation culture pelleted and the pellet was resuspended in 1 ml of PBS, and tested for activity in a phosphatase assay against pNPP (p-nitrophenyl polyphosphate) as described above in Example 48. The AcpC overexpressing spores had a much higher phosphatase activity, as illustrated in FIG. **8**. In FIG. **8**, the y-axis shows units of phosphatase activity, indicated by the release of p-nitrophenol.

(1174) The increased acid phosphatase activity observed for the *Bacillus mycoides* EE155 spores modified to overexpress AcpC can solubilize nutrients in the environment upon the addition of such spores to a plant growth medium or application of such spores to a plant seed, a plant, or an area surrounding a plant or a plant seed. Since phosphate is a very important nutrient for plant growth and development, this can increase plant growth and provide beneficial effects on plant health.

(1175) Similarly, superoxide dismutase is a very powerful antioxidant protein. Overexpression of a superoxide dismutase in a *Bacillus cereus* family member would provide spores having the ability to degrade free radicals, which exert stress on plants. Removal of the free radicals would alleviate some of this stress and lead to increased plant vigor under stressful conditions. *Bacillus thuringiensis* BT013A spores overexpressing SODA1 and SODA2 (i.e., spores transformed with the pHP13-SODA1 and pHP13-SODA2 plasmids, respectively) can be subjected to enzymatic analysis. One milliliter of the sporulation culture can be pelleted and the pellet and resuspended in 1 ml of dH.sub.2O containing xanthine. Xanthine oxidase can then be added to the reaction mixture, as well as cytochrome C. Inhibition of the degradation of cytochrome C in this assay indicates activity of the superoxide dismutase.

(1176) *Bacillus mycoides* EE155 spores overexpressing a zinc metalloprotease (i.e., spores transformed with the pHP13-InhA2 plasmid) were subjected to enzymatic analysis. One milliliter of the sporulation culture was pelleted and the pellet was resuspended in 1 ml of PBS. The spores were then reacted with 0.5% azocasein, a protease substrate, for 5 minutes. These reaction mixtures were precipitated with TCA (trichloroacetic acid) to remove undigested casein, and the absorbance of the remaining free azo dye was read at ABS595. The spores overexpressing InhA2 generated 211% more protease activity as compared to non-recombinant *Bacillus mycoides* EE155 spores.

(1177) Examples 3 and 7 above illustrate that expression of a protease on the exosporium of a *Bacillus cereus* family member can provide beneficial effects on plants. The *Bacillus thuringiensis* BT013A spores InhA1, InhA2, or InhA3 would have similar effects upon introduction into a plant growth medium, or application to plant seeds, plants, or an area surrounding a plant or a plant seed.

Example 51. Expression of Fusion Proteins in an Endophytic *Bacillus cereus* Family Strain

(1178) *Bacillus cereus* family member EE349 was found to have the ability to grow endophytically and to be capable as serving as a host strain for the BEMD system. To demonstrate the ability of *Bacillus cereus* family member EE349 to grow endophytically and to serve as a host strain for the BEMD system, *Bacillus cereus* family member EE349 was transformed with the pSUPER-BclA 20-35-endoglucanase plasmid (described above in Example 44). Spores were made and purified as described above in Example 40.

(1179) These spores were diluted to a concentration of 1×10.sup.5 spores/50 ml water, and the 50 ml of water was then added to commercial hybrid corn seed in potting soil at planting. The corn seeds were coated with a fungicide and a biological inoculant. The corn hybrid variety was BECK 5475RR, which contains the ROUNDUP READY glyphosate resistance gene and AQUAMAX drought resistance gene. Plants were grown under artificial light for 14 hours a day and plant growth over a ten day period was determined. Plants were watered every three days over the course of the experiment. After ten days, the plants were measured for height and normalized against the height of untreated corn plants. The results of these experiments are shown in Table 53.

(1180) TABLE-US-00053 TABLE 53 Effects of an endophytic *Bacillus cereus* family member expressing the BclA 20-35-endoglucanase fusion protein on corn seedling growth Corn Growth Plasmid Expression Strain (Normalized) None (Control) None 100% None *Bacillus cereus* family 104.1% member EE349 pSUPER-BclA 20-35- *Bacillus cereus* family 111.5% endoglucanase member EE349

(1181) As can be seen from the data shown in Table 53, expression of the pSUPER-BclA 20-35-endoglucanase in the endophytic strain *Bacillus cereus* family member EE349 resulted in increased corn growth as compared to untreated plants, or plants treated with *Bacillus cereus* family member EE349 alone.

(1182) *Bacillus cereus* family member 349 expressing the BclA 20-35-endoglucanase was then isolated from the inside of the corn plants. The ten day old plants were extracted from the soil and washed to remove excess debris. The plants were then inverted, exposed to 5% bleach for ten minutes, washed in water, exposed to hydrogen peroxide (10%) for ten minutes, washed again in water, and the stalks split with a sterile razor blade. The split halves of the stalks were placed face down onto nutrient agar plates for two hours. After two hours, the stalks were removed, and the agar plates incubated at 30° C. for 48 hours. After 48 hours, the plates were examined for colony morphology, and *Bacillus cereus* family member colonies found internal to the plant were toothpicked onto nutrient agar and nutrient agar plus tetracycline plates (to select for bacteria containing the pSUPER-20-35 BclA-endoglucanase plasmid). The resultant increase in *Bacillus cereus* family member 349 colony numbers is indicated shown in Table 54. These results demonstrate the ability of the BEMD system to be introduced into the target plant by expression in an endophytic strain of the *Bacillus cereus* family.

(1183) TABLE-US-00054 TABLE 54 Endophytic assay on *Bacillus cereus* family member EE349 *Bacillus* Tetracycline *cereus* resistant Endophytic family *Bacillus cereus* Bacteria bacteria family Treatment (Total) (all strains) members H.sub.2O (Control) 156 31 0 *Bacillus cereus* family 221 64 21 member EE349 transformed with pSUPER-20-35 BclA-endoglucanase

(1184) Tetracycline resistant *Bacillus* clones were grown overnight at 30° C. in brain heart infusion broth plus tetracycline, and spun down at 10,000×g for 5 minutes. The supernatant was removed, and the pellet frozen overnight at −20 C. Chromosomal DNA was then extracted from each clone, and the presence of the pSUPER-20-35 BclA-endoglucanase plasmid determined by transformation of the chromosomal DNA (containing the plasmid) into DH5*α E. coli* cells and plating on LB plus ampicillin plates. Correct clones were subjected to DNA sequence analysis, which verified that *Bacillus cereus* family member 349 was internal to the plant (endophytic) and contained the plasmid.

(1185) Many endophytic bacteria were found in the corn seedlings, with a number of different strains and species within the *Bacillus cereus* family found inside both the control and the EE349 treated plants. The tetracycline resistant *Bacillus cereus* family members (indicating the presence of the pSUPER-20-35 BclA-endoglucanase plasmid) were only found in the treated corn seedlings, and all had the same colony morphology of the original expression host, *Bacillus cereus* family members EE349. The presence of the pSUPER 20-35 BclA-endoglucanase plasmid was verified by PCR amplification using unique primers.

Example 52. Isolation, Identification, and Characterization of Endophytic *Bacillus cereus* Family Bacterial Strains

(1186) In addition to the endophytic strain *Bacillus cereus* family member 349 discussed above in the immediately preceding example, several other *Bacillus cereus* family members that have the ability to grow endophytically were also identified: *Bacillus cereus* family member EE439, *Bacillus thuringiensis* EE417, *Bacillus cereus* EE444, *Bacillus thuringiensis* EE319, *Bacillus thuringiensis* EE-B00184, *Bacillus mycoides* EE-B00363, *Bacillus pseudomycoides* EE-B00366, and *Bacillus cereus* family member EE-B00377.

(1187) To obtain these additional *Bacillus cereus* family members, commercial hybrid corn seed was planted in potting soil and allowed to grow. The corn seeds were coated with a fungicide and a biological inoculant. Plants were grown under artificial light for 14 hours a day and plant growth over a 14 day period was determined. Plants were watered every three days over the course of the experiment. After 14 days, the plants were extracted from the soil and washed to remove excess debris. The plants were then inverted, exposed to 5% bleach for ten minutes, washed in water, exposed to hydrogen peroxide (10%) for ten minutes, washed again in water, and the stalks split with a sterile razor blade. The split halves of the stalks were placed face down onto nutrient agar plates for two hours. After two hours, the stalks were removed, and the agar plates incubated at 30° C. for 48 hours. After 48 hours, the plates were examined for colony morphology, and *Bacillus cereus* family member colonies found internal to the plant were toothpicked onto nutrient agar. These were then were grown overnight at 30° C. in brain heart infusion broth, and spun down at 10,000×g for 5 minutes. The supernatant was removed, and the pellet frozen overnight at −20° C. Chromosomal DNA was then extracted from each clone, and the identity of each colony verified by PCR using 16S rRNA primers and amplicons were sent for DNA sequencing and identification. The 16S rRNA sequences for these strains are provided above in Table 13.

Example 53. Isolation, Identification, and Characterization of Additional Endophytic Bacterial Strains (Non-*Bacillus cereus* Family Members)

(1188) The endophytic bacterial strains *Bacillus megaterium* EE385, *Bacillus* sp. EE387, *Bacillus circulans* EE388, *Bacillus subtilis* EE405, *Lysinibacillus fusiformis* EE442, *Lysinibacillus* spp. EE443, and *Bacillus pumilus* EE-B00143 were isolated from corn seedlings. Two week old corn seedlings were first sterilized. The plants were extracted them from the soil and washed them to remove excess debris. The plants were then inverted, exposed to 5% bleach for ten minutes, washed in water, exposed to hydrogen peroxide (10%) for ten minutes, and washed again in water. The stalks were then split with a sterile razor blade. The split halves of the stalks were placed face down onto nutrient agar plates for two hours. After two hours, the plant stems were removed from the plates, and the plates were then incubated at 30° C. for 48 hours. Bacilli colonies that were endophytic were selected for further

analysis. These strains were grown up in brain heart infusion broth overnight at 30° C., and the cultures subjected to extraction of DNA using a Qiagen Chromosomal DNA Kit. The DNA was PCR amplified to obtain the 16S rRNA gene, which was sent for DNA sequencing. The resultant sequences BLAST searched using the NCBI databases to establish the identity of the Bacilli species. The 16S rRNA sequences are provided above in Table 14.

Example 54. Expression of Fusion Proteins Comprising a Spore Coat Protein in Endophytic *Bacillus* Bacterial Strains

(1189) The endophytic bacterial strains *Bacillus thuringiensis* EE319, *Bacillus firmus* A30, and *Bacillus lichenformis* A4 were transformed to contain plasmids encoding various spore coat proteins fused to endoglucanase. The plasmids pHP13-CotC-endoglucanase and pHP13-CgeA-endoglucanase were created. Each of these plasmids encoded the spore coat protein (CotC or CgeA) fused in frame with to a polyalanine linker containing eight alanine residues and endoglucanase. The polyalanine linker and endoglucanase were fused to the carboxy terminus of the spore coat proteins.

(1190) To create the plasmids encoding the fusion proteins, the endoglucanase gene from *Bacillus subtilis* 168 was PCR amplified. The genes encoding the spore coat proteins CotC and CgeA were also PCR amplified from the chromosomal DNA of *Bacillus subtilis* 168 (CotC) or *Bacillus amyloliquefaciens* (CgeA). Correct amplicons were then subject to splicing by overlapping extension PCR to generate the fusion protein DNA fragment through annealing of homologous 15 bp overhangs. External primers were each engineered to contain XhoI sites. The amplicons were cleaned up with a Promega PCR clean up kit, and the DNA digested with XhoI and ligated into the SalI site of pHP13. The plasmid DNAs were then sequenced, transformed into *E. coli* cells, and the DNA introduced into the various endophytic *Bacillus* strains.

(1191) Spores of each of the recombinant *Bacillus* species expressing the fusion proteins were generated by swabbing overnight cultures onto nutrient agar plates, which were then incubated at 30° C. for 72 hours. After 72 hours, bacterial spores were collected from the plates by swabbing into sterile PBS. Spores were purified by density centrifugation three times, diluted to 1×10.sup.8 CFU/ml, and assayed for endogloconase activity as described above in Example 45. The results of this assay are shown in Table 55 below and in FIG. **9**.

(1192) TABLE-US-00055 TABLE 55 Endogloconase activity in *Bacillus* spores expressing fusion proteins CotC-endoglucanase or CgeA-endoglucanase Enzyme Reading/ Plasmid Expression Strain Activity Spore Control *Bacillus firmus* A30 .201 Spore Control *Bacillus thuringiensis* .206 BT013A pHP13-CgeA- *Bacillus firmus* A30 .818 endoglucanase pHP13-CotC- *Bacillus thuringiensis* 1.738 endoglucanase EE319 pHP13-CotC- *Bacillus licheniformis* 0.414 endoglucanase A4

(1193) In FIG. **9**, CotC1, CotC2, and CotC3 are three separate experimental sporulation cultures of *Bacillus thuringiensis* EE319 with pHP13-CotC-Endo.

Example 55. Effects of *Bacillus* Spores Expressing Fusion Proteins CotC-Endoglucanase, CotB-Endoglucanase, or CgeA-Endoglucanase on Growth of Corn and Soy Seeds

(1194) Spores of the recombinant *Bacillus* species expressing the fusion proteins comprising a spore coat protein and endoglucanase (e.g., the CotC-endoglucanase, CotB-endoglucanase, or CgeA-endoglucanase fusion proteins described above in the immediately preceding example) can be tested for their effects on the growth of plants (e.g., corn and soy) as follows. Spores can be generated as described above in the immediately preceding example, washed, diluted to 1×10.sup.8 CFU/ml in water, and applied to plant seeds (e.g., corn and soy seeds) at a rate of 1×10.sup.5-7 spores/seed. The spores can then be applied either as a seed treatment or as a soil drench. The plants can be planted 1″ deep in 4″ pots, and grown at 18.3° C. with a 13 hour light/dark cycle. After two weeks, plant height and root length can be determined.

Example 56. Delivery of Probiotic Bacteria to Animals by Feeding Plants Comprising Such Bacteria to the Animal

(1195) Probiotic bacteria can be delivered to animals (e.g., livestock, fish, or other animals) by applying the probiotic bacteria to a plant seed, to a plant growth medium (e.g., by in furrow application to soil), to a plant (e.g., by foliar application) or to an area surrounding a plant or a plant seed, and subsequently feeding such plants or plants grown from the plant seeds to the animal. Bacteria can be applied to plant leaves or stems while plants are growing, and will colonize the phylloplane (leaf and stem surface). The plants can be subjected to processing into animal feed prior to feeding to the animal.

(1196) The use of endophytic strains of bacteria in such methods allows the bacteria to survive and persist in plant tissue, such that they will be ingested in significant numbers by the animal upon ingestion of plant matter from the plant. For example, the strains *Bacillus cereus* family member EE349, *Bacillus cereus* family member EE439, *Bacillus thuringiensis* EE417, *Bacillus cereus* EE444, *Bacillus megaterium* EE385, *Bacillus* sp. EE387, *Bacillus circulans* EE388, *Bacillus subtilis* EE405, *Lysinibacillus fusiformis* EE442, *Lysinibacillus* spp. EE443, and *Bacillus pumilus* EE-B00143 are thought to be probiotic and are endophytic and can be used in these methods.

(1197) Any of these strains or other probiotic and endophytic strains can be grown and spores generated as described above in Example 40. The spores can then be applied to a plant growth medium, a plant seed, a plant, or an area surrounding a plant or a plant seed. Plants grown in the plant growth medium, plants grown from the plant

seeds, plants to which the bacteria were applied, or plants or plant seeds grown in an area to which the bacteria were applied can grow and subsequently be fed to an animal. Endophytic bacteria can colonize the internal tissue of the plant, and replicate to great numbers inside the plant. The bacteria will sporulate upon the use of traditional harvesting methods, allowing for prolonged storage of plant matter (e.g., as hay or silage) that can later be fed to a target animal.

(1198) Only a small amount of bacteria needs to be used in these methods, since the endophytic bacteria will naturally colonize and proliferate on and in the plants.

Example 57. Delivery of Beneficial Enzymes to Animals by Feeding to the Animals Plants Comprising a Recombinant *Bacillus cereus* Family Member or Other Recombinant Bacteria Expressing a Fusion Protein Comprising the Beneficial Enzyme

(1199) The recombinant *Bacillus cereus* family members expressing a fusion protein comprising a protein or peptide of interest and a targeting sequence, exosporium protein, or exosporium protein fragment that targets the fusion protein to the exosporium that are described herein can also be used to deliver beneficial enzymes to animals. The recombinant *Bacillus cereus* family members can be fed directly to the animals (e.g., by mixing a recombinant *Bacillus cereus* family member into animal feed that is subsequently fed to the animal). Alternatively, the methods described above in the immediately preceding example for delivering bacteria to animals can be used in connection with recombinant *Bacillus cereus* family member expressing a fusion protein that comprises a protein or peptide that has beneficial effects in an animal (e.g., an enzyme that aids digestion of plant matter).

(1200) Enzymes present in feed for livestock, fish, and other animals can impact the nutrient uptake, yield, and health of the animal that ingests the enzymes. Enzymes that are beneficial for animal health include, for example, xylanases, phytases, phosphatases, proteases, cellulases, endoglucanases, glucanases, amylases, lipases, phospholipases, glycosylases, galactanases, α-galactosidases, amylases, pectinases, biotinases, and polygalacturonases, among others. The BEMD system can be used to express such enzymes on the surface of the exosporium. Recombinant *Bacillus cereus* family members expressing a fusion protein comprising one of these enzymes can be applied to a plant growth medium, a plant seed, a plant, or an area surrounding a plant or a plant seed. Similarly, the recombinant bacteria that express a fusion protein comprising one of these enzymes and a spore coat protein that targets the fusion protein to a surface of a spore of the bacterium can be used in these methods. The recombinant bacteria can be applied to a plant growth medium, a plant seed, a plant, or an area surrounding a plant or a plant seed. Plants grown in the plant growth medium, plants grown from the plant seeds, plants to which the bacteria were applied, or plants or plant seeds grown in an area to which the bacteria were applied can be grown and subsequently fed to an animal, and the beneficial enzyme thereby delivered to the animal. The bacteria will sporulate upon the use of traditional harvesting methods, allowing for prolonged storage of plant matter (e.g., as hay or silage) that can later be fed to a target animal.

(1201) Endophytic strains of *Bacillus cereus* family members can be used as hosts for expression of the fusion proteins comprising a protein or peptide of interest (e.g., an enzyme having beneficial effects in animals) and a targeting sequence, exosporium protein, or exosporium protein fragment that targets the fusion protein to the exosporium. For example, the endophytic strains *Bacillus cereus* family member EE349, *Bacillus cereus* family member EE439, *Bacillus thuringiensis* EE417, *Bacillus cereus* EE444, and *Bacillus thuringiensis* EE319 described herein can be used as hosts.

(1202) Additional *Bacillus cereus* family members can be selected to be applied to the aerial portions of the plant, as these bacteria do not have to be endophytic to colonize the phylloplane. For example, *Bacillus mycoides* BT155, *Bacillus mycoides* EE118, *Bacillus mycoides* EE141, *Bacillus mycoides* BT46-3, *Bacillus cereus* family member EE218, *Bacillus thuringiensis* BT013A, *Bacillus thuringiensis* EE-B00184, *Bacillus mycoides* EE-B00363, *Bacillus pseudomycoides* EE-B00366, or *Bacillus cereus* family member EE-B00377 can be used for this purpose.

(1203) Similarly, endophytic strains of recombinant bacteria can be used as hosts for the expression of fusion proteins comprising a protein or peptide of interest and a spore coat protein that targets the fusion protein to a surface of a spore of the bacterium. For example, the endophytic strains *Bacillus megaterium* EE385, *Bacillus* sp. EE387, *Bacillus circulans* EE388, *Bacillus subtilis* EE405, *Lysinibacillus fusiformis* EE442, *Lysinibacillus* spp. EE443, or *Bacillus pumilus* EE-B00143 can be used as hosts.

(1204) The use of endophytic strains of bacteria in these methods allows the bacteria to survive and persist in plant tissue, such that both the bacteria and the fusion proteins expressed by the bacteria will be ingested in significant numbers by the animal upon ingestion of plant matter from the plant. Thus, through a simple addition of the recombinant *Bacillus cereus* family member or other recombinant bacteria at planting, beneficial enzymes can be spread throughout the plant tissue and delivered to animals upon ingestion of plant matter.

Example 58: Use of Various Targeting Sequences to Express Endoglucanase on the Surface of *Bacillus cereus* Family Member Spores, and Use of Such Spores for Promoting Plant Growth

(1205) The pSUPER plasmid was modified by cloning of a PCR generated fragment through homologous recombination that fused the BclA promoter, start codon, and amino acids 20-35 of BclA (amino acids 20-35 of

SEQ ID NO: 1) in frame with *Bacillus subtilis* 168 endoglucanase (pSUPER-BclA 20-35-Endo) as described above in Example 44. This plasmid was then subjected to inverse PCR to amplify the entire plasmid backbone, but leaving out the sequence corresponding to amino acids 20-35 of BclA. This inverse PCR product was combined with a PCR product that amplified the equivalent region from each of SEQ ID NOs. 5, 15, 25, 81, 85, 87, or amino acids 20-33 of SEQ ID NO: 1. Thus, constructs were created that contained each of the following targeting sequences fused in frame with *Bacillus subtilis* 168 endoglucanase: (1) amino acids 20-35 of SEQ ID NO: 1; (2) amino acids 23-38 of SEQ ID NO: 5; (3) amino acids 28-43 of SEQ ID NO: 15; (4) amino acids 9-24 of SEQ ID NO: 25; (5) amino acids 23-38 of SEQ ID NO: 81; (6) amino acids 13-28 of SEQ ID NO: 85; (7) amino acids 13-28 of SEQ ID NO: 87; and (8) amino acids 20-33 of SEQ ID NO: 1. Each construct contained the wildtype BclA promoter and a methionine at the start codon, followed by the targeting sequence fused in frame to the *Bacillus subtilis* endoglucanase gene. Each of these constructs was transformed into *E. coli* and plated to obtain single colonies on Luria plates plus ampicillin (100 μg/ml). Plasmids from each single colony were grown up in overnight cultures in Luria broth plus ampicillin, and purified using a WIZARD SV miniprep kit, and sequences were verified by Sanger sequencing. DNA was also quantified via spectrophotometry, and the DNA was introduced into *Bacillus thuringiensis* BT013A. In addition, the pSUPER-BclA-20-35 Endo construct was introduced into *Bacillus thuringiensis* BT013A which had the native BclA protein removed from its genome through homologous recombination (BclA knockout, “BclA KO”). Correct colonies were screened by plating on nutrient broth plate containing antibiotic (tetracycline at 10 μg/ml). Each positive colony was grown up in brain heart infusion broth at 30° C. overnight at 300 rpm, with antibiotic, and genomic DNA was purified and re-sequenced to verify genetic purity. Verified colonies were grown overnight in brain heart infusion broth with 10 μg/ml tetracycline, and induced to sporulate through sporulation in a yeast extract-based media.

(1206) Each of the production runs in the yeast extract-based media were collected at 48 hours post production of spores, and subjected to enzyme comparison of the resultant spores using the methodology described above in Example 45. The absorbance was determined at 540 nm using an IMPLEN nanophotometer model P330. There were three samples and a blank for each reaction. The results from the enzyme readings are shown in Table 56.

(1207) For corn, 1 μl of each of the whole broth for each of the constructs was placed onto each seed. For summer squash, 2 μl of whole broth for each construct was placed onto each seed. To accomplish this, 50 seeds were placed in a 50 ml conical bottom polypropylene tube and vortexed lightly using a vortex mixer. To this swirling of seeds, 50 μl (for corn) or 100 μl (for squash) of broth containing the recombinant spores was slowly pipetted into the tube, and the vortexing action coated the seeds with an even coating of the whole cell broth from each construct. These seeds were then planted at 1″ deep into native soil using a 39.6 cm.sup.3 (15.6 in.sup.3) planting pot, with two seeds per pot. The pots were then watered to saturation, and the plants allowed to germinate. The plants were grown in a controlled growth room, set to 70° F. during the day, and 60° F. during the evening, with a light period of 14 hours/day, under artificial light conditions, for 14 days. After 14 days, the plants were measured for height, and results were normalized to a control group that received only water as treatment on the seeds.

(1208) TABLE-US-00056 TABLE 56 Enzyme levels and plant growth phenotypes. Endo Sequence Sequence Average Enzyme Identity to Identity to Com Squash Plant Targeting Levels AA 20-35 AA 25-35 Growth Growth Phenotype Sequence (mU/ml) of BclA of BclA Phenotype Phenotype Change Control (H.sub.2O) 0 mU/ml N/A N/A 100% 100% 100% AA 20-35 of 38.2 100% 100% 112% 94.7% 103.4% BclA (SEQ ID NO: 1) AA 23-38 of 33.5 50.0% 72.7% 106.7% 102.3% 104.5% SEQ ID NO: 5 AA 28-43 of 16.7 68.8% 81.8% 115.7% 103.4% 109.6% SEQ ID NO: 15 AA 9-24 of 25.7 56.3% 63.6% 118.4% 107.1% 112.8% SEQ ID NO: 25 AA 23-38 of 21.5 50.0% 72.7% 106.7% 98.3% 102.5% SEQ ID NO: 81 AA 13-28 of 38.3 43.8% 54.5% 99.7% 100.5% 100.1% SEQ ID NO: 85 AA 13-28 of 14.4 43.8% 54.5% 102.6% 104.1% 103.4% SEQ ID NO: 87 AA 20-33 of 30.5 N/A 100% 104.6% 100.7% 102.7% SEQ ID NO: 1 AA 20-35 of 100.8 100% 100% ND ND ND SEQ ID NO: 1 in BT013A BclA KO AA = amino acids ND = not determined

(1209) The above data show that each of these constructs was able to stimulate plant growth and show that the use of different targeting sequences allows for control of the expression level of the enzyme on the outside of the spore.

(1210) Use of amino acids 20-35 of SEQ ID NO: 1 or AA 13-28 of SEQ ID NO: 85 as the targeting sequence resulted in the highest levels of enzyme production. This is surprising considering the low degree of identity between these targeting sequences (43.8% identity over the entire length of the targeting sequence). Use of amino acids 28-43 of SEQ ID NO: 15 or amino acids 9-24 of SEQ ID NO: 25 resulted in the largest plant response across the two plant types. Expression of the fusion protein containing amino acids 20-25 of SEQ ID NO: 1 as the targeting sequence in the BT013A BclA KO host led to very large (263.8%) increase in the amount of enzyme activity on the surface of the spores as compared to expression of the same fusion protein in the wild-type strain.

Example 59: Use of Various Targeting Sequences and Exosporium Proteins to Express Phospholipase, Lipase, and Endoglucanase on the Surface of *Bacillus cereus* Family Member Spores, and Use of Such Spores for Promoting Plant Growth

(1211) The pSUPER plasmid was modified by cloning of a PCR generated fragment (XhoI digestion and ligation)

that fused the BclA promoter, start codon, and amino acids 20-35 of BclA (amino acids 20-35 of SEQ ID NO: 1) followed by a six alanine linker sequence in frame with either *Bacillus thuringiensis* phosphatidylcholine-specific phospholipase C gene (PC-PLC) (pSUPER-BclA 20-35-PL) or *Bacillus subtilis* lipase LipA (pSUPER-BclA-20-35-Lipase), or *Bacillus subtilis* endoglucanase eglS (pSUPER-BclA-20-35-Endo) as described above in Example 44. These plasmids were then subjected to inverse PCR to amplify the entire plasmid backbone, but leaving out the sequence corresponding to the amino acids 20-35 of BclA. This inverse PCR product was combined with a PCR product that amplified the equivalent region from each of SEQ ID NOs. 5 (i.e., amino acids 23-38 of SEQ ID NO: 5), 15 (i.e., amino acids 28-43 of SEQ ID NO: 15), and 25 (i.e., amino acids 9-24 of SEQ ID NO: 25; the full-length exosporium proteins of SEQ ID NOs. 120, 111, 121, 108, and 114; or amino acids 20-33, 20-31, 21-33, 23-33, or 23-31 of SEQ ID NO: 1. Each of these constructs contained the wild-type BclA promoter, a methionine at the start codon, followed by the targeting sequence or exosporium protein fused in frame to the *Bacillus cereus* phosphatidylcholine-specific phospholipase C, *Bacillus subtilis* 168 Lipase LipA, or *Bacillus subtilis* 168 eglS endoglucanase gene. Each of these constructs was screened for correct transformants as described in Example 58 above.

(1212) Each of the production runs in the yeast extract-based media were collected at 48 hours post production of spores, and subjected to enzyme comparison of the resultant spores. Determination of enzyme data for endoglucanase was performed as described above in Example 58. For the phospholipase C enzyme assay, 1 ml of recombinant spores was pelleted at 10,000×g for 3 minutes, and supernatant removed and discarded. The spore pellet was then resuspended in 500 µl reaction buffer (0.25 mM Tris-HCL, 60% glycerol, 20 mM o-nitrophenyl phosphorylcholine, pH 7.2). A negative control for enzyme assays contained BT013A spores with no enzyme expression. Each sample was incubated at 37° C. for 18 hours, centrifuged again to remove the spores, diluted 1:1 in water, and the Abs540 read using a spectrophotometer. This was compared to a standard curve against commercially purchased phospholipase and lipase controls to establish the U/ml of activity. The results from the enzyme readings are shown in Tables 57 and Table 58.

(1213) TABLE-US-00057 TABLE 57 Endoglucanase Enzyme Levels Endoglucanase Levels (mU/ml) Targeting Sequence, Experiment #1 Control (H.sub.2O) 0 mU/ml AA 20-35 SEQ ID NO: 1 38.2 SEQ ID NO: 120 25.7 SEQ ID NO: 111 29.7 SEQ ID NO: 121 24.4 SEQ ID NO: 108 24.0 SEQ ID NO: 114 11.0 AA 20-33 of SEQ ID NO: 1 30.5 Targeting Sequence, Experiment #2 AA 20-31 of SEQ ID NO: 1 48.22 AA 21-33 of SEQ ID NO: 1 60.86 AA 23-33 of SEQ ID NO: 1 19.93 AA 23-31 of SEQ ID NO: 1 45.31 AA 20-35 of SEQ ID NO: 1 54.1 AA = Amino acids

(1214) Many of the targeting sequences and exosporium proteins were able to display a large amount of active enzymes on the surface of the spores, including SEQ ID NOs. 108, 111, 114, 120, and 121. Amino acids 20-31, 21-33, and 23-31 of SEQ ID NO: 1 provided similar enzyme expression levels to amino acids 20-35 of SEQ ID NO: 1, indicating that smaller fragments are adequate for the display of enzymes on the surface of the spores. Only amino acids 23-33 of SEQ ID NO: 1 exhibited a diminished enzyme display level on the spores.

(1215) TABLE-US-00058 TABLE 58 Phospholipase Enzyme levels Targeting PC-PLC Enzyme Lipase Enzyme Sequence Levels Levels Control (H.sub.2O) 0.0 0.0 AA 20-35 SEQ .787 .436 ID NO: 1 AA 23-38 of .688 .602 SEQ ID NO: 5 AA 28-43 of .372 .228 SEQ ID NO: 15 AA 9-24 of SEQ .247 .359 ID NO: 25 SEQ ID NO: 114 .446 .798 SEQ ID NO: 120 3.612 .753 SEQ ID NO: 111 .738 .329 AA = Amino acids

(1216) Similar to the results shown above in Table 57, the highest levels of phospholipase or lipase on the spore surface were observed when amino acids 20-35 of SEQ ID NO: 1, amino acids 23-38 of SEQ ID NO: 5, or the exosporium protein sequence of SEQ ID NO: 120 were used.

(1217) The effects of these spores expressing several of these constructs on nodulation in soybeans are shown below in Table 59.

(1218) TABLE-US-00059 TABLE 59 Phospholipase Plant Responses Nodulation per Plant Targeting Sequence (Soybean) Control (H.sub.2O) 9.8 Strain Control (*Bacillus thuringiensis* BT013A) 8.2 *Bacillus thuringiensis* BT013A expressing a fusion 14.0 protein of AA 20-35 of SEQ ID NO: 1 and phospholipase

(1219) Soybeans plants were coated as above, but the assay was run out to 3 weeks' time. Plants were carefully removed, dirt washed gently off of the roots, and nodules counted for each plant. As shown in Table 59, addition of spores displaying phospholipase onto the seeds of soybean allows for an accelerated number of nodules on the plants, which is a positive indication for both early growth as well as eventual increases in yield in soybeans.

Example 60: Binding of MIR319 RNA and Random RNA 1 to *Bacillus cereus* Spores Expressing a Fusion Protein Containing a Nucleic Acid Binding Protein, and Use of Such Spores to Deliver RNA to Plants

(1220) DNA and RNA can be bound to *Bacillus cereus* family member spores that express fusion proteins containing a targeting sequence and a nucleic acid binding protein or peptide on their exosporium, as described in the above Examples and in the Description. The spores act as a delivery mechanism, delivering the target nucleic acid (e.g., a miRNA) to the target plant. To demonstrate this ability of the recombinant *Bacillus cereus* family member spores, a common miRNA, MIR319 was delivered to soybeans using spores expressing a fusion protein

containing amino acids 20-35 of SEQ ID NO: 1 fused in frame to the known DNA binding gene SspC. MIR319 has different effects on plant phenotype in different plants, and even within different parts of the same plant. For example, in some species, treatment of leaves with MIR319 leads to curling of leaves, whereas in other species, application of MIR319 leads to stress resistance. MIR319 is ubiquitous across plant genomes, is a global regulator of pathways, and its delivery into various plants leads to various phenotypes.

(1221) TABLE-US-00060 TABLE 60 RNAs used in this study RNA 3′ Sequence 5′ Sequence MIR319 UUGGACUGAAGGGUGCUCCC GAGCUCUCUUCAGUCCACUC (SEQ ID NO: 306) (SEQ ID NO: 307) or AGAGCGUCCUUCAGUCCACUC (SEQ ID NO: 308) Random GAGCCCATGGTTGAATGAGT ACTCATTCAACCATGGGCTC RNA #1 (SEQ ID NO: 309) (SEQ ID NO: 310)

(1222) Synthetic MIR319 microRNA from *Glycine max* (soybean) was designed to match the MIR319 sequence available in miRBase (miRBase.org, central repository for microRNA sequences). Two partially complementary single stranded sequences were synthesized by Integrated DNA Technologies (IDT, Iowa) to represent the 3′ and 5′ mature gene products known to exist in vivo (two different versions of the 5′ sequence were used). Likewise, two single stranded RNAs were synthesized with random sequences not matching anything in the soy genome as a control. The double stranded (ds) gene products were made by combining the two single stranded (ss) products at 95° C. for 10 min and then cooling slowly at room temperature to allow for annealing. *Bacillus thuringiensis* expressing a fusion protein containing the BclA promoter, a methionine residue as the start codon, and amino acids 20-35 of SEQ ID NO: 1 fused in frame to the known DNA binding gene SspC (an α/β type SASP, Small Acid-soluble Spore Protein C of *Bacillus thuringiensis* BT013A) was engineered by standard cloning procedures as described above in Example 58. This construct (SspC-BclA) was created in *E. coli*, transformed into *Bacillus thuringiensis* BT013A and clones verified by DNA sequencing. *B. thuringiensis* spores expressing SspC-BclA were obtained by an overnight growth of transformed bacteria in brain heart infusion broth (BHI) for 2 days in a yeast extract-based media until a density of 2×10.sup.8 spores per milliliter (ml) was achieved with less than 1% vegetative cells. DNA was extracted from an aliquot of the parent BHI culture and sent for sequencing to confirm incorporation of the SspC-BclA plasmid. To prepare spores for seed treatments, 1 ml of spore culture in the yeast extract-based media was pelleted by centrifugation and resuspended in 100 μl of water. This concentrated suspension was counted and spores were used at 6×10.sup.8 spores/ml. For each soy seed, 1 μl of spores was combined with 10 μl of RNA at 10 UM and incubated at 30° C. for 2 hours (scaled up for multiple seeds). After this incubation spores were pelleted (carrying bound RNA) and unbound excess RNA in the supernatant was discarded and the pellet was resuspended in 10 μl of water. Samples were applied to the seeds as follows: 39.6 cm.sup.3 (15.6 in.sup.3) of Timberline brand commercial top soil was prepared in each pot and a 1 inch indentation was made where 2 ml of water was applied and a single seed was set on top. The 10 μl spore+bound RNA sample was applied by micropipetting directly on to the top of the seed. Seeds were allowed to sit for 30 min and then the adjacent soil was pushed to loosely cover the seed. The seeds were allowed to germinate for 4 days in an artificial light plant growth room with a 13/11 hour light/day cycle, and at 21° C. day/15° C. night temperature range. On day 14 soy plants were uprooted, photographed and measured. Heights were normalized to water control treated plants (Sec Table 61).

(1223) Example 41 above describes the ability of the SspC-BclA recombinant *Bacillus cereus* family member spores to bind to and hold DNA. To assess RNA binding ability of the SspC-BclA expressing spores, biotin labeled random RNA sequences were synthesized by IDT and incubated with the spores exactly as was done for the treatments described above (1 μl of spores at 6×10.sup.8 spores/ml+10 μl of 10 μM RNA for 2 hours at 30° C., pelleted and resuspended in 10 μl of water). Avidin conjugated to Fluorescein (FITC) (Life Technologies) was added to the 10 μl spore+RNA sample at 20 μg/ml final concentration and incubated for 1 hour at room temperature in the dark. Avidin is known to bind biotin and FITC is a fluorescent tracer. Spores were pelleted once again to remove excess unbound avidin-FITC and resuspended in 4% paraformaldehyde made in PBS and stored at 4° C. overnight in the dark. Spores were inspected for fluorescence and photographed (See Table 62). In addition, as shown in FIG. **10**, the Sspc-BclA tagged spores were able to bind and retain both ssRNA and dsRNA, as shown by the FITC-avidin labeling of spores in the presence of the ssRNA or dsRNA bound with biotin. To generate the results shown in FIG. **10**, spores were incubated with either double or single stranded RNA (of a random sequence) tagged with biotin and detected with avidin conjugated to fluorescein (FITC). No fluorescence was detected on spores incubated with water only. Brightfield and corresponding fluorescent images were taken with 40× objective and 10× ocular lenses.

(1224) As can be seen in Table 61 below, the major effect of MIR319 as a seed treatment on soybeans is on root growth and overall height. Curly roots were defined as having at least two 180° turns. Heights were measured along the main stalk. When soybean plants were uprooted and assayed for the presence of “curly roots”, a phenotype observed by our group specific to soybeans, no evidence of curly roots was found in the water control, the BT013A strain control, the double stranded (dsRNA) RNA alone control, or the spores alone (carrier control). The only

evidence of curly roots is noted when both the SspC-BclA spores (the carrier) was delivered to the seed with the dsRNA (60% curly roots) (also see FIG. **10**). FIG. **11** also shows the phenotypic changes in the soybean plants when exposed to SspC-BclA spores combined with ds MIR319 RNA. When the spores are used to deliver the RNA, the impact of the RNA is amplified, leading to an increased stunting and curly root phenotype in FIG. **11**. To generate the results shown in FIG. **11**, soy seeds were treated with double stranded (ds) MIR319 with or without prior binding to *B. thuringiensis* spores expressing SspC-BclA. Application of dsMIR319 resulted in slightly taller plants on average; however, application of dsMIR319 bound to spores resulted in "curly" roots defined as having at least two 180° turns and overall less height. The median sample from each experimental condition is shown. Images were taken using a digital camera with plants together in a single image.

(1225) As an RNA control, a random set of ssRNA (single-stranded) and dsRNA was applied to soybeans. In these experiments, the random ssRNA had no effect when applied alone, while the dsRNA had a stunting effect on the height of the plants when delivered to the seeds. In both cases, when the spores (carrier) were used in conjunction with either the random ssRNA or the dsRNA version, the stunting phenotype was increased significantly (33% and 27.8% stunted, respectively). This stunting is not evident in the spore (carrier control) alone samples. These data, when taken together, demonstrate the ability of the spores to amplify and specifically deliver ssRNA and dsRNA to plants by application to the seed, and demonstrate the ability of two different RNAs (Random #1 and MIR319) to affect phenotype when delivered via *Bacillus cereus* spores expressing a fusion protein containing a DNA/RNA binding protein.

(1226) TABLE-US-00061 TABLE 61 Root and Height effect of MIR319 on soybean development Height Seed Treatment % Curly (Normalized (5 replicates each) Roots to Control) Water (Control) 0 100% Water + Spores (Control) 0 105.21% Random ssRNA #1 no 0 102.62% spores Random ssRNA #1 + spores 0 69.62% dsMIR319 no spores 0 125.30% dsMIR319 + spores 60% 67.10% Random dsRNA #1 no 0 82.40% spores Random dsRNA #1 + spores 0 54.61%

(1227) TABLE-US-00062 TABLE 62 Fluorescence detection on SspC-BclA expression spores with bound biotin labeled RNA Fluoresence Detected Spore Treatment on Spores Spores + Water (control for background Not detected spore fluorescence) Spores + Water + Avidin-Fitc (control for Not Detected background spore + FITC fluorescence) Spores + biotin labeled ssRNA + Avidin-FITC Detected Spores + biotin labeled dsRNA + Avidin-FITC Detected

(1228) As can be seen in Table 62, no fluorescence was detected on the spores without the presence of RNA. Both single stranded (ss) and double stranded (ds) RNA was detected on the spores.

Example 61: Delivery of Nucleic Acids to *Caenorhabditis elegans* Nematodes by Ingestion of Recombinant *Bacillus thuringiensis* Spores Expressing a Fusion Protein

(1229) Delivery of RNA and DNA to nematodes has a great deal of applications in both plant science, animal health, and in basic research. Nematodes cause a great deal of damage and yield loss to commercial and non-commercial growing operations for key crops, and parasitic nematodes cause high morbidity in humans and other animals in many impoverished areas of the world. Delivery of RNA and DNA has the potential to alleviate and treat many nematode problems, and delivery of RNA and DNA constructs has been demonstrated to be useful in impacting target nematodes. This example illustrates the utility of the RNA/DNA delivery mechanism described above in Example 60 in delivering spores to nematodes.

(1230) Wild type *C. elegans* nematodes were purchased from Carolina Biological (North Carolina) and maintained at 23° C. on NGM-Lite agar plates coated with OP50 *E. coli* for food. Two different *Bacillus thuringiensis* BT013A strains were engineered by standard cloning procedures to express amino acids 20-35 of SEQ ID NO: 1 fused in frame to green fluorescent protein (GFP) or mCherry to trace the presence of spores in the gut. These green or red fluorescently tagged spores were obtained by an overnight growth in BHI (brain heart infusion) medium, followed by three days in a yeast extract-based media until a density of approximately 2×10.sup.8 spores per milliliter (ml) was achieved with less than 1% vegetative cells. To prepare spores for feeding to nematodes, 1 ml of spore culture in media was pelleted by centrifugation and resuspended in 100 μl of water to remove excess media. This concentrated suspension was counted and diluted to 1×10.sup.8 spores/ml. To feed spores to the worms, 1 μl of the spore suspension containing both the red and green fluorescently tagged spores was added to a 60 mm NGM-lite agar plate with 10 μl of PBS (phosphate buffered saline) to aid in spreading. No other food source was made available. Twenty wild type nematodes of various ages were transferred to the plates immediately. Living nematodes were checked 5 hours later for ingestion of spores using standard fluorescence microscopy.

(1231) As can be seen in FIG. **12** and Table 63, the centralized gut of the nematodes fluoresced when fed recombinant *Bacillus cereus* family member spores expressing the fusion protein containing the targeting sequence and GFP, whereas the gut did not fluoresce when fed OP50 *E. coli* (standard food). Images were taken of live nematodes with 4× objective and 10× ocular lenses. This demonstrates the ability of these spores to be both ingested and delivery of a "cargo" of target proteins, exemplified by the green fluorescence protein. Other Exosporium proteins and targeting proteins can also be used interchangeably with a targeting sequence to deliver RNA and DNA

to a nematode or other target organism. Other recombinant *Bacillus cereus* family member spores can also be used due to the high degree of conserved nature of the exosporium and its creation on the surface of the spore.

(1232) TABLE-US-00063 TABLE 63 *C. elegans* fluorescence detected in the gut Green Fluorescence Food Source Detected in gut OP50 *E. coli* (control) No GFP expressing *B.* Yes (High) *thuringiensis* BT013A

Example 62: Construction, Purification, and Uses of Exosporium Fragments

(1233) Knock out (KO) Mutants: To make exsY and cotE knockout (KO) mutant strains of *Bacillus thuringiensis* BT013A, the plasmid pKOKI shuttle and integration vector was constructed that contained the pUC57 backbone, which is able to replicate in *E. coli*, as well as the origin of replication erythromycin resistance cassette from pE194. This construct is able to replicate in both *E. coli* and *Bacillus* spp. A 1 kb DNA region that corresponded to the upstream region of the cotE gene and a 1 kb region that corresponded to the downstream region of the gene cotE were PCR amplified from *Bacillus thuringiensis* BT013A. A second construct was made that contained the 1 kb DNA region that corresponded to the upstream region of the exsY gene and a 1 kb region that corresponded to the downstream region of the gene exsY, both of which were PCR amplified from *Bacillus thuringiensis* BT013A. For each construct, the two 1 kb regions were then spliced together using homologous recombination with overlapping regions with the pKOKI plasmid. This plasmid construct was verified by digestion and DNA sequencing. Clones were screened by looking for erythromycin resistance.

(1234) Clones were passaged under high temperature (40° C.) in brain heart infusion broth. Individual colonies were toothpicked onto LB agar plates containing erythromycin 5 μg/ml, grown at 30° C., and screened for the presence of the pKOKI plasmid as a free plasmid by colony PCR. Colonies that had an integration event were continued through passaging to screen for single colonies that lost erythromycin resistance (signifying loss of the plasmid but recombination and removal of the exsY or cotE gene). Verified deletions were confirmed by PCR amplification and sequencing of the target region of the chromosome. The pSUPER-BclA 20-35 Endo plasmid (described above in Example 58) was transformed into each of the exsY and cotE KO mutants. As described above in Example 48, the cotE KO mutant was also transformed with the pSUPER BclA 20-35 eGFP plasmid (made as described above in Example 44, but with endoglucanase swapped for cGFP by homologous recombination).

(1235) Dominant Negative Mutants: To create a dominant negative mutant, we PCR amplified the N-terminal half and the C-terminal half of CotO (Seq ID NO: 126), containing the amino acids 1-81 and 81-199 respectively, and cloned these fragments into the pHP13 vector using homologous recombination (the pHP13 vector is described above in Example 1). Correct clones were verified by Sanger sequencing. Each of the two CotO dominant negative mutants was introduced into *Bacillus thuringiensis* BT013A that contained the pSUPER-BclA 20-35 Endo construct, which produces endoglucanase on the surface of the spore as illustrated above in Example 58.

(1236) Exosporium Fragment Creation: For each of the two KO mutants, and both of the dominant negative mutants, an overnight culture was grown in BHI media at 30° C., 300 rpm, in baffled flasks with antibiotic selection. One milliliter of this overnight culture was inoculated into a yeast extract-based media (50 ml) in a baffled flask and grown at 30° C. for 3 days. An aliquot of spores was removed, 1% Tween was added, and the spores were agitated by vortexing for one minute. The spores were collected via centrifugation at 10,000×g for 5 minutes, and supernatant containing the exosporium fragments was filtered through a 0.22 μM filter to remove any residual spores. The supernatant (containing the broken exosporium fragments) was filtered through a 100,000 Da membrane filter to obtain purified exosporium fragments containing the fusion proteins. Smaller MW proteins were removed by passaging through the 100 kDa filter. No spores were found in the filtrate or retentate of the supernatant.

(1237) Transmission electron micrographs are provided in FIG. **15** showing intact spores of *Bacillus thuringiensis* BT013A (panel A) surrounded by attached exosporium, and spores of the *Bacillus thuringiensis* BT013A CotE knock-out mutant (panel B), from which the exosporium has detached. Arrows in panel A of FIG. **15** indicate the exosporium of intact spores, while arrows in panel B of FIG. **15** indicate exosporium that has detached from the spores. Panel C of FIG. **15** shows a transmission electron micrograph of a purified exosporium fragment preparation of derived from the *Bacillus thuringiensis* BT013A CotE knock-out (prepared as described above by vortexing, centrifugation, and filtration of the supernatant), visualized by negative staining. Images were taken on a JEOL JEM 1400 transmission electron microscope. No visible exosporium fragments were observed when control spores (*Bacillus thuringiensis* BT013A without the CotE knockout, expressing the BclA 20-35 Endo fusion protein, data not shown) were subjected to same vortexing, centrifugation, filtration procedures described above.

(1238) Presence of BclA 20-35 Endoglucanase in Exosporium Fragments collection from the CotE and ExsY Knockout and CotO Dominant Negative Mutants: Exosporium fragments were created and purified as described above that contained the pSUPER BclA 20-35-Endo plasmid that creates an exosporium that contains the endoglucanase enzymes on the surface of the spores. Exosporium fragments containing this construct were created from the cotE knockout mutant spores, exsY knockout mutant spores, CotO N-terminal dominant mutant spores, or CotO C-terminal dominant mutant spores. In each of these experiments, the amount of activity for the endoglucanase on the exosporium fragments was quantified as a percentage of the total enzyme levels. These results

were compared against a wildtype construct that did not contain any mutants, but did contain the pSUPER BclA 20-35-Endo plasmid.

(1239) Effects of Exosporium Fragments on Plant Growth: These exosporium fragments were then delivered as a seed treatment onto soybean seeds (as described in Example 59 above). A wild-type control (*B. thuringiensis* BT013A expressing the BclA 20-35 Endo construct) was also coated onto soybeans seeds. For each experiment, 1 μl of exosporium fragments from each construct, or a 1:2, a 1:4, or a 1:8 dilution of the fragments was applied to each seed.

(1240) TABLE-US-00064 TABLE 64 Exosporium Fragment Enzyme Activity and Plant Growth Response Endoglucanase Activity, Soy Plant Soy Plant Soy Plant Exosporium Growth Growth Growth Fragments Response, Response, Response, Presence of Mutation Construct (mU/ml) 1:2 dilution 1:4 dilution 1:8 dilution Spores? Wild-type BclA 20-35 10.3 93.1% 92.2% 83.4% No BT013A Endo cotE KO BclA 20-35 269.0 121.4% 110.7% 90.7% No Endo exsY KO BclA 20-35 238.0 107.7% 89.1% 90.7% No Endo CotO NTD BclA 20-35 22.4 99.6% N/A N/A No dominant Endo CotO CTD BclA 20-35 27.5 95.8% N/A N/A No dominant Endo

(1241) These results demonstrate that mutations that disrupt the exosporium, such as a knock-out mutation in the cotE or exsY gene, or a dominant negative mutation in the CotO protein, can be used to generate exosporium fragments that are substantially free of spores, and demonstrates that these exosporium fragments contain fusion proteins that are targeted to the exosporium. These fragments can be utilized to promote plant growth and in other applications. There was a small amount of background endoglucanase activity in the exosporium fragment preparation from the BT013 strain having no mutations and expressing the BclA 20-25 Endo construct (BT013A BclA 20-35 Endo). This was unexpected and may represent a low level of unstable exosporium that is being released from spores and captured during the exosporium fragment collection process. CotE and ExsY KO strains contain the highest amount of enzyme in the exosporium fragment fraction. The CotO dominant negative mutants that express a fusion protein also have an elevated level of enzyme in the exosporium fragment fraction as well.

(1242) The exosporium fragments from the CotE and ExsY mutants (not expressing BclA 20-35 Endo) applied directly to plants had a negative effect on growth and were removed from this experiment. When the exosporium fragments from BT013A BclA 20-35 Endo were applied to soybeans, there was a negative growth phenotype. When exosporium fragments from the CotE or ExsY mutants expressing the BclA 20-35 Endo fusion protein were added to soybeans, a substantial increase in growth rate occurred (+28.3% and +14.8% over BT013A BclA 20-35 Endo fragments). The CotE mutant exosporium fragments were still active at the 1:4 dilution, but the ExsY exosporium fragments were no longer giving a growth benefit to the soybeans at this dilution. The CotO dominant negative mutants expressing the BclA 20-35 Endo fusion protein gave a small increase in soybean growth compared to the fragments from BT013A BclA 20-35 Endo, giving +6.5% and +2.7% growth, respectively.

Example 63: Additional Demonstration of the Utility of Endophytic *Bacillus cereus* Family Members and Other Recombinant *Bacillus* Species to Deliver Peptides, Proteins, and Enzymes Endophytically to the Plant

(1243) *Bacillus thuringiensis* EE417, *Bacillus thuringiensis* EE-B00184, *Bacillus cereus* EE439, and *Bacillus* sp. EE387 were found to have the ability to grow endophytically and to be capable as serving as a host strain for the BEMD system (See Examples 52 and 53). To demonstrate the ability of these Bacilli to grow endophytically and to serve as a host strain for the BEMD system, each of these strains was transformed with the pMK4-BclA 20-35-cGFP plasmid (described above in Example 62). Spores were made and purified as described above in Example 40.

(1244) These spores were diluted to a concentration of 1×10.sup.8/ml, and 1 μl of whole cell broth was then added to commercial hybrid corn seed in potting soil at planting. The corn seeds were coated with a fungicide and a biological inoculant. The corn hybrid variety was BECK 6175YE, which contains the ROUNDUP READY glyphosate resistance gene and AQUAMAX drought resistance gene. Plants were grown under artificial light for 14 hours a day and plant growth over a ten day period was determined. Plants were watered every three days over the course of the experiment.

(1245) *Bacillus thuringiensis* EE417, *Bacillus thuringiensis* EE-B00184, *Bacillus cereus* EE439, and *Bacillus* sp. EE387, expressing the BclA 20-35-eGFP were then isolated from the inside of the corn plants. The ten day old plants were extracted from the soil and washed to remove excess debris. The plants were then inverted, washed in water, exposed to 5% bleach for ten minutes, washed in water, exposed to 70% ethanol for ten minutes, washed again in water, and the stalks split with a sterile razor blade. The split halves of the stalks were placed face down onto nutrient agar plates for two hours at 30° C. After two hours, the stalks were removed, and the agar plates incubated at 30° C. for 48 hours. After 48 hours, the plates were examined for colony morphology, and *Bacillus* colonies found internal to the plant were toothpicked onto nutrient agar and nutrient agar plus chloramphenicol plates (to select for bacteria containing the pMK4-20-35 BclA-eGFP plasmid). Results are shown in Table 65. These results demonstrate the ability of the BEMD system to be introduced into the target plant by expression in an endophytic strain of the *Bacillus cereus* family. FIG. **13** also demonstrates the ability of *Bacillus thuringiensis* EE-B00184 to express eGFP on the spores, as evidenced by fluorescent microscopy. In FIG. **13**, arrows denote single spores. FIG. **14** demonstrates the ability of the isolated bacterial colonies from plants to fluoresce green,

demonstrating that they do in fact deliver the protein of interest (herein eGFP) inside the plants. FIG. **14** shows fluorescence of colonies of endophytic bacteria isolated from inside corn plants on plates, illuminated with a GFP filtered lamp.

(1246) TABLE-US-00065 TABLE 65 Endophytic delivery of "cargo" proteins % Bacillus % Bacillus colonies + colonies + Strain Endophytic "Cargo" for plasmid for eGFP *Bacillus* Yes BclA 20-35 29.8% 29.8% *thuringiensis* eGFP EE417 *Bacillus* Yes BclA 20-35 38.9% 38.9% *thuringiensis* eGFP EE-B00184 *Bacillus* sp. Yes BclA 20-35 50% 50% EE387 eGFP *Bacillus cereus* Yes BclA 20-35 23.9% 23.9% EE439 eGFP

(1247) To further demonstrate the ability of these endophytic strains to express proteins on the surface of the spores, the following constructs were introduced into *Bacillus* sp. EE387: pHP13 plasmid with endoglucanase fused to either: BclA 20-35, CotB, CotG, CotC, CgeA, InhA, InhA2, InhA1, CotY, or AcpC (amino acids 20-25 of SEQ ID NO: 1 or SEQ ID NOs. 252, 256, 253, 254, 108, 121, 114, 111, and 120, respectively). The pSUPER BclA-20-35 Endo construct described above in Example 58 was also introduced into *Bacillus thuringiensis* EE-B00184, another endophytic strain. Transformed cells were screen by PCR and Sanger sequencing. Spores for each of these constructs was made by growing up an overnight culture in BHI plus selection (chloramphenicol), and 500 μl of each culture was swabbed onto nutrient broth agar plates and allowed to incubate at 30° C. for 3 days. After 3 days, the spores were swabbed off into PBS, diluted to a concentration of 1×10.sup.8/ml, spun down to recover the spores, and enzyme measurement of the spores was performed as described above in Example 58. The enzyme concentration was calculated as mU/ml for each construct. The ability of *Bacillus* sp. EE387 to express fusion proteins on its spore surface is indicated by the levels of enzyme. *Bacillus* sp. EE387 was able to express all of the spore fusion proteins on its surface, but AcpC (SEQ ID NO: 120) was a superior fusion protein for this strain. This finding was surprising since *Bacillus* sp. EE387 is not a *Bacillus cereus* family member strain and does not have an exosporium, yet exhibited surface expression of fusion proteins containing exosporium proteins or targeting sequences derived from exosporium proteins (e.g., CotY, AcpC, and amino acids 20-35 of SEQ ID NO: 1).

(1248) TABLE-US-00066 TABLE 66 Endophytic strains *Bacillus* sp. EE387 (EE387) and *Bacillus thuringiensis* EE-B00184 (EE-B00184) expressing fusion proteins Exosporium Protein or Host Endoglucanase Targeting Sequence Endophytic activity Fusion Partner Strain (mU/ml) CotB (SEQ ID NO: 252) EE387 4.0 CotG (SEQ ID NO: 256) EE387 4.2 CotC (SEQ ID NO: 253) EE387 4.4 CgeA (SEQ ID NO: 254) EE387 4.1 AA 20-35 of SEQ ID NO: 1 EE387 16.3 InhA (SEQ ID NO: 108) EE387 7.5 InhA2 (SEQ ID NO: 121) EE387 6.0 CotY (SEQ ID NO: 111) EE387 4.9 AcpC (SEQ ID NO: 120) EE387 36.0 InhA1 (SEQ ID NO: 114) EE387 4.5 AA 20-35 of SEQ ID NO: 1 EE-B00184 95.8

(1249) These endophytic strains can also be administered to the plant through addition into the plant growth medium, including soil, irrigation, and granular formulations. Endophytic strains can also enter the target plant through the aerial portions of the plants. These create a unique and effective delivery mechanism for delivering proteins and peptides of interest into the plant, or in the case of DNA and RNA binding proteins, delivering RNA and DNA into the plant.

(1250) These data, in *Bacillus* sp. EE387 also demonstrate demonstrates that amino acids 20-35 of BclA (SEQ ID NO: 1), and SEQ ID NOs. 108, 121, and 120 all have noticeably positive data in *Bacillus* strains outside of the *Bacillus cereus* family. *Bacillus thuringiensis* EE-B00184 is also an exceptional host expression system. These levels are both noticeable and positive, indicating a conserved mechanism for attachment may be present in other *Bacillus* species for these proteins.

(1251) Spore Surface Expression of *Bacillus thuringiensis* EE-B00184. *Bacillus thuringiensis* EE-B00184 was transformed with pSUPER BclA 20-35 eGFP, and allowed to sporulate as described above. Spores were pelleted, washed, and subjected to fluorescence microscopy to demonstrate the spore surface laden with eGFP proteins in FIG. **13**.

Example 64: Expression of Fusion Proteins in Herbicide- and Pesticide-Degrading *Bacillus cereus* Family Member Strains

(1252) Examples 49 and 51 above demonstrate the ability of the herbicide-degrading strain *Bacillus cereus* family member EE349 in both degrading herbicides and serving as a host strain for expression of a fusion protein attached the exosporium of its spores. To further demonstrate the ability of herbicide-degrading strains to produce enzyme laden exosporium on their spores, we introduced the pHP13 CotC-Endo (SEQ ID NO: 253), pSUPER AcpC-Endo (SEQ ID NO: 120), pSUPER InhA2-Endo (SEQ ID NO: 121) and pSUPER 23-38 SEQ ID NO:5-Endo) into *Bacillus cereus* family member EE-B00377. A description of pHP13 CotC-Endo can be found in Example 54, a description of pSUPER AcpC-Endo and pSUPER InhA2-Endo can be found in Example 59, and a description of pSUPER 23-38 SEQ ID NO:5-Endo can be found in Example 58. *Bacillus cereus* family member EE-B00377 was identified as a potent degrader of pyrethrin, dicamba, and 2,4-D. Herbicide and pesticide degradation was verified by both growth on the herbicide or pesticide as a nutrient source, as well as by reduction of dicamba and 2,4-D in the presence of the herbicide or pesticide-degrading strain. Plasmids were made and cells transformed identically to Example 48 above. Each construct was verified by Sanger sequencing. Spores were created by using the sporulation

media and conditions outlined in Example 48. Enzyme activity was also performed as in Example 58 above.
(1253) TABLE-US-00067 TABLE 67 Enzyme expression levels of fusion proteins in pesticide degrading strain *Bacillus cereus* family member EE-B00377. Endoglucanase Expression Construct activity (mU/ml) CotC-Endo (SEQ ID NO: 253) 46.9 AcpC-Endo (SEQ ID NO: 120) 4.3 pSUPER 23-38 SEQ ID NO: 5-Endo 108
(1254) As can be seen in Table 67, *Bacillus cereus* family member EE-B00377 is able to produce endoglucanase and display the endoglucanase on its exosporium using several different exosporium proteins or targeting sequences. Of the constructs tested, amino acids 23-38 of SEQ ID NO: 5 or SEQ ID NO: 253 gave the highest enzymes levels in this strain.
(1255) This example demonstrates the ability of the spore displayed system to be expressed in herbicide- and pesticide-degrading strains. This system can be used to express other target proteins on the surface of the spores, including those that act on herbicides or pesticides themselves, such as herbicide-degradation enzymes, pesticide-degradation enzymes, metabolic enzymes, reductases, oxidases, and other useful enzymes for the breakdown of pesticides alone or in the presence of plants.
Example 65: Use of Free Nitric Oxide Synthase (NOS) and Spore-Bound NOS to Enhance Plant Germination
(1256) Example 40 demonstrates the ability of nitric oxide synthatase (NOS) from *Bacillus subtilis* 168 to stimulate germination when attached to the exosporium of *Bacillus cereus* family members, and delivering that NOS-spore protein fusion to seeds or in the vicinity of seeds. In this example, free NOS from *Bacillus thuringiensis* BT013A (SEQ ID NO: 261) and free eNOS (epithelial NOS from bovine neutrophils, Sigma-Aldrich, Cat No N1533) can also help induce germination, or increased outgrowth of seeds exposed to NOS. The pHP13 BclA-BT NOS, pHP13 BclA-BS NOS, and pHP13 BclA-SODA (superoxide dismutase) plasmids were made in identical fashion to pHP13 BclA-BS NOS that described in Example 40 and were transformed into *Bacillus thuringiensis* BT013A. pHP13 BclA-BT NOS contains the BclA promoter, start codon, BclA amino acids 20-35, a 6 alanine linker, and the *Bacillus thuringiensis* BT013A NOS gene (see Table 9, SEQ ID NO: 263). pHP13 BclA-BS NOS contains the BclA promoter, start codon, BclA amino acids 20-35, a 6 alanine linker, and the *Bacillus subtilis* 168 NOS gene (see Table 9, SEQ ID NO: 264). pHP13 BclA-SODA contains the BclA promoter, BclA amino acids 20-35, a 6 alanine linker, and the *Bacillus cereus* superoxide dismutase 1 (SODA1) gene (SEQ ID NO: 155).
(1257) Table 68 shows the results of a soil germination assay. In this assay, commercial variety BECK'S 294NR (ROUNDUP READY) was coated with either 1 μl water (control) or 1 μl of water spiked with 34.2 mU of Bovine Neutrophil eNOS. 50 seeds of each were then planted and grown as described in Example 58, but with 4 seeds per pot. After 7 days, the plants were measured for height. As can be seen in Table 68, the presence of the eNOS allowed for increased outgrowth of the seeds, leading to a 30.7% increase in shoot height of the treated soybeans.
(1258) TABLE-US-00068 TABLE 68 Influence of free eNOS on plant height in soybeans. Height Normalized Treatment to Control H.sub.2O, 1 μl/seed 100.0% H.sub.2O with 34.2 mU eNOS/seed 130.7%
(1259) In addition to the soil germination test described above, standard germination assays were performed as described in Example 40. For soybeans, we choose 2 year old soybean seed with a lower germination rate, and coated 1 μl on each of 50 seeds with the treatments. Treatments were H.sub.2O control (water), L-arginine, *Bacillus thuringiensis* BT013A (strain control), *Bacillus thuringiensis* BT013A with pHP13 BclA-BT NOS, and *Bacillus thuringiensis* BT013A with pHP13 BclA BS NOS. The results for soybeans are shown in Table 69 below.
(1260) TABLE-US-00069 TABLE 69 Influence of spore-displayed NOS on germination rate in soybeans. Treatment Germination Rate H.sub.2O, 1 μl/seed 38% H.sub.2O Control with L-Arginine 58% *Bacillus thuringiensis* BT013A (strain 52% control) *Bacillus thuringiensis* BT013A with BS NOS 82% *Bacillus thuringiensis* BT013A with BT NOS 54%
(1261) Standard germination assays were also performed as described above for commercial hybrids of sorghum. Each sorghum seed was coated with 0.5 μl on each of 50 seeds with the treatments. Treatments were H.sub.2O control (water), L-arginine, *Bacillus thuringiensis* BT013A (strain control), *Bacillus thuringiensis* BT013A with pHP13 BclA-BS NOS, *Bacillus thuringiensis* BT013A with pHP13 BclA-BT NOS. After 4 days, the seeds were measured for shoot height and root length to examine increased outgrowth of the seeds, and all data were normalized to the water control. The results are shown in Table 70 below. The addition of either BT NOS or BS NOS led to a greatly increased root length and shoot growth, with the difference most evident in the BS NOS treatment.
(1262) TABLE-US-00070 TABLE 70 Spore bound NOS and increased sorghum outgrowth Root Growth Shoot Growth Treatment (Normalized) (Normalized) H.sub.2O, 1 μl/seed 100.0% 100.0% H.sub.2O Control with L-Arginine 109% 89% *Bacillus thuringiensis* BT013A 75% 145% (strain control) *Bacillus thuringiensis* BT013A 163% 293% with BS NOS *Bacillus thuringiensis* BT013A 141% 190% with BT NOS
(1263) The sorghum experiment above was repeated, but with slightly different treatments. Treatments were H.sub.2O control (water), *Bacillus thuringiensis* BT013A (strain control), *Bacillus thuringiensis* BT013A with pHP13 BclA-SODA, or free bovine eNOS. After 4 days, the seeds were measured for shoot height and root length to look at increased outgrowth of the seeds, and all data normalized to the water control. The results are shown in

Table 71 below. The addition of either BT SODA or free NOS (eNOS) leads to a greatly increased root length and shoot growth.

(1264) TABLE-US-00071 TABLE 71 Spore bound SODA and free NOS and increased sorghum outgrowth Root Growth Shoot Growth Treatment (Normalized) (Normalized) H.sub.2O, 0.5 μl/seed 100.0% 100.0% *Bacillus thuringiensis* BT013A 79% 117% (strain control) *Bacillus thuringiensis* BT013A 125% 228% with pHP13 BclA-SODA 1 Free eNOS, 34.2 mU/seed 123% 311%

(1265) Taken together, these results demonstrate that overexpression of nitric oxide synthatases from multiple sources can be added to seeds and increase their germination rate and outgrowth of seeds, in both soil and traditional germination methods. This effect can is also found when adding free NOS to seeds. The addition of superoxide dismutase with the spores also leads to an increase in the outgrowth of seeds. L-arginine assisted in the germination rate increases when utilized alone, or assisted in a lesser extent when mixed with NOS enzymes.

(1266) The NOS genes are prevalent in a variety of microorganisms, and these microorganisms can be genetically modified to enhance their ability to express NOS on the seed, or in the vicinity of the seed in plant growth media. Expression of NOS on a spore leads provides a superior delivery system, as vegetative microorganisms are more fragile and do not survive on the seed for long periods of time. Expression on spores using the targeting sequences, exosporium proteins, exosporium protein fragments, and spore coat proteins described herein would all be viable ways of delivery the NOS to seeds.

Example 66: Modulation of Enzyme Expression and Plant Growth

(1267) As demonstrated in Examples 44, 45, and 46, overexpression of a modulator protein in a recombinant *Bacillus cereus* family member that co-expresses a fusion protein can lead to increased and decreased levels of that fusion protein being incorporated into the exosporium. Fusion proteins and constructs were made and spores made as described above in Examples 44 and 45. Growth assays were performed as described above in Example 46.

(1268) As can be seen in Table 72, expression of the pSUPER BclA 20-35 Endo fusion proteins on the surface of the *Bacillus thuringiensis* BT013A spores using amino acids 20-35 of SEQ ID NO: 1 as the targeting sequence led to increased growth in corn, soy, and squash. This effect can be increased when a second exosporium protein is overexpressed. Each of the CotO, BxpB, and YjcB overexpression strains had a pronounced effect on corn, soy, and/or squash growth, with increases most prominent in corn.

(1269) TABLE-US-00072 TABLE 72 Spore bound SODA and free NOS and increased sorghum outgrowth Soy Squash Treatment Corn Growth Growth Growth H.sub.2O, 1.0 μl/seed 100.0% 100.0% 100.0% *Bacillus thuringiensis* 103.8% 108.8% 105.8% BT013A with pSUPER BclA 20-35 Endo (Base) Base with pHP13 BclA-CotO 109.6% 106.4% 105.2% Base with pHP13 BclA- 106.8% 117.2% 113.9% BxpB Base with pHP13 BclA-YjcB 110.4% 122.4% 106.7%

(1270) Overexpression of other modulator proteins can also modulate fusion protein expression levels as well as plant growth effects, including those described herein and in Examples 44 and 45 above. Each of these can be used to alter or tailor the enzyme levels to desired effective levels.

Example 67: Overexpression of Exosporium Proteins and Effects of on Plants

(1271) Overexpression of naturally occurring spore and exosporium proteins can impact the effect that plant growth promoting, endophytic, and other *Bacillus cereus* family members have on plants. Expression of various exosporium proteins as part of a fusion protein or as free enzyme can have beneficial effects on plants, as illustrated above for phosphatases (Examples 11 and 36), nitric oxide synthatase (Example 65), and proteases such as InhA (Examples 3, 6, 7, 13). Other exosporium and spore proteins, such as alanine racemase and inosine uridine preferring hydrolases, can prevent or delay germination of spores, and their overexpression will make spore less prone to quick germination, an unwanted side effect in the use of many types of spores. Lastly, spores that overexpress certain exosporium proteins can alter the overall assembly of the exosporium, leading to alterations in the binding of spores to plants. An example of this can be seen in Table 73 below.

(1272) Spores were created as described for *Bacillus thuringiensis* BT013A in Example 58. Growth assays were performed by placement of 1 μl of whole cell broth from each construct per corn seed, or 2 μl per squash seed. Treatment of seeds, planting, and data recording was performed as in Example 58.

(1273) *Bacillus mycoides* strain EE155, a plant growth promoting strain of the *Bacillus cereus* family, was transformed with overexpression plasmids as described in Example 44. Overexpression of exosporium proteins in this strain directly led to an increase in the binding of the spores to the plant, and leads to higher plant growth promotion. Specifically, overexpression of BclB, BclA, CotO, CotE led to enhanced plant growth promotion. Other exosporium proteins can be overexpressed that can lead to alterations in the structure of the exosporium, including ExsY, ExsFA/BxpB, CotY, CotO, ExsFB, InhA1, InhA2, ExsJ, ExsH, YjcA, YjcB, BclC, AcpC, InhA3, alanine racemase 1, alanine racemase 2, BclA, BclB, BxpA, BclE, BetA/BAS3290, CotE, ExsA, ExsK, ExsB, YabG, Tgl, superoxide dismutase 1 (SODA1), and superoxide dismutase 2 (SODA2). Overexpression or mutation of any of these genes will lead to alterations of exosporium structure, and lead to potentiating the plant growth benefits associated with members of the *Bacillus cereus* family.

(1274) TABLE-US-00073 TABLE 73 Overexpression of exosporium proteins in *Bacillus mycoides* EE155 Overexpression Squash Growth Corn Growth protein on plasmid (Normalized to (Normalized to Bacteria pHP13 control) control) *Bacillus mycoides* N/A (Control) 100% 100% B155 *Bacillus mycoides* BclB 116.3% 101.4% B155 *Bacillus mycoides* BclA 106.8% 108.5% B155 *Bacillus mycoides* CotE 134.5% 106.3% B155 *Bacillus mycoides* CotO 118.6% 111.7% B155

Example 68: Plant Tissues Binding Through Use of Exosporium Displayed Binding Proteins

(1275) Spores that are useful for the display of exogenous and endogenous proteins can be utilized as fusion partners to enhance spore binding to surfaces, including plant tissue. To demonstrate this attribute, *Bacillus thuringiensis* BT013A spores were transformed with plasmids pSUPER BclA 20-35 TasA, pSUPER BclA 20-35 Expansin, pSUPER BclA 20-35 Endo, and pSUPER BclA 20-35 Control. TasA and expansin are plant binding proteins. The control plasmid contained the BclA promoter, a start codon and amino acids 20-35 of SEQ ID NO: 1, but did not include a fusion partner. These constructs were prepared as in identical fashion to the others described in above in Example 58.

(1276) To perform the tissue binding assay, 2 week old corn plants and 3 week old soybean plants were grown as described in Example 58, but without any seed treatment. The primary leaf and first trifoliate of the plants was then swabbed with 1 ml of spores containing each of the above constructs. The leaves were allowed to dry, clipped from the plants and placed into a 50 ml conical tube with 10 ml of water, and vortexed heavily. The spores that were released from the leaf into the water were counted on a hemacytometer, and the counts compared to those expected if no spores bound to the leaves. This experiment was repeated in ten times, and a second experiment was performed which involved plating of the water onto antibiotic plates (tetracycline plus nutrient agar) overnight at 30° C. The final counts are shown in Table 74.

(1277) TABLE-US-00074 TABLE 74 Plant tissue binding is increased with binding protein expression on spores Overall Change in Change in Treatment Binding Binding Binding % Binding (Construct) Crop % from control (plate assay) from control Control (BclA Corn 42.9% N/A 0% N/A 20-35 Control) BclA 20-35 Corn 75.9% +33% 15.6% +15.6% Endoglucansae BclA 20-35 Corn 38.4% −4.5% 41.1% +41.1% Expansin BclA 20-35 Corn 54.9% +12% 100% +100% TasA Control (BclA Soy 58.3% N/A 65.2% N/A 20-35 Control) BclA 20-35 Soy 93.7% +35.4% 61% −4.2% Endoglucansae BclA 20-35 Soy 87.9% +29.6% 99.1% +33.9 Expansin BclA 20-35 Soy 75.7% +20.8% 91.7% +26.5% TasA

(1278) As can be seen from Table 74, the control BT013A spores have a high affinity for the BT013A spores for soybeans, with 58.3% and 65.2% of the spores bound for the controls. Despite this, expression of endoglucanase, expansin, or TasA on the surface of the spores led to an increase in binding of spores to the soy leaves, with many spore preparations approaching 100% bound to the leaves. In corn, there was much less binding for the control spores, especially in the plate assay. The results from the plate assay are the most striking, with an increase in each of the expression constructs, with TasA at 100% of spores bound in that assay.

(1279) These binding proteins can also be utilized in any of the recombinant spore forming microorganisms, utilizing any of the expression systems or fusion partners described herein. This system would also be useful in conjunction with the exosporium strips, to create a protein delivery system that is both cell free and binds tightly to leaves.

Example 69: Use of Recombinant Spore-Forming Bacteria Expressing Fusion Proteins Containing Cot/Cge Proteins and an Enzyme for Promoting Plant Growth

(1280) Coat proteins form protein layers that are found on all *Bacillus* species spores described to date, as well as related genera *Virginibacillus*, *Lysinibacillus*, *Clostridia*, and *Paenibacillus*. Fusion of proteins or peptides of interest to the coat proteins allows expression of foreign proteins on the surface of the spore, and delivery of these proteins or peptides of interest to plants. To demonstrate the ability of the coat proteins to deliver enzymes to plants, a series of constructs were created. The pHP13 plasmid from the *Bacillus* Genetic Stock Culture collection was used to clone each of the constructs described below into the multiple cloning site using homologous recombination utilizing their native promoter elements.

(1281) CotB, CotG, and CotC from *Bacillus subtilis* M01099 or CgeA from *Bacillus amyloliquefaciens* was fused in frame with the endoglucanase eglS gene from *Bacillus subtilis* 168, the lipA lipase gene from *Bacillus subtilis* 168, or the pe-plc gene from *Bacillus thuringiensis* BT013A. These constructs were cloned into pHP13 via homologous recombination, verified by Sanger sequencing, and transformed into *Bacillus subtilis* EE405, *Bacillus subtilis* A09, *Bacillus cereus* family member EE439, *Bacillus* sp. EE398, or *Bacillus thuringiensis* EE-B00184. Each transformant was also screened for correct clones by Sanger sequencing. After confirmation of the clones, each clone was grown up in brain heart infusion broth (BHI) plus tetracycline (10 μg/ml) overnight at 30° C., and 100 μl of the overnight culture was swabbed onto nutrient agar plates plus tetracycline. These plates were incubated at 30° C. for 3 days, and spores were collected by swabbing with a water-wettened cotton swab and resuspended in water.

(1282) Spores for endoglucanase assays were then diluted to 1×10.sup.8 CFU/ml in water, and assayed for enzyme

activity by utilizing the chromophore 4 chloro 2 nitrophenyl cellotetrose (4C2NC, 3 mM in water). For this method, 50 μl of spores was placed into a 96 well plate, and 50 μl of a 300 nM 4C2NC solution added to each plate. The plate was then incubated at 30° C. and absorbance at 410 nm read after 0.5 hours. In all cases, the respective strain control absorbance was subtracted out of the total absorbance of each clone to negate any background activity.
(1283) Spores for lipases assays were diluted to 1×10.sup.8 CFU/ml in water, and assayed for enzyme activity in a second method utilizing the chromophore 4-nitrophenyl palmitate (4NP, 3 mM in water). For this method, 50 μl of spores was placed into a 96 well plate, and 50 μl of a 300 nM 4NP solution added to each plate. The plate was then incubated at 30° C. and absorbance at 410 nm read after 0.5 hours. In all cases, the respective strain control absorbance was subtracted out of the total absorbance of each clone to negate any background activity.
(1284) Spores for phospholipase assays were diluted to 1×10.sup.8 CFU/ml in water, and assayed for enzyme activity as described above for phospholipase in Example 58. In all cases, the respective strain control absorbance was subtracted out of the total absorbance of each clone to negate any background activity.
(1285) Plant growth responses and treatments were applied and collected as described for squash in Example 58 above. All heights were normalized against a strain control with no enzyme displayed on the spores.
(1286) TABLE-US-00075 TABLE 75 Coat protein fusions and their enzyme expression levels. Endoglucanase Phospholipase Enzyme Lipase Enzyme Plant growth Activity, Enzyme Activity, Activity, response above Treatment Fusion partner (Absorbance (Absorbance (Absorbance strain control, (Construct) for endo minus control) minus control) minus control) Squash *Bacillus subtilis* N/A 0.0 0.0 0.0 100% A09 Strain Control A09 CotB 0.01 0.03 .198 101.5% A09 CotG ND .117 .196 101.0% A09 CotC 0.09 .069 .154 99.4% A09 CgeA 0.13 0 .218 ND *Bacillus subtilis* N/A 0.0 0.0 0.0 100% EE405 Strain Control EE405 CgeA 1.84 ND ND 104.7% *B. thuringiensis* N/A 0.0 0.0 0.0 100% EE184 strain control EE184 CotB 2.42 .262 1.37 95.5% EE184 CotG 2.41 0 .330 105% EE184 CotC 2.00 0.08 .373 119.4% EE184 CgeA 2.70 .520 0 110.1% *Bacillus* sp. N/A ND 0.0 0.0 100% EE387 strain control EE387 CotB ND .071 .163 108.0% EE387 CotG ND .104 .140 105.6% EE387 CotC ND .168 .196 106.3% EE387 CgeA ND .124 .187 108.5% N/A = not applicable ND = not determined
(1287) The data in Table 75 demonstrate that the coat proteins work broadly on a number of plant benefiting enzymes, in both *Bacillus cereus* family members (EE184, EE439) and non-*Bacillus cereus* family members (EE405, A09, and EE387 from here and Example 65). Addition of spore displayed enzymes, endoglucanase in this example, leads to an increased plant growth phenotype in most cases.
(1288) In view of the above, it will be seen that the several objects of the invention are achieved and other advantageous results attained.
(1289) As various changes could be made in the above products, compositions, and methods without departing from the scope of the invention, it is intended that all matter contained in the above description and shown in the accompanying drawings shall be interpreted as illustrative and not in a limiting sense.

Claims

1. A plant seed coated with a *Bacillus cereus* superoxide dismutase or a *Bacillus thuringiensis* superoxide dismutase.
2. The plant seed of claim 1, wherein the superoxide dismutase comprises superoxide dismutase 1 (SODA1) or superoxide dismutase 2 (SODA2).
3. The plant seed of claim 1, wherein the superoxide dismutase comprises an amino acid sequence having at least 85%, at least 90%, at least 95%, at least 98%, at least 99%, or 100% identity with SEQ ID NO: 155 or 156.
4. The plant seed of claim 1, wherein the seed is coated with a formulation comprising the superoxide dismutase and an agriculturally acceptable carrier.
5. The plant seed of claim 4, wherein the agriculturally acceptable carrier comprises a dispersant, a surfactant, an additive, water, a thickener, an anti-caking agent, residue breakdown, a composting formulation, a granular application, diatomaceous earth, an oil, a coloring agent, a stabilizer, a preservative, a polymer, a coating, or a combination thereof.
6. The plant seed of claim 5, wherein the agriculturally acceptable carrier comprises an additive, and the additive comprises an oil, a gum, a resin, a clay, a polyoxyethylene glycol, a terpene, a viscid organic, a fatty acid ester, a sulfated alcohol, an alkyl sulfonate, a petroleum sulfonate, an alcohol sulfate, a sodium alkyl butane diamate, a polyester of sodium thiobutane dioate, a benzene acetonitrile derivative, a proteinaceous material, or a combination thereof; the agriculturally acceptable carrier comprises a thickener, and the thickener comprises a long chain alkylsulfonate of polyethylene glycol, a polyoxyethylene oleate, or a combination thereof; the agriculturally acceptable carrier comprises a surfactant, and the surfactant comprises a heavy petroleum oil, a heavy petroleum distillate, a polyol fatty acid ester, a polyethoxylated fatty acid ester, an aryl alkyl polyoxyethylene glycol, an alkyl amine acetate, an alkyl aryl sulfonate, a polyhydric alcohol, an alkyl phosphate, or a combination thereof; or the agriculturally acceptable carrier comprises an anti-caking agent, and the anti-caking agent comprises a sodium salt,

a calcium carbonate, diatomaceous earth, or a combination thereof.
7. The plant seed of claim 6, wherein additive comprises a proteinaceous material, and the proteinaceous material comprises a milk product, wheat flour, soybean meal, blood, albumin, gelatin, alfalfa meal, yeast extract, or a combination thereof; or the anti-caking agent comprises a sodium salt, and the sodium salt comprises a sodium salt of monomethyl naphthalene sulfonate, a sodium salt of dimethyl naphthalene sulfonate, a sodium sulfite, a sodium sulfate, or a combination thereof.
8. The plant seed of claim 4, wherein the agriculturally acceptable carrier comprises vermiculite, charcoal, sugar factory carbonation press mud, rice husk, carboxymethyl cellulose, peat, perlite, fine sand, calcium carbonate, flour, alum, a starch, talc, polyvinyl pyrrolidone, or a combination thereof.
9. The plant seed of claim 4, wherein the seed coating formulation comprises an aqueous or oil-based solution for application to seeds or a powder or granular formulation for application to seeds.
10. The plant seed of claim 4, wherein the formulation further comprises an agrochemical, the agrochemical comprising a fertilizer, a micronutrient fertilizer material, an insecticide, an herbicide, a plant growth amendment, a fungicide, an insecticide, a molluscicide, an algicide, a bacterial inoculant, a fungal inoculant, or a combination thereof.
11. The plant seed of claim 10, wherein the formulation comprises a bacterial inoculant and the bacterial inoculant comprises a plant-growth promoting strain of bacteria.
12. The plant seed of claim 11, wherein the plant-growth promoting strain of bacteria produces an insecticidal toxin, produces a fungicidal compound, produces a nematocidal compound, produces a bactericidal compound, is resistant to one or more antibiotics, comprises one or more freely replicating plasmids, binds to plant roots, colonizes plant roots, forms biofilms, solubilizes nutrients, and/or secretes organic acids.
13. The plant seed of claim 12, wherein the insecticidal toxin comprises a Cry toxin; wherein the fungicidal compound comprises a β-1,3-glucanase, a chitosanase, a lyticase, or a combination thereof; or wherein the nematocidal compound comprises a Cry toxin.
14. The plant seed of claim 11, wherein the plant-growth promoting strain of bacteria comprises *Bacillus aryabhattai* CAP53 (NRRL No. B-50819), *Bacillus aryabhattai* CAP56 (NRRL No. B-50817), *Bacillus flexus* BT054 (NRRL No. B-50816), *Paracoccus kondratievae* NC35 (NRRL No. B-50820), *Bacillus mycoides* BT155 (NRRL No. B-50921), *Enterobacter cloacae* CAP12 (NRRL No. B-50822), *Bacillus nealsonii* BOBA57 (NRRL No. NRRL B-50821), *Bacillus mycoides* EE118 (NRRL No. B-50918), *Bacillus subtilis* EE148 (NRRL No. B-50927), *Alcaligenes faecalis* EE107 (NRRL No. B-50920), *Bacillus mycoides* EE141 (NRRL NO. B-50916), *Bacillus mycoides* BT46-3 (NRRL No. B-50922), *Bacillus cereus* family member EE128 (NRRL No. B-50917), *Bacillus thuringiensis* BT013A (NRRL No. B-50924), *Paenibacillus massiliensis* BT23 (NRRL No. B-50923), *Bacillus cereus* family member EE349 (NRRL No. B-50928), *Bacillus subtilis* EE218 (NRRL No. B-50926), *Bacillus megaterium* EE281 (NRRL No. B-50925), *Bacillus cereus* family member EE-B00377 (NRRL B-67119); *Bacillus pseudomycoides* EE-B00366 (NRRL B-67120), *Bacillus mycoides* EE-B00363 (NRRL B-67121), *Bacillus pumilus* EE-B00143 (NRRL B-67123), or *Bacillus thuringiensis* EE-B00184 (NRRL B-67122), or a combination thereof.
15. The plant seed of claim 10, wherein the agrochemical comprises a fertilizer, and the fertilizer comprises a liquid fertilizer; wherein the agrochemical comprises a micronutrient fertilizer material and the micronutrient fertilizer material comprises boric acid, a borate, a boron frit, copper sulfate, a copper frit, a copper chelate, a sodium tetraborate decahydrate, an iron sulfate, an iron oxide, iron ammonium sulfate, an iron frit, an iron chelate, a manganese sulfate, a manganese oxide, a manganese chelate, a manganese chloride, a manganese frit, a sodium molybdate, molybdic acid, a zinc sulfate, a zinc oxide, a zinc carbonate, a zinc frit, zinc phosphate, a zinc chelate, or a combination thereof; wherein the agrochemical comprises an insecticide, and the insecticide comprises an organophosphate, a carbamate, a pyrethroid, an acaricide, an alkyl phthalate, boric acid, a borate, a fluoride, sulfur, a haloaromatic substituted urea, a hydrocarbon ester, a biologically-based insecticide, or a combination thereof; wherein the agrochemical comprises an herbicide, and the herbicide comprises a chlorophenoxy compound, a nitrophenolic compound, a nitrocresolic compound, a dipyridyl compound, an acetamide, an aliphatic acid, an anilide, a benzamide, a benzoic acid, a benzoic acid derivative, anisic acid, an anisic acid derivative, a benzonitrile, benzothiadiazinone dioxide, a thiocarbamate, a carbamate, a carbanilate, chloropyridinyl, a cyclohexenone derivative, a dinitroaminobenzene derivative, a fluorodinitrotoluidine compound, isoxazolidinone, nicotinic acid, isopropylamine, an isopropylamine derivative, oxadiazolinone, a phosphate, a phthalate, a picolinic acid compound, a triazine, a triazole, a uracil, a urea derivative, endothall, sodium chlorate, or a combination thereof; wherein the agrochemical comprises a fungicide, and the fungicide comprises a substituted benzene, a thiocarbamate, an ethylene bis dithiocarbamate, a thiophthalidamide, a copper compound, an organomercury compound, an organotin compound, a cadmium compound, anilazine, benomyl, cyclohexamide, dodine, etridiazole, iprodione, metlaxyl, thiamimefon, triforine, or a combination thereof; wherein the agrochemical comprises a fungal inoculant and the fungal inoculant comprises a fungal inoculant of the family Glomeraceae, a fungal inoculant of the family

Claroidoglomeraceae, a fungal inoculant of the family Gigasporaceae, a fungal inoculant of the family Acaulosporaceae, a fungal inoculant of the family Sacculosporaceae, a fungal inoculant of the family Entrophosporaceae, a fungal inoculant of the family Pacidsporaceae, a fungal inoculant of the family Diversisporaceae, a fungal inoculant of the family Paraglomeraceae, a fungal inoculant of the family Archaeosporaceae, a fungal inoculant of the family Geosiphonaceae, a fungal inoculant of the family Ambisporaceae, a fungal inoculant of the family Scutellosporaceae, a fungal inoculant of the family Dentiscultataceae, a fungal inoculant of the family Racocetraceae, a fungal inoculant of the phylum Basidiomycota, a fungal inoculant of the phylum Ascomycota, a fungal inoculant of the phylum Zygomycota, or a combination thereof; or wherein the agrochemical comprises a bacterial inoculant and the bacterial inoculant comprises a bacterial inoculant of the genus *Rhizobium*, a bacterial inoculant of the genus *Bradyrhizobium*, a bacterial inoculant of the genus *Mesorhizobium*, a bacterial inoculant of the genus *Azorhizobium*, a bacterial inoculant of the genus *Allorhizobium*, a bacterial inoculant of the genus *Sinorhizobium*, a bacterial inoculant of the genus *Kluyvera*, a bacterial inoculant of the genus *Azotobacter*, a bacterial inoculant of the genus *Pseudomonas*, a bacterial inoculant of the genus *Azospirillium*, a bacterial inoculant of the genus *Bacillus*, a bacterial inoculant of the genus *Streptomyces*, a bacterial inoculant of the genus *Paenibacillus*, a bacterial inoculant of the genus *Paracoccus*, a bacterial inoculant of the genus *Enterobacter*, a bacterial inoculant of the genus *Alcaligenes*, a bacterial inoculant of the genus *Mycobacterium*, a bacterial inoculant of the genus *Trichoderma*, a bacterial inoculant of the genus *Gliocladium*, a bacterial inoculant of the genus *Glomus*, a bacterial inoculant of the genus *Klebsiella*, or a combination thereof.

16. The plant seed of claim 10, wherein the agrochemical comprises a fungicide, and the fungicide comprises aldimorph, ampropylfos, ampropylfos potassium, andoprim, anilazine, azaconazole, azoxystrobin, benalaxyl, benodanil, benomyl, benzamacril, benzamacryl-isobutyl, bialaphos, binapacryl, biphenyl, bitertanol, blasticidin-S, boscalid, bromuconazole, bupirimate, buthiobate, calcium polysulphide, capsimycin, captafol, captan, carbendazim, carvon, quinomethionate, chlobenthiazone, chlorfenazole, chloroneb, chloropicrin, chlorothalonil, chlozolinate, clozylacon, cufraneb, cymoxanil, cyproconazole, cyprodinil, cyprofuram, debacarb, dichlorophen, diclobutrazole, diclofluanid, diclomezine, dicloran, diethofencarb, dimethirimol, dimethomorph, dimoxystrobin, diniconazole, diniconazole-M, dinocap, diphenylamine, dipyrithione, ditalimfos, dithianon, dodemorph, dodine, drazoxolon, edifenphos, epoxiconazole, etaconazole, ethirimol, etridiazole, famoxadon, fenapanil, fenarimol, fenbuconazole, fenfuram, fenitropan, fenpiclonil, fenpropidin, fenpropimorph, fentin acetate, fentin hydroxide, ferbam, ferimzone, fluazinam, flumetover, fluoromide, fluquinconazole, flurprimidol, flusilazole, flusulfamide, flutolanil, flutriafol, folpet, fosetyl-aluminium, fosetyl-sodium, fthalide, fuberidazole, furalaxyl, furametpyr, furcarbonil, furconazole, furconazole-cis, furmecyclox, guazatine, hexachlorobenzene, hexaconazole, hymexazole, imazalil, imibenconazole, iminoctadine, iminoctadine albesilate, iminoctadine triacetate, iodocarb, iprobenfos (IBP), iprodione, irumamycin, isoprothiolane, isovaledione, kasugamycin, kresoxim-methyl, copper hydroxide, copper naphthenate, copper oxychloride, copper sulphate, copper oxide, oxine-copper, Bordeaux mixture, mancopper, mancozeb, maneb, meferimzone, mepanipyrim, mepronil, metconazole, methasulfocarb, methfuroxam, metiram, metomeclam, metsulfovax, mildiomycin, myclobutanil, myclozolin, nickel dimethyldithiocarbamate, nitrothal-isopropyl, nuarimol, ofurace, oxadixyl, oxamocarb, oxolinic acid, oxycarboxim, oxyfenthiin, paclobutrazole, pefurazoate, penconazole, pencycuron, phosdiphen, pimaricin, piperalin, polyoxin, polyoxorim, probenazole, prochloraz, procymidone, propamocarb, propanosine-sodium, propiconazole, propineb, prothioconazole, pyrazophos, pyrifenox, pyrimethanil, pyroquilon, pyroxyfur, quinconazole, quintozene (PCNB), sulphur and sulphur preparations, tebuconazole, tecloftalam, tecnazene, tetcyclasis, tetraconazole, thiabendazole, thicyofen, thifluzamide, thiophanate-methyl, tioxymid, tolclofos-methyl, tolylfluanid, triadimefon, triadimenol, triazbutil, triazoxide, trichlamide, tricyclazole, tridemorph, trifloxystrobin, triflumizole, triforine, uniconazole, validamycin A, vinclozolin, viniconazole, zarilamide, zineb, ziram, Dagger G, OK-8705, OK-8801, α-(1,1-dimethylethyl)-(3-(2-phenoxyethyl)-1H-1,2,4-triazole-1-ethanol, α-(2,4-dichlorophenyl)-[3-fluoro-3-propyl-1H-1,2,4-triazole-1-ethanol, α-(2,4-dichlorophenyl)-[3-methoxy-α-methyl-1H-1,2,4-triazole-1-ethanol, α-(5-methyl-1,3-dioxan-5-yl)-[3-[4-(trifluoromethyl)-phenyl]-methylene]-1H-1,2,4-triazole-1-ethanol, (E)-α-(methoxyimino)-N-methyl-2-phenoxy-phenylacetamide, 1-isopropyl{2-methyl-1-[[[1-(4-methylphenyl)-ethyl]-amino]-carbonyl]-propyl}carbamate, 1-(2,4-dichlorophenyl)-2-(1H-1,2,4-triazol-1-yl)-ethanone-O-(phenyl methyl)-oxime, 1-(2-methyl-1-naphthalenyl)-1H-pyrrole-2,5-dione, 1-(3,5-dichlorophenyl)-3-(2-propenyl)-2,5-pyrrolidindione, 1-[(diiodomethyl)-sulphonyl]-4-methyl-benzene, 1-[[2-(2,4-dichlorophenyl)-1, 3-dioxolan-2-yl]-methyl]-1H-imidazole, 1-[[2-(4-chlorophenyl)-3-phenyloxiranyl]-methyl]-1H-1,2,4-triazole, 1-[1-[2-[(2,4-dichlorophenyl)-methoxy]-phenyl]-ethenyl]-1H-imidazole, 1-methyl-5-nonyl-2-(phenylmethyl)-3-pyrrolidinole, 2′,6′-dibromo-2-methyl-4′-trifluoromethoxy-4′-trifluoro-methyl-1, 3-thiazole-carboxanilide, 2,2-dichloro-N-[1-(4-chlorophenyl)-ethyl]-1-ethyl-3-methyl-cyclopropanecarboxamide, 2,6-dichloro-5-(methylthio)-4-pyrimidinyl-thiocyanate, 2,6-dichloro-N-(4-trifluoromethylbenzyl)-benzamide, 2,6-dichloro-N-[[4-(trifluoromethyl)-phenyl]-methyl]-benzamide, 2-(2,3,3-triiodo-2-propenyl)-2H-tetrazole, 2-[(1-methylethyl)-sulphonyl]-5-(trichloromethyl)-1,3,4-thiadiazole, 2-[[6-deoxy-

4-O-(4-O-methyl-β-D-glucopyranosyl)-α-D-glucopyranosyl]amino]-4-methoxy-1H-pyrrolo[2,3-d]pyrimidine-5-carbonitrile, 2-aminobutane, 2-bromo-2-(bromomethyl)-pentanedinitrile, 2-chloro-N-(2,3-dihydro-1,1,3-trimethyl-1H-inden-4-yl)-3-pyridinecarboxamide, 2-chloro-N-(2,6-dimethylphenyl)-N-(isothiocyanatomethyl)-acetamide, 2-phenylphenol (OPP), 3,4-dichloro-1-[4-(difluoromethoxy)-phenyl]-pyrrole-2,5-dione, 3,5-dichloro-N-[cyano[(1-methyl-2-propynyl)-oxy]-methyl]-benzamide, 3-(1,1-dimethylpropyl-1-oxo-1H-indene-2-carbonitrile, 3-[2-(4-chlorophenyl)-5-ethoxy-3-isoxazolidinyl]-pyridine, 4-chloro-2-cyano-N,N-dimethyl-5-(4-methylphenyl)-1H-imidazole-1-sulphonamide, 4-methyl-tetrazolo[1,5-a]quinazolin-5 (4H)-one, 8-(1,1-dimethylethyl)-N-ethyl-N-propyl-1,4-dioxaspiro[4, 5]decane-2-methanamine, 8-hydroxyquinoline sulphate, 9H-xanthene-2-[(phenylamino)-carbonyl]-9-carboxylic hydrazide, bis-(1-methylethyl)-3-methyl-4-[(3-methylbenzoyl)-oxy]-2,5-thiophenedicarboxylate, cis-1-(4-chlorophenyl)-2-(1H-1,2,4-triazol-1-yl)-cycloheptanol, cis-4-[3-[4-(1,1-dimethylpropyl)-phenyl-2-methylpropyl]-2,6-dimethyl-morpholine hydrochloride, ethyl[(4-chlorophenyl)-azo]-cyanoacetate, potassium bicarbonate, methanetetrathiol-sodium salt, methyl 1-(2,3-dihydro-2,2-dimethyl-inden-1-yl)-1H-imidazole-5-carboxylate, methyl N-(2,6-dimethylphenyl)-N-(5-isoxazolylcarbonyl)-DL-alaninate, methyl N-(chloroacetyl)-N-(2,6-dimethylphenyl)-DL-alaninate, N-(2,3-dichloro-4-hydroxyphenyl)-1-methyl-cyclohexanecarboxamide, N-(2,6-dimethyl phenyl)-2-methoxy-N-(tetra hydro-2-oxo-3-furanyl)-acetamide, N-(2,6-dimethyl phenyl)-2-methoxy-N-(tetrahydro-2-oxo-3-thienyl)-acetamide, N-(2-chloro-4-nitrophenyl)-4-methyl-3-nitro-benzenesulphonamide, N-(4-cyclohexylphenyl)-1,4,5,6-tetrahydro-2-pyrimidinamine, N-(4-hexylphenyl)-1,4,5,6-tetrahydro-2-pyrimidinamine, N-(5-chloro-2-methylphenyl)-2-methoxy-N-(2-oxo-3-oxazolidinyl)-acetamide, N-(6-methoxy)-3-pyridinyl)-cyclopropanecarboxamide, N-[2,2,2-trichloro-1-[(chloroacetyl)-amino]-ethyl]-benzamide, N-[3-chloro-4,5-bis(2-propinyloxy)-phenyl]-N′-methoxy-methanimidamide, N-formyl-N-hydroxy-DL-alanine-sodium salt, O,O-diethyl[2-(dipropylamino)-2-oxoethyl]-ethylphosphoramidothioate, O-methyl S-phenyl phenylpropylphosphoramidothioate, S-methyl 1,2,3-benzothiadiazole-7-carbothioate, and spiro[2H]-1-benzopyrane-2,1′(3′H)-isobenzofuran]-3′-one, tetramethylthioperoxydicarbonic diamide, methyl N-(2,6-dimethylphenyl)-N-(methoxyacetyl)-DL-alaninate, 4-(2,2-difluoro-1,3-benzodioxol-4-yl)-1-H-pyrrol-3-carbonitril, or a combination thereof.

17. The plant seed of claim 10, wherein the agrochemical comprises a bacterial inoculant of the genus *Bacillus*, and the bacterial inoculant of the genus *Bacillus* comprises *Bacillus argri, Bacillus aizawai, Bacillus albolactis, Bacillus amyloliquefaciens, Bacillus cereus, Bacillus coagulans, Bacillus endoparasiticus, Bacillus endorhythmos, Bacillus kurstaki, Bacillus lacticola, Bacillus lactimorbus, Bacillus lactis, Bacillus laterosporus, Bacillus lentimorbus, Bacillus licheniformis, Bacillus megaterium, Bacillus medusa, Bacillus metiens, Bacillus natto, Bacillus nigrificans, Bacillus popillae, Bacillus pumilus, Bacillus siamensis, Bacillus sphearicus, Bacillus* spp., *Bacillus subtilis, Bacillus thuringiensis, Bacillus unifagellatu*, or a combination thereof.

18. The plant seed of claim 10, wherein the agrochemical comprises an herbicide, and the herbicide comprises 2,4-D, 2,4-DB, acetochlor, acifluorfen, alachlor, ametryn, atrazine, aminopyralid, benefin, bensulfuron, bensulide, bentazon, bromacil, bromoxynil, butylate, carfentrazone, chlorimuron, chlorsulfuron, clethodim, clomazone, clopyralid, cloransulam, cycloate, DCPA, desmedipham, dicamba, dichlobenil, diclofop, diclosulam, diflufenzopyr, dimethenamid, diquat, diuron, DSMA, endothall, EPTC, ethalfluralin, ethofumesate, fenoxaprop, fluazifop-P, flucarbazone, flufenacet, flumetsulam, flumiclorac, flumioxazin, fluometuron, fluroxypyr, fomesafen, foramsulfuron, glufosinate, glyphosate, halosulfuron, hexazinone, imazamethabenz, imazamox, imazapic, imazaquin, imazethapyr, isoxaben, isoxaflutole, lactofen, linuron, MCPA, MCPB, mesotrione, metolachlor-s, metribuzin, metsulfuron, molinate, MSMA, napropamide, naptalam, nicosulfuron, norflurazon, oryzalin, oxadiazon, oxyfluorfen, paraquat, pelargonic acid, pendimethalin, phenmedipham, picloram, primisulfuron, prodiamine, prometryn, pronamide, propanil, prosulfuron, pyrazon, pyrithiobac, quinclorac, quizalofop, rimsulfuron, sethoxydim, siduron, simazine, sulfentrazone, sulfometuron, sulfosulfuron, tebuthiuron, terbacil, thiazopyr, thifensulfuron, thiobencarb, tralkoxydim, triallate, triasulfuron, tribenuron, triclopyr, trifluralin, triflusulfuron, or a combination thereof.

19. The plant seed of claim 10, wherein the formulation comprises the herbicide and the bacterial inoculum, wherein the bacterial inoculum comprises a strain of bacteria capable of degrading the herbicide.

20. The plant seed of claim 19, wherein the strain of bacteria that is capable of degrading an herbicide comprises *Bacillus cereus* family member EE349 (NRRL No. B-50928), *Bacillus cereus* family member EE-B00377 (NRRL B-67119), *Bacillus pseudomycoides* EE-B00366 (NRRL B-67120), or *Bacillus mycoides* EE-B00363 (NRRL B-67121), or a combination thereof.

21. The plant seed of claim 19, wherein the herbicide comprises a sulfonylurea, an aryl triazine, dicamba, a phenoxy herbicide, 2,4-D, a pyrethrin, a pyrethroid, or a combination thereof.

22. The plant seed of claim 21, wherein the sulfonylurea comprises sulfentrazone.

23. The plant seed of claim 10, wherein the fertilizer comprises ammonium sulfate, ammonium nitrate, ammonium sulfate nitrate, ammonium chloride, ammonium bisulfate, ammonium polysulfide, ammonium thiosulfate, aqueous ammonia, anhydrous ammonia, ammonium polyphosphate, aluminum sulfate, calcium nitrate, calcium ammonium

nitrate, calcium sulfate, calcined magnesite, calcitic limestone, calcium oxide, calcium nitrate, dolomitic limestone, hydrated lime, calcium carbonate, diammonium phosphate, monoammonium phosphate, magnesium nitrate, magnesium sulfate, potassium nitrate, potassium chloride, potassium magnesium sulfate, potassium sulfate, sodium nitrates, magnesian limestone, magnesia, urea, urea-formaldehydes, urea ammonium nitrate, sulfur-coated urea, polymer-coated urea, isobutylidene diurea, langbeinite, kainite, sylvinite, kieserite, Epsom salts, elemental sulfur, marl, ground oyster shells, fish meal, oil cakes, fish manure, blood meal, rock phosphate, super phosphates, slag, bone meal, wood ash, manure, bat guano, peat moss, compost, green sand, cottonseed meal, feather meal, crab meal, fish emulsion, humic acid, or a combination thereof.

24. The plant seed of claim 4, wherein the formulation comprises a salt of iron, manganese, boron, copper, cobalt, molybdenum, zinc, or a combination of any thereof.

25. A method for stimulating germination of a plant seed comprising: introducing into a plant growth medium comprising a seed, or applying to a plant seed, or an area surrounding a plant seed a *Bacillus cereus* superoxide dismutase or a *Bacillus thuringiensis* superoxide dismutase.