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Temporally and spatially targeted dynamic nitrogen delivery by remodeled microbes

Abstract

The present disclosure provides non-intergeneric remodeled microbes that are able to fix atmospheric nitrogen and deliver such to plants in a targeted, efficient, and environmentally sustainable manner. The utilization of the taught microbial products will enable farmers to realize more productive and predictable crop yields without the nutrient degradation, leaching, or toxic runoff associated with traditional synthetically derived nitrogen fertilizer, by mitigating or eliminating the need for exogenous nitrogen-containing fertilizers. The remodeled microbes have unique colonization and nitrogen fixation abilities, which enable the microbes to deliver nitrogen to a cereal plant in a spatially targeted (e.g. rhizospheric) and temporally targeted (e.g. during advantageous stages of plants life cycle) manner. The microbes are able to replace the standard agricultural practice of sidedressing and enable a more environmentally sustainable form of farming. The present disclosure also provides methods of using non-intergeneric remodeled microbes, for example, to fix atmospheric nitrogen by reducing or eliminating the need for exogenous nitrogen-containing fertilizers, to increase yield, and to reduce infield variability in the yield.

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Background/Summary

CROSS-REFERENCE TO RELATED APPLICATIONS (1) This application claims the benefit of International PCT Application No. PCT/US2019/041429, filed Jul. 11, 2019, which claims priority to U.S. Provisional Application No. 62/696,452, filed Jul. 11, 2018, and U.S. Provisional Application No. 62/801,504, filed Feb. 5, 2019, the contents of each of which are herein incorporated by reference in their entirety.

STATEMENT REGARDING SEQUENCE LISTING
(1) The contents of the text file submitted electronically herewith are incorporated herein by reference in their entirety: A computer readable format copy of the Sequence Listing filename: PIVO_006_01WO_SeqList_ST25.txt, date created, Jul. 10, 2019, file size 632 kilobytes.
BACKGROUND OF THE DISCLOSURE
(2) By 2050 the United Nations' Food and Agriculture Organization projects that total food production must increase by 70% to meet the needs of a growing population, a challenge that is exacerbated by numerous factors, including: diminishing freshwater resources, increasing competition for arable land, rising energy prices, increasing input costs, and the likely need for crops to adapt to the pressures of a drier, hotter, and more extreme global climate.
(3) Current agricultural practices are not well equipped to meet this growing demand for food production, while simultaneously balancing the environmental impacts that result from increased agricultural intensity.
(4) One of the major agricultural inputs needed to satisfy global food demand is nitrogen fertilizer. However, the current industrial standard utilized to produce nitrogen fertilizer, is an artificial nitrogen fixation method called the Haber-Bosch process, which converts atmospheric nitrogen (N.sub.2) to ammonia (NH.sub.3) by a reaction with hydrogen (H.sub.2) using a metal catalyst under high temperatures and pressures. This process is resource intensive and deleterious to the environment.
(5) In contrast to the synthetic Haber-Bosch process, certain biological systems have evolved to fix atmospheric nitrogen. These systems utilize an enzyme called nitrogenase that catalyzes the reaction between N.sub.2 and H.sub.2, and results in nitrogen fixation. For example, *rhizobia* are

diazotrophic bacteria that fix nitrogen after becoming established inside root nodules of legumes. An important goal of nitrogen fixation research is the extension of this phenotype to non-leguminous plants, particularly to important agronomic grasses such as wheat, rice, and corn. However, despite the significant progress made in understanding the development of the nitrogen-fixing symbiosis between *rhizobia* and legumes, the path to use that knowledge to induce nitrogen-fixing nodules on non-leguminous crops is still not clear.

(6) Consequently, the vast majority of modern row crop agriculture utilizes nitrogen fertilizer that is produced via the resource intensive and environmentally deleterious Haber-Bosch process. For instance, the USDA indicates that the average U.S. corn farmer typically applies between 130 and 200 lb. of nitrogen per acre (146 to 224 kg/ha). This nitrogen is not only produced in a resource intensive synthetic process, but is applied by heavy machinery crossing/impacting the field's soil, burning petroleum, and requiring hours of human labor.

(7) Furthermore, the nitrogen fertilizer produced by the industrial Haber-Bosch process is not well utilized by the target crop. Rain, runoff, heat, volatilization, and the soil microbiome degrade the applied chemical fertilizer. This equates to not only wasted money, but also adds to increased pollution instead of harvested yield. To this end, the United Nations has calculated that nearly 80% of fertilizer is lost before a crop can utilize it. Consequently, modern agricultural fertilizer production and delivery is not only deleterious to the environment, but it is extremely inefficient.

(8) In order to meet the world's growing food supply needs—while also balancing resource utilization and providing minimal impacts upon environmental systems—a better approach to nitrogen fixation and delivery to plants is urgently needed.

SUMMARY OF THE DISCLOSURE

(9) In some embodiments, provided herein is a method of providing fixed atmospheric nitrogen to a cereal plant, comprising: providing to a locus a plurality of non-intergeneric remodeled bacteria that each produce fixed N of at least about 5.49×10.sup.−13 mmol of N per CFU per hour; and providing to the locus a plurality of cereal plants, wherein said plurality of non-intergeneric remodeled bacteria colonize the root surface of said plurality of cereal plants and supply the cereal plants with fixed N, and wherein said plurality of non-intergeneric remodeled bacteria produce in the aggregate at least about 15 pounds of fixed N per acre over the course of at least about 10 days to about 60 days.

(10) In some embodiments, provided herein is a method of providing fixed atmospheric nitrogen to a corn plant that eliminates the need for the addition of in-season exogenous nitrogen application, comprising: providing to a locus a plurality of non-intergeneric remodeled bacteria that each produce fixed N of at least about 5.49×10.sup.−13 mmol of N per CFU per hour; and providing to the locus a plurality of corn plants, wherein said plurality of non-intergeneric remodeled bacteria colonize the root surface of said plurality of corn plants and supply the corn plants with fixed N, and wherein said plurality of non-intergeneric remodeled bacteria produce in the aggregate at least about 15 pounds of fixed N per acre over the course of at least about 10 days to about 60 days, and wherein exogenous nitrogen is not applied to said locus after the plurality of non-intergeneric remodeled bacteria and cereal plants are applied.

(11) In some embodiments, provided herein is a method for increasing corn yield per acre, comprising: providing to a locus a plurality of non-intergeneric remodeled bacteria that each produce fixed N of at least about 5.49×10.sup.−13 mmol of N per CFU per hour; and providing to the locus a plurality of corn plants at a density of at least 35,000 seeds per acre, wherein said plurality of non-intergeneric remodeled bacteria colonize the root surface of said plurality of corn plants and supply the corn plants with fixed N, and wherein said plurality of non-intergeneric remodeled bacteria produce in the aggregate at least about 15 pounds of fixed N per acre over the course of at least about 10 days to about 60 days, and wherein exogenous nitrogen is not applied to said locus after the plurality of non-intergeneric remodeled bacteria and cereal plants are applied.

(12) In some embodiments, provided herein is a method for increasing corn yield per acre in

agriculturally challenging soil, comprising: providing to a locus located in an agriculturally challenging soil a plurality of non-intergeneric remodeled bacteria that each produce fixed N of at least about 5.49×10.sup.−13 mmol of N per CFU per hour; and providing to the locus located in an agriculturally challenging soil a plurality of corn plants, wherein said plurality of non-intergeneric remodeled bacteria colonize the root surface of said plurality of corn plants and supply the corn plants with fixed N, and wherein said agriculturally challenging soil comprises a soil that comprises at least about 30% sand, and wherein said plurality of corn plants achieve at least a 1 bushel per acre yield increase, as compared to a control plurality of corn plants when the control plurality of corn plants is provided to the locus. In some of these embodiments, said plurality of corn plants achieve at least a 5 bushel per acre yield increase or at least a 10 bushel per acre yield increase, as compared to a control plurality of corn plants. In some of these embodiments, said control plurality of corn plants have exogenous nitrogen applied to said plants after said control plurality of corn plants have been planted. In some of these embodiments, exogenous nitrogen is not applied to said locus after the plurality of non-intergeneric remodeled bacteria and corn plants are supplied. In some of these embodiments, the control plurality of corn plants is provided to the locus without the plurality of non-intergeneric remodeled bacteria. In some of these embodiments, exogenous nitrogen is not applied to said locus after the plurality of non-intergeneric remodeled bacteria and corn plants are supplied, but wherein exogenous nitrogen is applied to the control plurality of corn plants, said control plurality of corn plants not being provided with a plurality of non-intergeneric remodeled bacteria. In some of these embodiments, exogenous nitrogen is not applied to said locus after the plurality of non-intergeneric remodeled bacteria and corn plants are supplied, but wherein exogenous nitrogen is applied to the control plurality of corn plants, said control plurality of corn plants not being provided with a plurality of non-intergeneric remodeled bacteria, wherein said plurality of corn plants with the remodeled bacteria achieve at least about a 17 bushel per acre yield increase, as compared to the control plurality of corn plants without said remodeled bacteria.

(13) In some embodiments, provided herein is a method for reducing infield variability for corn yield per acre, comprising: providing to a locus a plurality of non-intergeneric remodeled bacteria that each produce fixed N of at least about 5.49×10.sup.−13 mmol of N per CFU per hour; and providing to the locus a plurality of corn plants, wherein said plurality of non-intergeneric remodeled bacteria colonize the root surface of said plurality of corn plants and supply the corn plants with fixed N, and wherein the standard deviation of corn mean yield measured across the locus as measured in bushels per acre is lower for the plurality of corn plants colonized by said non-intergeneric remodeled bacteria, as compared to a control plurality of corn plants when the control plurality of corn plants is provided to the locus. In some of these embodiments, the standard deviation for the plurality of corn plants colonized by said non-intergeneric remodeled bacteria is less than 19 bushels per acre, as compared to the control plurality of corn plants, said control plurality of corn plants not being colonized by non-intergeneric remodeled bacteria. In some of these embodiments, the yield between the plurality of corn plants colonized by the non-intergeneric remodeled bacteria is within 1-10% of the yield of the control plurality of corn plants, said control plurality of corn plants not being colonized by non-intergeneric remodeled bacteria. In some of these embodiments, said control plurality of corn plants have exogenous nitrogen applied to said plants after said control plurality of corn plants have been planted. In some of these embodiments, exogenous nitrogen is not applied to said locus after the plurality of non-intergeneric remodeled bacteria and corn plants are supplied. In some of these embodiments, the control plurality of corn plants is provided to the locus without the plurality of non-intergeneric remodeled bacteria. In some of these embodiments, exogenous nitrogen is not applied to said locus after the plurality of non-intergeneric remodeled bacteria and corn plants are supplied, but wherein exogenous nitrogen is applied to the control plurality of corn plants, said control plurality of corn plants not being provided with a plurality of non-intergeneric remodeled bacteria. In some of these embodiments,

exogenous nitrogen is not applied to said locus after the plurality of non-intergeneric remodeled bacteria and corn plants are supplied, but wherein exogenous nitrogen is applied to the control plurality of corn plants, said control plurality of corn plants not being provided with a plurality of non-intergeneric remodeled bacteria, wherein the yield between the plurality of corn plants colonized by the non-intergeneric remodeled bacteria is within 1-10% of the yield of the control plurality of corn plants.

(14) In some embodiments, the plurality of non-intergeneric remodeled bacteria each produce fixed N of at least about 2.75×10.sup.−12 mmol of N per CFU per hour.

(15) In some embodiments, the plurality of non-intergeneric remodeled bacteria each produce fixed N of at least about 4.03×10.sup.−13 mmol of N per CFU per hour.

(16) In some embodiments, the plurality of non-intergeneric remodeled bacteria each produce fixed N of at least about 2.75×10.sup.−12 mmol of N per CFU per hour and wherein said plurality of bacteria produce in the aggregate at least about 25 pounds of fixed N per acre over the course of at least about 10 days to about 60 days.

(17) In some embodiments, the plurality of non-intergeneric remodeled bacteria each produce fixed N of at least about 2.75×10.sup.−12 mmol of N per CFU per hour and wherein said plurality of bacteria produce in the aggregate at least about 50 pounds of fixed N per acre over the course of at least about 10 days to about 60 days.

(18) In some embodiments, the plurality of non-intergeneric remodeled bacteria each produce fixed N of at least about 2.75×10.sup.−12 mmol of N per CFU per hour and wherein said plurality of bacteria produce in the aggregate at least about 75 pounds of fixed N per acre over the course of at least about 10 days to about 60 days.

(19) In some embodiments, the plurality of non-intergeneric remodeled bacteria each produce fixed N of at least about 2.75×10.sup.−12 mmol of N per CFU per hour and wherein said plurality of bacteria produce in the aggregate at least about 100 pounds of fixed N per acre over the course of at least about 10 days to about 60 days.

(20) In some embodiments, the plurality of non-intergeneric remodeled bacteria colonize the root surface of the plurality of cereal plants at a total aggregate CFU per acre concentration according to FIG. **31**, **32**, **33**, **34**, or **35**.

(21) In some embodiments, the plurality of non-intergeneric remodeled bacteria colonize the root surface of the plurality of cereal plants at a total aggregate CFU per acre concentration of about 5×10.sup.13 for at least about 20 days.

(22) In some embodiments, the plurality of non-intergeneric remodeled bacteria colonize the root surface of the plurality of cereal plants at a total aggregate CFU per acre concentration of about 5×10.sup.13 for at least about 30 days.

(23) In some embodiments, the plurality of non-intergeneric remodeled bacteria colonize the root surface of the plurality of cereal plants at a total aggregate CFU per acre concentration of about 5×10.sup.13 for at least about 60 days.

(24) In some embodiments, the plurality of non-intergeneric remodeled bacteria colonize the root surface of peripheral roots of the plurality of cereal plants equally as well as they colonize other roots.

(25) In some embodiments, the plurality of non-intergeneric remodeled bacteria colonize the root surface of peripheral roots of the plurality of cereal plants to a higher degree than they colonize other roots.

(26) In some embodiments, exogenous nitrogen is not applied to said locus after the plurality of non-intergeneric remodeled bacteria and cereal plants are applied.

(27) In some embodiments, a sidedressing containing exogenous nitrogen is not applied to said locus after the plurality of non-intergeneric remodeled bacteria and cereal plants are applied.

(28) In some embodiments, the methods provided herein further comprise applying exogenous nitrogen to said locus before steps a) and b).

(29) In some embodiments, the methods provided herein further comprise applying exogenous nitrogen to said locus before steps a) and b), and not applying exogenous nitrogen to said locus after steps a) and b).
(30) In some embodiments, exogenous nitrogen is not applied as a sidedressing.
(31) In some embodiments, the cereal plant is corn, rice, wheat, barley, *sorghum*, millet, oat, rye, or triticale.
(32) In some embodiments, the cereal plant is a corn plant and the plurality of non-intergeneric remodeled bacteria produce in the aggregate at least about 15 pounds of fixed N per acre before the corn plant's VT stage.
(33) In some embodiments, the cereal plant is a corn plant and the plurality of non-intergeneric remodeled bacteria produce in the aggregate at least about 15 pounds of fixed N per acre before the corn plant's R1 stage.
(34) In some embodiments, the cereal plant is a corn plant and the plurality of non-intergeneric remodeled bacteria produce in the aggregate at least about 15 pounds of fixed N per acre before the corn plant's R6 stage.
(35) In some embodiments, the plurality of non-intergeneric remodeled bacteria produce 1% or more of the fixed nitrogen in an individual cereal plant of said plurality exposed thereto.
(36) In some embodiments, the plurality of non-intergeneric remodeled bacteria are capable of fixing atmospheric nitrogen in the presence of exogenous nitrogen.
(37) In some embodiments, each member of the plurality of non-intergeneric remodeled bacteria comprises at least one genetic variation introduced into at least one gene, or non-coding polynucleotide, of the nitrogen fixation or assimilation genetic regulatory network.
(38) In some embodiments, each member of the plurality of non-intergeneric remodeled bacteria comprises an introduced control sequence operably linked to at least one gene of the nitrogen fixation or assimilation genetic regulatory network.
(39) In some embodiments, each member of the plurality of non-intergeneric remodeled bacteria comprises a heterologous promoter operably linked to at least one gene of the nitrogen fixation or assimilation genetic regulatory network.
(40) In some embodiments, each member of the plurality of non-intergeneric remodeled bacteria comprises at least one genetic variation introduced into a member selected from the group consisting of: nifA, nifL, ntrB, ntrC, polynucleotide encoding glutamine synthetase, glnA, glnB, glnK, drat, amtB, polynucleotide encoding glutaminase, glnD, glnE, nifJ, nifH, nifD, nifK, nifY, nifE, nifN, nifU, nifS, nifV, nifW, nifZ, nifM, nifF; nifB, nifQ, a gene associated with biosynthesis of a nitrogenase enzyme, and combinations thereof.
(41) In some embodiments, each member of the plurality of non-intergeneric remodeled bacteria comprises at least one genetic variation introduced into at least one gene, or non-coding polynucleotide, of the nitrogen fixation or assimilation genetic regulatory network that results in one or more of: increased expression or activity of NifA or glutaminase; decreased expression or activity of NifL, NtrB, glutamine synthetase, GlnB, GlnK, DraT, AmtB; decreased adenylyl-removing activity of GlnE; or decreased uridylyl-removing activity of GlnD.
(42) In some embodiments, each member of the plurality of non-intergeneric remodeled bacteria comprises a mutated nifL gene that has been altered to comprise a heterologous promoter inserted into said nifL gene.
(43) In some embodiments, each member of the plurality of non-intergeneric remodeled bacteria comprises a mutated glnE gene that results in a truncated GlnE protein lacking an adenylyl-removing (AR) domain.
(44) In some embodiments, each member of the plurality of non-intergeneric remodeled bacteria comprises a mutated amtB gene that results in the lack of expression of said amtB gene.
(45) In some embodiments, each member of the plurality of non-intergeneric remodeled bacteria comprises at least one of: a mutated nifL gene that has been altered to comprise a heterologous

promoter inserted into said nifL gene; a mutated glnE gene that results in a truncated GlnE protein lacking an adenylyl-removing (AR) domain; a mutated amtB gene that results in the lack of expression of said amtB gene; and combinations thereof.
(46) In some embodiments, each member of the plurality of non-intergeneric remodeled bacteria comprises a mutated nifL gene that has been altered to comprise a heterologous promoter inserted into said nifL gene and a mutated glnE gene that results in a truncated GlnE protein lacking an adenylyl-removing (AR) domain.
(47) In some embodiments, each member of the plurality of non-intergeneric remodeled bacteria comprises a mutated nifL gene that has been altered to comprise a heterologous promoter inserted into said nifL gene and a mutated glnE gene that results in a truncated GlnE protein lacking an adenylyl-removing (AR) domain and a mutated amtB gene that results in the lack of expression of said amtB gene.
(48) In some embodiments, each member of the plurality of non-intergeneric remodeled bacteria comprises at least one genetic variation introduced into genes involved in a pathway selected from the group consisting of: exopolysaccharide production, endo-polygalaturonase production, trehalose production, and glutamine conversion.
(49) In some embodiments, each member of the plurality of non-intergeneric remodeled bacteria comprises at least one genetic variation introduced into genes selected from the group consisting of: bcsii, bcsiii, yjbE, fhaB, pehA, otsB, treZ, glsA2, and combinations thereof.
(50) In some embodiments, the plurality of non-intergeneric remodeled bacteria comprise at least two different species of bacteria.
(51) In some embodiments, the plurality of non-intergeneric remodeled bacteria comprise at least two different strains of the same species of bacteria.
(52) In some embodiments, the plurality of non-intergeneric remodeled bacteria comprise bacteria selected from: *Rahnella aquatilis, Klebsiella variicola, Achromobacter spiritinus, Achromobacter marplatensis, Microbacterium murale, Kluyvera intermedia, Kosakonia pseudosacchari, Enterobacter* sp., *Azospirillum lipoferum, Kosakonia sacchari*, and combinations thereof.
(53) In some embodiments, the plurality of non-intergeneric remodeled bacteria are epiphytic or rhizospheric.
(54) In some embodiments, the plurality of non-intergeneric remodeled bacteria comprise bacteria selected from: a bacteria deposited as NCMA 201701002, a bacteria deposited as NCMA 201708004, a bacteria deposited as NCMA 201708003, a bacteria deposited as NCMA 201708002, a bacteria deposited as NCMA 201712001, a bacteria deposited as NCMA 201712002, and combinations thereof.
(55) In some embodiments, the plurality of non-intergeneric remodeled bacteria comprise bacteria comprising a nucleic acid sequence that shares at least about 90% sequence identity to a nucleic acid sequence selected from SEQ ID NOs: 177-260, 296-303, and 458-469.
(56) In some embodiments, the plurality of non-intergeneric remodeled bacteria comprise bacteria comprising a nucleic acid sequence that shares at least about 95% sequence identity to a nucleic acid sequence selected from SEQ ID NOs: 177-260, 296-303, and 458-469.
(57) In some embodiments, the plurality of non-intergeneric remodeled bacteria comprise bacteria comprising a nucleic acid sequence that shares at least about 99% sequence identity to a nucleic acid sequence selected from SEQ ID NOs: 177-260, 296-303, and 458-469.
(58) In some embodiments, the plurality of non-intergeneric remodeled bacteria comprise bacteria comprising a nucleic acid sequence selected from SEQ ID NOs: 177-260, 296-303, and 458-469.
(59) In some embodiments, the agriculturally challenging soil comprises at least about 40% sand.
(60) In some embodiments, the agriculturally challenging soil comprises at least about 50% sand.
(61) In some embodiments, the agriculturally challenging soil comprises less than about 30% silt.
(62) In some embodiments, the agriculturally challenging soil comprises less than about 20% clay.
(63) In some embodiments, the agriculturally challenging soil comprises a pH of about 5 to about

8.
(64) In some embodiments, the agriculturally challenging soil comprises a pH of about 6.8.
(65) In some embodiments, the agriculturally challenging soil comprises an organic matter content of about 0.40 to about 2.8.
(66) In some embodiments, the agriculturally challenging soil comprises an organic matter content of about 1.42.
(67) In some embodiments, the agriculturally challenging soil is a sandy loam or loam soil.
(68) In some embodiments, the agriculturally challenging soil comprises at least one of the soil variables in the approximate amounts as listed in Table 35.
(69) In some embodiments, the plurality of non-intergeneric remodeled bacteria produce in the aggregate at least about 15 pounds of fixed N per acre over the course of at least about 10 days to about 60 days.

Description

BRIEF DESCRIPTION OF THE DRAWINGS
(1) FIG. **1**A depicts an overview of the guided microbial remodeling process, in accordance with embodiments.
(2) FIG. **1**B depicts an expanded view of the measurement of microbiome composition as shown in FIG. **1**A.
(3) FIG. **1**C depicts a problematic “traditional bioprospecting” approach, which has several drawbacks compared to the taught guided microbial remodeling (GMR) platform.
(4) FIG. **1**D depicts a problematic “field-first approach to bioprospecting” system, which has several drawbacks compared to the taught guided microbial remodeling (GMR) platform.
(5) FIG. **1**E depicts the time period in the corn growth cycle, at which nitrogen is needed most by the plant.
(6) FIG. **1**F depicts an overview of a field development process for a remodeled microbe.
(7) FIG. **1**G depicts an overview of a guided microbial remodeling platform embodiment.
(8) FIG. **1**H depicts an overview of a computationally-guided microbial remodeling platform.
(9) FIG. **1**I depicts the use of field data combined with modeling in aspects of the guided microbial remodeling platform.
(10) FIG. **1**J depicts 5 properties that can be possessed by remodeled microbes of the present disclosure.
(11) FIG. **1**K depicts a schematic of a remodeling approach for a microbe, PBC6.1.
(12) FIG. **1**L depicts decoupled nifA expression from endogenous nitrogen regulation in remodeled microbes.
(13) FIG. **1**M depicts improved assimilation and excretion of fixed nitrogen by remodeled microbes.
(14) FIG. **1**N depicts corn yield improvement attributable to remodeled microbes.
(15) FIG. **1**O illustrates the inefficiency of current nitrogen delivery systems, which result in underfertilized fields, over fertilized fields, and environmentally deleterious nitrogen runoff.
(16) FIG. **2** illustrates PBC6.1 colonization to nearly 21% abundance of the root-associated microbiota in corn roots. Abundance data is based on 16S amplicon sequencing of the rhizosphere and endosphere of corn plants inoculated with PBC6.1 and grown in greenhouse conditions.
(17) FIGS. **3**A-**3**E illustrate derivative microbes that fix and excrete nitrogen in vitro under conditions similar to high nitrate agricultural soils. FIG. **3**A illustrates the regulatory network controlling nitrogen fixation and assimilation in PBC6.1 is shown, including the key nodes NifL, NifA, GS, GlnE depicted as the two-domain ATase-AR enzyme, and AmtB. FIG. **3**B illustrates the genome of *Kosakonia sacchari* isolate PBC6.1 is shown. The three tracks circumscribing the

genome convey transcription data from PBC6.1, PBC6.38, and the differential expression between the strains respectively. FIG. **3**C illustrates the nitrogen fixation gene cluster and transcription data is expanded for finer detail. FIG. **3**D illustrates nitrogenase activity under varying concentrations of exogenous nitrogen is measured with the acetylene reduction assay. The wild type strain exhibits repression of nitrogenase activity as glutamine concentrations increase, while derivative strains show varying degrees of robustness. In the line graph, triangles represent strain PBC6.22; circles represent strain PBC6.1; squares represent strain PBC6.15; and diamonds represent strain PBC6.14. Error bars represent standard error of the mean of at least three biological replicates. FIG. **3**E illustrates temporal excretion of ammonia by derivative strains is observed at mM concentrations. Wild type strains are not observed to excrete fixed nitrogen, and negligible ammonia accumulates in the media. Error bars represent standard error of the mean.

(18) FIG. **4** illustrates transcriptional rates of nifA in derivative strains of PBC6.1 correlated with acetylene reduction rates. An ARA assay was performed as described in the Methods, after which cultures were sampled and subjected to qPCR analysis to determine nifA transcript levels. Error bars show standard error of the mean of at least three biological replicates in each measure.

(19) FIGS. **5**A-**5**C illustrate greenhouse experiments that demonstrate microbial nitrogen fixation in corn. FIG. **5**A illustrates microbe colonization six weeks after inoculation of corn plants by PBC6.1 derivative strains. Error bars show standard error of the mean of at least eight biological replicates. FIG. **5**B illustrates in planta transcription of nifH measured by extraction of total RNA from roots and subsequent Nanostring analysis. Only derivative strains show nifH transcription in the root environment. Error bars show standard error of the mean of at least 3 biological replicates. FIG. **5**C illustrates microbial nitrogen fixation measured by the dilution of isotopic tracer in plant tissues. Derivative microbes exhibit substantial transfer of fixed nitrogen to the plant. Error bars show standard error of the mean of at least ten biological replicates.

(20) FIG. **6** depicts the lineage of modified strains that were derived from strain CI006.

(21) FIG. **7** depicts the lineage of modified strains that were derived from strain CI019.

(22) FIG. **8** depicts a heatmap of the pounds of nitrogen delivered per acre-season by microbes of the present disclosure recorded as a function of microbes per g-fresh weight by mmol of nitrogen/microbe-hr. Below the thin line that transects the larger image are the microbes that deliver less than one pound of nitrogen per acre-season, and above the line are the microbes that deliver greater than one pound of nitrogen per acre-season. The table below the heatmap gives the precise value of mmol N produced per microbe per hour (mmol N/Microbe hr) along with the precise CFU per gram of fresh weight (CFU/g fw) for each microbe shown in the heatmap. The microbes utilized in the heatmap were assayed for N production in corn. For the WT strains CI006 and CI019, corn root colonization data was taken from a single field site. For the remaining strains, colonization was assumed to be the same as the WT field level. N-fixation activity was determined using an in vitro ARA assay at 5 mM glutamine.

(23) FIG. **9** depicts the plant yield of plants having been exposed to strain CI006. The area of the circles corresponds to the relative yield, while the shading corresponds to the particular MRTN treatment. The x-axis is the p value and the y-axis is the win rate.

(24) FIG. **10** depicts the plant yield of plants having been exposed to strain CM029. The area of the circles corresponds to the relative yield, while the shading corresponds to the particular MRTN treatment. The x-axis is the p value and the y-axis is the win rate.

(25) FIG. **11** depicts the plant yield of plants having been exposed to strain CM038. The area of the circles corresponds to the relative yield, while the shading corresponds to the particular MRTN treatment. The x-axis is the p value and the y-axis is the win rate.

(26) FIG. **12** depicts the plant yield of plants having been exposed to strain CI019. The area of the circles corresponds to the relative yield, while the shading corresponds to the particular MRTN treatment. The x-axis is the p value and the y-axis is the win rate.

(27) FIG. **13** depicts the plant yield of plants having been exposed to strain CM081. The area of the

circles corresponds to the relative yield, while the shading corresponds to the particular MRTN treatment. The x-axis is the p value and the y-axis is the win rate.
(28) FIG. **14** depicts the plant yield of plants having been exposed to strains CM029 and CM081. The area of the circles corresponds to the relative yield, while the shading corresponds to the particular MRTN treatment. The x-axis is the p value and the y-axis is the win rate.
(29) FIG. **15** depicts the plant yield of plants as the aggregated bushel gain/loss. The area of the circles corresponds to the relative yield, while the shading corresponds to the particular MRTN treatment. The x-axis is the p value and the y-axis is the win rate.
(30) FIG. **16** illustrates results from a summer 2017 field testing experiment. The yield results obtained demonstrate that the microbes of the disclosure can serve as a potential fertilizer replacement. For instance, the utilization of a microbe of the disclosure (i.e. 6-403) resulted in a higher yield than the wild type strain (WT) and a higher yield than the untreated control (UTC). The "−25 lbs N" treatment utilizes 25 lbs less N per acre than standard agricultural practices of the region. The "100% N" UTC treatment is meant to depict standard agricultural practices of the region, in which 100% of the standard utilization of N is deployed by the farmer. The microbe "6-403" was deposited as NCMA 201708004 and can be found in Table 1. This is a mutant *Kosakonia sacchari* (also called CM037) and is a progeny mutant strain from CI006 WT.
(31) FIG. **17** illustrates results from a summer 2017 field testing experiment. The yield results obtained demonstrate that the microbes of the disclosure perform consistently across locations. Furthermore, the yield results demonstrate that the microbes of the disclosure perform well in both a nitrogen stressed environment, as well as an environment that has sufficient supplies of nitrogen. The microbe "6-881" (also known as CM094, PBC6.94), and which is a progeny mutant *Kosakonia sacchari* strain from CI006 WT, was deposited as NCMA 201708002 and can be found in Table 1. The microbe "137-1034," which is a progeny mutant *Klebsiella variicola* strain from CI137 WT, was deposited as NCMA 201712001 and can be found in Table 1. The microbe "137-1036," which is a progeny mutant *Klebsiella variicola* strain from CI137 WT, was deposited as NCMA 201712002 and can be found in Table 1. The microbe "6-404" (also known as CM38, PBC6.38), and which is a progeny mutant *Kosakonia sacchari* strain from CI006 WT, was deposited as NCMA 201708003 and can be found in Table 1. The "Nutrient Stress" condition corresponds to the 0% nitrogen regime. The "Sufficient Fertilizer" condition corresponds to the 100% nitrogen regime.
(32) FIG. **18** depicts the lineage of modified strains that were derived from strain CI006 (also termed "6", *Kosakonia sacchari* WT).
(33) FIG. **19** depicts the lineage of modified strains that were derived from strain CI019 (also termed "19", *Rahnella aquatilis* WT).
(34) FIG. **20** depicts the lineage of modified strains that were derived from strain CI137 (also termed ("137", *Klebsiella variicola* WT).
(35) FIG. **21** depicts the lineage of modified strains that were derived from strain 1021 (*Kosakonia pseudosacchari* WT).
(36) FIG. **22** depicts the lineage of modified strains that were derived from strain 910 (*Kluyvera intermedia* WT).
(37) FIG. **23** depicts the lineage of modified strains that were derived from strain 63 (*Rahnella aquatilis* WT).
(38) FIG. **24** depicts a heatmap of the pounds of nitrogen delivered per acre-season by microbes of the present disclosure recorded as a function of microbes per g-fresh weight by mmol of nitrogen/microbe-hr. Below the thin line that transects the larger image are the microbes that deliver less than one pound of nitrogen per acre-season, and above the line are the microbes that deliver greater than one pound of nitrogen per acre-season. The Table 28 in Example 5 gives the precise value of mmol N produced per microbe per hour (mmol N/Microbe hr) along with the precise CFU per gram of fresh weight (CFU/g fw) for each microbe shown in the heatmap. The

data in FIG. **24** is derived from microbial strains assayed for N production in corn in field conditions. Each point represents lb N/acre produced by a microbe using corn root colonization data from a single field site. N-fixation activity was determined using in vitro ARA assay at 5 mM N in the form of glutamine or ammonium phosphate.

(39) FIG. **25** depicts a heatmap of the pounds of nitrogen delivered per acre-season by microbes of the present disclosure recorded as a function of microbes per g-fresh weight by mmol of nitrogen/microbe-hr. Below the thin line that transects the larger image are the microbes that deliver less than one pound of nitrogen per acre-season, and above the line are the microbes that deliver greater than one pound of nitrogen per acre-season. The Table 29 in Example 5 gives the precise value of mmol N produced per microbe per hour (mmol N/Microbe hr) along with the precise CFU per gram of fresh weight (CFU/g fw) for each microbe shown in the heatmap. The data in FIG. **25** is derived from microbial strains assayed for N production in corn in laboratory and greenhouse conditions. Each point represents lb N/acre produced by a single strain. White points represent strains in which corn root colonization data was gathered in greenhouse conditions. Black points represent mutant strains for which corn root colonization levels are derived from average field corn root colonization levels of the wild-type parent strain. Hatched points represent the wild type parent strains at their average field corn root colonization levels. In all cases, N-fixation activity was determined by in vitro ARA assay at 5 mM N in the form of glutamine or ammonium phosphate.

(40) FIG. **26** depicts the type, energy source, and fixation capabilities of biological N.sub.2 fixation systems in soils.

(41) FIG. **27** depicts the nitrogen needs of a corn plant throughout the growing season. In order for a nitrogen fixing microbe to supply a corn plant with all of its nitrogen needs over a growing season, and thus completely replace synthetic fertilizer, then the microbes (in the aggregate) need to produce about 200 pounds of nitrogen per acre. FIG. **27** also illustrates that strain PBC 137-1036 (i.e. the remodeled *Klebsiella variicola*) supplies about 20 pounds of nitrogen per acre.

(42) FIG. **28**A provides a scenario whereby fertilizer could be replaced by the remodeled microbes of the disclosure. As aforementioned in FIG. **27**, the large dashed line is the nitrogen required by the corn (about 200 pounds per acre). The solid line, as already discussed, is the current nitrogen amount that can be supplied by the remodeled 137-1036 strain (about 20 pounds per acre). In the "A" bubble scenario, the inventors expect to increase the activity of the 137-1036 strain by 5 fold (see FIG. **29** for GMR campaign strategy to achieve such). In the "B" scenario, the inventors expect to utilize a remodeled microbe with a particular colonization profile that is complementary to that of the 137-1036 strain, and which will supply nitrogen to the plant at later stages of the growth cycle.

(43) FIG. **28**B shows the nitrogen production by a further remodeled strain 137-3890 at the time of the present application relative to the nitrogen production by the strain 137-1036 from the time of the provisional application. The dashed line indicates the nitrogen needs of a corn plant throughout the growing season.

(44) FIG. **29**A illustrates genetic features (i.e. non-intergeneric genetic modifications) that were used with respect to a GMR campaign for PBC6.1 (*Kosakonia sacchari*). As can be seen, the predicted N produced (lbs of N per acre) increased with each additional feature engineered into the microbial strain. In addition to the GMR campaign for PBC6.1 depicted in FIG. **29**A, one can also see the GMR campaign being executed for the PBC137 (*Klebsiella variicola*). At the time of the provisional application, the nitrogenase expression feature (F1) had been engineered into the host strain. Features 2-6 were being executed and their expected contribution to N produced (lbs of N per acre) at the time the provisional application was filed is depicted by the dashed bar graphs. These expectations were informed by the data from the PBC6.1 GMR campaign. As can be seen in FIG. **28**A scenario "A", once the GMR campaign is completed in PBC137, it is anticipated that the non-intergeneric remodeled strain (in the aggregate, considering all microbes/colonized plants in an

acre) will be capable of supplying nearly all of the nitrogen needs of a corn plant throughout the plant's early growth cycle.
(45) FIG. **29**B illustrates genetic features (i.e. non-intergeneric genetic modifications) that were used with respect to a GMR campaign for PBC6.1 (*Kosakonia sacchari*). As can be seen, the predicted N produced (lbs of N per acre) increased with each additional feature engineered into the microbial strain. In addition to the GMR campaign for PBC6.1 depicted in FIG. **29**A, one can also see the GMR campaign being executed for the PBC137 (*Klebsiella variicola*). Currently, features F1-F3 have been engineered into the host strain and features F4-F6 are being executed. As can be seen in FIG. **28**A scenario "A", once the GMR campaign is completed in PBC137, it is anticipated that the non-intergeneric remodeled strain (in the aggregate, considering all microbes/colonized plants in an acre) will be capable of supplying nearly all of the nitrogen needs of a corn plant throughout the plant's early growth cycle.
(46) FIG. **30**A depicts the same expectation as presented in FIG. **29**A, and maps the expected gains in nitrogen production to the applicable feature set.
(47) FIG. **30**B depicts N produced as mmol of N/CFU per hour by the remodeled strains of PBC137 once the features F1 (nitrogenase expression), F2 (nitrogen assimilation), and F3 (ammonium excretion) were incorporated.
(48) FIG. **31** depicts the colonization days 1-130 and the total CFU per acre of the non-intergeneric remodeled microbe of 137-1036
(49) FIG. **32** depicts the colonization days 1-130 and the total CFU per acre of the proposed non-intergeneric remodeled microbe (progeny of 137-1036, see FIG. **29** and FIG. **30** for proposed genetic alteration features),
(50) FIG. **33** depicts the colonization days 1-130 and the total CFU per acre of a proposed non-intergeneric remodeled microbe that has a complimentary colonization profile to the 137-1036 microbe. As mentioned, this microbe is expected to produce about 100 pounds of nitrogen per acre (in the aggregate) (scenario "B" in FIG. **28**), and should start colonizing at about the same time that the 137-1036 microbe begins to decline.
(51) FIG. **34** provides the colonization profile of the 137-1036 in the top panel and the colonization profile of the microbe with a later stage/complimentary colonization dynamic in the bottom panel.
(52) FIG. **35** depicts two scenarios: (1) the colonization days 1-130 and the total CFU per acre of a proposed consortia of non-intergeneric remodeled microbes that have a colonization profile as depicted, or (2) the colonization days 1-130 and the total CFU per acre of a proposed single non-intergeneric remodeled microbe that has the depicted colonization profile.
(53) FIG. **36** sets forth the general experimental design utilized in Example 9, which entailed collecting colonization and transcript samples from corn over the course of 10 weeks. These samples allowed for the calculation of colonization ability of the microbes, as well as activity of the microbes.
(54) FIG. **37** provides a visual representation of aspects of the sampling scheme utilized in Example 9, which allows for differentiation of colonization patterns between a "standard" seminal node root sample and a more "peripheral" root sample.
(55) FIG. **38** provides a visual representation of aspects of the sampling scheme utilized in Example 9.
(56) FIG. **39** illustrates that the WT 137 (*Klebsiella variicola*), 019 (*Rahnella aquatilis*), and 006 (*Kosakonia sacchari*), all have a similar colonization pattern.
(57) FIG. **40** depicts the experimental scheme utilized to sample the corn roots in Example 9. The plots: each square is a time point, the Y axis is the distance, and the X axis is the node. The standard sample was always collected along with the leading edge of growth. The periphery and intermediate samples changed week to week, but an attempt at consistency was made.
(58) FIG. **41** depicts the overall results from the Example 9, which utilized and averaged all the data taken in the sampling scheme of FIG. **40**. As can be seen from FIG. **41**, strain 137 maintains

higher colonization in peripheral roots than strain 6 or strain 19. The ‘standard sample’ was most representative for this strain when compared to samples from other root locations.
(59) FIG. **42** depicts NDVI data illustrating that the microbes of the disclosure enable reduced infield variability of a corn crop exposed to said microbes, which translates into improved yield stability for the farmer.
(60) FIG. **43** depicts the amount of ammonium excreted from eight remodeled bacterial strains. Strain 137-1036 is estimated to produce 22.15 pounds of nitrogen per acre. Strain 137-2084 is estimated to produce 38.77 pounds of nitrogen per acre. Strain 137-2219 is estimated to produce 75.74 pounds of nitrogen per acre.
DETAILED DESCRIPTION OF THE DISCLOSURE
(61) While various embodiments of the disclosure have been shown and described herein, it will be obvious to those skilled in the art that such embodiments are provided by way of example only. Numerous variations, changes, and substitutions may occur to those skilled in the art without departing from the disclosure. It should be understood that various alternatives to the embodiments of the disclosure described herein may be employed.
(62) Increased fertilizer utilization brings with it environmental concerns and is also likely not possible for many economically stressed regions of the globe. Furthermore, many industry players in the microbial arena are focused on creating intergeneric microbes. However, there is a heavy regulatory burden placed on engineered microbes that are characterized/classified as intergeneric. These intergeneric microbes face not only a higher regulatory burden, which makes widespread adoption and implementation difficult, but they also face a great deal of public perception scrutiny.
(63) Currently, there are no engineered microbes on the market that are non-intergeneric and that are capable of increasing nitrogen fixation in non-leguminous crops. This dearth of such a microbe is a missing element in helping to usher in a truly environmentally friendly and more sustainable 21.sup.st century agricultural system.
(64) The present disclosure solves the aforementioned problems and provides a non-intergeneric microbe that has been engineered to readily fix nitrogen in crops. Further, the taught non-intergeneric microbes will serve to help 21.sup.st century farmers become less dependent upon utilizing ever increasing amounts of exogenous nitrogen fertilizer.
Definitions
(65) The use of the terms “a” and “an” and “the” and similar referents in the context of describing the disclosure (especially in the context of the following claims) are to be construed to cover both the singular and the plural, unless otherwise indicated herein or clearly contradicted by context. The terms “comprising,” “having,” “including,” and “containing” are to be construed as open-ended terms (i.e., meaning “including, but not limited to,”) unless otherwise noted. Recitation of ranges of values herein are merely intended to serve as a shorthand method of referring individually to each separate value falling within the range, unless otherwise indicated herein, and each separate value is incorporated into the specification as if it were individually recited herein. For example, if the range 10-15 is disclosed, then 11, 12, 13, and 14 are also disclosed. All methods described herein can be performed in any suitable order unless otherwise indicated herein or otherwise clearly contradicted by context. The use of any and all examples, or exemplary language (e.g., “such as”) provided herein, is intended merely to better illuminate the disclosure and does not pose a limitation on the scope of the disclosure unless otherwise claimed. No language in the specification should be construed as indicating any non-claimed element as essential to the practice of the disclosure.
(66) The terms “polynucleotide”, “nucleotide”, “nucleotide sequence”, “nucleic acid” and “oligonucleotide” are used interchangeably. They refer to a polymeric form of nucleotides of any length, either deoxyribonucleotides or ribonucleotides, or analogs thereof. Polynucleotides may have any three dimensional structure, and may perform any function, known or unknown. The following are non-limiting examples of polynucleotides: coding or non-coding regions of a gene or

gene fragment, loci (locus) defined from linkage analysis, exons, introns, messenger RNA (mRNA), transfer RNA (tRNA), ribosomal RNA (rRNA), short interfering RNA (siRNA), short-hairpin RNA (shRNA), micro-RNA (miRNA), ribozymes, cDNA, recombinant polynucleotides, branched polynucleotides, plasmids, vectors, isolated DNA of any sequence, isolated RNA of any sequence, nucleic acid probes, and primers. A polynucleotide may comprise one or more modified nucleotides, such as methylated nucleotides and nucleotide analogs. If present, modifications to the nucleotide structure may be imparted before or after assembly of the polymer. The sequence of nucleotides may be interrupted by non-nucleotide components. A polynucleotide may be further modified after polymerization, such as by conjugation with a labeling component.

(67) "Hybridization" refers to a reaction in which one or more polynucleotides react to form a complex that is stabilized via hydrogen bonding between the bases of the nucleotide residues. The hydrogen bonding may occur by Watson Crick base pairing, Hoogstein binding, or in any other sequence specific manner according to base complementarity. The complex may comprise two strands forming a duplex structure, three or more strands forming a multi stranded complex, a single self-hybridizing strand, or any combination of these. A hybridization reaction may constitute a step in a more extensive process, such as the initiation of PCR, or the enzymatic cleavage of a polynucleotide by an endonuclease. A second sequence that is complementary to a first sequence is referred to as the "complement" of the first sequence. The term "hybridizable" as applied to a polynucleotide refers to the ability of the polynucleotide to form a complex that is stabilized via hydrogen bonding between the bases of the nucleotide residues in a hybridization reaction.

(68) "Complementarity" refers to the ability of a nucleic acid to form hydrogen bond(s) with another nucleic acid sequence by either traditional Watson-Crick or other non-traditional types. A percent complementarity indicates the percentage of residues in a nucleic acid molecule which can form hydrogen bonds (e.g., Watson-Crick base pairing) with a second nucleic acid sequence (e.g., 5, 6, 7, 8, 9, 10 out of 10 being 50%, 60%, 70%, 80%, 90%, and 100% complementary, respectively). "Perfectly complementary" means that all the contiguous residues of a nucleic acid sequence will hydrogen bond with the same number of contiguous residues in a second nucleic acid sequence. "Substantially complementary" as used herein refers to a degree of complementarity that is at least 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, 97%, 98%, 99%, or 100% over a region of 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 30, 35, 40, 45, 50, or more nucleotides, or refers to two nucleic acids that hybridize under stringent conditions. Sequence identity, such as for the purpose of assessing percent complementarity, may be measured by any suitable alignment algorithm, including but not limited to the Needleman-Wunsch algorithm (see e.g. the EMBOSS Needle aligner available at www.ebi.ac.uk/Tools/psa/emboss needle/nucleotide.html, optionally with default settings), the BLAST algorithm (see e.g. the BLAST alignment tool available at blast.ncbi.nlm.nih.gov/Blast.cgi, optionally with default settings), or the Smith-Waterman algorithm (see e.g. the EMBOSS Water aligner available at www.ebi.ac.uk/Tools/psa/emboss_water/nucleotide.html, optionally with default settings). Optimal alignment may be assessed using any suitable parameters of a chosen algorithm, including default parameters.

(69) In general, "stringent conditions" for hybridization refer to conditions under which a nucleic acid having complementarity to a target sequence predominantly hybridizes with a target sequence, and substantially does not hybridize to non-target sequences. Stringent conditions are generally sequence-dependent and vary depending on a number of factors. In general, the longer the sequence, the higher the temperature at which the sequence specifically hybridizes to its target sequence. Non-limiting examples of stringent conditions are described in detail in Tijssen (1993), Laboratory Techniques In Biochemistry And Molecular Biology-Hybridization With Nucleic Acid Probes Part I, Second Chapter "Overview of principles of hybridization and the strategy of nucleic acid probe assay", Elsevier, N.Y.

(70) As used herein, "expression" refers to the process by which a polynucleotide is transcribed

from a DNA template (such as into and mRNA or other RNA transcript) and/or the process by which a transcribed mRNA is subsequently translated into peptides, polypeptides, or proteins. Transcripts and encoded polypeptides may be collectively referred to as “gene product.” If the polynucleotide is derived from genomic DNA, expression may include splicing of the mRNA in a eukaryotic cell.

(71) The terms “polypeptide”, “peptide” and “protein” are used interchangeably herein to refer to polymers of amino acids of any length. The polymer may be linear or branched, it may comprise modified amino acids, and it may be interrupted by non-amino acids. The terms also encompass an amino acid polymer that has been modified; for example, disulfide bond formation, glycosylation, lipidation, acetylation, phosphorylation, or any other manipulation, such as conjugation with a labeling component. As used herein the term “amino acid” includes natural and/or unnatural or synthetic amino acids, including glycine and both the D or L optical isomers, and amino acid analogs and peptidomimetics.

(72) As used herein, the term “about” is used synonymously with the term “approximately.” Illustratively, the use of the term “about” with regard to an amount indicates that values slightly outside the cited values, e.g., plus or minus 0.1% to 10%.

(73) The term “biologically pure culture” or “substantially pure culture” refers to a culture of a bacterial species described herein containing no other bacterial species in quantities sufficient to interfere with the replication of the culture or be detected by normal bacteriological techniques.

(74) “Plant productivity” refers generally to any aspect of growth or development of a plant that is a reason for which the plant is grown. For food crops, such as grains or vegetables, “plant productivity” can refer to the yield of grain or fruit harvested from a particular crop. As used herein, improved plant productivity refers broadly to improvements in yield of grain, fruit, flowers, or other plant parts harvested for various purposes, improvements in growth of plant parts, including stems, leaves and roots, promotion of plant growth, maintenance of high chlorophyll content in leaves, increasing fruit or seed numbers, increasing fruit or seed unit weight, reducing NO.sub.2 emission due to reduced nitrogen fertilizer usage and similar improvements of the growth and development of plants.

(75) Microbes in and around food crops can influence the traits of those crops. Plant traits that may be influenced by microbes include: yield (e.g., grain production, biomass generation, fruit development, flower set); nutrition (e.g., nitrogen, phosphorus, potassium, iron, micronutrient acquisition); abiotic stress management (e.g., drought tolerance, salt tolerance, heat tolerance); and biotic stress management (e.g., pest, weeds, insects, fungi, and bacteria). Strategies for altering crop traits include: increasing key metabolite concentrations; changing temporal dynamics of microbe influence on key metabolites; linking microbial metabolite production/degradation to new environmental cues; reducing negative metabolites; and improving the balance of metabolites or underlying proteins.

(76) As used herein, a “control sequence” refers to an operator, promoter, silencer, or terminator.

(77) As used herein, “in planta” may refer to in the plant, on the plant, or intimately associated with the plant, depending upon context of usage (e.g. endophytic, epiphytic, or rhizospheric associations). The plant may comprise plant parts, tissue, leaves, roots, root hairs, rhizomes, stems, seed, ovules, pollen, flowers, fruit, etc.

(78) In some embodiments, native or endogenous control sequences of genes of the present disclosure are replaced with one or more intrageneric control sequences.

(79) As used herein, “introduced” refers to the introduction by means of modern biotechnology, and not a naturally occurring introduction.

(80) In some embodiments, the bacteria of the present disclosure have been modified such that they are not naturally occurring bacteria.

(81) In some embodiments, the bacteria of the present disclosure are present in the plant in an amount of at least 10.sup.3 cfu, 10.sup.4 cfu, 10.sup.5 cfu, 10.sup.6 cfu, 10.sup.7 cfu, 10.sup.8 cfu,

10.sup.9 cfu, 10.sup.10 cfu, 10.sup.11 cfu, or 10.sup.12 cfu per gram of fresh or dry weight of the plant. In some embodiments, the bacteria of the present disclosure are present in the plant in an amount of at least about 10.sup.3 cfu, about 10.sup.4 cfu, about 10.sup.5 cfu, about 10.sup.6 cfu, about 10.sup.7 cfu, about 10.sup.8 cfu, about 10.sup.9 cfu, about 10.sup.10 cfu, about 10.sup.11 cfu, or about 10.sup.12 cfu per gram of fresh or dry weight of the plant. In some embodiments, the bacteria of the present disclosure are present in the plant in an amount of at least 10.sup.3 to 10.sup.9, 10.sup.3 to 10.sup.7, 10.sup.3 to 10.sup.5, 10.sup.5 to 10.sup.9, 10.sup.5 to 10.sup.7, 10.sup.6 to 10.sup.10, 10.sup.6 to 10.sup.7 cfu per gram of fresh or dry weight of the plant.

(82) Fertilizers and exogenous nitrogen of the present disclosure may comprise the following nitrogen-containing molecules: ammonium, nitrate, nitrite, ammonia, glutamine, etc. Nitrogen sources of the present disclosure may include anhydrous ammonia, ammonia sulfate, urea, diammonium phosphate, urea-form, monoammonium phosphate, ammonium nitrate, nitrogen solutions, calcium nitrate, potassium nitrate, sodium nitrate, etc.

(83) As used herein, “exogenous nitrogen” refers to non-atmospheric nitrogen readily available in the soil, field, or growth medium that is present under non-nitrogen limiting conditions, including ammonia, ammonium, nitrate, nitrite, urea, uric acid, ammonium acids, etc.

(84) As used herein, “non-nitrogen limiting conditions” refers to non-atmospheric nitrogen available in the soil, field, media at concentrations greater than about 4 mM nitrogen, as disclosed by Kant et al. (2010. J. Exp. Biol. 62(4):1499-1509), which is incorporated herein by reference.

(85) As used herein, an “intergeneric microorganism” is a microorganism that is formed by the deliberate combination of genetic material originally isolated from organisms of different taxonomic genera. An “intergeneric mutant” can be used interchangeably with “intergeneric microorganism”. An exemplary “intergeneric microorganism” includes a microorganism containing a mobile genetic element which was first identified in a microorganism in a genus different from the recipient microorganism. Further explanation can be found, inter alia, in 40 C.F.R. § 725.3.

(86) In aspects, microbes taught herein are “non-intergeneric,” which means that the microbes are not intergeneric.

(87) As used herein, an “intrageneric microorganism” is a microorganism that is formed by the deliberate combination of genetic material originally isolated from organisms of the same taxonomic genera. An “intrageneric mutant” can be used interchangeably with “intrageneric microorganism.”

(88) As used herein, “introduced genetic material” means genetic material that is added to, and remains as a component of, the genome of the recipient.

(89) As used herein, in the context of non-intergeneric microorganisms, the term “remodeled” is used synonymously with the term “engineered”. Consequently, a “non-intergeneric remodeled microorganism” has a synonymous meaning to “non-intergeneric engineered microorganism,” and will be utilized interchangeably. Further, the disclosure may refer to an “engineered strain” or “engineered derivative” or “engineered non-intergeneric microbe,” these terms are used synonymously with “remodeled strain” or “remodeled derivative” or “remodeled non-intergeneric microbe.”

(90) In some embodiments, the nitrogen fixation and assimilation genetic regulatory network comprises polynucleotides encoding genes and non-coding sequences that direct, modulate, and/or regulate microbial nitrogen fixation and/or assimilation and can comprise polynucleotide sequences of the nif cluster (e.g., nifA, nijB, nifC, . . . nifZ), polynucleotides encoding nitrogen regulatory protein C, polynucleotides encoding nitrogen regulatory protein B, polynucleotide sequences of the gln cluster (e.g. glnA and glnD), draT, and ammonia transporters/permeases. In some cases, the Nif cluster may comprise NifB, NifH, NifD, NifK, NifE, NifN, NifX, hesa, and NifV. In some cases, the Nif cluster may comprise a subset of NifB, NifH, NifD, NifK, NifE, NifN, NifX, hesa, and NifV.

(91) In some embodiments, fertilizer of the present disclosure comprises at least 5%, 6%, 7%, 8%,

9%, 10%, 11%, 12%, 13%, 14%, 15%, 16%, 17%, 18%, 19%, 20%, 21%, 22%, 23%, 24%, 25%, 26%, 27%, 28%, 29%, 30%, 31%, 32%, 33%, 34%, 35%, 36%, 37%, 38%, 39%, 40%, 41%, 42%, 43%, 44%, 45%, 46%, 47%, 48%, 49%, 50%, 51%, 52%, 53%, 54%, 55%, 56%, 57%, 58%, 59%, 60%, 61%, 62%, 63%, 64%, 65%, 66%, 67%, 68%, 69%, 70%, 71%, 72%, 73%, 74%, 75%, 76%, 77%, 78%, 79%, 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% nitrogen by weight.

(92) In some embodiments, fertilizer of the present disclosure comprises at least about 5%, about 6%, about 7%, about 8%, about 9%, about 10%, about 11%, about 12%, about 13%, about 14%, about 15%, about 16%, about 17%, about 18%, about 19%, about 20%, about 21%, about 22%, about 23%, about 24%, about 25%, about 26%, about 27%, about 28%, about 29%, about 30%, about 31%, about 32%, about 33%, about 34%, about 35%, about 36%, about 37%, about 38%, about 39%, about 40%, about 41%, about 42%, about 43%, about 44%, about 45%, about 46%, about 47%, about 48%, about 49%, about 50%, about 51%, about 52%, about 53%, about 54%, about 55%, about 56%, about 57%, about 58%, about 59%, about 60%, about 61%, about 62%, about 63%, about 64%, about 65%, about 66%, about 67%, about 68%, about 69%, about 70%, about 71%, about 72%, about 73%, about 74%, about 75%, about 76%, about 77%, about 78%, about 79%, about 80%, about 81%, about 82%, about 83%, about 84%, about 85%, about 86%, about 87%, about 88%, about 89%, about 90%, about 91%, about 92%, about 93%, about 94%, about 95%, about 96%, about 97%, about 98%, or about 99% nitrogen by weight.

(93) In some embodiments, fertilizer of the present disclosure comprises about 5% to 50%, about 5% to 75%, about 10% to 50%, about 10% to 75%, about 15% to 50%, about 15% to 75%, about 20% to 50%, about 20% to 75%, about 25% to 50%, about 25% to 75%, about 30% to 50%, about 30% to 75%, about 35% to 50%, about 35% to 75%, about 40% to 50%, about 40% to 75%, about 45% to 50%, about 45% to 75%, or about 50% to 75% nitrogen by weight.

(94) In some embodiments, the increase of nitrogen fixation and/or the production of 1% or more of the nitrogen in the plant are measured relative to control plants, which have not been exposed to the bacteria of the present disclosure. All increases or decreases in bacteria are measured relative to control bacteria. All increases or decreases in plants are measured relative to control plants.

(95) As used herein, a “constitutive promoter” is a promoter, which is active under most conditions and/or during most development stages. There are several advantages to using constitutive promoters in expression vectors used in biotechnology, such as: high level of production of proteins used to select transgenic cells or organisms; high level of expression of reporter proteins or scorable markers, allowing easy detection and quantification; high level of production of a transcription factor that is part of a regulatory transcription system; production of compounds that requires ubiquitous activity in the organism; and production of compounds that are required during all stages of development. Non-limiting exemplary constitutive promoters include, CaMV 35S promoter, opine promoters, ubiquitin promoter, alcohol dehydrogenase promoter, etc.

(96) As used herein, a “non-constitutive promoter” is a promoter which is active under certain conditions, in certain types of cells, and/or during certain development stages. For example, tissue specific, tissue preferred, cell type specific, cell type preferred, inducible promoters, and promoters under development control are non-constitutive promoters. Examples of promoters under developmental control include promoters that preferentially initiate transcription in certain tissues.

(97) As used herein, “inducible” or “repressible” promoter is a promoter which is under chemical or environmental factors control. Examples of environmental conditions that may affect transcription by inducible promoters include anaerobic conditions, certain chemicals, the presence of light, acidic or basic conditions, etc.

(98) As used herein, a “tissue specific” promoter is a promoter that initiates transcription only in certain tissues. Unlike constitutive expression of genes, tissue-specific expression is the result of several interacting levels of gene regulation. As such, in the art sometimes it is preferable to use promoters from homologous or closely related species to achieve efficient and reliable expression

of transgenes in particular tissues. This is one of the main reasons for the large amount of tissue-specific promoters isolated from particular tissues found in both scientific and patent literature.
(99) As used herein, the term “operably linked” refers to the association of nucleic acid sequences on a single nucleic acid fragment so that the function of one is regulated by the other. For example, a promoter is operably linked with a coding sequence when it is capable of regulating the expression of that coding sequence (i.e., that the coding sequence is under the transcriptional control of the promoter). Coding sequences can be operably linked to regulatory sequences in a sense or antisense orientation. In another example, the complementary RNA regions of the disclosure can be operably linked, either directly or indirectly, 5′ to the target mRNA, or 3′ to the target mRNA, or within the target mRNA, or a first complementary region is 5′ and its complement is 3′ to the target mRNA.
(100) In aspects, “applying to the plant a plurality of non-intergeneric bacteria,” includes any means by which the plant (including plant parts such as a seed, root, stem, tissue, etc.) is made to come into contact (i.e. exposed) with said bacteria at any stage of the plant's life cycle. Consequently, “applying to the plant a plurality of non-intergeneric bacteria,” includes any of the following means of exposing the plant (including plant parts such as a seed, root, stem, tissue, etc.) to said bacteria: spraying onto plant, dripping onto plant, applying as a seed coat, applying to a field that will then be planted with seed, applying to a field already planted with seed, applying to a field with adult plants, etc.
(101) As used herein “MRTN” is an acronym for maximum return to nitrogen and is utilized as an experimental treatment in the Examples. MRTN was developed by Iowa State University and information can be found at: cnrc.agron.iastate.edu/. The MRTN is the nitrogen rate where the economic net return to nitrogen application is maximized. The approach to calculating the MRTN is a regional approach for developing corn nitrogen rate guidelines in individual states. The nitrogen rate trial data was evaluated for Illinois, Iowa, Michigan, Minnesota, Ohio, and Wisconsin where an adequate number of research trials were available for corn plantings following soybean and corn plantings following corn. The trials were conducted with spring, sidedress, or split preplant/sidedress applied nitrogen, and sites were not irrigated except for those that were indicated for irrigated sands in Wisconsin. MRTN was developed by Iowa State University due to apparent differences in methods for determining suggested nitrogen rates required for corn production, misperceptions pertaining to nitrogen rate guidelines, and concerns about application rates. By calculating the MRTN, practitioners can determine the following: (1) the nitrogen rate where the economic net return to nitrogen application is maximized, (2) the economic optimum nitrogen rate, which is the point where the last increment of nitrogen returns a yield increase large enough to pay for the additional nitrogen, (3) the value of corn grain increase attributed to nitrogen application, and the maximum yield, which is the yield where application of more nitrogen does not result in a corn yield increase. Thus the MRTN calculations provide practitioners with the means to maximize corn crops in different regions while maximizing financial gains from nitrogen applications.
(102) The term mmol is an abbreviation for millimole, which is a thousandth (10.sup.−3) of a mole, abbreviated herein as mol.
(103) As used herein the term “plant” can include plant parts, tissue, leaves, roots, root hairs, rhizomes, stems, seeds, ovules, pollen, flowers, fruit, etc. Thus, when the disclosure discusses providing a plurality of corn plants to a particular locus, it is understood that this may entail planting a corn seed at a particular locus.
(104) As used herein the terms “microorganism” or “microbe” should be taken broadly. These terms, used interchangeably, include but are not limited to, the two prokaryotic domains, Bacteria and Archaea. The term may also encompass eukaryotic fungi and protists.
(105) As used herein, when the disclosure discuses a particular microbial deposit by accession number, it is understood that the disclosure also contemplates a microbial strain having all of the identifying characteristics of said deposited microbe, and/or a mutant thereof.

(106) The term “microbial consortia” or “microbial consortium” refers to a subset of a microbial community of individual microbial species, or strains of a species, which can be described as carrying out a common function, or can be described as participating in, or leading to, or correlating with, a recognizable parameter, such as a phenotypic trait of interest.
(107) The term “microbial community” means a group of microbes comprising two or more species or strains. Unlike microbial consortia, a microbial community does not have to be carrying out a common function, or does not have to be participating in, or leading to, or correlating with, a recognizable parameter, such as a phenotypic trait of interest.
(108) As used herein, “isolate,” “isolated,” “isolated microbe,” and like terms, are intended to mean that the one or more microorganisms has been separated from at least one of the materials with which it is associated in a particular environment (for example soil, water, plant tissue, etc.). Thus, an “isolated microbe” does not exist in its naturally occurring environment; rather, it is through the various techniques described herein that the microbe has been removed from its natural setting and placed into a non-naturally occurring state of existence. Thus, the isolated strain or isolated microbe may exist as, for example, a biologically pure culture, or as spores (or other forms of the strain). In aspects, the isolated microbe may be in association with an acceptable carrier, which may be an agriculturally acceptable carrier.
(109) In certain aspects of the disclosure, the isolated microbes exist as “isolated and biologically pure cultures.” It will be appreciated by one of skill in the art, that an isolated and biologically pure culture of a particular microbe, denotes that said culture is substantially free of other living organisms and contains only the individual microbe in question. The culture can contain varying concentrations of said microbe. The present disclosure notes that isolated and biologically pure microbes often “necessarily differ from less pure or impure materials.” See, e.g. In re Bergstrom, 427 F.2d 1394, (CCPA 1970)(discussing purified prostaglandins), see also, In re Bergy, 596 F.2d 952 (CCPA 1979)(discussing purified microbes), see also, Parke-Davis & Co. v. H. K. Mulford & Co., 189 F. 95 (S.D.N.Y. 1911) (Learned Hand discussing purified adrenaline), aff'd in part, rev'd in part, 196 F. 496 (2d Cir. 1912), each of which are incorporated herein by reference. Furthermore, in some aspects, the disclosure provides for certain quantitative measures of the concentration, or purity limitations, that must be found within an isolated and biologically pure microbial culture. The presence of these purity values, in certain embodiments, is a further attribute that distinguishes the presently disclosed microbes from those microbes existing in a natural state. See, e.g., Merck & Co. v. Olin Mathieson Chemical Corp., 253 F.2d 156 (4th Cir. 1958) (discussing purity limitations for vitamin B12 produced by microbes), incorporated herein by reference.
(110) As used herein, “individual isolates” should be taken to mean a composition, or culture, comprising a predominance of a single genera, species, or strain, of microorganism, following separation from one or more other microorganisms.
(111) Microbes of the present disclosure may include spores and/or vegetative cells. In some embodiments, microbes of the present disclosure include microbes in a viable but non-culturable (VBNC) state. As used herein, “spore” or “spores” refer to structures produced by bacteria and fungi that are adapted for survival and dispersal. Spores are generally characterized as dormant structures; however, spores are capable of differentiation through the process of germination. Germination is the differentiation of spores into vegetative cells that are capable of metabolic activity, growth, and reproduction. The germination of a single spore results in a single fungal or bacterial vegetative cell. Fungal spores are units of asexual reproduction, and in some cases are necessary structures in fungal life cycles. Bacterial spores are structures for surviving conditions that may ordinarily be nonconducive to the survival or growth of vegetative cells.
(112) As used herein, “microbial composition” refers to a composition comprising one or more microbes of the present disclosure. In some embodiments, a microbial composition is administered to plants (including various plant parts) and/or in agricultural fields.
(113) As used herein, “carrier,” “acceptable carrier,” or “agriculturally acceptable carrier” refers to

a diluent, adjuvant, excipient, or vehicle with which the microbe can be administered, which does not detrimentally effect the microbe.

(114) In some embodiments, the microbes and/or genetic modifications disclosed herein are not the microbes taught in PCT/US2018/013671 (WO 2018/132774 A1), filed Jan. 12, 2018, and entitled: Methods and Compositions for Improving Plant Traits. In some embodiments, the methods disclosed herein are not the methods taught in PCT/US2018/013671 (WO 2018/132774 A1), filed Jan. 12, 2018, and entitled: Methods and Compositions for Improving Plant Traits. Thus, the present disclosure contemplates embodiments, which have a negative proviso of the microbes, methods, and gene modifications disclosed in said application.

(115) Regulation of Nitrogen Fixation

(116) In some cases, nitrogen fixation pathway may act as a target for genetic engineering and optimization. One trait that may be targeted for regulation by the methods described herein is nitrogen fixation. Nitrogen fertilizer is the largest operational expense on a farm and the biggest driver of higher yields in row crops like corn and wheat. Described herein are microbial products that can deliver renewable forms of nitrogen in non-leguminous crops. While some endophytes have the genetics necessary for fixing nitrogen in pure culture, the fundamental technical challenge is that wild-type endophytes of cereals and grasses stop fixing nitrogen in fertilized fields. The application of chemical fertilizers and residual nitrogen levels in field soils signal the microbe to shut down the biochemical pathway for nitrogen fixation.

(117) Changes to the transcriptional and post-translational levels of components of the nitrogen fixation regulatory network may be beneficial to the development of a microbe capable of fixing and transferring nitrogen to corn in the presence of fertilizer. To that end, described herein is Host-Microbe Evolution (HoME) technology to precisely evolve regulatory networks and elicit novel phenotypes. Also described herein are unique, proprietary libraries of nitrogen-fixing endophytes isolated from corn, paired with extensive omics data surrounding the interaction of microbes and host plant under different environmental conditions like nitrogen stress and excess. In some embodiments, this technology enables precision evolution of the genetic regulatory network of endophytes to produce microbes that actively fix nitrogen even in the presence of fertilizer in the field. Also described herein are evaluations of the technical potential of evolving microbes that colonize corn root tissues and produce nitrogen for fertilized plants and evaluations of the compatibility of endophytes with standard formulation practices and diverse soils to determine feasibility of integrating the microbes into modern nitrogen management strategies.

(118) In order to utilize elemental nitrogen (N) for chemical synthesis, life forms combine nitrogen gas (N.sub.2) available in the atmosphere with hydrogen in a process known as nitrogen fixation. Because of the energy-intensive nature of biological nitrogen fixation, diazotrophs (bacteria and archaea that fix atmospheric nitrogen gas) have evolved sophisticated and tight regulation of the nif gene cluster in response to environmental oxygen and available nitrogen. Nif genes encode enzymes involved in nitrogen fixation (such as the nitrogenase complex) and proteins that regulate nitrogen fixation. Shamseldin (2013. Global J. Biotechnol. Biochem. 8(4):84-94) discloses detailed descriptions of nif genes and their products, and is incorporated herein by reference. Described herein are methods of producing a plant with an improved trait comprising isolating bacteria from a first plant, introducing a genetic variation into a gene of the isolated bacteria to increase nitrogen fixation, exposing a second plant to the variant bacteria, isolating bacteria from the second plant having an improved trait relative to the first plant, and repeating the steps with bacteria isolated from the second plant.

(119) In Proteobacteria, regulation of nitrogen fixation centers around the 654-dependent enhancer-binding protein NifA, the positive transcriptional regulator of the nif cluster. Intracellular levels of active NifA are controlled by two key factors: transcription of the nifLA operon, and inhibition of NifA activity by protein-protein interaction with NifL. Both of these processes are responsive to intracellular glutamine levels via the PII protein signaling cascade. This cascade is mediated by

GlnD, which directly senses glutamine and catalyzes the uridylylation or deuridylylation of two PII regulatory proteins—GlnB and GlnK—in response the absence or presence, respectively, of bound glutamine. Under conditions of nitrogen excess, unmodified GlnB signals the deactivation of the nifLA promoter. However, under conditions of nitrogen limitation, GlnB is post-translationally modified, which inhibits its activity and leads to transcription of the nifLA operon. In this way, nifLA transcription is tightly controlled in response to environmental nitrogen via the PII protein signaling cascade. On the post-translational level of NifA regulation, GlnK inhibits the NifL/NifA interaction in a matter dependent on the overall level of free GlnK within the cell.

(120) NifA is transcribed from the nifLA operon, whose promoter is activated by phosphorylated NtrC, another 654-dependent regulator. The phosphorylation state of NtrC is mediated by the histidine kinase NtrB, which interacts with deuridylylated GlnB but not uridylylated GlnB. Under conditions of nitrogen excess, a high intracellular level of glutamine leads to deuridylylation of GlnB, which then interacts with NtrB to deactivate its phosphorylation activity and activate its phosphatase activity, resulting in dephosphorylation of NtrC and the deactivation of the nifLA promoter. However, under conditions of nitrogen limitation, a low level of intracellular glutamine results in uridylylation of GlnB, which inhibits its interaction with NtrB and allows the phosphorylation of NtrC and transcription of the nifLA operon. In this way, nifLA expression is tightly controlled in response to environmental nitrogen via the PII protein signaling cascade. nifA, ntrB, ntrC, and glnB, are all genes that can be mutated in the methods described herein. These processes may also be responsive to intracellular or extracellular levels of ammonia, urea or nitrates.

(121) The activity of NifA is also regulated post-translationally in response to environmental nitrogen, most typically through NifL-mediated inhibition of NifA activity. In general, the interaction of NifL and NifA is influenced by the PII protein signaling cascade via GlnK, although the nature of the interactions between GlnK and NifL/NifA varies significantly between diazotrophs. In *Klebsiella pneumoniae*, both forms of GlnK inhibit the NifL/NifA interaction, and the interaction between GlnK and NifL/NifA is determined by the overall level of free GlnK within the cell. Under nitrogen-excess conditions, deuridylylated GlnK interacts with the ammonium transporter AmtB, which serves to both block ammonium uptake by AmtB and sequester GlnK to the membrane, allowing inhibition of NifA by NifL. On the other hand, in *Azotobacter vinelandii*, interaction with deuridylylated GlnK is required for the NifL/NifA interaction and NifA inhibition, while uridylylation of GlnK inhibits its interaction with NifL. In diazotrophs lacking the nifL gene, there is evidence that NifA activity is inhibited directly by interaction with the deuridylylated forms of both GlnK and GlnB under nitrogen-excess conditions. In some bacteria the Nif cluster may be regulated by glnR, and further in some cases this may comprise negative regulation. Regardless of the mechanism, post-translational inhibition of NifA is an important regulator of the nif cluster in most known diazotrophs. Additionally, nifL, amtB, glnK, and glnR are genes that can be mutated in the methods described herein.

(122) In addition to regulating the transcription of the nif gene cluster, many diazotrophs have evolved a mechanism for the direct post-translational modification and inhibition of the nitrogenase enzyme itself, known as nitrogenase shutoff. This is mediated by ADP-ribosylation of the Fe protein (NifH) under nitrogen-excess conditions, which disrupts its interaction with the MoFe protein complex (NifDK) and abolishes nitrogenase activity. DraT catalyzes the ADP-ribosylation of the Fe protein and shutoff of nitrogenase, while DraG catalyzes the removal of ADP-ribose and reactivation of nitrogenase. As with nifLA transcription and NifA inhibition, nitrogenase shutoff is also regulated via the PII protein signaling cascade. Under nitrogen-excess conditions, deuridylylated GlnB interacts with and activates DraT, while deuridylylated GlnK interacts with both DraG and AmtB to form a complex, sequestering DraG to the membrane. Under nitrogen-limiting conditions, the uridylylated forms of GlnB and GlnK do not interact with DraT and DraG, respectively, leading to the inactivation of DraT and the diffusion of DraG to the Fe protein, where

it removes the ADP-ribose and activates nitrogenase. The methods described herein also contemplate introducing genetic variation into the nifH, nifD, nifK, and draT genes.

(123) Although some endophytes have the ability to fix nitrogen in vitro, often the genetics are silenced in the field by high levels of exogenous chemical fertilizers. One can decouple the sensing of exogenous nitrogen from expression of the nitrogenase enzyme to facilitate field-based nitrogen fixation. Improving the integral of nitrogenase activity across time further serves to augment the production of nitrogen for utilization by the crop. Specific targets for genetic variation to facilitate field-based nitrogen fixation using the methods described herein include one or more genes selected from the group consisting of nifA, nifL, ntrB, ntrC, glnA, glnB, glnK, draT, amtB, glnD, glnE, nifJ, nifH, nifD, nifK, nifY, nifE, nifN, nifU, nifS, nifV, nifW, nifZ, nifM, nifF, nifB, and nifQ.

(124) An additional target for genetic variation to facilitate field-based nitrogen fixation using the methods described herein is the NifA protein. The NifA protein is typically the activator for expression of nitrogen fixation genes. Increasing the production of NifA (either constitutively or during high ammonia condition) circumvents the native ammonia-sensing pathway. In addition, reducing the production of NifL proteins, a known inhibitor of NifA, also leads to an increased level of freely active NifA. In addition, increasing the transcription level of the nifAL operon (either constitutively or during high ammonia condition) also leads to an overall higher level of NifA proteins. Elevated level of nifAL expression is achieved by altering the promoter itself or by reducing the expression of NtrB (part of ntrB and ntrC signaling cascade that originally would result in the shutoff of nifAL operon during high nitrogen condition). High level of NifA achieved by these or any other methods described herein increases the nitrogen fixation activity of the endophytes.

(125) Another target for genetic variation to facilitate field-based nitrogen fixation using the methods described herein is the GlnD/GlnB/GlnK PII signaling cascade. The intracellular glutamine level is sensed through the GlnD/GlnB/GlnK PII signaling cascade. Active site mutations in GlnD that abolish the uridylyl-removing activity of GlnD disrupt the nitrogen-sensing cascade. In addition, reduction of the GlnB concentration short circuits the glutamine-sensing cascade. These mutations “trick” the cells into perceiving a nitrogen-limited state, thereby increasing the nitrogen fixation level activity. These processes may also be responsive to intracellular or extracellular levels of ammonia, urea or nitrates.

(126) The amtB protein is also a target for genetic variation to facilitate field-based nitrogen fixation using the methods described herein. Ammonia uptake from the environment can be reduced by decreasing the expression level of amtB protein. Without intracellular ammonia, the endophyte is not able to sense the high level of ammonia, preventing the down-regulation of nitrogen fixation genes. Any ammonia that manages to get into the intracellular compartment is converted into glutamine. Intracellular glutamine level is the major currency of nitrogen sensing. Decreasing the intracellular glutamine level prevents the cells from sensing high ammonium levels in the environment. This effect can be achieved by increasing the expression level of glutaminase, an enzyme that converts glutamine into glutamate. In addition, intracellular glutamine can also be reduced by decreasing glutamine synthase (an enzyme that converts ammonia into glutamine). In diazotrophs, fixed ammonia is quickly assimilated into glutamine and glutamate to be used for cellular processes. Disruptions to ammonia assimilation may enable diversion of fixed nitrogen to be exported from the cell as ammonia. The fixed ammonia is predominantly assimilated into glutamine by glutamine synthetase (GS), encoded by glnA, and subsequently into glutamine by glutamine oxoglutarate aminotransferase (GOGAT). In some examples, glnS encodes a glutamine synthetase. GS is regulated post-translationally by GS adenylyl transferase (GlnE), a bi-functional enzyme encoded by glnE that catalyzes both the adenylylation and de-adenylylation of GS through activity of its adenylyl-transferase (AT) and adenylyl-removing (AR) domains, respectively. Under nitrogen limiting conditions, glnA is expressed, and GlnE's AR domain de-adynylylates GS,

allowing it to be active. Under conditions of nitrogen excess, glnA expression is turned off, and GlnE's AT domain is activated allosterically by glutamine, causing the adenylylation and deactivation of GS.
(127) Furthermore, the draT gene may also be a target for genetic variation to facilitate field-based nitrogen fixation using the methods described herein. Once nitrogen fixing enzymes are produced by the cell, nitrogenase shut-off represents another level in which cell downregulates fixation activity in high nitrogen condition. This shut-off could be removed by decreasing the expression level of DraT.
(128) Methods for imparting new microbial phenotypes can be performed at the transcriptional, translational, and post-translational levels. The transcriptional level includes changes at the promoter (such as changing sigma factor affinity or binding sites for transcription factors, including deletion of all or a portion of the promoter) or changing transcription terminators and attenuators. The translational level includes changes at the ribosome binding sites and changing mRNA degradation signals. The post-translational level includes mutating an enzyme's active site and changing protein-protein interactions. These changes can be achieved in a multitude of ways. Reduction of expression level (or complete abolishment) can be achieved by swapping the native ribosome binding site (RBS) or promoter with another with lower strength/efficiency. ATG start sites can be swapped to a GTG, TTG, or CTG start codon, which results in reduction in translational activity of the coding region. Complete abolishment of expression can be done by knocking out (deleting) the coding region of a gene. Frameshifting the open reading frame (ORF) likely will result in a premature stop codon along the ORF, thereby creating a non-functional truncated product. Insertion of in-frame stop codons will also similarly create a non-functional truncated product. Addition of a degradation tag at the N or C terminal can also be done to reduce the effective concentration of a particular gene.
(129) Conversely, expression level of the genes described herein can be achieved by using a stronger promoter. To ensure high promoter activity during high nitrogen level condition (or any other condition), a transcription profile of the whole genome in a high nitrogen level condition could be obtained and active promoters with a desired transcription level can be chosen from that dataset to replace the weak promoter. Weak start codons can be swapped out with an ATG start codon for better translation initiation efficiency. Weak ribosomal binding sites (RBS) can also be swapped out with a different RBS with higher translation initiation efficiency. In addition, site-specific mutagenesis can also be performed to alter the activity of an enzyme.
(130) Increasing the level of nitrogen fixation that occurs in a plant can lead to a reduction in the amount of chemical fertilizer needed for crop production and reduce greenhouse gas emissions (e.g., nitrous oxide).
(131) Regulation of Colonization Potential
(132) One trait that may be targeted for regulation by the methods described herein is colonization potential. Accordingly, in some embodiments, pathways and genes involved in colonization may act as a target for genetic engineering and optimization.
(133) In some cases, exopolysaccharides may be involved in bacterial colonization of plants. In some cases, plant colonizing microbes may produce a biofilm. In some cases, plant colonizing microbes secrete molecules which may assist in adhesion to the plant, or in evading a plant immune response. In some cases, plant colonizing microbes may excrete signaling molecules which alter the plants response to the microbes. In some cases, plant colonizing microbes may secrete molecules which alter the local microenvironment. In some cases, a plant colonizing microbe may alter expression of genes to adapt to a plant said microbe is in proximity to. In some cases, a plant colonizing microbe may detect the presence of a plant in the local environment and may change expression of genes in response.
(134) In some embodiments, to improve colonization, a gene involved in a pathway selected from the group consisting of: exopolysaccharide production, endo-polygalaturonase production,

trehalose production, and glutamine conversion may be targeted for genetic engineering and optimization.
(135) In some embodiments, an enzyme or pathway involved in production of exopolysaccharides may be genetically modified to improve colonization. Exemplary genes encoding an exopolysaccharide producing enzyme that may be targeted to improve colonization include, but are not limited to, bcsii, bcsiii, and yjbE.
(136) In some embodiments, an enzyme or pathway involved in production of a filamentous hemagglutinin may be genetically modified to improve colonization. For example, a fhaB gene encoding a filamentous hemagglutinin may be targeted to improve colonization.
(137) In some embodiments, an enzyme or pathway involved in production of an endo-polygalaturonase may be genetically modified to improve colonization. For example, a pehA gene encoding an endo-polygalaturonase precursor may be targeted to improve colonization.
(138) In some embodiments, an enzyme or pathway involved in production of trehalose may be genetically modified to improve colonization. Exemplary genes encoding a trehalose producing enzyme that may be targeted to improve colonization include, but are not limited to, otsB and treZ.
(139) In some embodiments, an enzyme or pathway involved in conversion of glutamine may be genetically modified to improve colonization. For example, the glsA2 gene encodes a glutaminase which converts glutamine into ammonium and glutamate. Upregulating glsA2 improves fitness by increasing the cell's glutamate pool, thereby increasing available N to the cells. Accordingly, in some embodiments, the glsA2 gene may be targeted to improve colonization.
(140) In some embodiments, colonization genes selected from the group consisting of: bcsii, bcsiii, yjbE, fhaB, pehA, otsB, treZ, glsA2, and combinations thereof, may be genetically modified to improve colonization.
(141) Colonization genes that may be targeted to improve the colonization potential are also described in a PCT publication, WO/2019/032926, which is incorporated by reference herein in its entirety.
(142) Generation of Bacterial Populations
(143) Isolation of Bacteria
(144) Microbes useful in methods and compositions disclosed herein can be obtained by extracting microbes from surfaces or tissues of native plants. Microbes can be obtained by grinding seeds to isolate microbes. Microbes can be obtained by planting seeds in diverse soil samples and recovering microbes from tissues. Additionally, microbes can be obtained by inoculating plants with exogenous microbes and determining which microbes appear in plant tissues. Non-limiting examples of plant tissues may include a seed, seedling, leaf, cutting, plant, bulb, or tuber.
(145) A method of obtaining microbes may be through the isolation of bacteria from soils. Bacteria may be collected from various soil types. In some example, the soil can be characterized by traits such as high or low fertility, levels of moisture, levels of minerals, and various cropping practices. For example, the soil may be involved in a crop rotation where different crops are planted in the same soil in successive planting seasons. The sequential growth of different crops on the same soil may prevent disproportionate depletion of certain minerals. The bacteria can be isolated from the plants growing in the selected soils. The seedling plants can be harvested at 2-6 weeks of growth. For example, at least 400 isolates can be collected in a round of harvest. Soil and plant types reveal the plant phenotype as well as the conditions, which allow for the downstream enrichment of certain phenotypes.
(146) Microbes can be isolated from plant tissues to assess microbial traits. The parameters for processing tissue samples may be varied to isolate different types of associative microbes, such as rhizospheric bacteria, epiphytes, or endophytes. The isolates can be cultured in nitrogen-free media to enrich for bacteria that perform nitrogen fixation. Alternatively, microbes can be obtained from global strain banks.
(147) In planta analytics are performed to assess microbial traits. In some embodiments, the plant

tissue can be processed for screening by high throughput processing for DNA and RNA. Additionally, non-invasive measurements can be used to assess plant characteristics, such as colonization. Measurements on wild microbes can be obtained on a plant-by-plant basis. Measurements on wild microbes can also be obtained in the field using medium throughput methods. Measurements can be done successively over time. Model plant system can be used including, but not limited to, *Setaria.*

(148) Microbes in a plant system can be screened via transcriptional profiling of a microbe in a plant system. Examples of screening through transcriptional profiling are using methods of quantitative polymerase chain reaction (qPCR), molecular barcodes for transcript detection, Next Generation Sequencing, and microbe tagging with fluorescent markers. Impact factors can be measured to assess colonization in the greenhouse including, but not limited to, microbiome, abiotic factors, soil conditions, oxygen, moisture, temperature, inoculum conditions, and root localization. Nitrogen fixation can be assessed in bacteria by measuring 15N gas/fertilizer (dilution) with IRMS or NanoSIMS as described herein NanoSIMS is high-resolution secondary ion mass spectrometry. The NanoSIMS technique is a way to investigate chemical activity from biological samples. The catalysis of reduction of oxidation reactions that drive the metabolism of microorganisms can be investigated at the cellular, subcellular, molecular and elemental level. NanoSIMS can provide high spatial resolution of greater than 0.1 μm. NanoSIMS can detect the use of isotope tracers such as .sup.13C, .sup.15N, and .sup.18O. Therefore, NanoSIMS can be used to the chemical activity nitrogen in the cell.

(149) Automated greenhouses can be used for planta analytics. Plant metrics in response to microbial exposure include, but are not limited to, biomass, chloroplast analysis, CCD camera, volumetric tomography measurements.

(150) One way of enriching a microbe population is according to genotype. For example, a polymerase chain reaction (PCR) assay with a targeted primer or specific primer. Primers designed for the nifH gene can be used to identity diazotrophs because diazotrophs express the nifH gene in the process of nitrogen fixation. A microbial population can also be enriched via single-cell culture-independent approaches and chemotaxis-guided isolation approaches. Alternatively, targeted isolation of microbes can be performed by culturing the microbes on selection media. Premeditated approaches to enriching microbial populations for desired traits can be guided by bioinformatics data and are described herein.

(151) Enriching for Microbes with Nitrogen Fixation Capabilities Using Bioinformatics

(152) Bioinformatic tools can be used to identify and isolate plant growth promoting rhizobacteria (PGPRs), which are selected based on their ability to perform nitrogen fixation. Microbes with high nitrogen fixing ability can promote favorable traits in plants. Bioinformatic modes of analysis for the identification of PGPRs include, but are not limited to, genomics, metagenomics, targeted isolation, gene sequencing, transcriptome sequencing, and modeling.

(153) Genomics analysis can be used to identify PGPRs and confirm the presence of mutations with methods of Next Generation Sequencing as described herein and microbe version control.

(154) Metagenomics can be used to identify and isolate PGPR using a prediction algorithm for colonization. Metadata can also be used to identify the presence of an engineered strain in environmental and greenhouse samples.

(155) Transcriptomic sequencing can be used to predict genotypes leading to PGPR phenotypes. Additionally, transcriptomic data is used to identify promoters for altering gene expression. Transcriptomic data can be analyzed in conjunction with the Whole Genome Sequence (WGS) to generate models of metabolism and gene regulatory networks.

(156) Domestication of Microbes

(157) Microbes isolated from nature can undergo a domestication process wherein the microbes are converted to a form that is genetically trackable and identifiable. One way to domesticate a microbe is to engineer it with antibiotic resistance. The process of engineering antibiotic resistance can

begin by determining the antibiotic sensitivity in the wild type microbial strain. If the bacteria are sensitive to the antibiotic, then the antibiotic can be a good candidate for antibiotic resistance engineering. Subsequently, an antibiotic resistant gene or a counterselectable suicide vector can be incorporated into the genome of a microbe using recombineering methods. A counterselectable suicide vector may consist of a deletion of the gene of interest, a selectable marker, and the counterselectable marker sacB. Counterselection can be used to exchange native microbial DNA sequences with antibiotic resistant genes. A medium throughput method can be used to evaluate multiple microbes simultaneously allowing for parallel domestication. Alternative methods of domestication include the use of homing nucleases to prevent the suicide vector sequences from looping out or from obtaining intervening vector sequences.

(158) DNA vectors can be introduced into bacteria via several methods including electroporation and chemical transformations. A standard library of vectors can be used for transformations. An example of a method of gene editing is CRISPR preceded by Cas9 testing to ensure activity of Cas9 in the microbes.

(159) Non-Transgenic Engineering of Microbes

(160) A microbial population with favorable traits can be obtained via directed evolution. Direct evolution is an approach wherein the process of natural selection is mimicked to evolve proteins or nucleic acids towards a user-defined goal. An example of direct evolution is when random mutations are introduced into a microbial population, the microbes with the most favorable traits are selected, and the growth of the selected microbes is continued. The most favorable traits in growth promoting rhizobacteria (PGPRs) may be in nitrogen fixation. The method of directed evolution may be iterative and adaptive based on the selection process after each iteration.

(161) Plant growth promoting rhizobacteria (PGPRs) with high capability of nitrogen fixation can be generated. The evolution of PGPRs can be carried out via the introduction of genetic variation. Genetic variation can be introduced via polymerase chain reaction mutagenesis, oligonucleotide-directed mutagenesis, saturation mutagenesis, fragment shuffling mutagenesis, homologous recombination, CRISPR/Cas9 systems, chemical mutagenesis, and combinations thereof. These approaches can introduce random mutations into the microbial population. For example, mutants can be generated using synthetic DNA or RNA via oligonucleotide-directed mutagenesis. Mutants can be generated using tools contained on plasmids, which are later cured. Genes of interest can be identified using libraries from other species with improved traits including, but not limited to, improved PGPR properties, improved colonization of cereals, increased oxygen sensitivity, increased nitrogen fixation, and increased ammonia excretion. Intrageneric genes can be designed based on these libraries using software such as Geneious or Platypus design software. Mutations can be designed with the aid of machine learning. Mutations can be designed with the aid of a metabolic model. Automated design of the mutation can be done using a la Platypus and will guide RNAs for Cas-directed mutagenesis.

(162) The intra-generic genes can be transferred into the host microbe. Additionally, reporter systems can also be transferred to the microbe. The reporter systems characterize promoters, determine the transformation success, screen mutants, and act as negative screening tools.

(163) The microbes carrying the mutation can be cultured via serial passaging. A microbial colony contains a single variant of the microbe. Microbial colonies are screened with the aid of an automated colony picker and liquid handler. Mutants with gene duplication and increased copy number express a higher genotype of the desired trait.

(164) Selection of Plant Growth Promoting Microbes Based on Nitrogen Fixation

(165) The microbial colonies can be screened using various assays to assess nitrogen fixation. One way to measure nitrogen fixation is via a single fermentative assay, which measures nitrogen excretion. An alternative method is the acetylene reduction assay (ARA) with in-line sampling over time. ARA can be performed in high throughput plates of microtube arrays. ARA can be performed with live plants and plant tissues. The media formulation and media oxygen concentration can be

varied in ARA assays. Another method of screening microbial variants is by using biosensors. The use of NanoSIMS and Raman microspectroscopy can be used to investigate the activity of the microbes. In some cases, bacteria can also be cultured and expanded using methods of fermentation in bioreactors. The bioreactors are designed to improve robustness of bacteria growth and to decrease the sensitivity of bacteria to oxygen. Medium to high TP plate-based microfermentors are used to evaluate oxygen sensitivity, nutritional needs, nitrogen fixation, and nitrogen excretion. The bacteria can also be co-cultured with competitive or beneficial microbes to elucidate cryptic pathways. Flow cytometry can be used to screen for bacteria that produce high levels of nitrogen using chemical, colorimetric, or fluorescent indicators. The bacteria may be cultured in the presence or absence of a nitrogen source. For example, the bacteria may be cultured with glutamine, ammonia, urea or nitrates.

(166) Guided Microbial Remodeling—An Overview

(167) Guided microbial remodeling is a method to systematically identify and improve the role of species within the crop microbiome. In some aspects, and according to a particular methodology of grouping/categorization, the method comprises three steps: 1) selection of candidate species by mapping plant-microbe interactions and predicting regulatory networks linked to a particular phenotype, 2) pragmatic and predictable improvement of microbial phenotypes through intra-species crossing of regulatory networks and gene clusters within a microbe's genome, and 3) screening and selection of new microbial genotypes that produce desired crop phenotypes.

(168) To systematically assess the improvement of strains, a model is created that links colonization dynamics of the microbial community to genetic activity by key species. The model is used to predict genetic targets for non-intergeneric genetic remodeling (i.e. engineering the genetic architecture of the microbe in a non-transgenic fashion). See, FIG. **1**A for a graphical representation of an embodiment of the process.

(169) As illustrated in FIG. **1**A, rational improvement of the crop microbiome may be used to increase soil biodiversity, tune impact of keystone species, and/or alter timing and expression of important metabolic pathways.

(170) To this end, the inventors have developed a platform to identify and improve the role of strains within the crop microbiome. In some aspects, the inventors call this process microbial breeding.

(171) The aforementioned “Guided Microbial Remodeling” process will be further elaborated upon in the Examples, for instance in Example 1, entitled: “Guided Microbial Remodeling—A Platform for the Rational Improvement of Microbial Species for Agriculture.”

(172) Serial Passage

(173) Production of bacteria to improve plant traits (e.g., nitrogen fixation) can be achieved through serial passage. The production of these bacteria can be done by selecting plants, which have a particular improved trait that is influenced by the microbial flora, in addition to identifying bacteria and/or compositions that are capable of imparting one or more improved traits to one or more plants. One method of producing a bacteria to improve a plant trait includes the steps of: (a) isolating bacteria from tissue or soil of a first plant; (b) introducing a genetic variation into one or more of the bacteria to produce one or more variant bacteria; (c) exposing a plurality of plants to the variant bacteria; (d) isolating bacteria from tissue or soil of one of the plurality of plants, wherein the plant from which the bacteria is isolated has an improved trait relative to other plants in the plurality of plants; and (e) repeating steps (b) to (d) with bacteria isolated from the plant with an improved trait (step (d)). Steps (b) to (d) can be repeated any number of times (e.g., once, twice, three times, four times, five times, ten times, or more) until the improved trait in a plant reaches a desired level. Further, the plurality of plants can be more than two plants, such as 10 to 20 plants, or 20 or more, 50 or more, 100 or more, 300 or more, 500 or more, or 1000 or more plants.

(174) In addition to obtaining a plant with an improved trait, a bacterial population comprising bacteria comprising one or more genetic variations introduced into one or more genes (e.g., genes

regulating nitrogen fixation) is obtained. By repeating the steps described above, a population of bacteria can be obtained that include the most appropriate members of the population that correlate with a plant trait of interest. The bacteria in this population can be identified and their beneficial properties determined, such as by genetic and/or phenotypic analysis. Genetic analysis may occur of isolated bacteria in step (a). Phenotypic and/or genotypic information may be obtained using techniques including: high through-put screening of chemical components of plant origin, sequencing techniques including high throughput sequencing of genetic material, differential display techniques (including DDRT-PCR, and DD-PCR), nucleic acid microarray techniques, RNA-sequencing (Whole Transcriptome Shotgun Sequencing), and qRT-PCR (quantitative real time PCR). Information gained can be used to obtain community profiling information on the identity and activity of bacteria present, such as phylogenetic analysis or microarray-based screening of nucleic acids coding for components of rRNA operons or other taxonomically informative loci. Examples of taxonomically informative loci include 16S rRNA gene, 23S rRNA gene, 5S rRNA gene, 5.8S rRNA gene, 12S rRNA gene, 18S rRNA gene, 28S rRNA gene, gyrB gene, rpoB gene, fusA gene, recA gene, coxl gene, nifD gene. Example processes of taxonomic profiling to determine taxa present in a population are described in US20140155283. Bacterial identification may comprise characterizing activity of one or more genes or one or more signaling pathways, such as genes associated with the nitrogen fixation pathway. Synergistic interactions (where two components, by virtue of their combination, increase a desired effect by more than an additive amount) between different bacterial species may also be present in the bacterial populations.

(175) Genetic Variation—Locations and Sources of Genomic Alteration

(176) The genetic variation may be a gene selected from the group consisting of: nifA, nifL, ntrB, ntrC, glnA, glnB, glnK, draT, amtB, glnD, glnE, nifJ, nifH, nifD, nifK, nifY, nifE, nifN, nifU, nifS, nifV, nifW, nifZ, nifM, nifF, nifB, and nifQ. The genetic variation may be a variation in a gene encoding a protein with functionality selected from the group consisting of: glutamine synthetase, glutaminase, glutamine synthetase adenylyltransferase, transcriptional activator, anti-transcriptional activator, pyruvate flavodoxin oxidoreductase, flavodoxin, and NAD+-dinitrogen-reductase aDP-D-ribosyltransferase. The genetic variation may be a mutation that results in one or more of: increased expression or activity of NifA or glutaminase; decreased expression or activity of NifL, NtrB, glutamine synthetase, GlnB, GlnK, DraT, AmtB; decreased adenylyl-removing activity of GlnE; or decreased uridylyl-removing activity of GlnD. The genetic variation may be a variation in a gene selected from the group consisting of: bcsii, bcsiii, yjbE, fhaB, pehA, otsB, treZ, glsA2, and combinations thereof. In some embodiments, a genetic variation may be a variation in any of the genes described throughout this disclosure.

(177) Introducing a genetic variation may comprise insertion and/or deletion of one or more nucleotides at a target site, such as 1, 2, 3, 4, 5, 10, 25, 50, 100, 250, 500, or more nucleotides. The genetic variation introduced into one or more bacteria of the methods disclosed herein may be a knock-out mutation (e.g. deletion of a promoter, insertion or deletion to produce a premature stop codon, deletion of an entire gene), or it may be elimination or abolishment of activity of a protein domain (e.g. point mutation affecting an active site, or deletion of a portion of a gene encoding the relevant portion of the protein product), or it may alter or abolish a regulatory sequence of a target gene. One or more regulatory sequences may also be inserted, including heterologous regulatory sequences and regulatory sequences found within a genome of a bacterial species or genus corresponding to the bacteria into which the genetic variation is introduced. Moreover, regulatory sequences may be selected based on the expression level of a gene in a bacterial culture or within a plant tissue. The genetic variation may be a pre-determined genetic variation that is specifically introduced to a target site. The genetic variation may be a random mutation within the target site. The genetic variation may be an insertion or deletion of one or more nucleotides. In some cases, a plurality of different genetic variations (e.g. 2, 3, 4, 5, 10, or more) are introduced into one or more

of the isolated bacteria before exposing the bacteria to plants for assessing trait improvement. The plurality of genetic variations can be any of the above types, the same or different types, and in any combination. In some cases, a plurality of different genetic variations are introduced serially, introducing a first genetic variation after a first isolation step, a second genetic variation after a second isolation step, and so forth so as to accumulate a plurality of genetic variations in bacteria imparting progressively improved traits on the associated plants.

(178) Genetic Variation—Methods of Introducing Genomic Alteration

(179) In general, the term "genetic variation" refers to any change introduced into a polynucleotide sequence relative to a reference polynucleotide, such as a reference genome or portion thereof, or reference gene or portion thereof. A genetic variation may be referred to as a "mutation," and a sequence or organism comprising a genetic variation may be referred to as a "genetic variant" or "mutant". Genetic variations can have any number of effects, such as the increase or decrease of some biological activity, including gene expression, metabolism, and cell signaling. Genetic variations can be specifically introduced to a target site, or introduced randomly. A variety of molecular tools and methods are available for introducing genetic variation. For example, genetic variation can be introduced via polymerase chain reaction mutagenesis, oligonucleotide-directed mutagenesis, saturation mutagenesis, fragment shuffling mutagenesis, homologous recombination, recombineering, lambda red mediated recombination, CRISPR/Cas9 systems, chemical mutagenesis, and combinations thereof. Chemical methods of introducing genetic variation include exposure of DNA to a chemical mutagen, e.g., ethyl methanesulfonate (EMS), methyl methanesulfonate (MMS), N-nitrosourea (EN U), N-methyl-N-nitro-N′-nitrosoguanidine, 4-nitroquinoline N-oxide, diethylsulfate, benzopyrene, cyclophosphamide, bleomycin, triethylmelamine, acrylamide monomer, nitrogen mustard, vincristine, diepoxyalkanes (for example, diepoxybutane), ICR-170, formaldehyde, procarbazine hydrochloride, ethylene oxide, dimethylnitrosamine, 7,12 dimethylbenz(a)anthracene, chlorambucil, hexamethylphosphoramide, bisulfan, and the like. Radiation mutation-inducing agents include ultraviolet radiation, γ-irradiation, X-rays, and fast neutron bombardment. Genetic variation can also be introduced into a nucleic acid using, e.g., trimethylpsoralen with ultraviolet light. Random or targeted insertion of a mobile DNA element, e.g., a transposable element, is another suitable method for generating genetic variation. Genetic variations can be introduced into a nucleic acid during amplification in a cell-free in vitro system, e.g., using a polymerase chain reaction (PCR) technique such as error-prone PCR. Genetic variations can be introduced into a nucleic acid in vitro using DNA shuffling techniques (e.g., exon shuffling, domain swapping, and the like). Genetic variations can also be introduced into a nucleic acid as a result of a deficiency in a DNA repair enzyme in a cell, e.g., the presence in a cell of a mutant gene encoding a mutant DNA repair enzyme is expected to generate a high frequency of mutations (i.e., about 1 mutation/100 genes-1 mutation/10,000 genes) in the genome of the cell. Examples of genes encoding DNA repair enzymes include but are not limited to Mut H, Mut S, Mut L, and Mut U, and the homologs thereof in other species (e.g., MSH 1 6, PMS 1 2, MLH 1, GTBP, ERCC-1, and the like). Example descriptions of various methods for introducing genetic variations are provided in e.g., Stemple (2004) Nature 5:1-7; Chiang et al. (1993) PCR Methods Appl 2(3): 210-217; Stemmer (1994) Proc. Natl. Acad. Sci. USA 91:10747-10751; and U.S. Pat. Nos. 6,033,861, and 6,773,900.

(180) Genetic variations introduced into microbes may be classified as transgenic, cisgenic, intragenomic, intrageneric, intergeneric, synthetic, evolved, rearranged, or SNPs.

(181) Genetic variation may be introduced into numerous metabolic pathways within microbes to elicit improvements in the traits described above. Representative pathways include sulfur uptake pathways, glycogen biosynthesis, the glutamine regulation pathway, the molybdenum uptake pathway, the nitrogen fixation pathway, ammonia assimilation, ammonia excretion or secretion, Nitrogen uptake, glutamine biosynthesis, colonization pathways, annamox, phosphate solubilization, organic acid transport, organic acid production, agglutinins production, reactive

oxygen radical scavenging genes, Indole Acetic Acid biosynthesis, trehalose biosynthesis, plant cell wall degrading enzymes or pathways, root attachment genes, exopolysaccharide secretion, glutamate synthase pathway, iron uptake pathways, siderophore pathway, chitinase pathway, ACC deaminase, glutathione biosynthesis, phosphorous signaling genes, quorum quenching pathway, cytochrome pathways, hemoglobin pathway, bacterial hemoglobin-like pathway, small RNA rsmZ, rhizobitoxine biosynthesis, lapA adhesion protein, AHL quorum sensing pathway, phenazine biosynthesis, cyclic lipopeptide biosynthesis, and antibiotic production.

(182) CRISPR/Cas9 (Clustered regularly interspaced short palindromic repeats)/CRISPR-associated (Cas) systems can be used to introduce desired mutations. CRISPR/Cas9 provide bacteria and archaea with adaptive immunity against viruses and plasmids by using CRISPR RNAs (crRNAs) to guide the silencing of invading nucleic acids. The Cas9 protein (or functional equivalent and/or variant thereof, i.e., Cas9-like protein) naturally contains DNA endonuclease activity that depends on the association of the protein with two naturally occurring or synthetic RNA molecules called crRNA and tracrRNA (also called guide RNAs). In some cases, the two molecules are covalently link to form a single molecule (also called a single guide RNA ("sgRNA"). Thus, the Cas9 or Cas9-like protein associates with a DNA-targeting RNA (which term encompasses both the two-molecule guide RNA configuration and the single-molecule guide RNA configuration), which activates the Cas9 or Cas9-like protein and guides the protein to a target nucleic acid sequence. If the Cas9 or Cas9-like protein retains its natural enzymatic function, it will cleave target DNA to create a double-stranded break, which can lead to genome alteration (i.e., editing: deletion, insertion (when a donor polynucleotide is present), replacement, etc.), thereby altering gene expression. Some variants of Cas9 (which variants are encompassed by the term Cas9-like) have been altered such that they have a decreased DNA cleaving activity (in some cases, they cleave a single strand instead of both strands of the target DNA, while in other cases, they have severely reduced to no DNA cleavage activity). Further exemplary descriptions of CRISPR systems for introducing genetic variation can be found in, e.g. U.S. Pat. No. 8,795,965.

(183) As a cyclic amplification technique, polymerase chain reaction (PCR) mutagenesis uses mutagenic primers to introduce desired mutations. PCR is performed by cycles of denaturation, annealing, and extension. After amplification by PCR, selection of mutated DNA and removal of parental plasmid DNA can be accomplished by: 1) replacement of dCTP by hydroxymethylated-dCTP during PCR, followed by digestion with restriction enzymes to remove non-hydroxymethylated parent DNA only; 2) simultaneous mutagenesis of both an antibiotic resistance gene and the studied gene changing the plasmid to a different antibiotic resistance, the new antibiotic resistance facilitating the selection of the desired mutation thereafter; 3) after introducing a desired mutation, digestion of the parent methylated template DNA by restriction enzyme Dpnl which cleaves only methylated DNA, by which the mutagenized unmethylated chains are recovered; or 4) circularization of the mutated PCR products in an additional ligation reaction to increase the transformation efficiency of mutated DNA. Further description of exemplary methods can be found in e.g. U.S. Pat. Nos. 7,132,265, 6,713,285, 6,673,610, 6,391,548, 5,789,166, 5,780,270, 5,354,670, 5,071,743, and US20100267147.

(184) Oligonucleotide-directed mutagenesis, also called site-directed mutagenesis, typically utilizes a synthetic DNA primer. This synthetic primer contains the desired mutation and is complementary to the template DNA around the mutation site so that it can hybridize with the DNA in the gene of interest. The mutation may be a single base change (a point mutation), multiple base changes, deletion, or insertion, or a combination of these. The single-strand primer is then extended using a DNA polymerase, which copies the rest of the gene. The gene thus copied contains the mutated site, and may then be introduced into a host cell as a vector and cloned. Finally, mutants can be selected by DNA sequencing to check that they contain the desired mutation.

(185) Genetic variations can be introduced using error-prone PCR. In this technique the gene of interest is amplified using a DNA polymerase under conditions that are deficient in the fidelity of

replication of sequence. The result is that the amplification products contain at least one error in the sequence. When a gene is amplified and the resulting product(s) of the reaction contain one or more alterations in sequence when compared to the template molecule, the resulting products are mutagenized as compared to the template. Another means of introducing random mutations is exposing cells to a chemical mutagen, such as nitrosoguanidine or ethyl methanesulfonate (Nestmann, Mutat Res 1975 June; 28(3):323-30), and the vector containing the gene is then isolated from the host.

(186) Saturation mutagenesis is another form of random mutagenesis, in which one tries to generate all or nearly all possible mutations at a specific site, or narrow region of a gene. In a general sense, saturation mutagenesis is comprised of mutagenizing a complete set of mutagenic cassettes (wherein each cassette is, for example, 1-500 bases in length) in defined polynucleotide sequence to be mutagenized (wherein the sequence to be mutagenized is, for example, from 15 to 100,000 bases in length). Therefore, a group of mutations (e.g. ranging from 1 to 100 mutations) is introduced into each cassette to be mutagenized. A grouping of mutations to be introduced into one cassette can be different or the same from a second grouping of mutations to be introduced into a second cassette during the application of one round of saturation mutagenesis. Such groupings are exemplified by deletions, additions, groupings of particular codons, and groupings of particular nucleotide cassettes.

(187) Fragment shuffling mutagenesis, also called DNA shuffling, is a way to rapidly propagate beneficial mutations. In an example of a shuffling process, DNAse is used to fragment a set of parent genes into pieces of e.g. about 50-100 bp in length. This is then followed by a polymerase chain reaction (PCR) without primers—DNA fragments with sufficient overlapping homologous sequence will anneal to each other and are then be extended by DNA polymerase. Several rounds of this PCR extension are allowed to occur, after some of the DNA molecules reach the size of the parental genes. These genes can then be amplified with another PCR, this time with the addition of primers that are designed to complement the ends of the strands. The primers may have additional sequences added to their 5′ ends, such as sequences for restriction enzyme recognition sites needed for ligation into a cloning vector. Further examples of shuffling techniques are provided in US20050266541.

(188) Homologous recombination mutagenesis involves recombination between an exogenous DNA fragment and the targeted polynucleotide sequence. After a double-stranded break occurs, sections of DNA around the 5′ ends of the break are cut away in a process called resection. In the strand invasion step that follows, an overhanging 3′ end of the broken DNA molecule then “invades” a similar or identical DNA molecule that is not broken. The method can be used to delete a gene, remove exons, add a gene, and introduce point mutations. Homologous recombination mutagenesis can be permanent or conditional. Typically, a recombination template is also provided. A recombination template may be a component of another vector, contained in a separate vector, or provided as a separate polynucleotide. In some embodiments, a recombination template is designed to serve as a template in homologous recombination, such as within or near a target sequence nicked or cleaved by a site-specific nuclease. A template polynucleotide may be of any suitable length, such as about or more than about 10, 15, 20, 25, 50, 75, 100, 150, 200, 500, 1000, or more nucleotides in length. In some embodiments, the template polynucleotide is complementary to a portion of a polynucleotide comprising the target sequence. When optimally aligned, a template polynucleotide might overlap with one or more nucleotides of a target sequences (e.g. about or more than about 1, 5, 10, 15, 20, 25, 30, 35, 40, 45, 50, 60, 70, 80, 90, 100 or more nucleotides). In some embodiments, when a template sequence and a polynucleotide comprising a target sequence are optimally aligned, the nearest nucleotide of the template polynucleotide is within about 1, 5, 10, 15, 20, 25, 50, 75, 100, 200, 300, 400, 500, 1000, 5000, 10000, or more nucleotides from the target sequence. Non-limiting examples of site-directed nucleases useful in methods of homologous recombination include zinc finger nucleases, CRISPR nucleases, TALE nucleases, and

meganuclease. For a further description of the use of such nucleases, see e.g. U.S. Pat. No. 8,795,965 and US20140301990.
(189) Mutagens that create primarily point mutations and short deletions, insertions, transversions, and/or transitions, including chemical mutagens or radiation, may be used to create genetic variations. Mutagens include, but are not limited to, ethyl methanesulfonate, methylmethane sulfonate, N-ethyl-N-nitrosurea, triethylmelamine, N-methyl-N-nitrosourea, procarbazine, chlorambucil, cyclophosphamide, diethyl sulfate, acrylamide monomer, melphalan, nitrogen mustard, vincristine, dimethylnitrosamine, N-methyl-N′-nitro-Nitrosoguanidine, nitrosoguanidine, 2-aminopurine, 7,12 dimethyl-benz(a)anthracene, ethylene oxide, hexamethylphosphoramide, bisulfan, diepoxyalkanes (diepoxyoctane, diepoxybutane, and the like), 2-methoxy-6-chloro-9[3-(ethyl-2-chloro-ethyl)aminopropylamino]acridine dihydrochloride and formaldehyde.
(190) Introducing genetic variation may be an incomplete process, such that some bacteria in a treated population of bacteria carry a desired mutation while others do not. In some cases, it is desirable to apply a selection pressure so as to enrich for bacteria carrying a desired genetic variation. Traditionally, selection for successful genetic variants involved selection for or against some functionality imparted or abolished by the genetic variation, such as in the case of inserting antibiotic resistance gene or abolishing a metabolic activity capable of converting a non-lethal compound into a lethal metabolite. It is also possible to apply a selection pressure based on a polynucleotide sequence itself, such that only a desired genetic variation need be introduced (e.g. without also requiring a selectable marker). In this case, the selection pressure can comprise cleaving genomes lacking the genetic variation introduced to a target site, such that selection is effectively directed against the reference sequence into which the genetic variation is sought to be introduced. Typically, cleavage occurs within 100 nucleotides of the target site (e.g. within 75, 50, 25, 10, or fewer nucleotides from the target site, including cleavage at or within the target site). Cleaving may be directed by a site-specific nuclease selected from the group consisting of a Zinc Finger nuclease, a CRISPR nuclease, a TALE nuclease (TALEN), and a meganuclease. Such a process is similar to processes for enhancing homologous recombination at a target site, except that no template for homologous recombination is provided. As a result, bacteria lacking the desired genetic variation are more likely to undergo cleavage that, left unrepaired, results in cell death. Bacteria surviving selection may then be isolated for use in exposing to plants for assessing conferral of an improved trait.
(191) A CRISPR nuclease may be used as the site-specific nuclease to direct cleavage to a target site. An improved selection of mutated microbes can be obtained by using Cas9 to kill non-mutated cells. Plants are then inoculated with the mutated microbes to re-confirm symbiosis and create evolutionary pressure to select for efficient symbionts. Microbes can then be re-isolated from plant tissues. CRISPR nuclease systems employed for selection against non-variants can employ similar elements to those described above with respect to introducing genetic variation, except that no template for homologous recombination is provided. Cleavage directed to the target site thus enhances death of affected cells.
(192) Other options for specifically inducing cleavage at a target site are available, such as zinc finger nucleases, TALE nuclease (TALEN) systems, and meganuclease. Zinc-finger nucleases (ZFNs) are artificial DNA endonucleases generated by fusing a zinc finger DNA binding domain to a DNA cleavage domain. ZFNs can be engineered to target desired DNA sequences and this enables zinc-finger nucleases to cleave unique target sequences. When introduced into a cell, ZFNs can be used to edit target DNA in the cell (e.g., the cell's genome) by inducing double stranded breaks. Transcription activator-like effector nucleases (TALENs) are artificial DNA endonucleases generated by fusing a TAL (Transcription activator-like) effector DNA binding domain to a DNA cleavage domain. TALENS can be quickly engineered to bind practically any desired DNA sequence and when introduced into a cell, TALENs can be used to edit target DNA in the cell (e.g., the cell's genome) by inducing double strand breaks. Meganucleases (homing endonuclease) are

endodeoxyribonucleases characterized by a large recognition site (double-stranded DNA sequences of 12 to 40 base pairs. Meganucleases can be used to replace, eliminate or modify sequences in a highly targeted way. By modifying their recognition sequence through protein engineering, the targeted sequence can be changed. Meganucleases can be used to modify all genome types, whether bacterial, plant or animal and are commonly grouped into four families: the LAGLIDADG family (SEQ ID NO: 1), the GIY-YIG family, the His-Cyst box family and the HNH family. Exemplary homing endonucleases include I-SceI, I-CeuI, PI-PspI, PI-Sce, I-SceIV, I-CsmI, I-PanI, I-SceII, I-PpoI, I-SceIII, I-CreI, I-TevI, I-TevII and I-TevIII.

(193) Genetic Variation—Methods of Identification

(194) The microbes of the present disclosure may be identified by one or more genetic modifications or alterations, which have been introduced into said microbe. One method by which said genetic modification or alteration can be identified is via reference to a SEQ ID NO that contains a portion of the microbe's genomic sequence that is sufficient to identify the genetic modification or alteration.

(195) Further, in the case of microbes that have not had a genetic modification or alteration (e.g. a wild type, WT) introduced into their genomes, the disclosure can utilize 16S nucleic acid sequences to identify said microbes. A 16S nucleic acid sequence is an example of a “molecular marker” or “genetic marker,” which refers to an indicator that is used in methods for visualizing differences in characteristics of nucleic acid sequences. Examples of other such indicators are restriction fragment length polymorphism (RFLP) markers, amplified fragment length polymorphism (AFLP) markers, single nucleotide polymorphisms (SNPs), insertion mutations, microsatellite markers (SSRs), sequence-characterized amplified regions (SCARs), cleaved amplified polymorphic sequence (CAPS) markers or isozyme markers or combinations of the markers described herein which defines a specific genetic and chromosomal location. Markers further include polynucleotide sequences encoding 16S or 18S rRNA, and internal transcribed spacer (ITS) sequences, which are sequences found between small-subunit and large-subunit rRNA genes that have proven to be especially useful in elucidating relationships or distinctions when compared against one another. Furthermore, the disclosure utilizes unique sequences found in genes of interest (e.g. nif H,D,K,L,A, glnE, amtB, etc.) to identify microbes disclosed herein.

(196) The primary structure of major rRNA subunit 16S comprise a particular combination of conserved, variable, and hypervariable regions that evolve at different rates and enable the resolution of both very ancient lineages such as domains, and more modern lineages such as genera. The secondary structure of the 16S subunit include approximately 50 helices which result in base pairing of about 67% of the residues. These highly conserved secondary structural features are of great functional importance and can be used to ensure positional homology in multiple sequence alignments and phylogenetic analysis. Over the previous few decades, the 16S rRNA gene has become the most sequenced taxonomic marker and is the cornerstone for the current systematic classification of bacteria and archaea (Yarza et al. 2014. *Nature Rev. Micro.* 12:635-45).

(197) Thus, in certain aspects, the disclosure provides for a sequence, which shares at least about 70%, 71%, 72%, 73%, 74%, 75%, 76%, 77%, 78%, 79%, 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% sequence identity to any sequence in Tables 23, 24, 30, 31, and 32.

(198) Thus, in certain aspects, the disclosure provides for a microbe that comprises a sequence, which shares at least about 70%, 71%, 72%, 73%, 74%, 75%, 76%, 77%, 78%, 79%, 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% sequence identity to SEQ ID NOs: 62-303. These sequences and their associated descriptions can be found in Tables 31 and 32.

(199) In some aspects, the disclosure provides for a microbe that comprises a 16S nucleic acid sequence, which shares at least about 70%, 71%, 72%, 73%, 74%, 75%, 76%, 77%, 78%, 79%, 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%,

97%, 98%, 99%, or 100% sequence identity to SEQ ID NOs: 85, 96, 111, 121, 122, 123, 124, 136, 149, 157, 167, 261, 262, 269, 277-283. These sequences and their associated descriptions can be found in Table 32.

(200) In some aspects, the disclosure provides for a microbe that comprises a nucleic acid sequence, which shares at least about 70%, 71%, 72%, 73%, 74%, 75%, 76%, 77%, 78%, 79%, 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% sequence identity to SEQ ID NOs: 86-95, 97-110, 112-120, 125-135, 137-148, 150-156, 158-166, 168-176, 263-268, 270-274, 275, 276, 284-295. These sequences and their associated descriptions can be found in Table 32.

(201) In some aspects, the disclosure provides for a microbe that comprises a nucleic acid sequence, which shares at least about 70%, 71%, 72%, 73%, 74%, 75%, 76%, 77%, 78%, 79%, 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% sequence identity to SEQ ID NOs: 177-260, 296-303. These sequences and their associated descriptions can be found in Table 32.

(202) In some aspects, the disclosure provides for a microbe that comprises, or primer that comprises, or probe that comprises, or non-native junction sequence that comprises, a nucleic acid sequence, which shares at least about 70%, 71%, 72%, 73%, 74%, 75%, 76%, 77%, 78%, 79%, 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% sequence identity to SEQ ID NOs: 304-424. These sequences and their associated descriptions can be found in Table 30.

(203) In some aspects, the disclosure provides for a microbe that comprises a non-native junction sequence that shares at least about 70%, 71%, 72%, 73%, 74%, 75%, 76%, 77%, 78%, 79%, 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% sequence identity to SEQ ID NOs: 372-405. These sequences and their associated descriptions can be found in Table 30.

(204) In some aspects, the disclosure provides for a microbe that comprises an amino acid sequence, which shares at least about 70%, 71%, 72%, 73%, 74%, 75%, 76%, 77%, 78%, 79%, 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% sequence identity to SEQ ID NOs: 77, 78, 81, 82, or 83. These sequences and their associated descriptions can be found in Table 31.

(205) Genetic Variation—Methods of Detection: Primers, Probes, and Assays

(206) The present disclosure teaches primers, probes, and assays that are useful for detecting the microbes taught herein. In some aspects, the disclosure provides for methods of detecting the WT parental strains. In other aspects, the disclosure provides for methods of detecting the non-intergeneric engineered microbes derived from the WT strains. In aspects, the present disclosure provides methods of identifying non-intergeneric genetic alterations in a microbe.

(207) In aspects, the genomic engineering methods of the present disclosure lead to the creation of non-natural nucleotide “junction” sequences in the derived non-intergeneric microbes. These non-naturally occurring nucleotide junctions can be used as a type of diagnostic that is indicative of the presence of a particular genetic alteration in a microbe taught herein.

(208) The present techniques are able to detect these non-naturally occurring nucleotide junctions via the utilization of specialized quantitative PCR methods, including uniquely designed primers and probes. In some aspects, the probes of the disclosure bind to the non-naturally occurring nucleotide junction sequences. In some aspects, traditional PCR is utilized. In other aspects, real-time PCR is utilized. In some aspects, quantitative PCR (qPCR) is utilized.

(209) Thus, the disclosure can cover the utilization of two common methods for the detection of PCR products in real-time: (1) non-specific fluorescent dyes that intercalate with any double-stranded DNA, and (2) sequence-specific DNA probes consisting of oligonucleotides that are labelled with a fluorescent reporter which permits detection only after hybridization of the probe with its complementary sequence. In some aspects, only the non-naturally occurring nucleotide

junction will be amplified via the taught primers, and consequently can be detected either via a non-specific dye, or via the utilization of a specific hybridization probe. In other aspects, the primers of the disclosure are chosen such that the primers flank either side of a junction sequence, such that if an amplification reaction occurs, then said junction sequence is present.

(210) Aspects of the disclosure involve non-naturally occurring nucleotide junction sequence molecules per se, along with other nucleotide molecules that are capable of binding to said non-naturally occurring nucleotide junction sequences under mild to stringent hybridization conditions. In some aspects, the nucleotide molecules that are capable of binding to said non-naturally occurring nucleotide junction sequences under mild to stringent hybridization conditions are termed “nucleotide probes.”

(211) In aspects, genomic DNA can be extracted from samples and used to quantify the presence of microbes of the disclosure by using qPCR. The primers utilized in the qPCR reaction can be primers designed by Primer Blast (www.ncbi.nlm.nih.gov/tools/primer-blast/) to amplify unique regions of the wild-type genome or unique regions of the engineered non-intergeneric mutant strains. The qPCR reaction can be carried out using the SYBR GreenER qPCR SuperMix Universal (Thermo Fisher P/N 11762100) kit, using only forward and reverse amplification primers; alternatively, the Kapa Probe Force kit (Kapa Biosystems P/N KK4301) can be used with amplification primers and a TaqMan probe containing a FAM dye label at the 5′ end, an internal ZEN quencher, and a minor groove binder and fluorescent quencher at the 3′ end (Integrated DNA Technologies).

(212) Certain primer, probe, and non-native junction sequences are listed in Table 30. qPCR reaction efficiency can be measured using a standard curve generated from a known quantity of gDNA from the target genome. Data can be normalized to genome copies per g fresh weight using the tissue weight and extraction volume.

(213) Quantitative polymerase chain reaction (qPCR) is a method of quantifying, in real time, the amplification of one or more nucleic acid sequences. The real time quantification of the PCR assay permits determination of the quantity of nucleic acids being generated by the PCR amplification steps by comparing the amplifying nucleic acids of interest and an appropriate control nucleic acid sequence, which may act as a calibration standard.

(214) TaqMan probes are often utilized in qPCR assays that require an increased specificity for quantifying target nucleic acid sequences. TaqMan probes comprise a oligonucleotide probe with a fluorophore attached to the 5′ end and a quencher attached to the 3′ end of the probe. When the TaqMan probes remain as is with the 5′ and 3′ ends of the probe in close contact with each other, the quencher prevents fluorescent signal transmission from the fluorophore. TaqMan probes are designed to anneal within a nucleic acid region amplified by a specific set of primers. As the Taq polymerase extends the primer and synthesizes the nascent strand, the 5′ to 3′ exonuclease activity of the Taq polymerase degrades the probe that annealed to the template. This probe degradation releases the fluorophore, thus breaking the close proximity to the quencher and allowing fluorescence of the fluorophore. Fluorescence detected in the qPCR assay is directly proportional to the fluorophore released and the amount of DNA template present in the reaction.

(215) The features of qPCR allow the practitioner to eliminate the labor-intensive post-amplification step of gel electrophoresis preparation, which is generally required for observation of the amplified products of traditional PCR assays. The benefits of qPCR over conventional PCR are considerable, and include increased speed, ease of use, reproducibility, and quantitative ability.

(216) Improvement of Traits

(217) Methods of the present disclosure may be employed to introduce or improve one or more of a variety of desirable traits. Examples of traits that may introduced or improved include: root biomass, root length, height, shoot length, leaf number, water use efficiency, overall biomass, yield, fruit size, grain size, photosynthesis rate, tolerance to drought, heat tolerance, salt tolerance, resistance to nematode stress, resistance to a fungal pathogen, resistance to a bacterial pathogen,

resistance to a viral pathogen, level of a metabolite, and proteome expression. The desirable traits, including height, overall biomass, root and/or shoot biomass, seed germination, seedling survival, photosynthetic efficiency, transpiration rate, seed/fruit number or mass, plant grain or fruit yield, leaf chlorophyll content, photosynthetic rate, root length, or any combination thereof, can be used to measure growth, and compared with the growth rate of reference agricultural plants (e.g., plants without the improved traits) grown under identical conditions.

(218) A preferred trait to be introduced or improved is nitrogen fixation, as described herein. A second preferred trait to be introduced or improved is colonization potential, as described herein. In some cases, a plant resulting from the methods described herein exhibits a difference in the trait that is at least about 5% greater, for example at least about 5%, at least about 8%, at least about 10%, at least about 15%, at least about 20%, at least about 25%, at least about 30%, at least about 40%, at least about 50%, at least about 60%, at least about 75%, at least about 80%, at least about 80%, at least about 90%, or at least 100%, at least about 200%, at least about 300%, at least about 400% or greater than a reference agricultural plant grown under the same conditions in the soil. In additional examples, a plant resulting from the methods described herein exhibits a difference in the trait that is at least about 5% greater, for example at least about 5%, at least about 8%, at least about 10%, at least about 15%, at least about 20%, at least about 25%, at least about 30%, at least about 40%, at least about 50%, at least about 60%, at least about 75%, at least about 80%, at least about 80%, at least about 90%, or at least 100%, at least about 200%, at least about 300%, at least about 400% or greater than a reference agricultural plant grown under similar conditions in the soil.

(219) The trait to be improved may be assessed under conditions including the application of one or more biotic or abiotic stressors. Examples of stressors include abiotic stresses (such as heat stress, salt stress, drought stress, cold stress, and low nutrient stress) and biotic stresses (such as nematode stress, insect herbivory stress, fungal pathogen stress, bacterial pathogen stress, and viral pathogen stress).

(220) The trait improved by methods and compositions of the present disclosure may be nitrogen fixation, including in a plant not previously capable of nitrogen fixation. In some cases, bacteria isolated according to a method described herein produce 1% or more (e.g. 2%, 3%, 4%, 5%, 6%, 7%, 8%, 9%, 10%, 15%, 20%, or more) of a plant's nitrogen, which may represent an increase in nitrogen fixation capability of at least 2-fold (e.g. 3-fold, 4-fold, 5-fold, 6-fold, 7-fold, 8-fold, 9-fold, 10-fold, 20-fold, 50-fold, 100-fold, 1000-fold, or more) as compared to bacteria isolated from the first plant before introducing any genetic variation. In some cases, the bacteria produce 5% or more of a plant's nitrogen. The desired level of nitrogen fixation may be achieved after repeating the steps of introducing genetic variation, exposure to a plurality of plants, and isolating bacteria from plants with an improved trait one or more times (e.g. 1, 2, 3, 4, 5, 10, 15, 25, or more times). In some cases, enhanced levels of nitrogen fixation are achieved in the presence of fertilizer supplemented with glutamine, ammonia, or other chemical source of nitrogen. Methods for assessing degree of nitrogen fixation are known, examples of which are described herein.

(221) Microbe breeding is a method to systematically identify and improve the role of species within the crop microbiome. The method comprises three steps: 1) selection of candidate species by mapping plant-microbe interactions and predicting regulatory networks linked to a particular phenotype, 2) pragmatic and predictable improvement of microbial phenotypes through intra-species crossing of regulatory networks and gene clusters, and 3) screening and selection of new microbial genotypes that produce desired crop phenotypes. To systematically assess the improvement of strains, a model is created that links colonization dynamics of the microbial community to genetic activity by key species. The model is used to predict genetic targets for breeding and improve the frequency of selecting improvements in microbiome-encoded traits of agronomic relevance.

(222) Measuring Nitrogen Delivered in an Agriculturally Relevant Field Context

(223) In the field, the amount of nitrogen delivered can be determined by the function of

colonization multiplied by the activity.

(224) $\text{Nitrogen}_{\text{delivered}} = \int_{\text{Time\&Space}} \text{Colonization} \times \text{Activity}$

(225) The above equation requires (1) the average colonization per unit of plant tissue, and (2) the activity as either the amount of nitrogen fixed or the amount of ammonia excreted by each microbial cell. To convert to pounds of nitrogen per acre, corn growth physiology is tracked over time, e.g., size of the plant and associated root system throughout the maturity stages.

(226) The pounds of nitrogen delivered to a crop per acre-season can be calculated by the following equation:

$$\text{Nitrogen delivered} = \int \text{Plant Tissue}(t) \times \text{Colonization}(t) \times \text{Activity}(t)\,dt$$

(227) The Plant Tissue (t) is the fresh weight of corn plant tissue over the growing time (t). Values for reasonably making the calculation are described in detail in the publication entitled Roots, Growth and Nutrient Uptake (Mengel. Dept. of Agronomy Pub. #AGRY-95-08 (Rev. May-95. p. 1-8.).

(228) The Colonization (t) is the amount of the microbes of interest found within the plant tissue, per gram fresh weight of plant tissue, at any particular time, t, during the growing season. In the instance of only a single timepoint available, the single timepoint is normalized as the peak colonization rate over the season, and the colonization rate of the remaining timepoints are adjusted accordingly.

(229) Activity (t) is the rate of which N is fixed by the microbes of interest per unit time, at any particular time, t, during the growing season. In the embodiments disclosed herein, this activity rate is approximated by in vitro acetylene reduction assay (ARA) in ARA media in the presence of 5 mM glutamine or Ammonium excretion assay in ARA media in the presence of 5 mM ammonium ions.

(230) The Nitrogen delivered amount is then calculated by numerically integrating the above function. In cases where the values of the variables described above are discretely measured at set timepoints, the values in between those timepoints are approximated by performing linear interpolation.

(231) Nitrogen Fixation

(232) Described herein are methods of increasing nitrogen fixation in a plant, comprising exposing the plant to bacteria comprising one or more genetic variations introduced into one or more genes regulating nitrogen fixation, wherein the bacteria produce 1% or more of nitrogen in the plant (e.g. 2%, 5%, 10%, or more), which may represent a nitrogen-fixation capability of at least 2-fold as compared to the plant in the absence of the bacteria. The bacteria may produce the nitrogen in the presence of fertilizer supplemented with glutamine, urea, nitrates or ammonia. Genetic variations can be any genetic variation described herein, including examples provided above, in any number and any combination. The genetic variation may be introduced into a gene selected from the group consisting of nifA, nifL, ntrB, ntrC, glutamine synthetase, glnA, glnB, glnK, draT, amtB, glutaminase, glnD, glnE, nifJ, nifH, nifD, nifK, nifY, nifE, nifN, nifU, nifS, nifV, nifW, nifZ, nifM, nifF, nifB, and nifQ. The genetic variation may be a mutation that results in one or more of: increased expression or activity of nifA or glutaminase; decreased expression or activity of nifL, ntrB, glutamine synthetase, glnB, glnK, draT, amtB; decreased adenylyl-removing activity of GlnE; or decreased uridylyl-removing activity of GlnD. The genetic variation introduced into one or more bacteria of the methods disclosed herein may be a knock-out mutation or it may abolish a regulatory sequence of a target gene, or it may comprise insertion of a heterologous regulatory sequence, for example, insertion of a regulatory sequence found within the genome of the same bacterial species or genus. The regulatory sequence can be chosen based on the expression level of a gene in a bacterial culture or within plant tissue. The genetic variation may be produced by chemical mutagenesis. The plants grown in step (c) may be exposed to biotic or abiotic stressors.

(233) In some embodiments, non-intergeneric remodeled bacteria of the present disclosure each

produce fixed N of at least about 2×10.sup.−13 mmol of N per CFU per hour, about 2.5×10.sup.−13 mmol of N per CFU per hour, about 3×10.sup.−13 mmol of N per CFU per hour, about 3.5×10.sup.−13 mmol of N per CFU per hour, about 4×10.sup.−13 mmol of N per CFU per hour, about 4.5×10.sup.−13 mmol of N per CFU per hour, about 5×10.sup.−13 mmol of N per CFU per hour, about 5.5×10.sup.−13 mmol of N per CFU per hour, about 6×10.sup.−13 mmol of N per CFU per hour, about 6.5×10.sup.−13 mmol of N per CFU per hour, about 7×10.sup.−13 mmol of N per CFU per hour, about 7.5×10.sup.−13 mmol of N per CFU per hour, about 8×10.sup.−13 mmol of N per CFU per hour, about 8.5×10.sup.−13 mmol of N per CFU per hour, about 9×10.sup.−13 mmol of N per CFU per hour, about 9.5×10.sup.−13 mmol of N per CFU per hour, or about 10×10.sup.−13 mmol of N per CFU per hour.

(234) In some embodiments, non-intergeneric remodeled bacteria of the present disclosure each produce fixed N of at least about 2×10.sup.−12 mmol of N per CFU per hour, about 2.25×10.sup.−12 mmol of N per CFU per hour, about 2.5×10.sup.−12 mmol of N per CFU per hour, about 2.75×10.sup.−12 mmol of N per CFU per hour, about 3×10.sup.−12 mmol of N per CFU per hour, about 3.25×10.sup.−12 mmol of N per CFU per hour, about 3.5×10.sup.−12 mmol of N per CFU per hour, about 3.75×10.sup.−12 mmol of N per CFU per hour, about 4×10.sup.−12 mmol of N per CFU per hour, about 4.25×10.sup.−12 mmol of N per CFU per hour, about 4.5×10.sup.−12 mmol of N per CFU per hour, about 4.75×10.sup.−12 mmol of N per CFU per hour, about 5×10.sup.−12 mmol of N per CFU per hour, about 5.25×10.sup.−12 mmol of N per CFU per hour, about 5.5×10.sup.−12 mmol of N per CFU per hour, about 5.75×10.sup.−12 mmol of N per CFU per hour, about 6×10.sup.−12 mmol of N per CFU per hour, about 6.25×10.sup.−12 mmol of N per CFU per hour, about 6.5×10.sup.−12 mmol of N per CFU per hour, about 6.75×10.sup.−12 mmol of N per CFU per hour, about 7×10.sup.−12 mmol of N per CFU per hour, about 7.25×10.sup.−12 mmol of N per CFU per hour, about 7.5×10.sup.−12 mmol of N per CFU per hour, about 7.75×10.sup.−12 mmol of N per CFU per hour, about 8×10.sup.−12 mmol of N per CFU per hour, about 8.25×10.sup.−12 mmol of N per CFU per hour, about 8.5×10.sup.−12 mmol of N per CFU per hour, about 8.75×10.sup.−12 mmol of N per CFU per hour, about 9×10.sup.−12 mmol of N per CFU per hour, about 9.25×10.sup.−12 mmol of N per CFU per hour, about 9.5×10.sup.−12 mmol of N per CFU per hour, about 9.75×10.sup.−12 mmol of N per CFU per hour, or about 10×10.sup.−12 mmol of N per CFU per hour.

(235) In some embodiments, non-intergeneric remodeled bacteria of the present disclosure each produce fixed N of at least about 5.49×10.sup.−13 mmol of N per CFU per hour. In some embodiments, non-intergeneric remodeled bacteria of the present disclosure produce fixed N of at least about 4.03×10.sup.−13 mmol of N per CFU per hour. In some embodiments, non-intergeneric remodeled bacteria of the present disclosure produce fixed N of at least about 2.75×10.sup.−12 mmol of N per CFU per hour.

(236) In some embodiments, non-intergeneric remodeled bacteria of the present disclosure in aggregate produce at least about 15 pounds of fixed N per acre, at least about 20 pounds of fixed N per acre, at least about 25 pounds of fixed N per acre, at least about 30 pounds of fixed N per acre, at least about 35 pounds of fixed N per acre, at least about 40 pounds of fixed N per acre, at least about 45 pounds of fixed N per acre, at least about 50 pounds of fixed N per acre, at least about 55 pounds of fixed N per acre, at least about 60 pounds of fixed N per acre, at least about 65 pounds of fixed N per acre, at least about 70 pounds of fixed N per acre, at least about 75 pounds of fixed N per acre, at least about 80 pounds of fixed N per acre, at least about 85 pounds of fixed N per acre, at least about 90 pounds of fixed N per acre, at least about 95 pounds of fixed N per acre, or at least about 100 pounds of fixed N per acre.

(237) In some embodiments, non-intergeneric remodeled bacteria of the present disclosure produce fixed N in the amounts disclosed herein over the course of at least about day 0 to about 80 days, at least about day 0 to about 70 days, at least about day 0 to about 60 days, at least about 1 day to about 80 days, at least about 1 day to about 70 days, at least about 1 day to about 60 days, at least

about 2 days to about 80 days, at least about 2 days to about 70 days, at least about 2 days to about 60 days, at least about 3 days to about 80 days, at least about 3 days to about 70 days, at least about 3 days to about 60 days, at least about 4 days to about 80 days, at least about 4 days to about 70 days, at least about 4 days to about 60 days, at least about 5 days to about 80 days, at least about 5 days to about 70 days, at least about 5 days to about 60 days, at least about 6 days to about 80 days, at least about 6 days to about 70 days, at least about 6 days to about 60 days, at least about 7 days to about 80 days, at least about 7 days to about 70 days, at least about 7 days to about 60 days, at least about 8 days to about 80 days, at least about 8 days to about 70 days, at least about 8 days to about 60 days, at least about 9 days to about 80 days, at least about 9 days to about 70 days, at least about 9 days to about 60 days, at least about 10 days to about 80 days, at least about 10 days to about 70 days, at least about 10 days to about 60 days, at least about 15 days to about 80 days, at least about 15 days to about 70 days, at least about 15 days to about 60 days, at least about 20 days to about 80 days, at least about 20 days to about 70 days, or at least about 20 days to about 60 days.

(238) In some embodiments, non-intergeneric remodeled bacteria of the present disclosure produce fixed N in any of the amounts disclosed herein over the course of at least about 80 days ±5 days, at least about 80 days ±10 days, at least about 80 days ±15 days, at least about 80 days ±20 days, at least about 75 days ±5 days, at least about 75 days ±10 days, at least about 75 days ±15 days, at least about 75 days ±20 days, at least about 70 days ±5 days, at least about 70 days ±10 days, at least about 70 days ±15 days, at least about 70 days ±20 days, at least about 60 days ±5 days, at least about 60 days ±10 days, at least about 60 days ±15 days, at least about 60 days ±20 days.

(239) In some embodiments, non-intergeneric remodeled bacteria of the present disclosure produce fixed N in any of the amounts disclosed herein over the course of at least about 10 days to about 80 days, at least about 10 days to about 70 days, or at least about 10 days to about 60 days.

(240) In some embodiments, non-intergeneric remodeled bacteria of the present disclosure produce fixed N in the amounts and time shown in FIG. **30**A, right panel.

(241) The amount of nitrogen fixation that occurs in the plants described herein may be measured in several ways, for example by an acetylene-reduction (AR) assay. An acetylene-reduction assay can be performed in vitro or in vivo. Evidence that a particular bacterium is providing fixed nitrogen to a plant can include: 1) total plant N significantly increases upon inoculation, preferably with a concomitant increase in N concentration in the plant; 2) nitrogen deficiency symptoms are relieved under N-limiting conditions upon inoculation (which should include an increase in dry matter); 3) N.sub.2 fixation is documented through the use of an .sup.15N approach (which can be isotope dilution experiments, .sup.15N.sub.2 reduction assays, or .sup.15N natural abundance assays); 4) fixed N is incorporated into a plant protein or metabolite; and 5) all of these effects are not be seen in non-inoculated plants or in plants inoculated with a mutant of the inoculum strain.

(242) The wild-type nitrogen fixation regulatory cascade can be represented as a digital logic circuit where the inputs O.sub.2 and NH.sub.4.sup.+ pass through a NOR gate, the output of which enters an AND gate in addition to ATP. In some embodiments, the methods disclosed herein disrupt the influence of NH.sub.4.sup.+ on this circuit, at multiple points in the regulatory cascade, so that microbes can produce nitrogen even in fertilized fields. However, the methods disclosed herein also envision altering the impact of ATP or O.sub.2 on the circuitry, or replacing the circuitry with other regulatory cascades in the cell, or altering genetic circuits other than nitrogen fixation. Gene clusters can be re-engineered to generate functional products under the control of a heterologous regulatory system. By eliminating native regulatory elements outside of, and within, coding sequences of gene clusters, and replacing them with alternative regulatory systems, the functional products of complex genetic operons and other gene clusters can be controlled and/or moved to heterologous cells, including cells of different species other than the species from which the native genes were derived. Once re-engineered, the synthetic gene clusters can be controlled by genetic circuits or other inducible regulatory systems, thereby controlling the products' expression as desired. The expression cassettes can be designed to act as logic gates, pulse generators, oscillators,

switches, or memory devices. The controlling expression cassette can be linked to a promoter such that the expression cassette functions as an environmental sensor, such as an oxygen, temperature, touch, osmotic stress, membrane stress, or redox sensor.

(243) As an example, the nifL, nifA, nifT, and nifX genes can be eliminated from the nif gene cluster. Synthetic genes can be designed by codon randomizing the DNA encoding each amino acid sequence. Codon selection is performed, specifying that codon usage be as divergent as possible from the codon usage in the native gene. Proposed sequences are scanned for any undesired features, such as restriction enzyme recognition sites, transposon recognition sites, repetitive sequences, sigma 54 and sigma 70 promoters, cryptic ribosome binding sites, and rho independent terminators. Synthetic ribosome binding sites are chosen to match the strength of each corresponding native ribosome binding site, such as by constructing a fluorescent reporter plasmid in which the 150 bp surrounding a gene's start codon (from −60 to +90) is fused to a fluorescent gene. This chimera can be expressed under control of the Ptac promoter, and fluorescence measured via flow cytometry. To generate synthetic ribosome binding sites, a library of reporter plasmids using 150 bp (−60 to +90) of a synthetic expression cassette is generated. Briefly, a synthetic expression cassette can consist of a random DNA spacer, a degenerate sequence encoding an RBS library, and the coding sequence for each synthetic gene. Multiple clones are screened to identify the synthetic ribosome binding site that best matched the native ribosome binding site. Synthetic operons that consist of the same genes as the native operons are thus constructed and tested for functional complementation. A further exemplary description of synthetic operons is provided in US20140329326.

(244) Bacterial Species

(245) Microbes useful in the methods and compositions disclosed herein may be obtained from any source. In some cases, microbes may be bacteria, archaea, protozoa or fungi. The microbes of this disclosure may be nitrogen fixing microbes, for example a nitrogen fixing bacteria, nitrogen fixing archaea, nitrogen fixing fungi, nitrogen fixing yeast, or nitrogen fixing protozoa. Microbes useful in the methods and compositions disclosed herein may be spore forming microbes, for example spore forming bacteria. In some cases, bacteria useful in the methods and compositions disclosed herein may be Gram positive bacteria or Gram negative bacteria. In some cases, the bacteria may be an endospore forming bacteria of the Firmicute phylum. In some cases, the bacteria may be a diazotroph. In some cases, the bacteria may not be a diazotroph.

(246) The methods and compositions of this disclosure may be used with an archaea, such as, for example, *Methanothermobacter thermoautotrophicus.*

(247) In some cases, bacteria which may be useful include, but are not limited to, *Agrobacterium radiobacter, Bacillus acidocaldarius, Bacillus acidoterrestris, Bacillus agri, Bacillus aizawai, Bacillus albolactis, Bacillus alcalophilus, Bacillus alvei, Bacillus aminoglucosidicus, Bacillus aminovorans, Bacillus amylolyticus* (also known as *Paenibacillus amylolyticus*) *Bacillus amyloliquefaciens, Bacillus aneurinolyticus, Bacillus atrophaeus, Bacillus azotoformans, Bacillus badius, Bacillus cereus* (synonyms: *Bacillus endorhythmos, Bacillus medusa*), *Bacillus chitinosporus, Bacillus circulars, Bacillus coagulans, Bacillus endoparasiticus Bacillus fastidiosus, Bacillus firmus, Bacillus kurstaki, Bacillus lacticola, Bacillus lactimorbus, Bacillus lactis, Bacillus laterosporus* (also known as *Brevibacillus laterosporus*), *Bacillus lautus, Bacillus lentimorbus, Bacillus lentus, Bacillus licheniformis, Bacillus maroccanus, Bacillus megaterium, Bacillus metiens, Bacillus mycoides, Bacillus natto, Bacillus nematocida, Bacillus nigrificans, Bacillus nigrum, Bacillus pantothenticus, Bacillus popillae, Bacillus psychrosaccharolyticus, Bacillus pumilus, Bacillus siamensis, Bacillus smithii, Bacillus sphaericus, Bacillus subtilis, Bacillus thuringiensis, Bacillus uniflagellatus, Bradyrhizobium japonicum, Brevibacillus brevis Brevibacillus laterosporus* (formerly *Bacillus laterosporus*), *Chromobacterium subtsugae, Delftia acidovorans, Lactobacillus acidophilus, Lysobacter antibioticus, Lysobacter enzymogenes, Paenibacillus alvei, Paenibacillus polymyxa, Paenibacillus popilliae* (formerly *Bacillus popilliae*),

Pantoea agglomerans, Pasteuria penetrans (formerly *Bacillus penetrans*), *Pasteuria usgae, Pectobacterium carotovorum* (formerly *Erwinia carotovora*), *Pseudomonas aeruginosa, Pseudomonas aureofaciens, Pseudomonas cepacia* (formerly known as *Burkholderia cepacia*), *Pseudomonas chlororaphis, Pseudomonas fluorescens, Pseudomonas proradix, Pseudomonas putida, Pseudomonas syringae, Serratia entomophila, Serratia marcescens, Streptomyces colombiensis, Streptomyces galbus, Streptomyces goshikiensis, Streptomyces griseoviridis, Streptomyces lavendulae, Streptomyces prasinus, Streptomyces saraceticus, Streptomyces venezuelae, Xanthomonas campestris, Xenorhabdus luminescens, Xenorhabdus nematophila, Rhodococcus globerulus* AQ719 (NRRL Accession No. B-21663), *Bacillus* sp. AQ175 (ATCC Accession No. 55608), *Bacillus* sp. AQ 177 (ATCC Accession No. 55609), *Bacillus* sp. AQ178 (ATCC Accession No. 53522), and *Streptomyces* sp. strain NRRL Accession No. B-30145. In some cases the bacterium may be *Azotobacter chroococcum, Methanosarcina barkeri, Klesiella pneumoniae, Azotobacter vinelandii, Rhodobacter spharoides, Rhodobacter capsulatus, Rhodobcter palustris, Rhodosporillum rubrum, Rhizobium leguminosarum* or *Rhizobium etli.*

(248) In some cases the bacterium may be a species of *Clostridium,* for example *Clostridium pasteurianum, Clostridium beijerinckii, Clostridium perfringens, Clostridium tetani, Clostridium acetobutylicum.*

(249) In some cases, bacteria used with the methods and compositions of the present disclosure may be cyanobacteria. Examples of cyanobacterial genuses include *Anabaena* (for example *Anagaena* sp. PCC7120), *Nostoc* (for example *Nostoc punctiforme*), or *Synechocystis* (for example *Synechocystis* sp. PCC6803).

(250) In some cases, bacteria used with the methods and compositions of the present disclosure may belong to the phylum Chlorobi, for example *Chlorobium tepidum.*

(251) In some cases, microbes used with the methods and compositions of the present disclosure may comprise a gene homologous to a known NifH gene. Sequences of known NifH genes may be found in, for example, the Zehr lab NifH database, (wwwzehr.pmc.ucsc.edu/nifH_Database_Public/, Apr. 4, 2014), or the Buckley lab NifH database (www.css.cornell.edu/faculty/buckley/nifh.htm, and Gaby, John Christian, and Daniel H. Buckley. "A comprehensive aligned nifH gene database: a multipurpose tool for studies of nitrogen-fixing bacteria." *Database* 2014 (2014): bau001.). In some cases, microbes used with the methods and compositions of the present disclosure may comprise a sequence which encodes a polypeptide with at least 60%, 70%, 80%, 85%, 90%, 95%, 96%, 96%, 98%, 99% or more than 99% sequence identity to a sequence from the Zehr lab NifH database, (wwwzehr.pmc.ucsc.edu/nifH_Database_Public/, Apr. 4, 2014). In some cases, microbes used with the methods and compositions of the present disclosure may comprise a sequence which encodes a polypeptide with at least 60%, 70%, 80%, 85%, 90%, 95%, 96%, 96%, 98%, 99% or more than 99% sequence identity to a sequence from the Buckley lab NifH database, (Gaby, John Christian, and Daniel H. Buckley. "A comprehensive aligned nifH gene database: a multipurpose tool for studies of nitrogen-fixing bacteria." *Database* 2014 (2014): bau001.).

(252) Microbes useful in the methods and compositions disclosed herein can be obtained by extracting microbes from surfaces or tissues of native plants; grinding seeds to isolate microbes; planting seeds in diverse soil samples and recovering microbes from tissues; or inoculating plants with exogenous microbes and determining which microbes appear in plant tissues. Non-limiting examples of plant tissues include a seed, seedling, leaf, cutting, plant, bulb, tuber, root, and rhizomes. In some cases, bacteria are isolated from a seed. The parameters for processing samples may be varied to isolate different types of associative microbes, such as rhizospheric, epiphytes, or endophytes. Bacteria may also be sourced from a repository, such as environmental strain collections, instead of initially isolating from a first plant. The microbes can be genotyped and phenotyped, via sequencing the genomes of isolated microbes; profiling the composition of communities in planta; characterizing the transcriptomic functionality of communities or isolated

microbes; or screening microbial features using selective or phenotypic media (e.g., nitrogen fixation or phosphate solubilization phenotypes). Selected candidate strains or populations can be obtained via sequence data; phenotype data; plant data (e.g., genome, phenotype, and/or yield data); soil data (e.g., pH, N/P/K content, and/or bulk soil biotic communities); or any combination of these.

(253) The bacteria and methods of producing bacteria described herein may apply to bacteria able to self-propagate efficiently on the leaf surface, root surface, or inside plant tissues without inducing a damaging plant defense reaction, or bacteria that are resistant to plant defense responses. The bacteria described herein may be isolated by culturing a plant tissue extract or leaf surface wash in a medium with no added nitrogen. However, the bacteria may be unculturable, that is, not known to be culturable or difficult to culture using standard methods known in the art. The bacteria described herein may be an endophyte or an epiphyte or a bacterium inhabiting the plant rhizosphere (rhizospheric bacteria). The bacteria obtained after repeating the steps of introducing genetic variation, exposure to a plurality of plants, and isolating bacteria from plants with an improved trait one or more times (e.g. 1, 2, 3, 4, 5, 10, 15, 25, or more times) may be endophytic, epiphytic, or rhizospheric. Endophytes are organisms that enter the interior of plants without causing disease symptoms or eliciting the formation of symbiotic structures, and are of agronomic interest because they can enhance plant growth and improve the nutrition of plants (e.g., through nitrogen fixation). The bacteria can be a seed-borne endophyte. Seed-borne endophytes include bacteria associated with or derived from the seed of a grass or plant, such as a seed-borne bacterial endophyte found in mature, dry, undamaged (e.g., no cracks, visible fungal infection, or prematurely germinated) seeds. The seed-borne bacterial endophyte can be associated with or derived from the surface of the seed; alternatively, or in addition, it can be associated with or derived from the interior seed compartment (e.g., of a surface-sterilized seed). In some cases, a seed-borne bacterial endophyte is capable of replicating within the plant tissue, for example, the interior of the seed. Also, in some cases, the seed-borne bacterial endophyte is capable of surviving desiccation.

(254) The bacterial isolated according to methods of the disclosure, or used in methods or compositions of the disclosure, can comprise a plurality of different bacterial taxa in combination. By way of example, the bacteria may include Proteobacteria (such as *Pseudomonas, Enterobacter, Stenotrophomonas, Burkholderia, Rhizobium, Herbaspirillum, Pantoea, Serratia, Rahnella, Azospirillum, Azorhizobium, Azotobacter, Duganella, Delftia, Bradyrhizobiun, Sinorhizobium* and *Halomonas*), Firmicutes (such as *Bacillus, Paenibacillus, Lactobacillus, Mycoplasma*, and *Acetabacterium*), and Actinobacteria (such as *Streptomyces, Rhodacoccus, Microbacterium*, and *Curtobacterium*). The bacteria used in methods and compositions of this disclosure may include nitrogen fixing bacterial consortia of two or more species. In some cases, one or more bacterial species of the bacterial consortia may be capable of fixing nitrogen. In some cases, one or more species of the bacterial consortia may facilitate or enhance the ability of other bacteria to fix nitrogen. The bacteria which fix nitrogen and the bacteria which enhance the ability of other bacteria to fix nitrogen may be the same or different. In some examples, a bacterial strain may be able to fix nitrogen when in combination with a different bacterial strain, or in a certain bacterial consortia, but may be unable to fix nitrogen in a monoculture. Examples of bacterial genuses which may be found in a nitrogen fixing bacterial consortia include, but are not limited to, *Herbaspirillum, Azospirillum, Enterobacter*, and *Bacillus.*

(255) Bacteria that can be produced by the methods disclosed herein include *Azotobacter* sp., *Bradyrhizobium* sp., *Klebsiella* sp., and *Sinorhizobium* sp. In some cases, the bacteria may be selected from the group consisting of: *Azotobacter vinelandii, Bradyrhizobium japonicum, Klebsiella pneumoniae*, and *Sinorhizobium meliloti*. In some cases, the bacteria may be of the genus *Enterobacter* or *Rahnella*. In some cases, the bacteria may be of the genus *Frankia*, or *Clostridium*. Examples of bacteria of the genus *Clostridium* include, but are not limited to,

Clostridium acetobutilicum, Clostridium pasteurianum, Clostridium beijerinckii, Clostridium perfringens, and *Clostridium tetani*. In some cases, the bacteria may be of the genus *Paenibacillus*, for example *Paenibacillus azotofixans, Paenibacillus borealis, Paenibacillus* durus, *Paenibacillus* macerans, *Paenibacillus* polymyxa, *Paenibacillus* alvei, *Paenibacillus* amylolyticus, *Paenibacillus* campinasensis, *Paenibacillus* chibensis, *Paenibacillus* glucanolyticus, *Paenibacillus* illinoisensis, *Paenibacillus larvae* subsp. Larvae, *Paenibacillus larvae* subsp. *Pulvifaciens, Paenibacillus* lautus, *Paenibacillus* macerans, *Paenibacillus* macquariensis, *Paenibacillus* macquariensis, *Paenibacillus* pabuli, *Paenibacillus peoriae*, or *Paenibacillus* polymyxa.

(256) In some examples, bacteria isolated according to methods of the disclosure can be a member of one or more of the following taxa: *Achromobacter, Acidithiobacillus, Acidovorax, Acidovoraz, Acinetobacter, Actinoplanes, Adlercreutzia, Aerococcus, Aeromonas, Afipia, Agromyces, Ancylobacter, Arthrobacter, Atopostipes, Azospirillum, Bacillus, Bdellovibrio, Beijerinckia, Bosea, Bradyrhizobium, Brevibacillus, Brevundimonas, Burkholderia, Candidatus Haloredivivus, Caulobacter, Cellulomonas, Cellvibrio, Chryseobacterium, Citrobacter, Clostridium, Coraliomargarita, Corynebacterium, Cupriavidus, Curtobacterium, Curvibacter, Deinococcus, Delftia, Desemzia, Devosia, Dokdonella, Dyella, Enhydrobacter, Enterobacter, Enterococcus, Erwinia, Escherichia, Escherichia/Shigella, Exiguobacterium, Ferroglobus, Filimonas, Finegoldia, Flavisolibacter, Flavobacterium, Frigoribacterium, Gluconacetobacter, Hafnia, Halobaculum, Halomonas, Halosimplex, Herbaspirillum, Hymenobacter, Klebsiella, Kocuria, Kosakonia, Lactobacillus, Leclercia, Lentzea, Luteibacter, Luteimonas, Massilia, Mesorhizobium, Methylobacterium, Microbacterium, Micrococcus, Microvirga, Mycobacterium, Neisseria, Nocardia, Oceanibaculum, Ochrobactrum, Okibacterium, Oligotropha, Oryzihumus, Oxalophagus, Paenibacillus, Panteoa, Pantoea, Pelomonas, Perlucidibaca, Plantibacter, Polynucleobacter, Propionibacterium, Propioniciclava, Pseudoclavibacter, Pseudomonas, Pseudonocardia, Pseudoxanthomonas, Psychrobacter, Rahnella, Ralstonia, Rheinheimera, Rhizobium, Rhodococcus, Rhodopseudomonas, Roseateles, Ruminococcus, Sebaldella, Sediminibacillus, Sediminibacterium, Serratia, Shigella, Shinella, Sinorhizobium, Sinosporangium, Sphingobacterium, Sphingomonas, Sphingopyxis, Sphingosinicella, Staphylococcus,* 25 *Stenotrophomonas, Strenotrophomonas, Streptococcus, Streptomyces, Stygiolobus, Sulfurisphaera, Tatumella, Tepidimonas, Thermomonas, Thiobacillus, Variovorax*, WPS-2 genera incertae *sedis, Xanthomonas*, and *Zimmermannella*.

(257) In some cases, a bacterial species selected from at least one of the following genera are utilized: *Enterobacter, Klebsiella, Kosakonia*, and *Rahnella*. In some cases, a combination of bacterial species from the following genera are utilized: *Enterobacter, Klebsiella, Kosakonia*, and *Rahnella*. In some cases, the species utilized can be one or more of: *Enterobacter sacchari, Klebsiella variicola, Kosakonia sacchari*, and *Rahnella* aquatilis.

(258) In some cases, a Gram positive microbe may have a Molybdenum-Iron nitrogenase system comprising: nifH, nifD, nifK, nifB, nifE, nifN, nifX, hesA, MTV, nifW, nifS, nifI1, and nifI2. In some cases, a Gram positive microbe may have a vanadium nitrogenase system comprising: vnfDG, vnfK, vnfE, vnfN, vupC, vupB, vupA, vnfV, vnfR1, vnfH, vnfR2, vnfA (transcriptional regulator). In some cases, a Gram positive microbe may have an iron-only nitrogenase system comprising: anfK, anfG, anfD, anfH, anfA (transcriptional regulator). In some cases, a Gram positive microbe may have a nitrogenase system comprising glnB, and glnK (nitrogen signaling proteins). Some examples of enzymes involved in nitrogen metabolism in Gram positive microbes include glnA (glutamine synthetase), gdh (glutamate dehydrogenase), bdh (3-hydroxybutyrate dehydrogenase), glutaminase, gltAB/gltB/gltS (glutamate synthase), asnA/asnB (aspartate-ammonia ligase/asparagine synthetase), and ansA/ansZ (asparaginase). Some examples of proteins involved in nitrogen transport in Gram positive microbes include amtB (ammonium transporter), glnK (regulator of ammonium transport), glnPHQ/glnQHMP (ATP-dependent glutamine/glutamate transporters), glnT/alsT/yrbD/ylfA (glutamine-like proton symport transporters), and gltP/gltT/yhcl/nqt (glutamate-like proton symport transporters).

(259) Examples of Gram positive microbes which may be of particular interest include *Paenibacillus polymixa, Paenibacillus riograndensis, Paenibacillus* sp., *Frankia* sp., *Heliobacterium* sp., *Heliobacterium chlorum, Heliobacillus* sp., *Heliophilum* sp., *Heliorestis* sp., *Clostridium acetobutylicum, Clostridium* sp., *Mycobacterium flaum, Mycobacterium* sp., *Arthrobacter* sp., *Agromyces* sp., *Corynebacterium autitrophicum, Corynebacterium* sp., *Micromonspora* sp., *Propionibacteria* sp., *Streptomyces* sp., and *Microbacterium* sp.,
(260) Some examples of genetic alterations which may be made in Gram positive microbes include: deleting glnR to remove negative regulation of BNF in the presence of environmental nitrogen, inserting different promoters directly upstream of the nif cluster to eliminate regulation by GlnR in response to environmental nitrogen, mutating glnA to reduce the rate of ammonium assimilation by the GS-GOGAT pathway, deleting amtB to reduce uptake of ammonium from the media, mutating glnA so it is constitutively in the feedback-inhibited (FBI-GS) state, to reduce ammonium assimilation by the GS-GOGAT pathway.
(261) In some cases, glnR is the main regulator of N metabolism and fixation in *Paenibacillus* species. In some cases, the genome of a *Paenibacillus* species may not contain a gene to produce glnR. In some cases, the genome of a *Paenibacillus* species may not contain a gene to produce glnE or glnD. In some cases, the genome of a *Paenibacillus* species may contain a gene to produce glnB or glnK. For example, *Paenibacillus* sp. WLY78 doesn't contain a gene for glnB, or its homologs found in the archaeon *Methanococcus maripaludis*, nifI1 and nifI2. In some cases, the genomes of *Paenibacillus* species may be variable. For example, *Paenibacillus polymixa* E681 lacks glnK and gdh, has several nitrogen compound transporters, but only amtB appears to be controlled by GlnR. In another example, *Paenibacillus* sp. JDR2 has glnK, gdh and most other central nitrogen metabolism genes, has many fewer nitrogen compound transporters, but does have ginPHQ controlled by GlnR. *Paenibacillus riograndensis* SBR5 contains a standard glnRA operon, an fdx gene, a main nif operon, a secondary nif operon, and an anf operon (encoding iron-only nitrogenase). Putative glnR/tnrA sites were found upstream of each of these operons. GlnR may regulate all of the above operons, except the anf operon. GlnR may bind to each of these regulatory sequences as a dimer.
(262) *Paenibacillus* N-fixing strains may fall into two subgroups: Subgroup I, which contains only a minimal nif gene cluster and subgroup II, which contains a minimal cluster, plus an uncharacterized gene between nifX and hesA, and often other clusters duplicating some of the nif genes, such as nifH, nifHDK, nifBEN, or clusters encoding vanadaium nitrogenase (vnf) or iron-only nitrogenase (anf) genes.
(263) In some cases, the genome of a *Paenibacillus* species may not contain a gene to produce glnB or glnK. In some cases, the genome of a *Paenibacillus* species may contain a minimal nif cluster with 9 genes transcribed from a sigma-70 promoter. In some cases, a *Paenibacillus* nif cluster may be negatively regulated by nitrogen or oxygen. In some cases, the genome of a *Paenibacillus* species may not contain a gene to produce sigma-54. For example, *Paenibacillus* sp. WLY78 does not contain a gene for sigma-54. In some cases, a nif cluster may be regulated by glnR, and/or TnrA. In some cases, activity of a nif cluster may be altered by altering activity of glnR, and/or TnrA.
(264) In Bacilli, glutamine synthetase (GS) is feedback-inhibited by high concentrations of intracellular glutamine, causing a shift in confirmation (referred to as FBI-GS). Nif clusters contain distinct binding sites for the regulators GlnR and TnrA in several Bacilli species. GlnR binds and represses gene expression in the presence of excess intracellular glutamine and AMP. A role of GlnR may be to prevent the influx and intracellular production of glutamine and ammonium under conditions of high nitrogen availability. TnrA may bind and/or activate (or repress) gene expression in the presence of limiting intracellular glutamine, and/or in the presence of FBI-GS. In some cases, the activity of a Bacilli nif cluster may be altered by altering the activity of GlnR.
(265) Feedback-inhibited glutamine synthetase (FBI-GS) may bind GlnR and stabilize binding of

GlnR to recognition sequences. Several bacterial species have a GlnR/TnrA binding site upstream of the nif cluster. Altering the binding of FBI-GS and GlnR may alter the activity of the nif pathway.

(266) Sources of Microbes

(267) The bacteria (or any microbe according to the disclosure) may be obtained from any general terrestrial environment, including its soils, plants, fungi, animals (including invertebrates) and other biota, including the sediments, water and biota of lakes and rivers; from the marine environment, its biota and sediments (for example, sea water, marine muds, marine plants, marine invertebrates (for example, sponges), marine vertebrates (for example, fish)); the terrestrial and marine geosphere (regolith and rock, for example, crushed subterranean rocks, sand and clays); the cryosphere and its meltwater; the atmosphere (for example, filtered aerial dusts, cloud and rain droplets); urban, industrial and other man-made environments (for example, accumulated organic and mineral matter on concrete, roadside gutters, roof surfaces, and road surfaces).

(268) The plants from which the bacteria (or any microbe according to the disclosure) are obtained may be a plant having one or more desirable traits, for example a plant which naturally grows in a particular environment or under certain conditions of interest. By way of example, a certain plant may naturally grow in sandy soil or sand of high salinity, or under extreme temperatures, or with little water, or it may be resistant to certain pests or disease present in the environment, and it may be desirable for a commercial crop to be grown in such conditions, particularly if they are, for example, the only conditions available in a particular geographic location. By way of further example, the bacteria may be collected from commercial crops grown in such environments, or more specifically from individual crop plants best displaying a trait of interest amongst a crop grown in any specific environment: for example the fastest-growing plants amongst a crop grown in saline-limiting soils, or the least damaged plants in crops exposed to severe insect damage or disease epidemic, or plants having desired quantities of certain metabolites and other compounds, including fiber content, oil content, and the like, or plants displaying desirable colors, taste or smell. The bacteria may be collected from a plant of interest or any material occurring in the environment of interest, including fungi and other animal and plant biota, soil, water, sediments, and other elements of the environment as referred to previously.

(269) The bacteria (or any microbe according to the disclosure) may be isolated from plant tissue. This isolation can occur from any appropriate tissue in the plant, including for example root, stem and leaves, and plant reproductive tissues. By way of example, conventional methods for isolation from plants typically include the sterile excision of the plant material of interest (e.g. root or stem lengths, leaves), surface sterilization with an appropriate solution (e.g. 2% sodium hypochlorite), after which the plant material is placed on nutrient medium for microbial growth. Alternatively, the surface-sterilized plant material can be crushed in a sterile liquid (usually water) and the liquid suspension, including small pieces of the crushed plant material spread over the surface of a suitable solid agar medium, or media, which may or may not be selective (e.g. contain only phytic acid as a source of phosphorus). This approach is especially useful for bacteria which form isolated colonies and can be picked off individually to separate plates of nutrient medium, and further purified to a single species by well-known methods. Alternatively, the plant root or foliage samples may not be surface sterilized but only washed gently thus including surface-dwelling epiphytic microorganisms in the isolation process, or the epiphytic microbes can be isolated separately, by imprinting and lifting off pieces of plant roots, stem or leaves onto the surface of an agar medium and then isolating individual colonies as above. This approach is especially useful for bacteria, for example. Alternatively, the roots may be processed without washing off small quantities of soil attached to the roots, thus including microbes that colonize the plant rhizosphere. Otherwise, soil adhering to the roots can be removed, diluted and spread out onto agar of suitable selective and non-selective media to isolate individual colonies of rhizospheric bacteria.

(270) Budapest Treaty on the International Recognition of the Deposit of Microorganisms for the

Purpose of Patent Procedures

(271) The microbial deposits of the present disclosure were made under the provisions of the Budapest Treaty on the International Recognition of the Deposit of Microorganisms for the Purpose of Patent Procedure (Budapest Treaty).

(272) Applicants state that pursuant to 37 C.F.R. § 1.808(a)(2) “all restrictions imposed by the depositor on the availability to the public of the deposited material will be irrevocably removed upon the granting of the patent.” This statement is subject to paragraph (b) of this section (i.e. 37 C.F.R. § 1.808(b)).

(273) The *Enterobacter sacchari* has now been reclassified as *Kosakonia sacchari*, the name for the organism may be used interchangeably throughout the manuscript.

(274) Many microbes of the present disclosure are derived from two wild-type strains, as depicted in FIG. **6** and FIG. **7**. Strain CI006 is a bacterial species previously classified in the genus *Enterobacter* (see aforementioned reclassification into *Kosakonia*), and FIG. **6** identifies the lineage of the mutants that have been derived from CI006. Strain CI019 is a bacterial species classified in the genus *Rahnella*, and FIG. **7** identifies the lineage of the mutants that have been derived from CI019. With regard to FIG. **6** and FIG. **7**, it is noted that strains comprising CM in the name are mutants of the strains depicted immediately to the left of said CM strain. The deposit information for the CI006 *Kosakonia* wild type (WT) and CI019 *Rahnella* WT are found in the below Table 1.

(275) Some microorganisms described in this application were deposited on Jan. 6, 2017 or Aug. 11, 2017 with the Bigelow National Center for Marine Algae and Microbiota (NCMA), located at 60 Bigelow Drive, East Boothbay, Maine 04544, USA. As aforementioned, all deposits were made under the terms of the Budapest Treaty on the International Recognition of the Deposit of Microorganisms for the Purposes of Patent Procedure. The Bigelow National Center for Marine Algae and Microbiota accession numbers and dates of deposit for the aforementioned Budapest Treaty deposits are provided in Table 1.

(276) Biologically pure cultures of *Kosakonia sacchari* (WT), *Rahnella aquatilis* (WT), and a variant/remodeled *Kosakonia sacchari* strain were deposited on Jan. 6, 2017 with the Bigelow National Center for Marine Algae and Microbiota (NCMA), located at 60 Bigelow Drive, East Boothbay, Maine 04544, USA, and assigned NCMA Patent Deposit Designation numbers 201701001, 201701003, and 201701002, respectively. The applicable deposit information is found below in Table 1.

(277) Biologically pure cultures of variant/remodeled *Kosakonia sacchari* strains were deposited on Aug. 11, 2017 with the Bigelow National Center for Marine Algae and Microbiota (NCMA), located at 60 Bigelow Drive, East Boothbay, Maine 04544, USA, and assigned NCMA Patent Deposit Designation numbers 201708004, 201708003, and 201708002, respectively. The applicable deposit information is found below in Table 1.

(278) A biologically pure culture of *Klebsiella variicola* (WT) was deposited on Aug. 11, 2017 with the Bigelow National Center for Marine Algae and Microbiota (NCMA), located at 60 Bigelow Drive, East Boothbay, Maine 04544, USA, and assigned NCMA Patent Deposit Designation number 201708001. Biologically pure cultures of two *Klebsiella variicola* variants/remodeled strains were deposited on Dec. 20, 2017 with the Bigelow National Center for Marine Algae and Microbiota (NCMA), located at 60 Bigelow Drive, East Boothbay, Maine 04544, USA, and assigned NCMA Patent Deposit Designation numbers 201712001 and 201712002, respectively. The applicable deposit information is found below in Table 1.

(279) TABLE-US-00001 TABLE 1 Microorganisms Deposited under the Budapest Treaty Pivot Strain Designation (some strains have multiple Accession Depository designations) Taxonomy Number Date of Deposit NCMA CI006, *Kosakonia sacchari* (WT) 201701001 Jan. 6, 2017 PBC6.1, 6 NCMA CI019, *Rahnella aquatilis* (WT) 201701003 Jan. 6, 2017 19 NCMA CM029, 6-412 *Kosakonia sacchari* 201701002 Jan. 6, 2017 NCMA 6-403 *Kosakonia sacchari* 201708004

Aug. 11, 2017 CM037 NCMA 6-404, *Kosakonia sacchari* 201708003 Aug. 11, 2017 CM38, PBC6.38 NCMA CM094, *Kosakonia sacchari* 201708002 Aug. 11, 2017 6-881, PBC6.94 NCMA CI137, 137, *Klebsiella variicola* (WT) 201708001 Aug. 11, 2017 PB137 NCMA 137-1034 *Klebsiella variicola* 201712001 Dec. 20, 2017 NCMA 137-1036 *Klebsiella variicola* 201712002 Dec. 20, 2017

Isolated and Biologically Pure Microorganisms

(280) The present disclosure, in certain embodiments, provides isolated and biologically pure microorganisms that have applications, inter alia, in agriculture. The disclosed microorganisms can be utilized in their isolated and biologically pure states, as well as being formulated into compositions (see below section for exemplary composition descriptions). Furthermore, the disclosure provides microbial compositions containing at least two members of the disclosed isolated and biologically pure microorganisms, as well as methods of utilizing said microbial compositions. Furthermore, the disclosure provides for methods of modulating nitrogen fixation in plants via the utilization of the disclosed isolated and biologically pure microbes.

(281) In some aspects, the isolated and biologically pure microorganisms of the disclosure are those from Table 1. In other aspects, the isolated and biologically pure microorganisms of the disclosure are derived from a microorganism of Table 1. For example, a strain, child, mutant, or derivative, of a microorganism from Table 1 are provided herein. The disclosure contemplates all possible combinations of microbes listed in Table 1, said combinations sometimes forming a microbial consortia. The microbes from Table 1, either individually or in any combination, can be combined with any plant, active molecule (synthetic, organic, etc.), adjuvant, carrier, supplement, or biological, mentioned in the disclosure.

(282) In some aspects, the disclosure provides microbial compositions comprising species as grouped in Tables 2-8. In some aspects, these compositions comprising various microbial species are termed a microbial consortia or consortium.

(283) With respect to Tables 2-8, the letters A through I represent a non-limiting selection of microorganisms of the present disclosure, defined as: A=Microbe with accession number 201701001 identified in Table 1; B=Microbe with accession number 201701003 identified in Table 1; C=Microbe with accession number 201701002 identified in Table 1; D=Microbe with accession number 201708004 identified in Table 1; E=Microbe with accession number 201708003 identified in Table 1; F=Microbe with accession number 201708002 identified in Table 1; G=Microbe with accession number 201708001 identified in Table 1; H=Microbe with accession number 201712001 identified in Table 1; and I=Microbe with accession number 201712002 identified in Table 1.

(284) TABLE-US-00002 TABLE 2 Eight and Nine Strain Compositions A, B, C, D, E, F, G, H A, B, C, D, E, F, G, I A, B, C, D, E, F, H, I A, B, C, D, E, G, H, I A, B, C, D, F, G, H, I A, B, C, E, F, G, H, I A, B, D, E, F, G, H, I A, C, D, E, F, G, H, I B, C, D, E, F, G, H, I A, B, C, D, E, F, G, H, I

(285) TABLE-US-00003 TABLE 3 Seven Strain Compositions A, B, C, D, E, F, G A, B, C, D, E, F, H A, B, C, D, E, F, I A, B, C, D, E, G, H A, B, C, D, E, G, I A, B, C, D, E, H, I A, B, C, D, F, G, H A, B, C, D, F, G, I A, B, C, D, F, H, I A, B, C, D, G, H, I A, B, C, E, F, G, H A, B, C, E, F, G, I A, B, C, E, F, H, I A, B, C, E, G, H, I A, B, C, F, G, H, I A, B, D, E, F, G, H A, B, D, E, F, G, I A, B, D, E, F, H, I A, B, D, E, G, H, I A, B, D, F, G, H, I A, B, E, F, G, H, I A, C, D, E, F, G, H A, C, D, E, F, G, I A, C, D, E, F, H, I A, C, D, E, G, H, I A, C, D, F, G, H, I A, C, E, F, G, H, I A, D, E, F, G, H, I B, C, D, E, F, G, H B, C, D, E, F, G, I B, C, D, E, F, H, I B, C, D, E, G, H, I B, C, D, F, G, H, I B, C, E, F, G, H, I B, D, E, F, G, H, I C, D, E, F, G, H, I

(286) TABLE-US-00004 TABLE 4 Six Strain Compositions A, B, C, D, E, F A, B, C, D, E, G A, B, C, D, E, H A, B, C, D, E, I A, B, C, D, F, G A, B, C, D, F, H A, B, C, D, F, I A, B, C, D, G, H A, B, C, D, G, I A, B, C, D, H, I A, B, C, E, F, G A, B, C, E, F, H A, B, C, E, F, I A, B, C, E, G, H A, B, C, E, G, I A, B, C, E, H, I A, B, C, F, G, H A, B, C, F, G, I A, B, C, F, H, I A, B, C, G, H, I A, B, D, E, F, G A, B, D, E, F, H A, B, D, E, F, I A, B, D, E, G, H A, B, D, E, G, I A, B, D, E, H, I A, B, D, F, G, H A, B, D, F, G, I D, E, F, G, H, I C, E, F, G, H, I A, B, D, F, H, I A, B, D, G, H, I A, B, E, F,

G, H A, B, E, F, G, I A, B, E, F, H, I A, B, E, G, H, I A, B, F, G, H, I A, C, D, E, F, G A, C, D, E, F, H A, C, D, E, F, I A, C, D, E, G, H A, C, D, E, G, I A, C, D, E, H, I A, C, D, F, G, H A, C, D, F, G, I A, C, D, F, H, I A, C, D, G, H, I A, C, E, F, G, H A, C, E, F, G, I A, C, E, F, H, I A, C, E, G, H, I A, C, F, G, H, I A, D, E, F, G, H A, D, E, F, G, I A, D, E, F, H, I A, D, E, G, H, I A, D, F, G, H, I A, E, F, G, H, I B, C, D, E, F, G B, C, D, E, F, H B, C, D, E, F, I B, C, D, E, G, H B, C, D, E, G, I B, C, D, E, H, I B, C, D, F, G, H B, C, D, F, G, I B, C, D, F, H, I B, C, D, G, H, I B, C, E, F, G, H B, C, E, F, G, I B, C, E, F, H, I B, C, E, G, H, I B, C, F, G, H, I B, D, E, F, G, H B, D, E, F, G, I B, D, E, F, H, I B, D, E, G, H, I B, D, F, G, H, I B, E, F, G, H, I C, D, E, F, G, H C, D, E, F, G, I C, D, E, F, H, I C, D, E, G, H, I C, D, F, G, H, I

(287) TABLE-US-00005 TABLE 5 Five Strain Compositions A, B, C, D, E A, B, C, D, F A, B, C, D, G A, B, C, D, H A, B, C, D, I A, B, C, E, F A, B, C, E, G A, B, C, E, H A, B, C, F, H A, B, C, F, G A, B, C, F, I A, B, C, G, H A, B, C, G, I A, B, C, H, I A, B, D, E, F A, B, D, E, G A, B, D, E, I A, B, D, F, G A, B, D, F, H A, B, D, F, I A, B, D, G, H A, B, D, G, I A, B, D, H, I A, B, E, F, G A, B, E, F, I A, B, E, G, H A, B, E, G, I A, B, E, H, I A, B, F, G, H A, B, F, G, I A, B, F, H, I A, B, G, H, I A, C, D, E, G A, C, D, E, H A, C, D, E, I A, C, D, F, G A, C, D, F, H A, C, D, F, I A, C, D, G, H A, C, D, G, I A, C, E, F, G A, C, E, F, H A, C, E, F, I A, C, E, G, H A, C, E, G, I A, C, E, H, I A, C, F, G, H A, C, F, G, I A, C, G, H, I A, D, E, F, G A, D, E, F, H A, D, E, F, I A, D, E, G, H A, D, E, G, I A, D, E, H, I A, D, F, G, H A, D, F, H, I A, D, G, H, I A, E, F, G, H A, E, F, G, I A, E, F, H, I A, E, G, H, I A, F, G, H, I B, C, D, E, F B, C, D, E, H B, C, D, E, I B, C, D, F, G B, C, D, F, H B, C, D, F, I B, C, D, G, H B, C, D, G, I B, C, D, H, I B, C, E, F, H B, C, E, F, I B, C, E, G, H B, C, E, G, I B, C, E, H, I B, C, F, G, H B, C, F, G, I B, C, F, H, I B, D, E, F, G B, D, E, F, H B, D, E, F, I B, D, E, G, H B, D, E, G, I B, D, E, H, I B, D, F, G, H B, D, F, G, I B, D, G, H, I B, E, F, G, H B, E, F, G, I B, E, F, H, I B, E, G, H, I B, F, G, H, I C, D, E, F, G C, D, E, F, H C, D, E, G, H C, D, E, G, I C, D, E, H, I C, D, F, G, H C, D, F, G, I C, D, F, H, I C, D, G, H, I C, E, F, G, H C, E, F, H, I C, E, G, H, I C, F, G, H, I D, E, F, G, H D, E, F, G, I D, E, F, H, I D, E, G, H, I D, F, G, H, I A, B, C, E, I A, B, D, E, H A, B, E, F, H A, C, D, E, F A, C, D, H, I A, C, F, H, I A, D, F, G, I B, C, D, E, G B, C, E, F, G B, C, G, H, I B, D, F, H, I C, D, E, F, I C, E, F, G, I E, F, G, H, I

(288) TABLE-US-00006 TABLE 6 Four Strain Compositions A, B, C, D A, B, C, E A, B, C, F A, B, C, G A, B, C, H A, B, C, I A, B, D, E A, B, D, F D, G, H, I A, B, D, G A, B, D, H A, B, D, I A, B, E, F A, B, E, G A, B, E, H A, B, E, I A, B, F, G E, F, G, H A, B, F, H A, D, F, H A, D, F, I A, D, G, H A, D, G, I A, D, H, I A, E, F, G A, E, F, H E, F, G, I A, B, F, I A, B, G, H A, B, G, I A, B, H, I A, C, D, E A, C, D, F A, C, D, G A, C, D, H E, F, H, I A, C, D, I A, C, E, F A, C, E, G A, C, E, H A, C, E, I A, C, F, G A, C, F, H A, C, F, I E, G, H, I A, C, G, H A, C, G, I A, C, H, I A, D, E, F A, D, E, G A, D, E, H A, D, E, I A, D, F, G F, G, H, I A, E, F, I A, E, G, H A, E, G, I A, E, H, I A, F, G, H A, F, G, I A, F, H, I A, G, H, I D, E, F, H B, C, D, E B, C, D, F B, C, D, G B, C, D, H B, C, D, I B, C, E, F B, C, E, G B, C, E, H D, E, F, I B, C, E, I B, C, F, G B, C, F, H B, C, F, I B, C, G, H B, C, G, I B, C, H, I B, D, E, F D, E, G, H B, D, E, G B, D, E, H B, D, E, I B, D, F, G B, D, F, H B, D, F, I B, D, G, H B, D, G, I D, E, G, I B, D, H, I B, E, F, G B, E, F, H B, E, F, I B, E, G, H B, E, G, I B, E, H, I B, F, G, H D, E, H, I B, F, G, I B, F, H, I B, G, H, I C, D, E, F C, D, E, G C, D, E, H C, D, E, I C, D, F, G D, F, G, H C, D, F, H C, D, F, I C, D, G, H C, D, G, I C, D, H, I C, E, F, G C, E, F, H C, E, F, I D, F, G, I C, E, G, H C, E, G, I C, E, H, I C, F, G, H C, F, G, I C, F, H, I C, G, H, I D, E, F, G D, F, H, I

(289) TABLE-US-00007 TABLE 7 Three Strain Compositions A, B, C A, B, D A, B, E A, B, F A, B, G A, B, H A, B, I A, C, D A, C, E G, H, I E, F, H A, C, F A, C, G A, C, H A, C, I A, D, E A, D, F A, D, G A, D, H A, D, I F, H, I E, F, G A, E, F A, E, G A, E, H A, E, I A, F, G A, F, H A, F, I A, G, H A, G, I F, G, I D, H, I A, H, I B, C, D B, C, E B, C, F B, C, G B, C, H B, C, I B, D, E B, D, F F, G, H D, G, I B, D, G B, D, H B, D, I B, E, F B, E, G B, E, H B, E, I B, F, G B, F, H E, H, I E, F, I B, F, I B, G, H B, G, I B, H, I C, D, E C, D, F C, D, G C, D, H C, D, I E, G, I D, G, H C, E, F C, E, G C, E, H C, E, I C, F, G C, F, H C, F, I C, G, H C, G, I E, G, H D, F, I C, H, I D, E, F D, E, G D, E, H D, E, I D, F, G D, F, H

(290) TABLE-US-00008 TABLE 8 Two Strain Compositions A, B A, C A, D A, E A, F A, G A, H A, I B, C B, D B, E B, F B, G B, H B, I C, D C, E C, F C, G C, H C, I D, E D, F D, G D, H D, I E, F E, G E, H E, I F, G F, H F, I G, H G, I H, I

(291) In some embodiments, microbial compositions may be selected from any member group from Tables 2-8.

(292) Agricultural Compositions

(293) Compositions comprising bacteria or bacterial populations produced according to methods described herein and/or having characteristics as described herein can be in the form of a liquid, a foam, or a dry product. Compositions comprising bacteria or bacterial populations produced according to methods described herein and/or having characteristics as described herein may also be used to improve plant traits. In some examples, a composition comprising bacterial populations may be in the form of a dry powder, a slurry of powder and water, or a flowable seed treatment. The compositions comprising bacterial populations may be coated on a surface of a seed, and may be in liquid form.

(294) The composition can be fabricated in bioreactors such as continuous stirred tank reactors, batch reactors, and on the farm. In some examples, compositions can be stored in a container, such as a jug or in mini bulk. In some examples, compositions may be stored within an object selected from the group consisting of a bottle, jar, ampule, package, vessel, bag, box, bin, envelope, carton, container, silo, shipping container, truck bed, and case.

(295) Compositions may also be used to improve plant traits. In some examples, one or more compositions may be coated onto a seed. In some examples, one or more compositions may be coated onto a seedling. In some examples, one or more compositions may be coated onto a surface of a seed. In some examples, one or more compositions may be coated as a layer above a surface of a seed. In some examples, a composition that is coated onto a seed may be in liquid form, in dry product form, in foam form, in a form of a slurry of powder and water, or in a flowable seed treatment. In some examples, one or more compositions may be applied to a seed and/or seedling by spraying, immersing, coating, encapsulating, and/or dusting the seed and/or seedling with the one or more compositions. In some examples, multiple bacteria or bacterial populations can be coated onto a seed and/or a seedling of the plant. In some examples, at least two, at least three, at least four, at least five, at least six, at least seven, at least eight, at least nine, at least ten, or more than ten bacteria of a bacterial combination can be selected from one of the following genera: *Acidovorax, Agrobacterium, Bacillus, Burkholderia, Chryseobacterium, Curtobacterium, Enterobacter, Escherichia, Methylobacterium, Paenibacillus, Pantoea, Pseudomonas, Ralstonia, Saccharibacillus, Sphingomonas,* and *Stenotrophomonas.*

(296) In some examples, at least two, at least three, at least four, at least five, at least six, at least seven, at least eight, at least nine, at least ten, or more than ten bacteria and bacterial populations of an endophytic combination are selected from one of the following families: Bacillaceae, Burkholderiaceae, Comamonadaceae, Enterobacteriaceae, Flavobacteriaceae, Methylobacteriaceae, Microbacteriaceae, Paenibacillileae, Pseudomonnaceae, Rhizobiaceae, Sphingomonadaceae, Xanthomonadaceae, Cladosporiaceae, Gnomoniaceae, Incertae *sedis*, Lasiosphaeriaceae, Netriaceae, and Pleosporaceae.

(297) In some examples, at least two, at least three, at least four, at least five, at least six, at least seven, at least eight, at least night, at least ten, or more than ten bacteria and bacterial populations of an endophytic combination are selected from one of the following families: Bacillaceae, Burkholderiaceae, Comamonadaceae, Enterobacteriaceae, Flavobacteriaceae, Methylobacteriaceae, Microbacteriaceae, Paenibacillileae, Pseudomonnaceae, Rhizobiaceae, Sphingomonadaceae, Xanthomonadaceae, Cladosporiaceae, Gnomoniaceae, Incertae *sedis*, Lasiosphaeriaceae, Netriaceae, Pleosporaceae.

(298) Examples of compositions may include seed coatings for commercially important agricultural crops, for example, *sorghum*, canola, tomato, strawberry, barley, rice, maize, and

wheat. Examples of compositions can also include seed coatings for corn, soybean, canola, *sorghum*, potato, rice, vegetables, cereals, and oilseeds. Seeds as provided herein can be genetically modified organisms (GMO), non-GMO, organic, or conventional. In some examples, compositions may be sprayed on the plant aerial parts, or applied to the roots by inserting into furrows in which the plant seeds are planted, watering to the soil, or dipping the roots in a suspension of the composition. In some examples, compositions may be dehydrated in a suitable manner that maintains cell viability and the ability to artificially inoculate and colonize host plants. The bacterial species may be present in compositions at a concentration of between 10.sup.8 to 10.sup.10 CFU/ml. In some examples, compositions may be supplemented with trace metal ions, such as molybdenum ions, iron ions, manganese ions, or combinations of these ions. The concentration of ions in examples of compositions as described herein may between about 0.1 mM and about 50 mM. Some examples of compositions may also be formulated with a carrier, such as beta-glucan, carboxylmethyl cellulose (CMC), bacterial extracellular polymeric substance (EPS), sugar, animal milk, or other suitable carriers. In some examples, peat or planting materials can be used as a carrier, or biopolymers in which a composition is entrapped in the biopolymer can be used as a carrier. The compositions comprising the bacterial populations described herein can improve plant traits, such as promoting plant growth, maintaining high chlorophyll content in leaves, increasing fruit or seed numbers, and increasing fruit or seed unit weight.

(299) The compositions comprising the bacterial populations described herein may be coated onto the surface of a seed. As such, compositions comprising a seed coated with one or more bacteria described herein are also contemplated. The seed coating can be formed by mixing the bacterial population with a porous, chemically inert granular carrier. Alternatively, the compositions may be inserted directly into the furrows into which the seed is planted or sprayed onto the plant leaves or applied by dipping the roots into a suspension of the composition. An effective amount of the composition can be used to populate the sub-soil region adjacent to the roots of the plant with viable bacterial growth, or populate the leaves of the plant with viable bacterial growth. In general, an effective amount is an amount sufficient to result in plants with improved traits (e.g. a desired level of nitrogen fixation).

(300) Bacterial compositions described herein can be formulated using an agriculturally acceptable carrier. The formulation useful for these embodiments may include at least one member selected from the group consisting of a tackifier, a microbial stabilizer, a fungicide, an antibacterial agent, a preservative, a stabilizer, a surfactant, an anti-complex agent, an herbicide, a nematicide, an insecticide, a plant growth regulator, a fertilizer, a rodenticide, a dessicant, a bactericide, a nutrient, and any combination thereof. In some examples, compositions may be shelf-stable. For example, any of the compositions described herein can include an agriculturally acceptable carrier (e.g., one or more of a fertilizer such as a non-naturally occurring fertilizer, an adhesion agent such as a non-naturally occurring adhesion agent, and a pesticide such as a non-naturally occurring pesticide). A non-naturally occurring adhesion agent can be, for example, a polymer, copolymer, or synthetic wax. For example, any of the coated seeds, seedlings, or plants described herein can contain such an agriculturally acceptable carrier in the seed coating. In any of the compositions or methods described herein, an agriculturally acceptable carrier can be or can include a non-naturally occurring compound (e.g., a non-naturally occurring fertilizer, a non-naturally occurring adhesion agent such as a polymer, copolymer, or synthetic wax, or a non-naturally occurring pesticide). Non-limiting examples of agriculturally acceptable carriers are described below. Additional examples of agriculturally acceptable carriers are known in the art.

(301) In some cases, bacteria are mixed with an agriculturally acceptable carrier. The carrier can be a solid carrier or liquid carrier, and in various forms including microspheres, powders, emulsions and the like. The carrier may be any one or more of a number of carriers that confer a variety of properties, such as increased stability, wettability, or dispersability. Wetting agents such as natural or synthetic surfactants, which can be nonionic or ionic surfactants, or a combination thereof can be

included in the composition. Water-in-oil emulsions can also be used to formulate a composition that includes the isolated bacteria (see, for example, U.S. Pat. No. 7,485,451). Suitable formulations that may be prepared include wettable powders, granules, gels, agar strips or pellets, thickeners, and the like, microencapsulated particles, and the like, liquids such as aqueous flowables, aqueous suspensions, water-in-oil emulsions, etc. The formulation may include grain or legume products, for example, ground grain or beans, broth or flour derived from grain or beans, starch, sugar, or oil.

(302) In some embodiments, the agricultural carrier may be soil or a plant growth medium. Other agricultural carriers that may be used include water, fertilizers, plant-based oils, humectants, or combinations thereof. Alternatively, the agricultural carrier may be a solid, such as diatomaceous earth, loam, silica, alginate, clay, bentonite, vermiculite, seed cases, other plant and animal products, or combinations, including granules, pellets, or suspensions. Mixtures of any of the aforementioned ingredients are also contemplated as carriers, such as but not limited to, pesta (flour and kaolin clay), agar or flour-based pellets in loam, sand, or clay, etc. Formulations may include food sources for the bacteria, such as barley, rice, or other biological materials such as seed, plant parts, sugar cane bagasse, hulls or stalks from grain processing, ground plant material or wood from building site refuse, sawdust or small fibers from recycling of paper, fabric, or wood.

(303) For example, a fertilizer can be used to help promote the growth or provide nutrients to a seed, seedling, or plant. Non-limiting examples of fertilizers include nitrogen, phosphorous, potassium, calcium, sulfur, magnesium, boron, chloride, manganese, iron, zinc, copper, molybdenum, and selenium (or a salt thereof). Additional examples of fertilizers include one or more amino acids, salts, carbohydrates, vitamins, glucose, NaCl, yeast extract, NH.sub.4H.sub.2PO.sub.4, (NH.sub.4).sub.2SO.sub.4, glycerol, valine, L-leucine, lactic acid, propionic acid, succinic acid, malic acid, citric acid, KH tartrate, xylose, lyxose, and lecithin. In one embodiment, the formulation can include a tackifier or adherent (referred to as an adhesive agent) to help bind other active agents to a substance (e.g., a surface of a seed). Such agents are useful for combining bacteria with carriers that can contain other compounds (e.g., control agents that are not biologic), to yield a coating composition. Such compositions help create coatings around the plant or seed to maintain contact between the microbe and other agents with the plant or plant part. In one embodiment, adhesives are selected from the group consisting of: alginate, gums, starches, lecithins, formononetin, polyvinyl alcohol, alkali formononetinate, hesperetin, polyvinyl acetate, cephalins, Gum Arabic, Xanthan Gum, Mineral Oil, Polyethylene Glycol (PEG), Polyvinyl pyrrolidone (PVP), Arabino-galactan, Methyl Cellulose, PEG 400, Chitosan, Polyacrylamide, Polyacrylate, Polyacrylonitrile, Glycerol, Triethylene glycol, Vinyl Acetate, Gellan Gum, Polystyrene, Polyvinyl, Carboxymethyl cellulose, Gum Ghatti, and polyoxyethylene-polyoxybutylene block copolymers.

(304) In some embodiments, the adhesives can be, e.g. a wax such as carnauba wax, beeswax, Chinese wax, shellac wax, spermaceti wax, candelilla wax, castor wax, ouricury wax, and rice bran wax, a polysaccharide (e.g., starch, dextrins, maltodextrins, alginate, and chitosans), a fat, oil, a protein (e.g., gelatin and zeins), gum arables, and shellacs. Adhesive agents can be non-naturally occurring compounds, e.g., polymers, copolymers, and waxes. For example, non-limiting examples of polymers that can be used as an adhesive agent include: polyvinyl acetates, polyvinyl acetate copolymers, ethylene vinyl acetate (EVA) copolymers, polyvinyl alcohols, polyvinyl alcohol copolymers, celluloses (e.g., ethylcelluloses, methylcelluloses, hydroxymethylcelluloses, hydroxypropylcelluloses, and carboxymethylcelluloses), polyvinylpyrolidones, vinyl chloride, vinylidene chloride copolymers, calcium lignosulfonates, acrylic copolymers, polyvinylacrylates, polyethylene oxide, acylamide polymers and copolymers, polyhydroxyethyl acrylate, methylacrylamide monomers, and polychloroprene.

(305) In some examples, one or more of the adhesion agents, anti-fungal agents, growth regulation agents, and pesticides (e.g., insecticide) are non-naturally occurring compounds (e.g., in any

combination). Additional examples of agriculturally acceptable carriers include dispersants (e.g., polyvinylpyrrolidone/vinyl acetate PVPIVA S-630), surfactants, binders, and filler agents.
(306) The formulation can also contain a surfactant. Non-limiting examples of surfactants include nitrogen-surfactant blends such as Prefer 28 (Cenex), Surf-N(US), Inhance (Brandt), P-28 (Wilfarm) and Patrol (Helena); esterified seed oils include Sun-It II (AmCy), MSO (UAP), Scoil (Agsco), Hasten (Wilfarm) and Mes-100 (Drexel); and organo-silicone surfactants include Silwet L77 (UAP), Silikin (Terra), Dyne-Amic (Helena), Kinetic (Helena), Sylgard 309 (Wilbur-Ellis) and Century (Precision). In one embodiment, the surfactant is present at a concentration of between 0.01% v/v to 10% v/v. In another embodiment, the surfactant is present at a concentration of between 0.1% v/v to 1% v/v.
(307) In certain cases, the formulation includes a microbial stabilizer. Such an agent can include a desiccant, which can include any compound or mixture of compounds that can be classified as a desiccant regardless of whether the compound or compounds are used in such concentrations that they in fact have a desiccating effect on a liquid inoculant. Such desiccants are ideally compatible with the bacterial population used, and should promote the ability of the microbial population to survive application on the seeds and to survive desiccation. Examples of suitable desiccants include one or more of trehalose, sucrose, glycerol, and Methylene glycol. Other suitable desiccants include, but are not limited to, non reducing sugars and sugar alcohols (e.g., mannitol or sorbitol). The amount of desiccant introduced into the formulation can range from about 5% to about 50% by weight/volume, for example, between about 10% to about 40%, between about 15% to about 35%, or between about 20% to about 30%. In some cases, it is advantageous for the formulation to contain agents such as a fungicide, an antibacterial agent, an herbicide, a nematicide, an insecticide, a plant growth regulator, a rodenticide, bactericide, or a nutrient. In some examples, agents may include protectants that provide protection against seed surface-borne pathogens. In some examples, protectants may provide some level of control of soil-borne pathogens. In some examples, protectants may be effective predominantly on a seed surface.
(308) In some examples, a fungicide may include a compound or agent, whether chemical or biological, that can inhibit the growth of a fungus or kill a fungus. In some examples, a fungicide may include compounds that may be fungistatic or fungicidal. In some examples, fungicide can be a protectant, or agents that are effective predominantly on the seed surface, providing protection against seed surface-borne pathogens and providing some level of control of soil-borne pathogens. Non-limiting examples of protectant fungicides include captan, maneb, thiram, or fludioxonil.
(309) In some examples, fungicide can be a systemic fungicide, which can be absorbed into the emerging seedling and inhibit or kill the fungus inside host plant tissues. Systemic fungicides used for seed treatment include, but are not limited to the following: azoxystrobin, carboxin, mefenoxam, metalaxyl, thiabendazole, trifloxystrobin, and various triazole fungicides, including difenoconazole, ipconazole, tebuconazole, and triticonazole. Mefenoxam and metalaxyl are primarily used to target the water mold fungi *Pythium* and *Phytophthora*. Some fungicides are preferred over others, depending on the plant species, either because of subtle differences in sensitivity of the pathogenic fungal species, or because of the differences in the fungicide distribution or sensitivity of the plants. In some examples, fungicide can be a biological control agent, such as a bacterium or fungus. Such organisms may be parasitic to the pathogenic fungi, or secrete toxins or other substances which can kill or otherwise prevent the growth of fungi. Any type of fungicide, particularly ones that are commonly used on plants, can be used as a control agent in a seed composition.
(310) In some examples, the seed coating composition comprises a control agent which has antibacterial properties. In one embodiment, the control agent with antibacterial properties is selected from the compounds described herein elsewhere. In another embodiment, the compound is Streptomycin, oxytetracycline, oxolinic acid, or gentamicin. Other examples of antibacterial compounds which can be used as part of a seed coating composition include those based on

dichlorophene and benzylalcohol hemi formal (Proxel® from ICI or Acticide® RS from Thor Chemie and Kathon® MK 25 from Rohm & Haas) and isothiazolinone derivatives such as alkylisothiazolinones and benzisothiazolinones (Acticide® MBS from Thor Chemie).

(311) In some examples, growth regulator is selected from the group consisting of: Abscisic acid, amidochlor, ancymidol, 6-benzylaminopurine, brassinolide, butralin, chlormequat (chlormequat chloride), choline chloride, cyclanilide, daminozide, dikegulac, dimethipin, 2,6-dimethylpuridine, ethephon, flumetralin, flurprimidol, fluthiacet, forchlorfenuron, gibberellic acid, inabenfide, indole-3-acetic acid, maleic hydrazide, mefluidide, mepiquat (mepiquat chloride), naphthaleneacetic acid, N-6-benzyladenine, paclobutrazol, prohexadione phosphorotrithioate, 2,3,5-tri-iodobenzoic acid, trinexapac-ethyl and uniconazole. Additional non-limiting examples of growth regulators include brassinosteroids, cytokinines (e.g., kinetin and zeatin), auxins (e.g., indolylacetic acid and indolylacetyl aspartate), flavonoids and isoflavanoids (e.g., formononetin and diosmetin), phytoaixins (e.g., glyceolline), and phytoalexin-inducing oligosaccharides (e.g., pectin, chitin, chitosan, polygalacuronic acid, and oligogalacturonic acid), and gibellerins. Such agents are ideally compatible with the agricultural seed or seedling onto which the formulation is applied (e.g., it should not be deleterious to the growth or health of the plant). Furthermore, the agent is ideally one which does not cause safety concerns for human, animal or industrial use (e.g., no safety issues, or the compound is sufficiently labile that the commodity plant product derived from the plant contains negligible amounts of the compound).

(312) Some examples of nematode-antagonistic biocontrol agents include ARF18; 30 *Arthrobotrys* spp.; *Chaetomium* spp.; *Cylindrocarpon* spp.; *Exophilia* spp.; *Fusarium* spp.; *Gliocladium* spp.; *Hirsutella* spp.; *Lecanicillium* spp.; *Monacrosporium* spp.; *Myrothecium* spp.; *Neocosmospora* spp.; *Paecilomyces* spp.; *Pochonia* spp.; *Stagonospora* spp.; vesicular-arbuscular mycorrhizal fungi, *Burkholderia* spp.; *Pasteuria* spp., *Brevibacillus* spp.; *Pseudomonas* spp.; and Rhizobacteria. Particularly preferred nematode-antagonistic biocontrol agents include ARF18, *Arthrobotrys oligospora, Arthrobotrys dactyloides, Chaetomium globosum, Cylindrocarpon heteronema, Exophilia jeanselmei, Exophilia pisciphila, Fusarium aspergilus, Fusarium solani, Gliocladium catenulatum, Gliocladium roseum, Gliocladium vixens, Hirsutella rhossiliensis, Hirsutella minnesotensis, Lecanicillium lecanii, Monacrosporium drechsleri, Monacrosporium gephyropagum, Myrotehcium verrucaria, Neocosmospora vasinfecta, Paecilomyces lilacinus, Pochonia chlamydosporia, Stagonospora heteroderae, Stagonospora phaseoli*, vesicular-arbuscular mycorrhizal fungi, *Burkholderia cepacia, Pasteuria penetrans, Pasteuria thomei, Pasteuria nishizawae, Pasteuria ramosa, Pastrueia usage, Brevibacillus laterosporus* strain G4, *Pseudomonas fluorescens* and Rhizobacteria.

(313) Some examples of nutrients can be selected from the group consisting of a nitrogen fertilizer including, but not limited to Urea, Ammonium nitrate, Ammonium sulfate, Non-pressure nitrogen solutions, Aqua ammonia, Anhydrous ammonia, Ammonium thiosulfate, Sulfur-coated urea, Urea-formaldehydes, IBDU, Polymer-coated urea, Calcium nitrate, Ureaform, and Methylene urea, phosphorous fertilizers such as Diammonium phosphate, Monoammonium phosphate, Ammonium polyphosphate, Concentrated superphosphate and Triple superphosphate, and potassium fertilizers such as Potassium chloride, Potassium sulfate, Potassium-magnesium sulfate, Potassium nitrate. Such compositions can exist as free salts or ions within the seed coat composition. Alternatively, nutrients/fertilizers can be complexed or chelated to provide sustained release over time.

(314) Some examples of rodenticides may include selected from the group of substances consisting of 2-isovalerylindan-1,3-dione, 4-(quinoxalin-2-ylamino) benzenesulfonamide, alpha-chlorohydrin, aluminum phosphide, antu, arsenous oxide, barium carbonate, bisthiosemi, brodifacoum, bromadiolone, bromethalin, calcium cyanide, chloralose, chlorophacinone, cholecalciferol, coumachlor, coumafuryl, coumatetralyl, crimidine, difenacoum, difethialone, diphacinone, ergocalciferol, flocoumafen, fluoroacetamide, flupropadine, flupropadine hydrochloride, hydrogen cyanide, iodomethane, lindane, magnesium phosphide, methyl bromide, norbormide, phosacetim,

phosphine, phosphorus, pindone, potassium arsenite, pyrinuron, scilliroside, sodium arsenite, sodium cyanide, sodium fluoroacetate, strychnine, thallium sulfate, warfarin and zinc phosphide.
(315) In the liquid form, for example, solutions or suspensions, bacterial populations can be mixed or suspended in water or in aqueous solutions. Suitable liquid diluents or carriers include water, aqueous solutions, petroleum distillates, or other liquid carriers.
(316) Solid compositions can be prepared by dispersing the bacterial populations in and on an appropriately divided solid carrier, such as peat, wheat, bran, vermiculite, clay, talc, bentonite, diatomaceous earth, fuller's earth, pasteurized soil, and the like. When such formulations are used as wettable powders, biologically compatible dispersing agents such as non-ionic, anionic, amphoteric, or cationic dispersing and emulsifying agents can be used.
(317) The solid carriers used upon formulation include, for example, mineral carriers such as kaolin clay, pyrophyllite, bentonite, montmorillonite, diatomaceous earth, acid white soil, vermiculite, and pearlite, and inorganic salts such as ammonium sulfate, ammonium phosphate, ammonium nitrate, urea, ammonium chloride, and calcium carbonate. Also, organic fine powders such as wheat flour, wheat bran, and rice bran may be used. The liquid carriers include vegetable oils such as soybean oil and cottonseed oil, glycerol, ethylene glycol, polyethylene glycol, propylene glycol, polypropylene glycol, etc.
(318) Pests
(319) Agricultural compositions of the disclosure, which may comprise any microbe taught herein, are sometimes combined with one or more pesticides.
(320) The pesticides that are combined with the microbes of the disclosure may target any of the pests mentioned below.
(321) “Pest” includes but is not limited to, insects, fungi, bacteria, nematodes, mites, ticks and the like. Insect pests include insects selected from the orders Coleoptera, Diptera, Hymenoptera, Lepidoptera, Mallophaga, Homoptera, Hemiptera Orthroptera, Thysanoptera, Dermaptera, Isoptera, Anoplura, Siphonaptera, Trichoptera, etc., particularly Lepidoptera and Coleoptera.
(322) Those skilled in the art will recognize that not all compounds are equally effective against all pests. Compounds that may be combined with microbes of the disclosure may display activity against insect pests, which may include economically important agronomic, forest, greenhouse, nursery ornamentals, food and fiber, public and animal health, domestic and commercial structure, household and stored product pests.
(323) As aforementioned, the agricultural compositions of the disclosure (which may comprise any microbe taught herein) are in embodiments combined with one or more pesticides. These pesticides may be active against any of the following pests:
(324) Larvae of the order Lepidoptera include, but are not limited to, armyworms, cutworms, loopers and heliothines in the family Noctuidae *Spodoptera frugiperda* J E Smith (fall armyworm); *S. exigua* Hubner (beet armyworm); *S. litura Fabricius* (tobacco cutworm, cluster caterpillar); *Mamestra configurata* Walker (bertha armyworm); *M. brassicae* Linnaeus (cabbage moth); *Agrotis ipsilon* Hufnagel (black cutworm); *A. orthogonia* Morrison (western cutworm); A. *subterranea Fabricius* (granulate cutworm); *Alabama argillacea* Hubner (cotton leaf worm); *Trichoplusia ni* Hubner (cabbage looper); *Pseudoplusia includens* Walker (soybean looper); *Anticarsia gemmatalis* Hubner (velvet bean caterpillar); *Hypena scabra Fabricius* (green clover worm); *Heliothis virescens Fabricius* (tobacco budworm); *Pseudaletia unipuncta* Haworth (armyworm); *Athetis mindara* Barnes and Mcdunnough (rough skinned cutworm); *Euxoa messoria* Harris (darksided cutworm); *Earias insulana* Boisduval (spiny bollworm); *E. vittella Fabricius* (spotted bollworm); *Helicoverpa armigera* Hubner (American bollworm); *H. zea* Boddie (corn earworm or cotton bollworm); Melanchra *picta* Harris (zebra caterpillar); *Egira* (Xylomyges) *curialis* Grote (citrus cutworm); borers, case bearers, webworms, coneworms, and skeletonizers from the family Pyralidae *Ostrinia nubilalis* Hubner (European corn borer); *Amyelois transitella* Walker (naval orangeworm); *Anagasta kuehniella* Zeller (Mediterranean flour moth); *Cadra cautella* Walker

(almond moth); *Chilo suppressalis* Walker (rice stem borer); *C. partellus*, (*sorghum* borer); *Corcyra cephalonica* Stainton (rice moth); *Crambus caliginosellus* Clemens (corn root webworm); *C. teterrellus* Zincken (bluegrass webworm); *Cnaphalocrocis medinalis* Guenee (rice leaf roller); *Desmia funeralis* Hubner (grape leaffolder); *Diaphania hyalinata* Linnaeus (melon worm); *D. nitidalis* Stoll (pickleworm); *Diatraea grandiosella* Dyar (southwestern corn borer), *D. saccharalis Fabricius* (sugarcane borer); *Eoreuma loftini* Dyar (Mexican rice borer); *Ephestia elutella* Hubner (tobacco (cacao) moth); *Galleria mellonella* Linnaeus (greater wax moth); *Herpetogramma licarsisalis* Walker (sod webworm); *Homoeosoma electellum* Hulst (sunflower moth); *Elasmopalpus lignosellus* Zeller (lesser cornstalk borer); *Achroia grisella Fabricius* (lesser wax moth); *Loxostege sticticalis* Linnaeus (beet webworm); *Orthaga thyrisalis* Walker (tea tree web moth); *Maruca testulalis* Geyer (bean pod borer); *Plodia interpunctella* Hubner (Indian meal moth); *Scirpophaga incertulas* Walker (yellow stem borer); *Udea rubigalis* Guenee (celery leaftier); and leafrollers, budworms, seed worms and fruit worms in the family Tortricidae *Acleris gloverana* Walsingham (Western blackheaded budworm); *A. variana* Fernald (Eastern blackheaded budworm); *Archips argyrospila* Walker (fruit tree leaf roller); *A. rosana* Linnaeus (European leaf roller); and other *Archips* species, *Adoxophyes orana* Fischer von Rosslerstamm (summer fruit *tortrix* moth); *Cochylis hospes* Walsingham (banded sunflower moth); *Cydia latiferreana* Walsingham (filbertworm); *C. pomonella* Linnaeus (colding moth); *Platynota flavedana* Clemens (variegated leafroller); *P. stultana* Walsingham (omnivorous leafroller); *Lobesia botrana* Denis & Schiffermuller (European grape vine moth); *Spilonota ocellana* Denis & Schiffermuller (eyespotted bud moth); *Endopiza viteana* Clemens (grape berry moth); *Eupoecilia ambiguella* Hubner (vine moth); *Bonagota salubricola* Meyrick (Brazilian apple leafroller); *Grapholita molesta* Busck (oriental fruit moth); *Suleima helianthana* Riley (sunflower bud moth); *Argyrotaenia* spp.; *Choristoneura* spp.

(325) Selected other agronomic pests in the order Lepidoptera include, but are not limited to, *Alsophila pometaria* Harris (fall cankerworm); *Anarsia lineatella* Zeller (peach twig borer); *Anisota senatoria* J. E. Smith (orange striped oakworm); *Antheraea pernyi* Guerin-Meneville (Chinese Oak Tussah Moth); *Bombyx mori* Linnaeus (Silkworm); *Bucculatrix thurberiella* Busck (cotton leaf perforator); *Colias eurytheme* Boisduval (alfalfa caterpillar); *Datana integerrima* Grote & Robinson (walnut caterpillar); *Dendrolimus sibiricus* Tschetwerikov (Siberian silk moth), *Ennomos subsignaria* Hubner (elm spanworm); *Erannis tiliaria* Harris (linden looper); *Euproctis chrysorrhoea* Linnaeus (browntail moth); *Harrisina americana* Guerin-Meneville (grapeleaf skeletonizer); *Hemileuca oliviae* Cockrell (range caterpillar); *Hyphantria cunea* Drury (fall web-worm); *Keiferia lycopersicella* Walsingham (tomato pinworm); *Lambdina fiscellaria fiscellaria* Hulst (Eastern hemlock looper); *L. fiscellaria lugubrosa* Hulst (Western hemlock looper); *Leucoma salicis* Linnaeus (satin moth); *Lymantria dispar* Linnaeus (gypsy moth); *Manduca quinquemaculata* Haworth (five spotted hawk moth, tomato hornworm); *M. sexta* Haworth (tomato homworm, tobacco hornworm); *Operophtera brumata* Linnaeus (winter moth); *Paleacrita vernata* Peck (spring cankerworm); *Papilio cresphontes* Cramer (giant swallowtail orange dog); Phryganidia *californica* Packard (California oakworm); *Phyllocnistis citrella* Stainton (citrus leafminer); *Phyllonorycter blancardella Fabricius* (spotted tentiform leafminer); *Pieris brassicae* Linnaeus (large white butterfly); *P. rapae* Linnaeus (small white butterfly); *P. napi* Linnaeus (green veined white butterfly); *Platyptilia carduidactyla* Riley (artichoke plume moth); *Plutella xylostella* Linnaeus (diamondback moth); *Pectinophora gossypiella* Saunders (pink bollworm); *Pontia protodice* Boisduval and Leconte (Southern cabbage-worm); *Sabulodes aegrotata* Guenee (onmivorous looper); *Schizura concinna* J. E. Smith (red humped caterpillar); *Sitotroga cerealella* Olivier (Angoumois grain moth); *Thaumetopoea pityocampa* Schiffermuller (pine processionary caterpillar); *Tineola bisselliella* Hummel (webbing clothes moth); *Tuta absoluta* Meyrick (tomato leafminer); *Yponomeuta padella* Linnaeus (ermine moth); *Heliothis subflexa* Guenee; *Malacosoma* spp. and *Orgyia* spp.; *Ostrinia nubilalis* (European corn borer); seed corn maggot; *Agrotis ipsilon*

(black cutworm).
(326) Larvae and adults of the order Coleoptera including weevils from the families Anthribidae, Bruchidae and Curculionidae (including, but not limited to: *Anthonomus grandis* Boheman (boll weevil); *Lissorhoptrus oryzophilus* Kuschel (rice water weevil); *Sitophilus granarius* Linnaeus (granary weevil); *S. oryzae* Linnaeus (rice weevil); *Hypera punctata Fabricius* (clover leaf weevil); *Cylindrocopturus adspersus* LeConte (sunflower stem weevil); *Smicronyx fulvus* LeConte (red sunflower seed weevil); *S. sordidus* LeConte (gray sunflower seed weevil); Sphenophorus *maidis* Chittenden (maize billbug)); flea beetles, cucumber beetles, rootworms, leaf beetles, potato beetles and leafminers in the family Chrysomelidae (including, but not limited to: *Leptinotarsa decemlineata* Say (Colorado potato beetle); *Diabrotica virgifera virgifera* LeConte (western corn rootworm); *D. barberi* Smith and Lawrence (northern corn rootworm); *D. undecimpunctata howardi* Barber (southern corn rootworm); *Chaetocnema pulicaria* Melsheimer (corn flea beetle); *Phyllotreta cruciferae* Goeze (Crucifer flea beetle); *Phyllotreta striolata* (stripped flea beetle); *Colaspis brunnea Fabricius* (grape *colaspis*); *Oulema melanopus* Linnaeus (cereal leaf beetle); *Zygogramma exclamationis Fabricius* (sunflower beetle)); beetles from the family Coccinellidae (including, but not limited to: *Epilachna varivestis* Mulsant (Mexican bean beetle)); chafers and other beetles from the family Scarabaeidae (including, but not limited to: *Popillia japonica* Newman (Japanese beetle); *Cyclocephala borealis* Arrow (northern masked chafer, white grub); *C. immaculata* Olivier (southern masked chafer, white grub); *Rhizotrogus majalis* Razoumowsky (European chafer); *Phyllophaga crinita* Burmeister (white grub); *Ligyrus gibbosus* De Geer (carrot beetle)); carpet beetles from the family Dermestidae; wireworms from the family Elateridae, *Eleodes* spp., *Melanotus* spp.; *Conoderus* spp.; *Limonius* spp.; *Agriotes* spp.; *Ctenicera* spp.; Aeolus spp.; bark beetles from the family Scolytidae and beetles from the family Tenebrionidae; *Cerotoma* trifurcate (bean leaf beetle); and wireworm.
(327) Adults and immatures of the order Diptera, including leafminers *Agromyza parvicornis* Loew (corn blotch leafminer); midges (including, but not limited to: *Contarinia sorghicola* Coquillett (*sorghum* midge); *Mayetiola destructor* Say (Hessian fly); *Sitodiplosis mosellana* Gehin (wheat midge); *Neolasioptera murtfeldtiana* Felt, (sunflower seed midge)); fruit flies (Tephritidae), *Oscinella frit* Linnaeus (fruit flies); maggots (including, but not limited to: *Delia platura* Meigen (seedcorn maggot); *D. coarctata* Fallen (wheat bulb fly) and other *Delia* spp., Meromyza *americana* Fitch (wheat stem maggot); *Musca domestica* Linnaeus (house flies); *Fannia canicularis* Linnaeus, *F. femoralis* Stein (lesser house flies); *Stomoxys calcitrans* Linnaeus (stable flies)); face flies, horn flies, blow flies, *Chrysomya* spp.; *Phormia* spp. and other muscoid fly pests, horse flies *Tabanus* spp.; bot flies *Gastrophilus* spp.; *Oestrus* spp.; cattle grubs *Hypoderma* spp.; deer flies *Chrysops* spp.; *Melophagus ovinus* Linnaeus (keds) and other Brachycera, mosquitoes *Aedes* spp.; *Anopheles* spp.; *Culex* spp.; black flies *Prosimulium* spp.; *Simulium* spp.; biting midges, sand flies, sciarids, and other Nematocera.
(328) Adults and nymphs of the orders Hemiptera and Homoptera such as, but not limited to, adelgids from the family Adelgidae, plant bugs from the family Miridae, cicadas from the family Cicadidae, leafhoppers, *Empoasca* spp.; from the family Cicadellidae, planthoppers from the families Cixiidae, Flatidae, Fulgoroidea, Issidae and Delphacidae, treehoppers from the family Membracidae, psyllids from the family Psyllidae, whiteflies from the family Aleyrodidae, aphids from the family Aphididae, *phylloxera* from the family Phylloxeridae, mealybugs from the family Pseudococcidae, scales from the families Asterolecanidae, Coccidae, Dactylopiidae, Diaspididae, Eriococcidae Ortheziidae, Phoenicococcidae and Margarodidae, lace bugs from the family Tingidae, stink bugs from the family Pentatomidae, cinch bugs, *Blissus* spp.; and other seed bugs from the family Lygaeidae, spittlebugs from the family Cercopidae squash bugs from the family Coreidae and red bugs and cotton stainers from the family Pyrrhocoridae.
(329) Agronomically important members from the order Homoptera further include, but are not limited to: *Acyrthisiphon pisum* Harris (pea aphid); *Aphis craccivora* Koch (cowpea aphid); *A.*

fabae Scopoli (black bean aphid); *A. gossypii* Glover (cotton aphid, melon aphid); *A. maidiradicis* Forbes (corn root aphid); *A. pomi* De Geer (apple aphid); *A. spiraecola* Patch (spirea aphid); *Aulacorthum solani* Kaltenbach (foxglove aphid); *Chaetosiphon fragaefolii* Cockerell (strawberry aphid); *Diuraphis noxia* Kurdjumov/Mordvilko (Russian wheat aphid); *Dysaphis plantaginea* Paaserini (rosy apple aphid); *Eriosoma lanigerum* Hausmann (woolly apple aphid); *Brevicoryne brassicae* Linnaeus (cabbage aphid); *Hyalopterus pruni* Geoffroy (mealy plum aphid); *Lipaphis erysimi* Kaltenbach (turnip aphid); *Metopolophium dirrhodum* Walker (cereal aphid); *Macrosiphum euphorbiae* Thomas (potato aphid); *Myzus persicae* Sulzer (peach potato aphid, green peach aphid); *Nasonovia ribisnigri* Mosley (lettuce aphid); Pemphigus spp. (root aphids and gall aphids); *Rhopalosiphum maidis* Fitch (corn leaf aphid); *R. padi* Linnaeus (bird cherry-oat aphid); *Schizaphis graminum* Rondani (greenbug); *Sipha flava* Forbes (yellow sugarcane aphid); *Sitobion avenae Fabricius* (English grain aphid); *Therioaphis maculata* Buckton (spotted alfalfa aphid); *Toxoptera aurantii* Boyer de Fonscolombe (black citrus aphid) and *T. citricida* Kirkaldy (brown citrus aphid); *Melanaphis sacchari* (sugarcane aphid); *Adelges* spp. (adelgids); *Phylloxera devastatrix* Pergande (pecan *phylloxera*); *Bemisia tabaci* Gennadius (tobacco whitefly, sweetpotato whitefly); *B. argentifolii* Bellows & Perring (silverleaf whitefly); *Dialeurodes citri* Ashmead (citrus whitefly); *Trialeurodes abutiloneus* (bandedwinged whitefly) and *T. vaporariorum* Westwood (greenhouse whitefly); *Empoasca fabae* Harris (potato leafhopper); *Laodelphax striatellus* Fallen (smaller brown planthopper); *Macrolestes quadrilineatus* Forbes (aster leafhopper); *Nephotettix cinticeps* Uhler (green leafhopper); *N. nigropictus* Stal (rice leafhopper); *Nilaparvata lugens* Stal (brown planthopper); *Peregrinus maidis* Ashmead (corn planthopper); *Sogatella furcifera* Horvath (white backed planthopper); *Sogatodes orizicola* Muir (rice delphacid); *Typhlocyba pomaria* McAtee (white apple leafhopper); *Erythroneoura* spp. (grape leafhoppers); Magicicada *septendecim* Linnaeus (periodical cicada); *Icerya purchasi* Maskell (cottony cushion scale); *Quadraspidiotus perniciosus* Comstock (San Jose scale); *Planococcus citri* Risso (citrus mealybug); *Pseudococcus* spp. (other mealybug complex); *Cacopsylla pyricola* Foerster (pear *psylla*); *Trioza diospyri* Ashmead (persimmon *psylla*).

(330) Species from the order Hemiptera include, but are not limited to: *Acrosternum hilare* Say (green stink bug); *Anasa tristis* De Geer (squash bug); *Blissus leucopterus leucopterus* Say (chinch bug); *Corythuca gossypii Fabricius* (cotton lace bug); *Cyrtopeltis modesta* Distant (tomato bug); *Dysdercus suturellus* Herrich-Schaffer (cotton stainer); *Euschistus servus* Say (brown stink bug); *E. variolarius* Palisot de Beauvais (one spotted stink bug); *Graptostethus* spp. (complex of seed bugs); *Leptoglossus corculus* Say (leaf footed pine seed bug); *Lygus lineolaris* Palisot de Beauvais (tarnished plant bug); *L. Hesperus* Knight (Western tarnished plant bug); *L. pratensis* Linnaeus (common meadow bug); *L. rugulipennis* Poppius (European tarnished plant bug); *Lygocoris pabulinus* Linnaeus (common green capsid); *Nezara viridula* Linnaeus (southern green stink bug); *Oebalus pugnax Fabricius* (rice stink bug); *Oncopeltus fasciatus* Dallas (large milk-weed bug); *Pseudatomoscelis seriatus* Reuter (cotton flea hopper).

(331) Hemiptera such as, *Calocoris norvegicus* Gmelin (strawberry bug); *Orthops campestris* Linnaeus; *Plesiocoris rugicollis* Fallen (apple capsid); *Cyrtopeltis modestus* Distant (tomato bug); *Cyrtopeltis notatus* Distant (suckfly); *Spanagonicus albofasciatus* Reuter (whitemarked fleahopper); *Diaphnocoris chlorionis* Say (honeylocust plant bug); *Labopidicola allii* Knight (onion plant bug); *Pseudatomoscelis seriatus* Reuter (cotton fleahopper); *Adelphocoris rapidus* Say (rapid plant bug); *Poecilocapsus lineatus Fabricius* (four lined plant bug); *Nysius ericae* Schilling (false chinch bug); *Nysius raphanus* Howard (false chinch bug); *Nezara viridula* Linnaeus (Southern green stink bug); *Eurygaster* spp.; Coreidae spp.; Pyrrhocoridae spp.; Tinidae spp.; Blostomatidae spp.; Reduviidae spp. and Cimicidae spp.

(332) Adults and larvae of the order Acari (mites) such as *Aceria tosichella* Keifer (wheat curl mite); *Petrobia latens* Muller (brown wheat mite); spider mites and red mites in the family Tetranychidae, *Panonychus ulmi* Koch (European red mite); *Tetranychus urticae* Koch (two spotted

spider mite); (*T. mcdanieli* McGregor (McDaniel mite); *T. cinnabarinus* Boisduval (carmine spider mite); *T. turkestani* Ugarov & Nikolski (strawberry spider mite); flat mites in the family Tenuipalpidae, *Brewpalpus lewisi* McGregor (citrus flat mite); rust and bud mites in the family Eriophyidae and other foliar feeding mites and mites important in human and animal health, i.e., dust mites in the family Epidermoptidae, follicle mites in the family Demodicidae, grain mites in the family Glycyphagidae, ticks in the order Ixodidae. *Ixodes scapularis* Say (deer tick); *I. holocyclus* Neumann (Australian paralysis tick); *Dermacentor variabilis* Say (American dog tick); *Amblyomma americanum* Linnaeus (lone star tick) and scab and itch mites in the families Psoroptidae, Pyemotidae and Sarcoptidae.

(333) Insect pests of the order *Thysanura*, such as *Lepisma saccharina* Linnaeus (silverfish); *Thermobia domestica* Packard (firebrat).

(334) Additional arthropod pests include: spiders in the order Araneae such as *Loxosceles reclusa* Gertsch and Mulaik (brown recluse spider) and the *Latrodectus mactans Fabricius* (black widow spider) and centipedes in the order Scutigeromorpha such as *Scutigera coleoptrata* Linnaeus (house centipede).

(335) Superfamily of stink bugs and other related insects including but not limited to species belonging to the family Pentatomidae (*Nezara viridula, Halyomorpha halys, Piezodorus guildini, Euschistus servus, Acrosternum hilare, Euschistus heros, Euschistus tristigmus, Acrosternum hilare, Dichelops furcatus, Dichelops melacanthus*, and *Bagrada hilaris* (*Bagrada* Bug)), the family Plataspidae (*Megacopta cribraria*-Bean plataspid) and the family Cydnidae (*Scaptocoris castanea*-Root stink bug) and Lepidoptera species including but not limited to: diamond-back moth, e.g., *Helicoverpa zea* Boddie; soybean looper, e.g., *Pseudoplusia includens* Walker and velvet bean caterpillar e.g., *Anticarsia gemmatalis* Hubner.

(336) Nematodes include parasitic nematodes such as root-knot, cyst and lesion nematodes, including *Heterodera* spp., *Meloidogyne* spp. and *Globodera* spp.; particularly members of the cyst nematodes, including, but not limited to, *Heterodera glycines* (soybean cyst nematode); *Heterodera schachtii* (beet cyst nematode); *Heterodera avenae* (cereal cyst nematode) and *Globodera rostochiensis* and *Globodera pailida* (potato cyst nematodes). Lesion nematodes include *Pratylenchus* spp.

(337) Pesticidal Compositions Comprising a Pesticide and Microbe of the Disclosure

(338) As aforementioned, agricultural compositions of the disclosure, which may comprise any microbe taught herein, are sometimes combined with one or more pesticides. Pesticides can include herbicides, insecticides, fungicides, nematicides, etc.

(339) In some embodiments, the pesticides/microbial combinations can be applied in the form of compositions and can be applied to the crop area or plant to be treated, simultaneously or in succession, with other compounds. These compounds can be fertilizers, weed killers, cryoprotectants, surfactants, detergents, pesticidal soaps, dormant oils, polymers, and/or time release or biodegradable carrier formulations that permit long term dosing of a target area following a single application of the formulation. They can also be selective herbicides, chemical insecticides, virucides, microbicides, amoebicides, pesticides, fungicides, bacteriocides, nematicides, molluscicides or mixtures of several of these preparations, if desired, together with further agriculturally acceptable carriers, surfactants or application promoting adjuvants customarily employed in the art of formulation. Suitable carriers (i.e. agriculturally acceptable carriers) and adjuvants can be solid or liquid and correspond to the substances ordinarily employed in formulation technology, e.g. natural or regenerated mineral substances, solvents, dispersants, wetting agents, sticking agents, tackifiers, binders or fertilizers. Likewise, the formulations may be prepared into edible baits or fashioned into pest traps to permit feeding or ingestion by a target pest of the pesticidal formulation.

(340) Exemplary chemical compositions, which may be combined with the microbes of the disclosure, include:

(341) Fruits/Vegetables Herbicides: Atrazine, Bromacil, Diuron, Glyphosate, Linuron, Metribuzin, Simazine, Trifluralin, Fluazifop, Glufosinate, Halo sulfuron Gowan, Paraquat, Propyzamide, Sethoxydim, Butafenacil, Halosulfuron, Indaziflam; Fruits/Vegetables Insecticides: Aldicarb, *Bacillus thuringiensis*, Carbaryl, Carbofuran, Chlorpyrifos, Cypermethrin, Deltamethrin, Diazinon, Malathion, Abamectin, Cyfluthrin/betacyfluthrin, Esfenvalerate, Lambda-cyhalothrin, Acequinocyl, Bifenazate, Methoxyfenozide, Novaluron, Chromafenozide, Thiacloprid, Dinotefuran, FluaCrypyrim, Tolfenpyrad, Clothianidin, Spirodiclofen, Gamma-cyhalothrin, Spiromesifen, Spinosad, Rynaxypyr, Cyazypyr, Spinoteram, Triflumuron, Spirotetramat, Imidacloprid, Flubendiamide, Thiodicarb, Metaflumizone, Sulfoxaflor, Cyflumetofen, Cyanopyrafen, Imidacloprid, Clothianidin, Thiamethoxam, Spinotoram, Thiodicarb, Flonicamid, Methiocarb, Emamectin benzoate, Indoxacarb, Forthiazate, Fenamiphos, Cadusaphos, Pyriproxifen, Fenbutatin oxide, Hexthiazox, Methomyl, 4-[[(6-Chlorpyridin-3-yl)methyl](2, 2-difluorethyl)amino]furan-2(5H)-on; Fruits Vegetables Fungicides: Carbendazim, Chlorothalonil, EBDCs, Sulphur, Thiophanate-methyl, Azoxystrobin, Cymoxanil, Fluazinam, Fosetyl, Iprodione, Kresoxim-methyl, Metalaxyl/mefenoxam, Trifloxystrobin, Ethaboxam, Iprovalicarb, Trifloxystrobin, Fenhexamid, Oxpoconazole fumarate, Cyazofamid, Fenamidone, Zoxamide, Picoxystrobin, Pyraclostrobin, Cyflufenamid, Boscalid;
(342) Cereals Herbicides: Isoproturon, Bromoxynil, loxynil, Phenoxies, Chlorsulfuron, Clodinafop, Diclofop, Diflufenican, Fenoxaprop, Florasulam, Fluoroxypyr, Metsulfuron, Triasulfuron, Flucarbazone, lodosulfuron, Propoxycarbazone, Picolin-afen, Mesosulfuron, Beflubutamid, Pinoxaden, Amidosulfuron, Thifensulfuron Methyl, Tribenuron, Flupyrsulfuron, Sulfosulfuron, Pyrasulfotole, Pyroxsulam, Flufenacet, Tralkoxydim, Pyroxasulfon; Cereals Fungicides: Carbendazim, Chlorothalonil, Azoxystrobin, Cyproconazole, Cyprodinil, Fenpropimorph, Epoxiconazole, Kresoxim-methyl, Quinoxyfen, Tebuconazole, Trifloxystrobin, Simeconazole, Picoxystrobin, Pyraclostrobin, Dimoxystrobin, Prothioconazole, Fluoxastrobin; Cereals Insecticides: Dimethoate, Lambda-cyhalothrin, Deltamethrin, alpha-Cypermethrin, β-cyfluthrin, Bifenthrin, Imidacloprid, Clothianidin, Thiamethoxam, Thiacloprid, Acetamiprid, Dinetofuran, Clorphyriphos, Metamidophos, Oxidemethon methyl, Pirimicarb, Methiocarb;
(343) Maize Herbicides: Atrazine, Alachlor, Bromoxynil, Acetochlor, Dicamba, Clopyralid, S-Dimethenamid, Glufosinate, Glyphosate, Isoxaflutole, S-Metolachlor, Mesotrione, Nicosulfuron, Primisulfuron, Rimsulfuron, Sulcotrione, Foramsulfuron, Topramezone, Tembotrione, Saflufenacil, Thiencarbazone, Flufenacet, Pyroxasulfon; Maize Insecticides: Carbofuran, Chlorpyrifos, Bifenthrin, Fipronil, Imidacloprid, Lambda-Cyhalothrin, Tefluthrin, Terbufos, Thiamethoxam, Clothianidin, Spiromesifen, Flubendiamide, Triflumuron, Rynaxypyr, Deltamethrin, Thiodicarb, β-Cyfluthrin, Cypermethrin, Bifenthrin, Lufenuron, Triflumoron, Tefluthrin, Tebupirim-phos, Ethiprole, Cyazypyr, Thiacloprid, Acetamiprid, Dinetofuran, Avermectin, Methiocarb, Spirodiclofen, Spirotetramat; Maize Fungicides: Fenitropan, Thiram, Prothioconazole, Tebuconazole, Trifloxystrobin;
(344) Rice Herbicides: Butachlor, Propanil, Azimsulfuron, Bensulfuron, Cyhalo-fop, Daimuron, Fentrazamide, Imazosulfuron, Mefenacet, Oxaziclomefone, Pyrazosulfuron, Pyributicarb, Quinclorac, Thiobencarb, Indanofan, Flufenacet, Fentrazamide, Halosulfuron, Oxaziclomefone, Benzobicyclon, Pyriftalid, Penoxsulam, Bispyribac, Oxadiargyl, Ethoxysulfuron, Pretilachlor, Mesotrione, Tefuryltrione, Oxadiazone, Fenoxaprop, Pyrimisulfan; Rice Insecticides: Diazinon, Fenitro-thion, Fenobucarb, Monocrotophos, Benfuracarb, Buprofezin, Dinotefuran, Fipronil, Imidacloprid, Isoprocarb, Thiacloprid, Chromafenozide, Thiacloprid, Dinotefuran, Clothianidin, Ethiprole, Flubendiamide, Rynaxypyr, Deltamethrin, Acetamiprid, Thiamethoxam, Cyazypyr, Spinosad, Spinotoram, Emamectin-Benzoate, Cypermethrin, Chlorpyriphos, Cartap, Methamidophos, Etofen-prox, Triazophos, 4-[[(6-Chlorpyridin-3-yl)methyl](2,2-difluorethyl)amino]furan-2(5H)-on, Carbofuran, Benfuracarb; Rice Fungicides: Thiophanate-methyl, Azoxystrobin, Carpropamid, Edifenphos, Ferimzone, Iprobenfos, Isoprothiolane,

Pencycuron, Probenazole, Pyroquilon, Tricyclazole, Trifloxystrobin, Diclocymet, Fenoxanil, Simeconazole, Tiadinil;
(345) Cotton Herbicides: Diuron, Fluometuron, MSMA, Oxyfluorfen, Prometryn, Trifluralin, Carfentrazone, Clethodim, Fluazifop-butyl, Glyphosate, Norflurazon, Pendimethalin, Pyrithiobac-sodium, Trifloxysulfuron, Tepraloxydim, Glufosinate, Flumioxazin, Thidiazuron; Cotton Insecticides: Acephate, Aldicarb, Chlorpyrifos, Cypermethrin, Deltamethrin, Malathion, Monocrotophos, Abamectin, Acetamiprid, Emamectin Benzoate, Imidacloprid, Indoxacarb, Lambda-Cyhalothrin, Spinosad, Thiodicarb, Gamma-Cyhalothrin, Spiromesifen, Pyridalyl, Flonicamid, Flubendiamide, Triflumuron, Rynaxypyr, Beta-Cyfluthrin, Spirotetramat, Clothianidin, Thiamethoxam, Thiacloprid, Dinetofuran, Flubendiamide, Cyazypyr, Spinosad, Spinotoram, gamma Cyhalothrin, 4-[[(6-Chlorpyridin-3-yl) methyl](2,2-difluorethyl)amino]furan-2(5H)-on, Thiodicarb, Avermectin, Flonicamid, Pyridalyl, Spiromesifen, Sulfoxaflor, Profenophos, Thriazophos, Endosulfan; Cotton Fungicides: Etridiazole, Metalaxyl, Quintozene;
(346) Soybean Herbicides: Alachlor, Bentazone, Trifluralin, Chlorimuron-Ethyl, Cloransulam-Methyl, Fenoxaprop, Fomesafen, Flu-azifop, Glyphosate, Imazamox, Imazaquin, Imazethapyr, (S-)Metolachlor, Metribuzin, Pendimethalin, Tepraloxydim, Glufosinate; Soybean Insecticides: Lambda-cyhalothrin, Methomyl, Parathion, Thiocarb, Imidacloprid, Clothianidin, Thiamethoxam, Thiacloprid, Acetamiprid, Dinetofuran, Flubendiamide, Rynaxypyr, Cyazypyr, Spinosad, Spinotoram, Emamectin-Benzoate, Fipronil, Ethiprole, Deltamethrin, β-Cyfluthrin, gamma and lambda Cyhalothrin, 4-[[(6-Chlorpyridin-3-yl)methyl](2,2-difluorethyl)amino]furan-2(5H)-on, Spirotetramat, Spinodiclofen, Triflumuron, Flonicamid, Thiodicarb, beta-Cyfluthrin; Soybean Fungicides: Azoxystrobin, Cyproconazole, Epoxiconazole, Flutriafol, Pyraclostrobin, Tebuconazole, Trifloxystrobin, Prothioconazole, Tetraconazole;
(347) Sugarbeet Herbicides: Chloridazon, Desmedipham, Ethofumesate, Phenmedipham, Triallate, Clopyralid, Fluazifop, Lenacil, Metamitron, Quinmerac, Cycloxydim, Triflusulfuron, Tepral-oxydim, Quizalofop; Sugarbeet Insecticides: Imidacloprid, Clothianidin, Thiamethoxam, Thiacloprid, Acetamiprid, Dinetofuran, Deltamethrin, β-Cyfluthrin, gamma/lambda Cyhalothrin, 4-[[(6-Chlorpyridin-3-yl)methyl](2,2-difluor-ethyl)amino]furan-2(5H)-on, Tefluthrin, Rynaxypyr, Cyaxypyr, Fipronil, Carbofuran;
(348) Canola Herbicides: Clopyralid, Diclofop, Fluazifop, Glufosinate, Glyphosate, Metazachlor, Trifluralin Ethametsulfuron, Quinmerac, Quizalofop, Clethodim, Tepraloxydim; Canola Fungicides: Azoxystrobin, Carbendazim, Fludioxonil, Iprodione, Prochloraz, Vinclozolin; Canola Insecticides: Carbofuran organophos-phates, Pyrethroids, Thiacloprid, Deltamethrin, Imidacloprid, Clothianidin, Thiamethoxam, Acetamiprid, Dineto-furan, β-Cyfluthrin, gamma and lambda Cyhalothrin, tau-Fluvaleriate, Ethiprole, Spinosad, Spinotoram, Flubendiamide, Rynaxypyr, Cyazypyr, 4-[[(6-Chlorpyridin-3-yl)methyl] (2,2-difluorethyl)amino] furan-2(5H)-on.
(349) Insecticidal Compositions Comprising an Insecticide and Microbe of the Disclosure
(350) As aforementioned, agricultural compositions of the disclosure, which may comprise any microbe taught herein, are sometimes combined with one or more insecticides.
(351) In some embodiments, insecticidal compositions may be included in the compositions set forth herein, and can be applied to a plant(s) or a part(s) thereof simultaneously or in succession, with other compounds. Insecticides include ammonium carbonate, aqueous potassium silicate, boric acid, copper sulfate, elemental sulfur, lime sulfur, sucrose octanoate esters, 4-[[(6-Chlorpyridin-3-yl)methyl](2, 2-difluorethyl)amino]furan-2(5H)-on, abamectin, notenone, fenazaquin, fenpyroximate, pyridaben, pyrimedifen, tebufenpyrad, tolfenpyrad, acephate, emamectin benzoate, lepimectin, milbemectin, hdroprene, kinoprene, methoprene, fenoxycarb, pyriproxyfen, methryl bromide and other alkyl halides, fulfuryl fluoride, chloropicrin, borax, disodium octaborate, sodium borate, sodium metaborate, tartar emetic, dazomet, metam, pymetrozine, pyrifluquinazon, flofentezine, diflovidazin, hexythiazox, bifenazate, thiamethoxam, imidacloprid, fenpyroximate, azadirachtin, permethrin, esfenvalerate, acetamiprid, bifenthrin,

indoxacarb, azadirachtin, pyrethrin, imidacloprid, beta-cyfluthrin, sulfotep, tebupirimfos, temephos, terbufos, tetrachlorvinphos, thiometon, triazophos, alanycarb, aldicarb, bendiocarb, benfluracarb, butocarboxim, butoxycarboxim, carbaryl, carbofuran, carbosulfan, ethiofencarb, fenobucarb, formetanate, furathiocarb, isoprocarb, methiocarb, methymyl, metolcarb, oxamyl, primicarb, propoxur, thiodicarb, thiofanox, triazamate, trimethacarb, XMC, xylylcarb, acephate, azamethiphos, azinphos-ethyl, azinphos-methyl, cadusafos, chlorethoxyfox, trichlorfon, vamidothion, chlordane, endosulfan, ethiprole, fipronil, acrinathrin, allethrin, bifenthrin, bioallethrin, bioalletherin X-cyclopentenyl, bioresmethrin, cyclorothrin, cyfluthrin, cyhalothrin, cypermethrin, cyphenothrin [(1R)-trans-isomers], deltamethrin, empenthrin [(EZ)-(1R)-isomers], esfenvalerate, etofenprox, fenpropathrin, fenvalerate, flucythrinate, flumethrin, halfenprox, kadathrin, phenothrin [(1R)-trans-isomer] prallethrin, pyrethrins (pyrethrum), resmethrin, silafluofen, tefluthrin, tetramethrin, tetramethrin [(1R)-isomers], tralomethrin, transfluthrin, alpha-cypermethrin, beta-cyfluthrin, beta-cypermethrin, d-cis-trans allethrin, d-trans allethrin, gamma-cyhalothrin, lamda-cyhalothrin, tau-fluvalinate, theta-cypermethrin, zeta-cypermethrin, methoxychlor, nicotine, sulfoxaflor, acetamiprid, clothianidin, dinotefuran, imidacloprid, nitenpyram, thiacloprid, thiamethoxan, tebuprimphos, beta-cyfluthrin, clothianidin, flonicamid, hydramethylnon, amitraz, flubendiamide, blorantraniliprole, lambda cyhalothrin, spinosad, gamma cyhalothrin, *Beauveria bassiana*, *capsicum* oleoresin extract, garlic oil, carbaryl, chlorpyrifos, sulfoxaflor, lambda cyhalothrin, Chlorfenvinphos, Chlormephos, Chlorpyrifos, Chlorpyrifos-methyl, Coumaphos, Cyanophos, Demeton-S-methyl, Diazinon, Dichlorvos/DDVP, Dicrotophos, Dimethoate, Dimethylvinphos, Disulfoton, EPN, Ethion, Ethoprophos, Famphur, Fenamiphos, Fenitrothion, Fenthion, Fosthiazate, Heptenophos, Imicyafos, Isofenphos, Isopropyl O-(methoxyaminothio-phosphoryl) salicylate, Isoxathion, Malathion, Mecarbam, Methamidophos, Methidathion, Mevinphos, Monocrotophos, Naled, Omethoate, Oxydemeton-methyl, Parathion, Parathion-methyl, Phenthoate, Phorate, Phosalone, Phosmet, Phosphamidon, Phoxim, Pirimiphos-methyl, Profenofos, Propetamphos, Prothiofos, Pyraclofos, Pyridaphenthion, Quinalphosfluacrypyrim, tebufenozide, chlorantraniliprole, *Bacillus thuringiensis* subs. Kurstaki, terbufos, mineral oil, fenpropathrin, metaldehyde, deltamethrin, diazinon, dimethoate, diflubenzuron, pyriproxyfen, reosemary oil, peppermint oil, geraniol, azadirachtin, piperonyl butoxide, cyantraniliprole, alpha cypermethrin, tefluthrin, pymetrozine, malathion, *Bacillus thuringiensis* subsp. *israelensis*, dicofol, bromopropylate, benzoximate, azadirachtin, flonicamid, soybean oil, *Chromobacterium subtsugae* strain PRAA4-1, zeta cypermethrin, phosmet, methoxyfenozide, paraffinic oil, spirotetramat, methomyl, Metarhizium anisopliae strain F52, ethoprop, tetradifon, propargite, fenbutatin oxide, azocyclotin, cyhexatin, diafenthiuron, *Bacillus sphaericus*, etoxazole, flupyradifurone, azadirachtin, *Beauveria bassiana*, cyflumetofen, azadirachtin, chinomethionat, acephate, *Isaria fumosorosea* Apopka strain 97, sodium tetraborohydrate decahydrate, emamectin benzoate, cryolite, spinetoram, *Chenopodium ambrosioides* extract, novaluron, dinotefuran, carbaryl, acequinocyl, flupyradifurone, iron phosphate, kaolin, buprofezin, cyromazine, chromafenozide, halofenozide, methoxyfenozide, tebufenozide, bistrifluron, chlorfluazuron, diflubenzuron, flucycloxuron, flufenoxuron, hexaflumuron, lufenuron, nocaluron, noviflumuron, teflubenzuron, triflumuron, bensultap, cartap hydrochloride, thiocyclam, thiosultap-sodium, DNOC, chlorfenapyr, sulfuramid, phorate, tolfenpyrad, sulfoxaflor, neem oil, *Bacillus thuringiensis* subsp. tenebrionis strain SA-10, cyromazine, heat-killed *Burkholderia* spp., cyantraniliprole, cyenopyrafen, cyflumetofen, sodium cyanide, potassium cyanide, calcium cyanide, aluminum phosphide, calcium phosphide, phosphine, zinc phosphide, spriodiclofen, spiromesifen, spirotetramat, metaflumizone, flubendiamide, pyflubumide, oxamyl, *Bacillus thuringiensis* subsp. *aizawai*, etoxazole, and esfenvalerate

(352) TABLE-US-00009 TABLE 9 Exemplary insecticides associated with various modes of action, which can be combined with microbes of the disclosure Physiological function(s) Mode of Action Compound class Exemplary insecticides affected acetylcholinesterase carbamates

Alanycarb, Aldicarb, Nerve and (AChE) inhibitors Bendiocarb, Benfuracarb, muscle Butocarboxim, Butoxycarboxim, Carbaryl, Carbofuran, Carbosulfan, Ethiofencarb, Fenobucarb, Formetanate, Furathiocarb, Isoprocarb, Methiocarb, Methomyl, Metolcarb, Oxamyl, Pirimicarb, Propoxur, Thiodicarb, Thiofanox, Triazamate, Trimethacarb, XMC, Xylylcarb acetylcholinesterase organophosphates Acephate, Azamethiphos, Nerve and (AChE) inhibitors Azinphos-ethyl, Azinphos- muscle methyl, Cadusafos, Chlorethoxyfos, Chlorfenvinphos, Chlormephos, Chlorpyrifos, Chlorpyrifos-methyl, Coumaphos, Cyanophos, Demeton-S-methyl, Diazinon, Dichlorvos/DDVP, Dicrotophos, Dimethoate, Dimethylvinphos, Disulfoton, EPN, Ethion, Ethoprophos, Famphur, Fenamiphos, Fenitrothion, Fenthion, Fosthiazate, Heptenophos, Imicyafos, Isofenphos, Isopropyl O- (methoxyaminothio- phosphoryl) salicylate, Isoxathion, Malathion, Mecarbam, Methamidophos, Methidathion, Mevinphos, Monocrotophos, Naled, Omethoate, Oxydemeton- methyl, Parathion, Parathion- methyl, Phenthoate, Phorate, Phosalone, Phosmet, Phosphamidon, Phoxim, Pirimiphos-methyl, Profenofos, Propetamphos, Prothiofos, Pyraclofos, Pyridaphenthion, Quinalphos, Sulfotep, Tebupirimfos, Temephos, Terbufos, Tetrachlorvinphos, Thiometon, Triazophos, Trichlorfon, Vamidothion GABA-gated cyclodiene Chlordane, Endosulfan Nerve and chloride channel organochlorines muscle blockers GABA-gated phenylpyrazoles Ethiprole, Fipronil Nerve and chloride channel (Fiproles) muscle blockers sodium channel pyrethroids, Acrinathrin, Allethrin, Nerve and modulators pyrethrins Bifenthrin, Bioallethrin, muscle Bioallethrin S-cyclopentenyl, Bioresmethrin, Cycloprothrin, Cyfluthrin, Cyhalothrin, Cypermethrin, Cyphenothrin [(1R)-trans-isomers], Deltamethrin, Empenthrin [(EZ)-(1R)-isomers], Esfenvalerate, Etofenprox, Fenpropathrin, Fenvalerate, Flucythrinate, Flumethrin, Halfenprox, Kadathrin, Phenothrin [(1R)-trans- isomer], Prallethrin, Pyrethrins (pyrethrum), Resmethrin, Silafluofen, Tefluthrin, Tetramethrin, Tetramethrin [(1R)- isomers], Tralomethrin, Transfluthrin, alpha- Cypermethrin, beta-Cyfluthrin, beta-Cypermethrin, d-cis-trans Allethrin, d-trans Allethrin, gamma-Cyhalothrin, lambda- Cyhalothrin, tau-Fluvalinate, theta-Cypermethrin, zeta-Cypermethrin sodium channel DDT, DDT, methoxychlor Nerve and modulators methoxychlor muscle nicotinic neonicotinoids Acetamiprid, Clothianidin, Nerve and acetylcholine Dinotefuran, Imidacloprid, muscle receptor (nAChR) Nitenpyram, Thiacloprid, competitive Thiamethoxam modulators nicotinic nicotine nicotine Nerve and acetylcholine muscle receptor (nAChR) competitive modulators nicotinic sulfoximines sulfoxaflor Nerve and acetylcholine muscle receptor (nAChR) competitive modulators nicotinic butenolides Flupyradifurone Nerve and acetylcholine muscle receptor (nAChR) competitive modulators nicotinic spinosyns Spinetoram, Spinosad Nerve and acetylcholine muscle receptor (nAChR) allosteric modulators Glutamate-gated avermectins, Abamectin, Emamectin Nerve and chloride channel milbemycins benzoate, Lepimectin, muscle (GluCl) allosteric Milbemectin modulators juvenile hormone juvenile hormone Hydroprene, Kinoprene, Growth mimics analogues Methoprene juvenile hormone Fenoxycarb Fenoxycarb Growth mimics juvenile hormone Pyriproxyfen Pyriproxyfen Growth mimics miscellaneous non-alkyl halides Methyl bromide and other Unknown or specific (multi-site) alkyl halides non-specific inhibitors miscellaneous non- Chloropicrin Chloropicrin Unknown or specific (multi-site) non-specific inhibitors miscellaneous non- fluorides Cryolite, sulfuryl fluoride Unknown or specific (multi-site) non-specific inhibitors miscellaneous non- borates Borax, Boric acid, Disodium Unknown or specific (multi-site) octaborate, Sodium borate, non-specific inhibitors Sodium metaborate miscellaneous non- tartar emetic tartar emetic Unknown or specific (multi-site) non-specific inhibitors miscellaneous non- methyl Dazomet, Metam Unknown or specific (multi-site) isothiocyanate non-specific inhibitors generators modulators of Pyridine Pymetrozine, Pyrifluquinazon Nerve and chordotonal organs azomethine muscle derivatives mite growth Clofentezine, Clofentezine, Diflovidazin, Growth inhibitors Diflovidazin, Hexythiazox Hexythiazox mite growth Etoxazole Etoxazole Growth inhibitors microbial *Bacillus* Bt var. *aizawai*, Bt var. Midgut disruptors of insect *thuringiensis* and *israelensis*, Bt var. *kurstaki*, Bt midgut membranes the insecticidal var. *tenebrionensis* proteins they produce microbial *Bacillus*

Bacillus sphaericus Midgut disruptors of insect *sphaericus* midgut membranes inhibitors of Diafenthiuron Diafenthiuron Respiration mitochondrial ATP synthase inhibitors of organotin Azocyclotin, Cyhexatin, Respiration mitochondrial ATP miticides Fenbutatin oxide synthase inhibitors of Propargite Propargite Respiration mitochondrial ATP synthase inhibitors of Tetradifon Tetradifon Respiration mitochondrial ATP synthase uncouplers of Chlorfenapyr, Chlorfenapyr, DNOC, Respiration oxidative DNOC, Sulfuramid phosphorylation via Sulfuramid disruption of the proton gradient Nicotinic nereistoxin Bensultap, Cartap Nerve and acetylcholine analogues hydrochloride, Thiocyclam, muscle receptor (nAChR) Thiosultap-sodium channel blockers inhibitors of chitin benzoylureas Bistrifluron, Chlorfluazuron, Growth biosynthesis, type 0 Diflubenzuron, Flucycloxuron, Flufenoxuron, Hexaflumuron, Lufenuron, Novaluron, Noviflumuron, Teflubenzuron, Triflumuron inhibitors of chitin Buprofezin Buprofezin Growth biosynthesis, type 1 moulting disruptor, Cyromazine Cyromazine Growth Dipteran ecdysone receptor diacylhydrazines Chromafenozide, Growth agonists Halofenozide, Methoxyfenozide, Tebufenozide octopamine Amitraz Amitraz Nerve and receptor agonists muscle mitochondrial Hydramethylnon Hydramethylnon Respiration complex III electron transport inhibitors mitochondrial Acequinocyl Acequinocyl Respiration complex III electron transport inhibitors mitochondrial Fluacrypyrim Fluacrypyrim Respiration complex III electron transport inhibitors mitochondrial Bifenazate Bifenazate Respiration complex III electron transport inhibitors mitochondrial Meti acaricides Fenazaquin, Fenpyroximate, Respiration complex I electron and insecticides Pyridaben, Pyrimidifen, transport inhibitors Tebufenpyrad, Tolfenpyrad mitochondrial Rotenone Rotenone Respiration complex I electron transport inhibitors voltage-dependent oxadiazines Indoxacarb Nerve and sodium channel muscle blockers voltage-dependent semicarbazones Metaflumizone Nerve and sodium channel muscle blockers inhibitors of acetyl tetronic and Spirodiclofen, Spiromesifen, Growth CoA carboxylase tetramic acid Spirotetramat derivatives mitochondrial phosphides Aluminium phosphide, Respiration complex IV Calcium phosphide, electron transport Phosphine, Zinc phosphide inhibitors mitochondrial cyanides Calcium cyanide, Potassium Respiration complex IV cyanide, Sodium cyanide electron transport inhibitors mitochondrial beta-ketonitrile Cyenopyrafen, Cyflumetofen Respiration complex II electron derivatives transport inhibitors mitochondrial carboxanilides Pyflubumide Respiration complex II electron transport inhibitors ryanodine receptor diamides Chlorantraniliprole, Nerve and modulators Cyantraniliprole, muscle Flubendiamide Chordotonal organ Flonicamid Flonicamid Nerve and modulators - muscle undefined target site compounds of Azadirachtin Azadirachtin Unknown unknown or uncertain mode of action compounds of Benzoximate Benzoximate Unknown unknown or uncertain mode of action compounds of Bromopropylate Bromopropylate Unknown unknown or uncertain mode of action compounds of Chinomethionat Chinomethionat Unknown unknown or uncertain mode of action compounds of Dicofol Dicofol Unknown unknown or uncertain mode of action compounds of lime sulfur lime sulfur Unknown unknown or uncertain mode of action compounds of Pyridalyl Pyridalyl Unknown unknown or uncertain mode of action compounds of sulfur sulfur Unknown unknown or uncertain mode of action

(353) TABLE-US-00010 TABLE 10 Exemplary list of pesticides, which can be combined with microbes of the disclosure Category Compounds INSECTICIDES arsenical insecticides calcium arsenate copper acetoarsenite copper arsenate lead arsenate potassium arsenite sodium arsenite botanical insecticides allicin anabasine azadirachtin carvacrol d-limonene matrine nicotine nornicotine oxymatrine pyrethrins cinerins cinerin I cinerin II jasmolin I jasmolin II pyrethrin I pyrethrin II quassia rhodojaponin-III rotenone ryania sabadilla sanguinarine triptolide carbamate insecticides bendiocarb carbaryl benzofuranyl methylcarbamate benfuracarb insecticides carbofuran carbosulfan decarbofuran furathiocarb dimethylcarbamate insecticides dimetan dimetilan hyquincarb isolan pirimicarb pyramat pyrolan oxime carbamate insecticides alanycarb aldicarb aldoxycarb butocarboxim butoxycarboxim methomyl nitrilacarb oxamyl tazimcarb thiocarboxime thiodicarb thiofanox phenyl methylcarbamate insecticides allyxycarb aminocarb

bufencarb butacarb carbanolate cloethocarb CPMC dicresyl dimethacarb dioxacarb EMPC ethiofencarb fenethacarb fenobucarb isoprocarb methiocarb metolcarb mexacarbate promacyl promecarb propoxur trimethacarb XMC xylylcarb diamide insecticides broflanilide chlorantraniliprole cyantraniliprole cyclaniliprole cyhalodiamide flubendiamide tetraniliprole dinitrophenol insecticides dinex dinoprop dinosam DNOC fluorine insecticides barium hexafluorosilicate cryolite flursulamid sodium fluoride sodium hexafluorosilicate sulfluramid formamidine insecticides amitraz chlordimeform formetanate formpar anate medimeform semiamitraz fumigant insecticides acrylonitrile carbon disulfide carbon tetrachloride carbonyl sulfide chloroform chloropicrin cyanogen para-dichlorobenzene 1,2-dichloropropane dithioether ethyl formate ethylene dibromide ethylene dichloride ethylene oxide hydrogen cyanide methyl bromide methyl iodide methylchloroform methylene chloride naphthalene phosphine sodium tetrathiocarbonate sulfuryl fluoride tetrachloroethane inorganic insecticides borax boric acid calcium polysulfide copper oleate diatomaceous earth mercurous chloride potassium thiocyanate silica gel sodium thiocyanate insect growth regulators chitin synthesis inhibitors buprofezin cyromazine benzoylphenylurea chitin synthesis bistrifluron inhibitors chlorbenzuron chlorfluazuron dichlorbenzuron diflubenzuron flucycloxuron flufenoxuron hexaflumuron lufenuron novaluron noviflumuron penfluron teflubenzuron triflumuron juvenile hormone mimics dayoutong epofenonane fenoxycarb hydroprene kinoprene methoprene pyriproxyfen triprene juvenile hormones juvenile hormone I juvenile hormone II juvenile hormone III moulting hormone agonists chromafenozide furan tebufenozide halofenozide methoxyfenozide tebufenozide yishijing moulting hormones α-ecdysone ecdysterone moulting inhibitors diofenolan precocenes precocene I precocene II precocene III unclassified insect growth regulators dicyclanil macrocyclic lactone insecticides avermectin insecticides abamectin doramectin emamectin eprinomectin ivermectin selamectin milbemycin insecticides lepimectin milbemectin milbemycin oxime moxidectin spinosyn insecticides spinetoram spinosad neonicotinoid insecticides nitroguanidine neonicotinoid clothianidin insecticides dinotefuran imidacloprid imidaclothiz thiamethoxam nitromethylene neonicotinoid nitenpyram insecticides nithiazine pyridylmethylamine neonicotinoid acetamiprid insecticides imidacloprid nitenpyram paichongding thiacloprid nereistoxin analogue insecticides bensultap cartap polythialan thiocyclam thiosultap organochlorine insecticides bromo-DDT camphechlor DDT pp′-DDT ethyl-DDD HCH gamma-HCH lindane methoxychlor pentachlorophenol TDE cyclodiene insecticides aldrin bromocyclen chlorbicyclen chlordane chlordecone dieldrin dilor endosulfan alpha-endosulfan endrin HEOD heptachlor HHDN isobenzan isodrin kelevan mirex organophosphorus insecticides organophosphate insecticides bromfenvinfos calvinphos chlorfenvinphos crotoxyphos dichlorvos dicrotophos dimethylvinphos fospirate heptenophos methocrotophos mevinphos monocrotophos naled naftalofos phosphamidon propaphos TEPP tetrachlorvinphos organothiophosphate insecticides dioxabenzofos fosmethilan phenthoate aliphatic organothiophosphate acethion insecticides acetophos amiton cadusafos chlorethoxyfos chlormephos demephion demephion-O demephion-S demeton demeton-O demeton-S demeton-methyl demeton-O-methyl demeton-S-methyl demeton-S-methylsulphon disulfoton ethion ethoprophos IPSP isothioate malathion methacrifos methylacetophos oxydemeton-methyl oxydeprofos oxydisulfoton phorate sulfotep terbufos thiometon aliphatic amide amidithion organothiophosphate insecticides cyanthoate dimethoate ethoate-methyl formothion mecarbam omethoate prothoate sophamide vamidothion oxime organothiophosphate chlorphoxim insecticides phoxim phoxim-methyl heterocyclic organothiophosphate azamethiphos insecticides colophonate coumaphos coumithoate dioxathion endothion menazon morphothion phosalone pyraclofos pyrazothion pyridaphenthion quinothion benzothiopyran dithicrofos organothiophosphate insecticides thicrofos benzotriazine organothiophosphate azinphos-ethyl insecticides azinphos-methyl isoindole organothiophosphate dialifos insecticides phosmet isoxazole organothiophosphate isoxathion insecticides zolaprofos pyrazolopyrimidine chlorprazophos organothiophosphate insecticides pyrazophos pyridine organothiophosphate chlorpyrifos insecticides chlorpyrifos-

methyl pyrimidine organothiophosphate butathiofos insecticides diazinon etrimfos lirimfos pirimioxyphos pirimiphos-ethyl pirimiphos-methyl primidophos pyrimitate tebupirimfos quinoxaline organothiophosphate quinalphos insecticides quinalphos-methyl thiadiazole organothiophosphate athidathion insecticides lythidathion methidathion prothidathion triazole organothiophosphate isazofos insecticides triazophos phenyl organothiophosphate azothoate insecticides bromophos bromophos-ethyl carbophenothion chlorthiophos cyanophos cythioate dicapthon dichlofenthion etaphos famphur fenchlorphos fenitrothion fensulfothion fenthion fenthion-ethyl heterophos jodfenphos mesulfenfos parathion parathion-methyl phenkapton phosnichlor profenofos prothiofos sulprofos temephos trichlormetaphos-3 trifenofos xiaochongliulin phosphonate insecticides butonate trichlorfon phosphonothioate insecticides mecarphon phenyl ethylphosphonothioate fonofos insecticides trichloronat phenyl phenylphosphonothioate cyanofenphos insecticides EPN leptophos phosphoramidate insecticides crufomate fenamiphos fosthietan mephosfolan phosfolan phosfolan-methyl pirimetaphos phosphoramidothioate insecticides acephate chloramine phosphorus isocarbophos isofenphos isofenphos-methyl methamidophos phosglycin propetamphos phosphorodiamide insecticides dimefox mazidox mipafox schradan oxadiazine insecticides indoxacarb oxadiazolone insecticides metoxadiazone phthalimide insecticides dialifos phosmet tetramethrin physical insecticides maltodextrin desiccant insecticides boric acid diatomaceous earth silica gel pyrazole insecticides chlorantraniliprole cyantraniliprole cyclaniliprole dimetilan isolan tebufenpyrad tetraniliprole tolfenpyrad phenylpyrazole insecticides acetoprole ethiprole fipronil flufiprole pyraclofos pyrafluprole pyriprole pyrolan vaniliprole pyrethroid insecticides pyrethroid ester insecticides acrinathrin allethrin bioallethrin esdépalléthrine barthrin bifenthrin kappa-bifenthrin bioethanomethrin brofenvalerate brofluthrinate bromethrin butethrin chlorempenthrin cyclethrin cycloprothrin cyfluthrin beta-cyfluthrin cyhalothrin gamma-cyhalothrin lambda-cyhalothrin cypermethrin alpha-cypermethrin beta-cypermethrin theta-cypermethrin zeta-cypermethrin cyphenothrin deltamethrin dimefluthrin dimethrin empenthrin d-fanshiluquebingjuzhi chloroprallethrin fenfluthrin fenpirithrin fenpropathrin fenvalerate esfenvalerate flucythrinate fluvalinate tau-fluvalinate furamethrin furethrin heptafluthrin imiprothrin japothrins kadethrin methothrin metofluthrin epsilon-metofluthrin momfluorothrin epsilon-momfluorothrin pentmethrin permethrin biopermethrin transpermethrin phenothrin prallethrin profluthrin proparthrin pyresmethrin renofluthrin meperfluthrin resmethrin bioresmethrin cismethrin tefluthrin kappa-tefluthrin terallethrin tetramethrin tetramethylfluthrin tralocythrin tralomethrin transfluthrin valerate pyrethroid ether insecticides etofenprox flufenprox halfenprox protrifenbute silafluofen pyrethroid oxime insecticides sulfoxime thiofluoximate pyrimidinamine insecticides flufenerim pyrimidifen pyrrole insecticides chlorfenapyr quaternary ammonium insecticides sanguinarine sulfoximine insecticides sulfoxaflor tetramic acid insecticides spirotetramat tetronic acid insecticides spiromesifen thiazole insecticides clothianidin imidaclothiz thiamethoxam thiapronil thiazolidine insecticides tazimcarb thiacloprid thiourea insecticides diafenthiuron urea insecticides flucofuron sulcofuron zwitterionic insecticides dicloromezotiaz triflumezopyrim unclassified insecticides afidopyropen afoxolaner allosamidin closantel copper naphthenate crotamiton EXD fenazaflor fenoxacrim flometoquin flonicamid fluhexafon flupyradifurone Aural aner Auxametamide hydramethylnon isoprothiolane jiahuangchongzong malonoben metaflumizone nifluridide plifenate pyridaben pyridalyl pyrifluquinazon rafoxanide thuringiensin triarathene triazamate ACARICIDES botanical acaricides carvacrol sanguinarine bridged diphenyl acaricides azobenzene benzoximate benzyl benzoate bromopropylate chlorbenside chlorfenethol chlorfenson chlorfensulphide chlorobenzilate chloropropylate cyflumetofen DDT dicofol diphenyl sulfone dofenapyn fenson fentrifanil fluorbenside genit hexachlorophene phenproxide proclonol tetradifon tetrasul carbamate acaricides benomyl carbanolate carbaryl carbofuran methiocarb metolcarb promacyl propoxur oxime carbamate acaricides aldicarb butocarboxim oxamyl thiocarboxime thiofanox carbazate acaricides bifenazate dinitrophenol acaricides binapacryl dinex dinobuton

dinocap dinocap-4 dinocap-6 dinocton dinopenton dinosulfon dinoterbon DNOC formamidine acaricides amitraz chlordimeform chloromebuform formetanate formparanate medimeform semi amitraz macrocyclic lactone acaricides tetranactin avermectin acaricides abamectin doramectin eprinomectin ivermectin selamectin milbemycin acaricides milbemectin milbemycin oxime moxidectin mite growth regulators clofentezine cyromazine diflovidazin dofenapyn fluazuron flubenzimine flucycloxuron flufenoxuron hexythiazox organochlorine acaricides bromocyclen camphechlor DDT dienochlor endosulfan lindane organophosphorus acaricides organophosphate acaricides chlorfenvinphos crotoxyphos dichlorvos heptenophos mevinphos monocrotophos naled TEPP tetrachlorvinphos organothiophosphate acaricides amidithion amiton azinphos-ethyl azinphos-methyl azothoate benoxafos bromophos bromophos-ethyl carbophenothion chlorpyrifos chlorthiophos coumaphos cyanthoate demeton demeton-O demeton-S demeton-methyl demeton-O-methyl demeton-S-methyl demeton-S-methylsulphon dialifos diazinon dimethoate dioxathion disulfoton endothion ethion ethoate-methyl formothion malathion mecarbam methacrifos omethoate oxydeprofos oxydisulfoton parathion phenkapton phorate phosalone phosmet phostin phoxim pirimiphos-methyl prothidathion prothoate pyrimitate quinalphos quintiofos sophamide sulfotep thiometon triazophos trifenofos vamidothion phosphonate acaricides trichlorfon phosphoramidothioate acaricides isocarbophos methamidophos propetamphos phosphorodiamide acaricides dimefox mipafox schradan organotin acaricides azocyclotin cyhexatin fenbutatin oxide phostin phenylsulfamide acaricides dichlofluanid phthalimide acaricides dialifos phosmet pyrazole acaricides cyenopyrafen fenpyroximate pyflubumide tebufenpyrad phenylpyrazole acaricides acetoprole fipronil vaniliprole pyrethroid acaricides pyrethroid ester acaricides acrinathrin bifenthrin brofluthrinate cyhalothrin cypermethrin alpha-cypermethrin fenpropathrin fenvalerate flucythrinate flumethrin fluvalinate tau-fluvalinate permethrin pyrethroid ether acaricides halfenprox pyrimidinamine acaricides pyrimidifen pyrrole acaricides chlorfenapyr quaternary ammonium acaricides sanguinarine quinoxaline acaricides chinomethionat thioquinox strobilurin acaricides methoxyacrylate strobilurin acaricides bifujunzhi fluacrypyrim flufenoxystrobin pyriminostrobin sulfite ester acaricides aramite propargite tetronic acid acaricides spirodiclofen tetrazine acaricides clofentezine diflovidazin thiazolidine acaricides flubenzimine hexythiazox thiocarbamate acaricides fenothiocarb thiourea acaricides chloromethiuron diafenthiuron unclassified acaricides acequinocyl afoxolaner amidoflumet arsenous oxide clenpirin closantel crotamiton cycloprate cymiazole disulfiram etoxazole fenazaflor fenazaquin fluenetil fluralaner mesulfen MNAF nifluridide nikkomycins pyridaben sulfiram sulfluramid sulfur thuringiensin triarathene CHEMOSTERILANTS apholate bisazir busulfan diflubenzuron dimatif hemel hempa metepa methiotepa methyl apholate morzid penfluron tepa thiohempa thiotepa tretamine uredepa INSECT REPELLENTS acrep butopyronoxyl camphor d-camphor carboxide dibutyl phthalate diethyltoluamide dimethyl carbate dimethyl phthalate dibutyl succinate ethohexadiol hexamide icaridin methoquin-butyl methylneodecanamide 2-(octylthio)ethanol oxamate quwenzhi quyingding rebemide zengxiaoan NEMATICIDES avermectin nematicides abamectin botanical nematicides carvacrol carbamate nematicides benomyl carbofuran carbosulfan cloethocarb oxime carbamate nematicides alanycarb aldicarb aldoxycarb oxamyl tirpate fumigant nematicides carbon disulfide cyanogen 1,2-dichloropropane 1,3-dichloropropene dithioether methyl bromide methyl iodide sodium tetrathiocarbonate organophosphorus nematicides organophosphate nematicides diamidafos fenamiphos fosthietan phosphamidon organothiophosphate nematicides cadusafos chlorpyrifos dichlofenthion dimethoate ethoprophos fensulfothion fosthiazate heterophos isamidofos isazofos phorate phosphocarb terbufos thionazin triazophos phosphonothioate nematicides imicyafos mecarphon unclassified nematicides acetoprole benclothiaz chloropicrin dazomet DBCP DCIP fluazaindolizine fluensulfone furfural metam methyl isothiocyanate tioxazafen xylenols

(354) Insecticides also include synergists or activators that are not in themselves considered toxic or insecticidal, but are materials used with insecticides to synergize or enhance the activity of the

insecticides. Syngergists or activators include piperonyl butoxide.
(355) Biorational Pesticides
(356) Insecticides can be biorational, or can also be known as biopesticides or biological pesticides. Biorational refers to any substance of natural origin (or man-made substances resembling those of natural origin) that has a detrimental or lethal effect on specific target pest(s), e.g., insects, weeds, plant diseases (including nematodes), and vertebrate pests, possess a unique mode of action, are non-toxic to man, domestic plants and animals, and have little or no adverse effects on wildlife and the environment.
(357) Biorational insecticides (or biopesticides or biological pesticides) can be grouped as: (1) biochemicals (hormones, enzymes, pheromones and natural agents, such as insect and plant growth regulators), (2) microbial (viruses, bacteria, fungi, protozoa, and nematodes), or (3) Plant-Incorporated protectants (PIPs)—primarily transgenic plants, e.g., Bt corn.
(358) Biopesticides, or biological pesticides, can broadly include agents manufactured from living microorganisms or a natural product and sold for the control of plant pests. Biopesticides can be: microorganisms, biochemicals, and semiochemicals. Biopesticides can also include peptides, proteins and nucleic acids such as double-stranded DNA, single-stranded DNA, double-stranded RNA, single-stranded RNA and hairpin DNA or RNA.
(359) Bacteria, fungi, oomycetes, viruses and protozoa are all used for the biological control of insect pests. The most widely used microbial biopesticide is the insect pathogenic bacteria *Bacillus thuringiensis* (Bt), which produces a protein crystal (the Bt δ-endotoxin) during bacterial spore formation that is capable of causing lysis of gut cells when consumed by susceptible insects. Microbial Bt biopesticides consist of bacterial spores and δ-endotoxin crystals mass-produced in fermentation tanks and formulated as a sprayable product. Bt does not harm vertebrates and is safe to people, beneficial organisms and the environment. Thus, Bt sprays are a growing tactic for pest management on fruit and vegetable crops where their high level of selectivity and safety are considered desirable, and where resistance to synthetic chemical insecticides is a problem. Bt sprays have also been used on commodity crops such as maize, soybean and cotton, but with the advent of genetic modification of plants, farmers are increasingly growing Bt transgenic crop varieties.
(360) Other microbial insecticides include products based on entomopathogenic baculoviruses. Baculoviruses that are pathogenic to arthropods belong to the virus family and possess large circular, covalently closed, and double-stranded DNA genomes that are packaged into nucleocapsids. More than 700 baculoviruses have been identified from insects of the orders Lepidoptera, Hymenoptera, and Diptera. Baculoviruses are usually highly specific to their host insects and thus, are safe to the environment, humans, other plants, and beneficial organisms. Over 50 baculovirus products have been used to control different insect pests worldwide. In the US and Europe, the *Cydia pomonella* granulovirus (CpGV) is used as an inundative biopesticide against codlingmoth on apples. Washington State, as the biggest apple producer in the US, uses CpGV on 13% of the apple crop. In Brazil, the nucleopolyhedrovirus of the soybean caterpillar *Anticarsia gemmatalis* was used on up to 4 million ha (approximately 35%) of the soybean crop in the mid-1990s. Viruses such as Gemstar® (Certis USA) are available to control larvae of *Heliothis* and *Helicoverpa* species.
(361) At least 170 different biopesticide products based on entomopathogenic fungi have been developed for use against at least five insect and acarine orders in glasshouse crops, fruit and field vegetables as well as commodity crops. The majority of products are based on the ascomycetes *Beauveria bassiana* or Metarhizium anisopliae. M anisopliae has also been developed for the control of locust and grasshopper pests in Africa and Australia and is recommended by the Food and Agriculture Organization of the United Nations (FAO) for locust management.
(362) A number of microbial pesticides registered in the United States are listed in Table 16 of Kabaluk et al. 2010 (Kabaluk, J. T. et al. (ed.). 2010. The Use and Regulation of Microbial

Pesticides in Representative Jurisdictions Worldwide. IOBC Global. 99 pp.) and microbial pesticides registered in selected countries are listed in Annex 4 of Hoeschle-Zeledon et al. 2013 (Hoeschle-Zeledon, I., P. Neuenschwander and L. Kumar. (2013). Regulatory Challenges for biological control. SP-IPM Secretariat, International Institute of Tropical Agriculture (IITA), Ibadan, Nigeria. 43 pp.), each of which is incorporated herein in its entirety.

(363) Plants produce a wide variety of secondary metabolites that deter herbivores from feeding on them. Some of these can be used as biopesticides. They include, for example, pyrethrins, which are fast-acting insecticidal compounds produced by *Chrysanthemum cinerariaefolium*. They have low mammalian toxicity but degrade rapidly after application. This short persistence prompted the development of synthetic pyrethrins (pyrethroids). The most widely used botanical compound is neem oil, an insecticidal chemical extracted from seeds of *Azadirachta indica*. Two highly active pesticides are available based on secondary metabolites synthesized by soil actinomycetes, but they have been evaluated by regulatory authorities as if they were synthetic chemical pesticides. Spinosad is a mixture of two macrolide compounds from *Saccharopolyspora spinosa*. It has a very low mammalian toxicity and residues degrade rapidly in the field. Farmers and growers used it widely following its introduction in 1997 but resistance has already developed in some important pests such as western flower *thrips*. Abamectin is a macrocyclic lactone compound produced by *Streptomyces avermitilis*. It is active against a range of pest species but resistance has developed to it also, for example, in tetranychid mites.

(364) Peptides and proteins from a number of organisms have been found to possess pesticidal properties. Perhaps most prominent are peptides from spider venom (King, G. F. and Hardy, M. C. (2013) Spider-venom peptides: structure, pharmacology, and potential for control of insect pests. Annu. Rev. Entomol. 58: 475-496). A unique arrangement of disulfide bonds in spider venom peptides render them extremely resistant to proteases. As a result, these peptides are highly stable in the insect gut and hemolymph and many of them are orally active. The peptides target a wide range of receptors and ion channels in the insect nervous system. Other examples of insecticidal peptides include: sea anemone venom that act on voltage-gated Na+ channels (Bosmans, F. and Tytgat, J. (2007) Sea anemone venom as a source of insecticidal peptides acting on voltage-gated Na+ channels. Toxicon. 49(4): 550-560); the PA1b (Pea Albumin 1, subunit b) peptide from Legume seeds with lethal activity on several insect pests, such as mosquitoes, some aphids and cereal weevils (Eyraud, V. et al. (2013) Expression and Biological Activity of the Cystine Knot Bioinsecticide PAlb (Pea Albumin 1 Subunit b). PLoS ONE 8(12): e81619); and an internal 10 kDa peptide generated by enzymatic hydrolysis of *Canavalia ensiformis* (jack bean) urease within susceptible insects (Martinelli, A. H. S., et al. (2014) Structure—function studies on jaburetox, a recombinant insecticidal peptide derived from jack bean (*Canavalia ensiformis*) urease. Biochimica et Biophysica Acta 1840: 935-944). Examples of commercially available peptide insecticides include Spear™-T for the treatment of thrips in vegetables and ornamentals in greenhouses, Spear™-P to control the Colorado Potato Beetle, and Spear™-C to protect crops from lepidopteran pests (Vestaron Corporation, Kalamazoo, MI.). A novel insecticidal protein from *Bacillus bombysepticus*, called parasporal crystal toxin (PC), shows oral pathogenic activity and lethality towards silkworms and Cry 1 Ac-resistant *Helicoverpa armigera* strains (Lin, P. et al. (2015) PC, a novel oral insecticidal toxin from *Bacillus bombysepticus* involved in host lethality via APN and BtR-175. Sci. Rep. 5: 11101).

(365) A semiochemical is a chemical signal produced by one organism that causes a behavioral change in an individual of the same or a different species. The most widely used semiochemicals for crop protection are insect sex pheromones, some of which can now be synthesized and are used for monitoring or pest control by mass trapping, lure-and-kill systems and mating disruption. Worldwide, mating disruption is used on over 660,000 ha and has been particularly useful in orchard crops.

(366) As used herein, “transgenic insecticidal trait” refers to a trait exhibited by a plant that has

been genetically engineered to express a nucleic acid or polypeptide that is detrimental to one or more pests. In one embodiment, the plants of the present disclosure are resistant to attach and/or infestation from any one or more of the pests of the present disclosure. In one embodiment, the trait comprises the expression of vegetative insecticidal proteins (VIPs) from *Bacillus thuringiensis*, lectins and proteinase inhibitors from plants, terpenoids, cholesterol oxidases from *Streptomyces* spp., insect chitinases and fungal chitinolytic enzymes, bacterial insecticidal proteins and early recognition resistance genes. In another embodiment, the trait comprises the expression of a *Bacillus thuringiensis* protein that is toxic to a pest. In one embodiment, the Bt protein is a Cry protein (crystal protein). Bt crops include Bt corn, Bt cotton and Bt soy. Bt toxins can be from the Cry family (see, for example, Crickmore et al., 1998, Microbiol. Mol. Biol. Rev. 62: 807-812), which are particularly effective against Lepidoptera, Coleoptera and Diptera.

(367) Bt Cry and Cyt toxins belong to a class of bacterial toxins known as pore-forming toxins (PFT) that are secreted as water-soluble proteins undergoing conformational changes in order to insert into, or to translocate across, cell membranes of their host. There are two main groups of PFT: (i) the α-helical toxins, in which α-helix regions form the trans-membrane pore, and (ii) the β-barrel toxins, that insert into the membrane by forming a β-barrel composed of βsheet hairpins from each monomer. See, Parker M W, Feil S C, "Pore-forming protein toxins: from structure to function," Prog. Biophys. Mol. Biol. 2005 May; 88(1):91-142. The first class of PFT includes toxins such as the colicins, exotoxin A, diphtheria toxin and also the Cry three-domain toxins. On the other hand, aerolysin, α-hemolysin, anthrax protective antigen, cholesterol-dependent toxins as the perfringolysin O and the Cyt toxins belong to the β-barrel toxins. Id. In general, PFT producing-bacteria secrete their toxins and these toxins interact with specific receptors located on the host cell surface. In most cases, PFT are activated by host proteases after receptor binding inducing the formation of an oligomeric structure that is insertion competent. Finally, membrane insertion is triggered, in most cases, by a decrease in pH that induces a molten globule state of the protein. Id.

(368) The development of transgenic crops that produce Bt Cry proteins has allowed the substitution of chemical insecticides by environmentally friendly alternatives. In transgenic plants the Cry toxin is produced continuously, protecting the toxin from degradation and making it reachable to chewing and boring insects. Cry protein production in plants has been improved by engineering cry genes with a plant biased codon usage, by removal of putative splicing signal sequences and deletion of the carboxy-terminal region of the protoxin. See, Schuler T H, et al., "Insect-resistant transgenic plants," Trends Biotechnol. 1998; 16:168-175. The use of insect resistant crops has diminished considerably the use of chemical pesticides in areas where these transgenic crops are planted. See, Qaim M, Zilberman D, "Yield effects of genetically modified crops in developing countries," Science. 2003 Feb. 7; 299(5608):900-2.

(369) Known Cry proteins include: δ-endotoxins including but not limited to: the Cry1, Cry2, Cry3, Cry4, Cry5, Cry6, Cry7, Cry8, Cry9, Cry10, Cry11, Cry12, Cry13, Cry14, Cry15, Cry16, Cry17, Cry18, Cry19, Cry20, Cry21, Cry22, Cry23, Cry24, Cry25, Cry26, Cry27, Cry 28, Cry 29, Cry 30, Cry31, Cry32, Cry33, Cry34, Cry35, Cry36, Cry37, Cry38, Cry39, Cry40, Cry41, Cry42, Cry43, Cry44, Cry45, Cry 46, Cry47, Cry49, Cry 51, Cry52, Cry 53, Cry 54, Cry55, Cry56, Cry57, Cry58, Cry59. Cry60, Cry61, Cry62, Cry63, Cry64, Cry65, Cry66, Cry67, Cry68, Cry69, Cry70 and Cry71 classes of δ-endotoxin genes and the *B. thuringiensis* cytolytic cyt1 and cyt2 genes.

(370) Members of these classes of *B. thuringiensis* insecticidal proteins include, but are not limited to: Cry1Aa1 (Accession #AAA22353); Cry1Aa2 (Accession #Accession #AAA22552); Cry1Aa3 (Accession #BAA00257); Cry1Aa4 (Accession #CAA31886); Cry1Aa5 (Accession #BAA04468); Cry1Aa6 (Accession #AAA86265); Cry1Aa7 (Accession #AAD46139); Cry1Aa8 (Accession #126149); Cry1Aa9 (Accession #BAA77213); Cry1Aa10 (Accession #AAD55382); Cry1Aa11 (Accession #CAA70856); Cry1Aa12 (Accession #AAP80146); Cry1Aa13 (Accession #AAM44305); Cry1Aa14 (Accession #AAP40639); Cry1Aa15 (Accession #AAY66993);

Cry1Aa16 (Accession #HQ439776); Cry1Aa17 (Accession #HQ439788); Cry1Aa18 (Accession #HQ439790); Cry1Aa19 (Accession #HQ685121); Cry1Aa20 (Accession #JF340156); Cry1Aa21 (Accession #JN651496); Cry1Aa22 (Accession #KC158223); Cry1Ab1 (Accession #AAA22330); Cry1Ab2 (Accession #AAA22613); Cry1Ab3 (Accession #AAA22561); Cry1Ab4 (Accession #BAA00071); Cry1Ab5 (Accession #CAA28405); Cry1Ab6 (Accession #AAA22420); Cry1Ab7 (Accession #CAA31620); Cry1Ab8 (Accession #AAA22551); Cry1Ab9 (Accession #CAA38701); Cry1Ab10 (Accession #A29125); Cry1Ab11 (Accession #112419); Cry1Ab12 (Accession #AAC64003); Cry1Ab13 (Accession #AAN76494); Cry1Ab14 (Accession #AAG16877); Cry1Ab15 (Accession #AA013302); Cry1Ab16 (Accession #AAK55546); Cry1Ab17 (Accession #AAT46415); Cry1Ab18 (Accession #AAQ88259); Cry1Ab19 (Accession #AAW31761); Cry1Ab20 (Accession #ABB72460); Cry1Ab21 (Accession #ABS18384); Cry1Ab22 (Accession #ABW87320); Cry1Ab23 (Accession #HQ439777); Cry1Ab24 (Accession #HQ439778); Cry1Ab25 (Accession #HQ685122); Cry1Ab26 (Accession #HQ847729); Cry1Ab27 (Accession #JN135249); Cry1Ab28 (Accession #JN135250); Cry1Ab29 (Accession #JN135251); Cry1Ab30 (Accession #JN135252); Cry1Ab31 (Accession #JN135253); Cry1Ab32 (Accession #JN135254); Cry1Ab33 (Accession #AAS93798); Cry1Ab34 (Accession #KC156668); Cry1Ab-like (Accession #AAK14336); Cry1Ab-like (Accession #AAK14337); Cry1Ab-like (Accession #AAK14338); Cry1Ab-like (Accession #ABG88858); Cry1Ac1 (Accession #AAA22331); Cry1Ac2 (Accession #AAA22338); Cry1Ac3 (Accession #CAA38098); Cry1Ac4 (Accession #AAA73077); Cry1Ac5 (Accession #AAA22339); Cry1Ac6 (Accession #AAA86266); Cry1Ac7 (Accession #AAB46989); Cry1Ac8 (Accession #AAC44841); Cry1Ac9 (Accession #AAB49768); Cry1Ac10 (Accession #CAA05505); Cry1Ac11 (Accession #CAA10270); Cry1Ac12 (Accession #112418); Cry1Ac13 (Accession #AAD38701); Cry1Ac14 (Accession #AAQ06607); Cry1Ac15 (Accession #AAN07788); Cry1Ac16 (Accession #AAU87037); Cry1Ac17 (Accession #AAX18704); Cry1Ac18 (Accession #AAY88347); Cry1Ac19 (Accession #ABD37053); Cry1Ac20 (Accession #ABB89046); Cry1Ac21 (Accession #AAY66992); Cry1Ac22 (Accession #ABZ01836); Cry1Ac23 (Accession #CAQ30431); Cry1Ac24 (Accession #ABL01535); Cry1Ac25 (Accession #FJ513324); Cry1Ac26 (Accession #FJ617446); Cry1Ac27 (Accession #FJ617447); Cry1Ac28 (Accession #ACM90319); Cry1Ac29 (Accession #DQ438941); Cry1Ac30 (Accession #GQ227507); Cry1Ac31 (Accession #GU446674); Cry1Ac32 (Accession #HM061081); Cry1Ac33 (Accession #GQ866913); Cry1Ac34 (Accession #HQ230364); Cry1Ac35 (Accession #JF340157); Cry1Ac36 (Accession #JN387137); Cry1Ac37 (Accession #JQ317685); Cry1Ad1 (Accession #AAA22340); Cry1Ad2 (Accession #CAA01880); Cry1Ae1 (Accession #AAA22410); Cry1Af1 (Accession #AAB82749); Cry1Ag1 (Accession #AAD46137); Cry1Ah1 (Accession #AAQ14326); Cry1Ah2 (Accession #ABB76664); Cry1Ah3 (Accession #HQ439779); Cry1Ai1 (Accession #AA039719); Cry1Ai2 (Accession #HQ439780); Cry1A-like (Accession #AAK14339); Cry1Ba1 (Accession #CAA29898); Cry1Ba2 (Accession #CAA65003); Cry1Ba3 (Accession #AAK63251); Cry1Ba4 (Accession #AAK51084); Cry1Ba5 (Accession #AB020894); Cry1Ba6 (Accession #ABL60921); Cry1Ba7 (Accession #HQ439781); Cry1Bb1 (Accession #AAA22344); Cry1Bb2 (Accession #HQ439782); Cry1Bc1 (Accession #CAA86568); Cry1Bd1 (Accession #AAD10292); Cry1Bd2 (Accession #AAM93496); Cry1Be1 (Accession #AAC32850); Cry1Be2 (Accession #AAQ52387); Cry1Be3 (Accession #ACV96720); Cry1Be4 (Accession #HM070026); Cry1Bf1 (Accession #CAC50778); Cry1Bf2 (Accession #AAQ52380); Cry1Bg1 (Accession #AA039720); Cry1Bh1 (Accession #HQ589331); Cry1Bi1 (Accession #KC156700); Cry1Ca1 (Accession #CAA30396); Cry1Ca2 (Accession #CAA31951); Cry1Ca3 (Accession #AAA22343); Cry1Ca4 (Accession #CAA01886); Cry1Ca5 (Accession #CAA65457); Cry1Ca6 [1] (Accession #AAF37224); Cry1Ca7 (Accession #AAG50438); Cry1Ca8 (Accession #AAM00264); Cry1Ca9 (Accession #AAL79362); Cry1Ca10 (Accession #AAN16462); Cry1Ca11(Accession #AAX53094); Cry1Ca12 (Accession #HM070027); Cry1Ca13 (Accession #HQ412621); Cry1Ca14 (Accession #JN651493); Cry1Cb1 (Accession #M97880); Cry1Cb2

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#GU570697); Cry52Aa1 (Accession #EF613489); Cry52Ba1 (Accession #FJ361760); Cry53Aa1 (Accession #EF633476); Cry53Ab1 (Accession #FJ361759); Cry54Aa1 (Accession #ACA52194); Cry54Aa2 (Accession #GQ140349); Cry54Ba1 (Accession #GU446677); Cry55Aa1 (Accession #ABW88932); Cry54Ab1 (Accession #JQ916908); Cry55Aa2 (Accession #AAE33526); Cry56Aa1 (Accession #ACU57499); Cry56Aa2 (Accession #GQ483512); Cry56Aa3 (Accession #JX025567); Cry57Aa1 (Accession #ANC87261); Cry58Aa1 (Accession #ANC87260); Cry59Ba1 (Accession #JN790647); Cry59Aa1 (Accession #ACR43758); Cry60Aa1 (Accession #ACU24782); Cry60Aa2 (Accession #EA057254); Cry60Aa3 (Accession #EEM99278); Cry60Ba1 (Accession #GU810818); Cry60Ba2 (Accession #EA057253); Cry60Ba3 (Accession #EEM99279); Cry61Aa1 (Accession #HM035087); Cry61Aa2 (Accession #HM132125); Cry61Aa3 (Accession #EEM19308); Cry62Aa1 (Accession #HM054509); Cry63Aa1 (Accession #BA144028); Cry64Aa1 (Accession #BAJ05397); Cry65Aa1 (Accession #HM461868); Cry65Aa2 (Accession #ZP_04123838); Cry66Aa1 (Accession #HM485581); Cry66Aa2 (Accession #ZP_04099945); Cry67Aa1 (Accession #HM485582); Cry67Aa2 (Accession #ZP_04148882); Cry68Aa1 (Accession #HQ113114); Cry69Aa1 (Accession #HQ401006); Cry69Aa2 (Accession #JQ821388); Cry69Ab1 (Accession #JN209957); Cry70Aa1 (Accession #JN646781); Cry70Ba10 (Accession #AD051070); Cry70Bb1 (Accession #EEL67276); Cry71Aa1 (Accession #JX025568); Cry72Aa1 (Accession #JX025569); Cyt1Aa (GenBank Accession Number X03182); Cyt1Ab (GenBank Accession Number X98793); Cyt1B (GenBank Accession Number U37196); Cyt2A (GenBank Accession Number Z14147); and Cyt2B (GenBank Accession Number U52043).

(371) Examples of δ-endotoxins also include but are not limited to Cry1A proteins of U.S. Pat. Nos. 5,880,275, 7,858,849 8,530,411, 8,575,433, and 8,686,233; a DIG-3 or DIG-11 toxin (N-terminal deletion of α-helix 1 and/or α-helix 2 variants of cry proteins such as Cry1A, Cry3A) of U.S. Pat. Nos. 8,304,604, 8,304,605 and 8,476,226; Cry1B of U.S. patent application Ser. No. 10/525,318; Cry1C of U.S. Pat. No. 6,033,874; Cry1F of U.S. Pat. Nos. 5,188,960 and 6,218,188; Cry1A/F chimeras of U.S. Pat. Nos. 7,070,982; 6,962,705 and 6,713,063); a Cry2 protein such as Cry2Ab protein of U.S. Pat. No. 7,064,249); a Cry3A protein including but not limited to an engineered hybrid insecticidal protein (eHIP) created by fusing unique combinations of variable regions and conserved blocks of at least two different Cry proteins (US Patent Application Publication Number 2010/0017914); a Cry4 protein; a Cry5 protein; a Cry6 protein; Cry8 proteins of U.S. Pat. Nos. 7,329,736, 7,449,552, 7,803,943, 7,476,781, 7,105,332, 7,378,499 and 7,462,760; a Cry9 protein such as such as members of the Cry9A, Cry9B, Cry9C, Cry9D, Cry9E and Cry9F families, including but not limited to the Cry9D protein of U.S. Pat. No. 8,802,933 and the Cry9B protein of U.S. Pat. No. 8,802,934; a Cry15 protein of Naimov, et al., (2008), "Applied and Environmental Microbiology," 74:7145-7151; a Cry22, a Cry34Ab1 protein of U.S. Pat. Nos. 6,127,180, 6,624,145 and 6,340,593; a CryET33 and cryET34 protein of U.S. Pat. Nos. 6,248,535, 6,326,351, 6,399,330, 6,949,626, 7,385,107 and 7,504,229; a CryET33 and CryET34 homologs of US Patent Publication Number 2006/0191034, 2012/0278954, and PCT Publication Number WO 2012/139004; a Cry35Ab1 protein of U.S. Pat. Nos. 6,083,499, 6,548,291 and 6,340,593; a Cry46 protein, a Cry 51 protein, a Cry binary toxin; a TIC901 or related toxin; TIC807 of US Patent Application Publication Number 2008/0295207; ET29, ET37, TIC809, TIC810, TIC812, TIC127, TIC128 of PCT US 2006/033867; TIC853 toxins of U.S. Pat. No. 8,513,494, AXMI-027, AXMI-036, and AXMI-038 of U.S. Pat. No. 8,236,757; AXMI-031, AXMI-039, AXMI-040, AXMI-049 of U.S. Pat. No. 7,923,602; AXMI-018, AXMI-020 and AXMI-021 of WO 2006/083891; AXMI-010 of WO 2005/038032; AXMI-003 of WO 2005/021585; AXMI-008 of US Patent Application Publication Number 2004/0250311; AXMI-006 of US Patent Application Publication Number 2004/0216186; AXMI-007 of US Patent Application Publication Number 2004/0210965; AXMI-009 of US Patent Application Number 2004/0210964; AXMI-014 of US Patent Application Publication Number 2004/0197917; AXMI-004 of US Patent Application Publication Number 2004/0197916; AXMI-028 and AXMI-029 of WO 2006/119457; AXMI-007, AXMI-008, AXMI-

0080rf2, AXMI-009, AXMI-014 and AXMI-004 of WO 2004/074462; AXMI-150 of U.S. Pat. No. 8,084,416; AXMI-205 of US Patent Application Publication Number 2011/0023184; AXMI-011, AXMI-012, AXMI-013, AXMI-015, AXMI-019, AXMI-044, AXMI-037, AXMI-043, AXMI-033, AXMI-034, AXMI-022, AXMI-023, AXMI-041, AXMI-063 and AXMI-064 of US Patent Application Publication Number 2011/0263488; AXMI-R1 and related proteins of US Patent Application Publication Number 2010/0197592; AXMI221Z, AXMI222z, AXMI223z, AXMI224z and AXMI225z of WO 2011/103248; AXMI218, AXMI219, AXMI220, AXMI226, AXMI227, AXMI228, AXMI229, AXMI230 and AXMI231 of WO 2011/103247 and U.S. Pat. No. 8,759,619; AXMI-115, AXMI-113, AXMI-005, AXMI-163 and AXMI-184 of U.S. Pat. No. 8,334,431; AXMI-001, AXMI-002, AXMI-030, AXMI-035 and AXMI-045 of US Patent Application Publication Number 2010/0298211; AXMI-066 and AXMI-076 of US Patent Application Publication Number 2009/0144852; AXMI128, AXMI130, AXMI131, AXMI133, AXMI140, AXMI141, AXMI142, AXMI143, AXMI144, AXMI146, AXMI148, AXMI149, AXMI152, AXMI153, AXMI154, AXMI155, AXMI156, AXMI157, AXMI158, AXMI162, AXMI165, AXMI166, AXMI167, AXMI168, AXMI169, AXMI170, AXMI171, AXMI172, AXMI173, AXMI174, AXMI175, AXMI176, AXMI177, AXMI178, AXMI179, AXMI180, AXMI181, AXMI182, AXMI185, AXMI186, AXMI187, AXMI188, AXMI189 of U.S. Pat. No. 8,318,900; AXMI079, AXMI080, AXMI081, AXMI082, AXMI091, AXMI092, AXMI096, AXMI097, AXMI098, AXMI099, AXMI100, AXMI101, AXMI102, AXMI103, AXMI104, AXMI107, AXMI108, AXMI109, AXMI110, AXMI111, AXMI112, AXMI114, AXMI116, AXMI117, AXMI118, AXMI119, AXMI120, AXMI121, AXMI122, AXMI123, AXMI124, AXMI1257, AXMI1268, AXMI127, AXMI129, AXMI164, AXMI151, AXMI161, AXMI183, AXMI132, AXMI138, AXMI137 of US Patent Application Publication Number 2010/0005543, AXMI270 of US Patent Application Publication US20140223598, AXMI279 of US Patent Application Publication US20140223599, cry proteins such as Cry1A and Cry3A having modified proteolytic sites of U.S. Pat. No. 8,319,019; a Cry1Ac, Cry2Aa and Cry1Ca toxin protein from *Bacillus thuringiensis* strain VBTS 2528 of US Patent Application Publication Number 2011/0064710. Other Cry proteins are well known to one skilled in the art. See, N. Crickmore, et al., "Revision of the Nomenclature for the *Bacillus thuringiensis* Pesticidal Crystal Proteins," Microbiology and Molecular Biology Reviews," (1998) Vol 62: 807-813; see also, N. Crickmore, et al., "*Bacillus thuringiensis* toxin nomenclature" (2016), at www.btnomenclature.info/.

(372) The use of Cry proteins as transgenic plant traits is well known to one skilled in the art and Cry-transgenic plants including but not limited to plants expressing Cry1Ac, Cry1Ac+Cry2Ab, Cry1Ab, Cry1A.105, Cry1F, Cry1Fa2, Cry1F+Cry1Ac, Cry2Ab, Cry3A, mCry3A, Cry3Bb1, Cry34Ab1, Cry35Ab1, Vip3A, mCry3A, Cry9c and CBI-Bt have received regulatory approval. See, Sanahuja et al., "*Bacillus thuringiensis*: a century of research, development and commercial applications," (2011) Plant Biotech Journal, April 9(3):283-300 and the CERA (2010) GM Crop Database Center for Environmental Risk Assessment (CERA), ILSI Research Foundation, Washington D.C. at cera-gmc.org/index.php?action=gm crop database, which can be accessed on the world-wide web using the "www" prefix). More than one pesticidal proteins well known to one skilled in the art can also be expressed in plants such as Vip3Ab & Cry1Fa (US2012/0317682); Cry1BE & Cry1F (US2012/0311746); Cry1CA & Cry1AB (US2012/0311745); Cry1F & CryCa (US2012/0317681); Cry1DA& Cry1BE (US2012/0331590); Cry1DA & Cry1Fa (US2012/0331589); Cry1AB & Cry1BE (US2012/0324606); Cry1Fa & Cry2Aa and Cry11 & Cry1E (US2012/0324605); Cry34Ab/35Ab and Cry6Aa (US20130167269); Cry34Ab/VCry35Ab & Cry3Aa (US20130167268); Cry1Ab & Cry1F (US20140182018); and Cry3A and Cry1Ab or Vip3Aa (US20130116170). Pesticidal proteins also include insecticidal lipases including lipid acyl hydrolases of U.S. Pat. No. 7,491,869, and cholesterol oxidases such as from *Streptomyces* (Purcell et al. (1993) Biochem Biophys Res Commun 15:1406-1413).

(373) Pesticidal proteins also include VIP (vegetative insecticidal proteins) toxins.

Entomopathogenic bacteria produce insecticidal proteins that accumulate in inclusion bodies or parasporal crystals (such as the aforementioned Cry and Cyt proteins), as well as insecticidal proteins that are secreted into the culture medium. Among the latter are the Vip proteins, which are divided into four families according to their amino acid identity. The Vip1 and Vip2 proteins act as binary toxins and are toxic to some members of the Coleoptera and Hemiptera. The Vip1 component is thought to bind to receptors in the membrane of the insect midgut, and the Vip2 component enters the cell, where it displays its ADP-ribosyltransferase activity against actin, preventing microfilament formation. Vip3 has no sequence similarity to Vip1 or Vip2 and is toxic to a wide variety of members of the Lepidoptera. Its mode of action has been shown to resemble that of the Cry proteins in terms of proteolytic activation, binding to the midgut epithelial membrane, and pore formation, although Vip3A proteins do not share binding sites with Cry proteins. The latter property makes them good candidates to be combined with Cry proteins in transgenic plants (*Bacillus thuringiensis*-treated crops [Bt crops]) to prevent or delay insect resistance and to broaden the insecticidal spectrum. There are commercially grown varieties of Bt cotton and Bt maize that express the Vip3Aa protein in combination with Cry proteins. For the most recently reported Vip4 family, no target insects have been found yet. See, Chakroun et al., "Bacterial Vegetative Insecticidal Proteins (Vip) from Entomopathogenic Bacteria," Microbiol Mol Biol Rev. 2016 Mar. 2; 80(2):329-50. VIPs can be found in U.S. Pat. Nos. 5,877,012, 6,107,279 6,137,033, 7,244,820, 7,615,686, and 8,237,020 and the like. Other VIP proteins are well known to one skilled in the art (see, lifesci.sussex.ac.uk/home/Neil_Crickmore/Bt/vip.html, which can be accessed on the world-wide web using the "www" prefix).

(374) Pesticidal proteins also include toxin complex (TC) proteins, obtainable from organisms such as *Xenorhabdus, Photorhabdus* and *Paenibacillus* (see, U.S. Pat. Nos. 7,491,698 and 8,084,418). Some TC proteins have "stand alone" insecticidal activity and other TC proteins enhance the activity of the stand-alone toxins produced by the same given organism. The toxicity of a "stand-alone" TC protein (from *Photorhabdus, Xenorhabdus* or *Paenibacillus,* for example) can be enhanced by one or more TC protein "potentiators" derived from a source organism of a different genus. There are three main types of TC proteins. As referred to herein, Class A proteins ("Protein A") are stand-alone toxins. Class B proteins ("Protein B") and Class C proteins ("Protein C") enhance the toxicity of Class A proteins. Examples of Class A proteins are TcbA, TcdA, XptA1 and XptA2. Examples of Class B proteins are TcaC, TcdB, XptB1Xb and XptC1 Wi. Examples of Class C proteins are TccC, XptC1Xb and XptB1 Wi. Pesticidal proteins also include spider, snake and scorpion venom proteins. Examples of spider venom peptides include, but are not limited to lycotoxin-1 peptides and mutants thereof (U.S. Pat. No. 8,334,366).

(375) Some currently registered PIPs are listed in Table 11. Transgenic plants have also been engineered to express dsRNA directed against insect genes (Baum, J. A. et al. (2007) Control of coleopteran insect pests through RNA interference. Nature Biotechnology 25: 1322-1326; Mao, Y. B. et al. (2007) Silencing a cotton bollworm P450 monooxygenase gene by plant-mediated RNAi impairs larval tolerance of gossypol. Nature Biotechnology 25: 1307-1313). RNA interference can be triggered in the pest by feeding of the pest on the transgenic plant. Pest feeding thus causes injury or death to the pest.

(376) TABLE-US-00011 TABLE 11 List of exemplary Plant-incorporated Protectants, which can be combined with microbes of the disclosure Pesticide Company and Registration Plant-Incorporated Protectants (PIPs) Trade Names Numbers Potato Potato Cry3A Potato PC Code 006432 Naturemark 524-474 New Leaf Monsanto Cry3A & PLRV Potato Monsanto 524-498 PC Codes 006432, 006469 New Leaf Plus Corn Cry1Ab Corn Event 176 PC Code 006458 Mycogen Seeds/Dow 68467-1 Agro 66736-1 Syngenta Seeds Cry1Ab Corn Event Bill EPA PC Code Agrisure CB (with 67979-1 006444 OECD Unique Identifier SYN- Yieldgard) 65268-1 BTØ11-1, Attribute Insect Protected Sweet Corn Syngenta Seeds Cry1Ab Corn Event MON 801 Monsanto 524-492 Cry1Ab corn Event MON 810 PC Code Monsanto 524-489 006430 OECD Unique

Identifier MON- ØØ81Ø-6 Cry1Ac Corn PC Code 006463 Dekalb Genetics c/o 69575-2 Monsanto BT-XTRA Cry1F corn Event TC1507 PC Code Mycogen Seeds/Dow 68467-2 006481 OECD Unique Identifier DAS- Agro 29964-3 Ø15Ø7-1 Pioneer Hi- Bred/Dupont moCry1F corn Event DAS-Ø6275-8 PC Mycogen Seeds/Dow 68467-4 Code 006491 OECD Unique Identifier Agro DAS-Ø6275-8 Cry9C Corn Aventis 264-669 StarLink Cry3Bb1 corn Event MON863 PC Code Monsanto 524-528 006484 YielGard RW OECD Unique Identifier MON-ØØ863-5 Cry3Bb1 corn Event MON 88017 PC Monsanto 524-551 Code 006498 YieldGrad VT OECD Unique Identifier MON-88Ø17-3 Rootworm Cry34Ab1/Cry35Ab1 corn Event DAS- Mycogen Seeds/Dow 68467-5 591227-7 Agro 29964-4 PC Code 006490 Pioneer Hi- OECD Unique Identifier DAS-59122-7 Bred/Dupont Herculex Rootworm Cry34Ab1/Cry35Ab1 and Cry1F corn Pioneer Hi- 29964-17 Event 4114 Bred/Dupont PC Codes 006555, 006556 mCry3A corn Event MIR 604 Syngenta Seeds 67979-5 PC Code 006509 OECD Unique Identifier Agrisure RW SYN-IR604-8 Cry1A.105 and Cry2Ab2 corn Event Monsanto 524-575 MON 89034 PC Codes 006515 and Genuity VT Double 006514 Pro Vip3Aa20 corn Event MIR 162 Syngenta Seeds 67979-14 PC Code 006599 OECD Unique Identifier Agrisure Viptera SYN-IR162-4 eCry3.1Ab corn in Event 5307 PC Code Syngenta 67979-22 016483 OECD Unique Identifier SYN- Æ53Æ7-1 Stacked Events and Seed Blend Corn MON863 x MON810 with Cry3Bb1 + Monsanto YieldGard 524-545 Cry1Ab Plus DAS-59122-7 x TC1507 with Mycogen Seeds/Dow 68467-6 Cry34Ab1/Cry35Ab1 + Cry1F Agro Pioneer Hi- 29964-5 Bred/Dupont Herculex Xtra MON 88017 x MON 810 with Cry1AB + Monsanto 524-552 Cry3Bb YieldGard VT Triple YieldGard VT Plus MIR 604 x Bt11 with mCry3A + Cry1Ab Syngenta 67979-8 Agrisure CB/RW Agrisure 3000GT Mon 89034 x Mon 88017 with Cry1A.105 + Monsanto 524-576 Cry2Ab2 + Cry3Bb1 Genuity VT Triple PRO Bt11 x MIR 162 with Cry1Ab + Vip3Aa20 Syngenta Seeds 67979-12 Agrisure 2100 Bt11 x MIR 162 x MIR 604 with Cry1Ab + Syngenta Seeds 67979-13 Vip3Aa20 + mCry3A Agrisure 3100 MON 89034 x TC1507 x MON 88017 x Monsanto Company 524-581 DAS-59122-7 with Cry1A.105 + Mycogen Seeds/Dow 68467-7 Cry2Ab2 + Cry1F + Cry3Bb1 + Agro Cry34Ab1/Cry35Ab1 Genuity SmartStax SmartStax MON 89034 x TC1507 x MON 88017 x Monsanto Company 524-595 DAS-59122-7 Seed Blend Mycogen Seeds/Dow 68467-16 Agro Genuity SmartStax RIB Complete SmartStax Refuge Advanced; Refuge Advanced Powered by SmartStax Seed Blend of Herculex Xtra + Herculex I Pioneer Hi- 29964-6 Bred/Dupont Optimum AcreMax1 Insect Protection Seed Blend of Herculex RW + Non-Bt Pioneer Hi- 29964-10 corn Bred/Dupont Optimum AcreMax RW (Cry1F x Cry34/35 x Cry1Ab) - seed Pioneer Hi- 29964-11 blend Bred/Dupont Optimum AcreMax Xtra (Cry1F x Cry1Ab) - seed blend Pioneer Hi- 29964-12 Bred/Dupont Optimum AcreMax Insect Protection (Cry1F x mCry3A) Pioneer Hi- 29964-13 Bred/Dupont Optimum Trisect (Cry1F x Cry34/35 x Cry1Ab x mCry3A) Pioneer Hi- 29964-14 Bred/Dupont Optimum Intrasect Xtreme 59122 x MON 810 x MIR 604 (Cry34/35 Pioneer Hi- 29964-15 x Cry1Ab x mCry3A) Bred/Dupont Optimum AcreMax Xtreme (Cry1F x Pioneer Hi- 29964-16 Cry34/35 x Cry1Ab x mCry3A) - seed Bred/Dupont blend Optimum AcreMax Xtreme (seed blend) MON 810 x MIR 604 (Cry1Ab x Pioneer Hi- 29964-18 mCry3A) Bred/Dupont 1507 x MON810 x MIR 162 (Cry1F x Pioneer Hi- 29964-19 Cry1Ab x Vip 3Aa20) Bred/Dupont Optimum Intrasect Leptra 1507 x MIR 162 (Cry1F x Vip30Aa20) Pioneer Hi- 29964-20 Bred/Dupont 4114 x MON 810 x MIR 604 (Cry34/35 x Pioneer Hi- 29964-21 Cry1F x Cry1Ab x mCry3A) - seed blend Bred/Dupont 4114 x MON 810 x MIR 604 (Cry34/35 x Pioneer Hi- 29964-22 Cry1F x Cry1Ab x mCry3A) Bred/Dupont 1507 x MON810 x MIR 604 (Cry1F x Pioneer Hi- 29964-23 Cry1Ab x mCry3A) - seed blend Bred/Dupont Optimum AcreMax Trisect 1507 x MON810 x MIR 604 (Cry1F x Pioneer Hi- 29964-24 Cry1Ab x mCry3A) Bred/Dupont Optimum Intrasect Trisect 4114 x MON 810 (Cry34/35 x Cry1F x Pioneer Hi- 29964-25 Cry1Ab) Bred/Dupont 1507 x MON810 x MIR 162 (Cry1F x Pioneer Hi- 29964-26 Cry1Ab x Vip 3Aa20) - seed blend Bred/Dupont Optimum AcreMax Leptra SmartStax Intermediates (8 products) Monsanto 524-583, 524-584, 524-586, 524 -587, 524-588, 524-589, 524 -590 MON 89034 x 1507

(Cry1A.105 x Monsanto 524-585 Cry2Ab2 x Cry1F) Genuity Power Core MON 89034 (Cry1A.105 x Cry2Ab2) - Monsanto 524-597 seed blend Genuity VT Double PRO RIB Complete MON 89034 x 88017 RIB Complete Monsanto 524-606 (Cry1A.105 x Cry2Ab2 x Cry3Bb1) - Genuity VT Triple seed blend PRO RIB Complete MON 89034 x 1507 (Cry1A.105 x Monsanto 524-612 Cry2Ab2 x Cry1F) - seed blend Genuity PowerCore RIB Complete Bt11 x MIR162 x 1507 (Cry1Ab x Syngenta Seeds 67979-15 Vip3Aa20 x Cry1F) Agrisure Viptera 3220 Refuge Renew Bt11 x 59122-7 x MIR 604 x 1507 Syngenta Seeds 67979-17 (Cry1Ab x Cry34/35 x mCry3A x Cry1F) Agrisure 3122 Bt11 x MIR162 x TC1507 (Cry1Ab x Syngenta Seeds 67979-19 Vip3Aa20 x Cry1F) - seed blend Agisure Viptera 3220 (E-Z Refuge) (Refuge Advanced) Bt11 x DAS 59122-7 x MIR604 x Syngenta Seeds 67979-20 TC1507 (Cry1Ab x Cry34/35 x mCry3A Agisure Viptera 3122 x Cry1F) - seed blend (E-Z Refuge) (Refuge Advanced) Bt11 x MIR 162 x MIR 604 x TC1507 x Syngenta Seeds 67979-23 5307 (Cry1Ab x Vip3Aa20 x mCry3Ax Agrisure Duracade Cry1F x eCry3.1Ab) (Refuge Renew) 5222 Bt11 x MIR 604 x TC1507 x 5307 Syngenta Seeds 67979-24 (Cry1Ab x mCry3Ax Cry1F x Agrisure Duracade eCry 3.1 Ab) (Refuge Renew) 5122 Bt11 x MIR 604 x TC1507 x 5307 Syngenta Seeds 67979-25 (Cry1Ab x mCry3Ax Cry1F x Agisure Duracade eCry3.1Ab) - seed blend 5122 E-Z Refuge Bt11 x MIR 162 x MIR 604 x TC1507 x Syngenta Seeds 67979-26 5307 (Cry1Ab x Vip3Aa20 x mCry3Ax Agisure Duracade Cry1F x eCry3.1Ab) - seed blend 5222 E-Z Refuge Bt11 x MIR 162 x MIR 604 x TC1507 x Syngenta Seeds 67979-27 5307 (Cry1Ab x Vip3Aa20 x mCry3A x Agrisure Duracade Cry1F x eCry3.1Ab) (Refuge Renew) 5022 MIR604 x DAS-59122-7 x TC1507 Syngenta Seeds 67979-29 (mCry3A x Cry34/35 x Cry1F) SmartStax Intermediates (8 products) Mycogen Seeds/Dow 68467-8, 68467-9, Agro 68467-10, 68467-11, 68467-13, 68467-14, 68467-15 MON 89034 x 1507 (Cry1A.105 x Mycogen Seeds/Dow 68467-12 Cry2Ab2 x Cry1F) Agro PowerCore; PowerCore Enlist MON 89034 x 1507 (Cry1A.105 x Mycogen Seeds/Dow 68467-21 Cry2Ab2 x Cry1F) - seed blend Agro PowerCore Refuge Advanced; Refuge Advanced Powered by PowerCore 1507 x MON 810 Pioneer Hi- 29964-7 Bred/Dupont Optimum Intrasect 59122 x 1507 x MON 810 Pioneer Hi-29964-8 Bred/Dupont 59122 x MON 810 Pioneer Hi- 29964-9 Bred/Dupont Cotton Cry1Ac Cotton Monsanto 524-478 BollGard Cry1Ac and Cry2Ab2 in Event 15985 Monsanto 524-522 Cotton PC Codes 006445, 006487 BollGard II Bt cotton Event MON531 with Cry1Ac Monsanto 524-555 (breeding nursery use only) Bt cotton Event MON15947 with Monsanto 524-556 Cry2Ab2 (breeding nursery use only) COT102 x MON 15985 (Vip3Aa19 x Monsanto 524-613 Cry1Ac x Cry2Ab2) Bollgard III Cry1F and Cry1Ac (Events DAS-21023-5 Mycogen Seeds/Dow 68467-3 x DAS-24236-5) Cotton PC Codes Agro 006512, 006513 Widestrike Event 3006-210-23 (Cry1Ac) Mycogen Seeds/Dow 68467-17 Agro Event 281-24-236 (Cry1F) Mycogen Seeds/Dow 68467-18 Agro WideStrike x COT102 (Cry1F x Cry1Ac Mycogen Seeds/Dow 68467-19 x Vip3Aal9) Agro WideStrike 3 Vip3Aa19 and FLCry1Ab (Events Syngenta Seeds 67979-9 Cot102xCot67B) Cotton PC Codes (Formally VipCot) 016484, 016486 OECD Unique Identifier SYN-IR102-7 X SYN-IR67B-1 COT102 (Vip3Aal9) Syngenta Seeds 67979-18 COT67B (FLCry1Ab) Syngenta Seeds 67979-21 T304-40 (Cry1Ab) Bayer CropScience 264-1094 GHB119 (Cry2Ae) Bayer CropScience 264-1095 T304-40 GHB119 (Cry1Ab x Cry2Ae) Bayer CropScience 264-1096 OECD Unique Identifier: BCS-GHØØ4-7 TwinLink x BCS-GHØØ5-8 Soybean Cry1Ac in Event 87701 Soybean PC Monsanto 524-594 Code 006532 OECD Unique Identifier Inacta Cry1A.105 and Cry2Ab2 in Event 87751 Monsanto 524-619 Soybean PC Codes 006614, 006615 OECD Unique Identifier MON-87751-7 Cry1Ac x Cry1F in Event DAS 81419 Mycogen Seeds/Dow 68467-20 Soybean PC Codes 006527, 006528 Agro OECD Unique Identifier DAS 81419 (Cry1Ac x Cry1F)

(377) In some embodiments, any one or more of the pesticides set forth herein may be utilized with any one or more of the microbes of the disclosure and can be applied to plants or parts thereof, including seeds.

(378) Herbicides

(379) As aforementioned, agricultural compositions of the disclosure, which may comprise any microbe taught herein, are sometimes combined with one or more herbicides.
(380) Compositions comprising bacteria or bacterial populations produced according to methods described herein and/or having characteristics as described herein may further include one or more herbicides. In some embodiments, herbicidal compositions are applied to the plants and/or plant parts. In some embodiments, herbicidal compositions may be included in the compositions set forth herein, and can be applied to a plant(s) or a part(s) thereof simultaneously or in succession, with other compounds.
(381) Herbicides include 2,4-D, 2,4-DB, acetochlor, acifluorfen, alachlor, ametryn, atrazine, aminopyralid, benefin, bensulfuron, bensulide, bentazon, bicyclopyrone, 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, flucarbzone, flufenacet, flumetsulam, flumiclorac, flumioxazin, fluometuron, fluroxypyr, fomesafen, foramsulfuron, glufosinate, glyphosate, halosulfuron, hexazinone, imazamethabenz, imazamox, imazapic, imazaquin, imazethapyr, isoxaflutole, lactofen, linuron, MCPA, MCPB, mesotrione, metolachlor-s, metribuzin, indaziflam, metsulfuron, molinate, MSMA, napropamide, naptalam, nicosulfuron, norflurazon, oryzalin, oxadiazon, oxyfluorfen, paraquat, pelargonic acid, pendimethalin, phenmedipham, picloram, primisulfuron, prodiamine, prometryn, pronamide, propanil, prosulfuron, pyrazon, pyrithioac, quinclorac, quizalofop, rimsulfuron, S-metolachlor, sethoxydim, siduron, simazine, sulfentrazone, sulfometuron, sulfosulfuron, tebuthiuron, tembotrione, terbacil, thiazopyr, thifensulfuron, thiobencarb, topramezone, tralkoxydim, triallate, triasulfuron, tribenuron, triclopyr, trifluralin, and triflusulfuron.
(382) In some embodiments, any one or more of the herbicides set forth herein may be utilized with any one or more of the plants or parts thereof set forth herein.
(383) Herbicidal products may include CORVUS, BALANCE FLEXX, CAPRENO, DIFLEXX, LIBERTY, LAUDIS, AUTUMN SUPER, and DIFLEXX DUO.
(384) In some embodiments, any one or more of the herbicides set forth in the below Table 12 may be utilized with any one or more of the microbes taught herein, and can be applied to any one or more of the plants or parts thereof set forth herein.
(385) TABLE-US-00012 TABLE 12 List of exemplary herbicides, which can be combined with microbes of the disclosure Herbicide Group Site of Action Number Chemical Family Herbicide ACCase 1 Cyclohexanediones Sethoxydim (Poast, inhibitors Poast Plus) Clethodim (Select, Select Max, Arrow) Aryloxyphenoxypropionates Fluazifop (Fusilade DX, component in Fusion) Fenoxaprop (Puma, component in Fusion) Quizalofop (Assure II, Targa) Phenylpyrazolins Pinoxaden (Axial XL) ALS inhibitors 2 Imidazolinones Imazethapyr (Pursuit) Imazamox (Raptor) Sulfonylureas Chlorimuron (Classic) Halosulfuron (Permit, Sandea) Iodosulfuron (component in Autumn Super) Mesosulfuron (Osprey) Nicosulfuron (Accent Q) Primisulfuron (Beacon) Prosulfuron (Peak) Rimsulfuron (Matrix, Resolve) Thifensulfuron (Harmony) Tribenuron (Express) Triflusulfuron (UpBeet) Triazolopyrimidine Flumetsulam (Python) Cloransulam-methyl (FirstRate) Pyroxsulam (PowerFlex HL) Florasulam (component in Quelex) Sulfonylaminocarbonyltriazolinones Propoxycarbazone (Olympus) Thiencarbazone-methyl (component in Capreno) Microtubule 3 Dinitroanilines Trifluralin (many inhibitors (root names) inhibitors) Ethalfluralin (Sonalan) Pendimethalin (Prowl/Prowl H.sub.2O) Benzamide Pronamide (Kerb) Synthetic auxins 4 Arylpicolinate Halauxifen (Elevore, component in Quelex) Phenoxy acetic acids 2,4-D (Enlist One, others) 2,4-DB (Butyrac 200, Butoxone 200) MCPA Benzoic acids Dicamba (Banvel, Clarity, DiFlexx, Engenia, XtendiMax; component in Status) Pyridines Clopyralid (Stinger) Fluroxypyr (Starane Ultra) Photosystem II 5 Triazines Atrazine inhibitors Simazine (Princep, Sim- Trol) Triazinone Metribuzin (Metribuzin, others) Hexazinone (Velpar)

Phenyl-carbamates Desmedipham (Betenex) Phenmedipham (component in Betamix) Uracils Terbacil (Sinbar) 6 Benzothiadiazoles Bentazon (Basagran, others) Nitriles Bromoxynil (Buctril, Moxy, others) 7 Phenylureas Linuron (Lorox, Linex) Lipid synthesis 8 Thiocarbamates EPTC (Eptam) inhibitor EPSPS inhibitor 9 Organophosphorus Glyphosate Glutamine 10 Organophosphorus Glufosinate (Liberty, synthetase Rely) inhibitor Diterpene 13 Isoxazolidinone Clomazone (Command) biosynthesis inhibitor (bleaching) Protoporphyrinogen 14 Diphenylether Acifluorfen (Ultra oxidase Blazer) inhibitors (PPO) Fomesafen (Flexstar, Reflex) Lactofen (Cobra, Phoenix) N-phenylphthalimide Flumiclorac (Resource) Flumioxazin (Valor, Valor EZ, Rowel) Aryl triazolinone Sulfentrazone (Authority, Spartan) Carfentrazone (Aim) Fluthiacet-methyl (Cadet) Pyrazoles Pyraflufen-ethyl (Vida) Pyrimidinedione Saflufenacil (Sharpen) Long-chain fatty 15 Acetamides Acetochlor (Harness, acid inhibitors Surpass NXT, Breakfree NXT, Warrant) Dimethenamid-P (Outlook) Metolachlor (Parallel) Pyroxasulfone (Zidua, Zidua SC) s-metolachlor (Dual Magnum, Dual II Magnum, Cinch) Flufenacet (Define) Specific site 16 Benzofuranes Ethofumesate (Nortron) unknown Auxin transport 19 Semicarbazone diflufenzopyr inhibitor (component in Status) Photosystem I 22 Bipyridiliums Paraquat (Gramoxone, inhibitors Parazone) Diquat (Reglone) 4 HPPD 27 Isoxazole Isoxaflutole (Balance inhibitors Pyrazole Flexx) (bleaching) Pyrazolone Pyrasulfotole Triketone (component in Huskie) Topramezone (Armezon/Impact) Bicyclopyrone (component in Acuron) Mesotrione (Callisto) Tembotrione (Laudis)

Fungicides

(386) As aforementioned, agricultural compositions of the disclosure, which may comprise any microbe taught herein, are sometimes combined with one or more fungicides.

(387) Compositions comprising bacteria or bacterial populations produced according to methods described herein and/or having characteristics as described herein may further include one or more fungicides. In some embodiments, fungicidal compositions may be included in the compositions set forth herein, and can be applied to a plant(s) or a part(s) thereof simultaneously or in succession, with other compounds. The fungicides include azoxystrobin, captan, carboxin, ethaboxam, fludioxonil, mefenoxam, fludioxonil, thiabendazole, thiabendaz, ipconazole, mancozeb, cyazofamid, zoxamide, metalaxyl, PCNB, metaconazole, pyraclostrobin, *Bacillus subtilis* strain QST 713, sedaxane, thiamethoxam, fludioxonil, thiram, tolclofos-methyl, trifloxystrobin, *Bacillus subtilis* strain MBI 600, pyraclostrobin, fluoxastrobin, *Bacillus pumilus* strain QST 2808, chlorothalonil, copper, flutriafol, fluxapyroxad, mancozeb, gludioxonil, penthiopyrad, triazole, propiconaozole, prothioconazole, tebuconazole, fluoxastrobin, pyraclostrobin, picoxystrobin, qols, tetraconazole, trifloxystrobin, cyproconazole, flutriafol, SDHI, EBDCs, sedaxane, MAXIM QUATTRO (gludioxonil, mefenoxam, azoxystrobin, and thiabendaz), RAXIL (tebuconazole, prothioconazole, metalaxyl, and ethoxylated tallow alkyl amines), and benzovindiflupyr.

(388) In some embodiments, any one or more of the fungicides set forth herein may be utilized with any one or more of the plants or parts thereof set forth herein.

(389) Nematicides

(390) As aforementioned, agricultural compositions of the disclosure, which may comprise any microbe taught herein, are sometimes combined with one or more nematicides.

(391) Compositions comprising bacteria or bacterial populations produced according to methods described herein and/or having characteristics as described herein may further include one or more nematicide. In some embodiments, nematicidal compositions may be included in the compositions set forth herein, and can be applied to a plant(s) or a part(s) thereof simultaneously or in succession, with other compounds. The nematicides may be selected from D-D, 1,3-dichloropropene, ethylene dibromide, 1,2-dibromo-3-chloropropane, methyl bromide, chloropicrin, metam sodium, dazomet, methylisothiocyanate, sodium tetrathiocarbonate, aldicarb, aldoxycarb, carbofuran, oxamyl, ethoprop, fenamiphos, cadusafos, fosthiazate, terbufos, fensulfothion, phorate,

DiTera, clandosan, sincocin, methyl iodide, propargyl bromide, 2,5-dihydroxymethyl-3,4-dihydroxypyrrolidine (DMDP), any one or more of the avermectins, sodium azide, furfural, *Bacillus firmus*, abamectrin, thiamethoxam, fludioxonil, clothiandin, salicylic acid, and benzo-(1,2,3)-thiadiazole-7-carbothioic acid S-methyl ester.

(392) In some embodiments, any one or more of the nematicides set forth herein may be utilized with any one or more of the plants or parts thereof set forth herein.

(393) In some embodiments, any one or more of the nematicides, fungicides, herbicides, insecticides, and/or pesticides set forth herein may be utilized with any one or more of the plants or parts thereof set forth herein.

(394) Fertilizers, Nitrogen Stabilizers, and Urease Inhibitors

(395) As aforementioned, agricultural compositions of the disclosure, which may comprise any microbe taught herein, are sometimes combined with one or more of a: fertilizer, nitrogen stabilizer, or urease inhibitor.

(396) In some embodiments, fertilizers are used in combination with the methods and bacteria of the present disclosure. Fertilizers include anhydrous ammonia, urea, ammonium nitrate, and urea-ammonium nitrate (UAN) compositions, among many others. In some embodiments, pop-up fertilization and/or starter fertilization is used in combination with the methods and bacteria of the present disclosure.

(397) In some embodiments, nitrogen stabilizers are used in combination with the methods and bacteria of the present disclosure. Nitrogen stabilizers include nitrapyrin, 2-chloro-6-(trichloromethyl) pyridine, N-SERVE 24, INSTINCT, dicyandiamide (DCD).

(398) In some embodiments, urease inhibitors are used in combination with the methods and bacteria of the present disclosure. Urease inhibitors include N-(n-butyl)-thiophosphoric triamide (NBPT), AGROTAIN, AGROTAIN PLUS, and AGROTAIN PLUS SC. Further, the disclosure contemplates utilization of AGROTAIN ADVANCED 1.0, AGROTAIN DRI-MAXX, and AGROTAIN ULTRA.

(399) Further, stabilized forms of fertilizer can be used. For example, a stabilized form of fertilizer is SUPER U, containing 46% nitrogen in a stabilized, urea-based granule, SUPERU contains urease and nitrification inhibitors to guard from dentrification, leaching, and volatilization. Stabilized and targeted foliar fertilizer such as NITAMIN may also be used herein.

(400) Pop-up fertilizers are commonly used in corn fields. Pop-up fertilization comprises applying a few pounds of nutrients with the seed at planting. Pop-up fertilization is used to increase seedling vigor.

(401) Slow- or controlled-release fertilizer that may be used herein entails: A fertilizer containing a plant nutrient in a form which delays its availability for plant uptake and use after application, or which extends its availability to the plant significantly longer than a reference ‘rapidly available nutrient fertilizer’ such as ammonium nitrate or urea, ammonium phosphate or potassium chloride. Such delay of initial availability or extended time of continued availability may occur by a variety of mechanisms. These include controlled water solubility of the material by semi-permeable coatings, occlusion, protein materials, or other chemical forms, by slow hydrolysis of water-soluble low molecular weight compounds, or by other unknown means.

(402) Stabilized nitrogen fertilizer that may be used herein entails: A fertilizer to which a nitrogen stabilizer has been added. A nitrogen stabilizer is a substance added to a fertilizer which extends the time the nitrogen component of the fertilizer remains in the soil in the urea-N or ammoniacal-N form.

(403) Nitrification inhibitor that may be used herein entails: A substance that inhibits the biological oxidation of ammoniacal-N to nitrate-N. Some examples include: (1) 2-chloro-6-(trichloromethyl-pyridine), common name Nitrapyrin, manufactured by Dow Chemical; (2) 4-amino-1,2,4-6-triazole-HCl, common name ATC, manufactured by Ishihada Industries; (3) 2,4-diamino-6-trichloro-methyltriazine, common name CI-1580, manufactured by American Cyanamid; (4)

Dicyandiamide, common name DCD, manufactured by Showa Denko; (5) Thiourea, common name TU, manufactured by Nitto Ryuso; (6) 1-mercapto-1,2,4-triazole, common name MT, manufactured by Nippon; (7) 2-amino-4-chloro-6-methyl-pyramidine, common name AM, manufactured by Mitsui Toatsu; (8) 3,4-dimethylpyrazole phosphate (DMPP), from BASF; (9) 1-amide-2-thiourea (ASU), from Nitto Chemical Ind.; (10) Ammoniumthiosulphate (ATS); (11) 1H-1,2,4-triazole (HPLC); (12) 5-ethylene oxide-3-trichloro-methyl 1,2,4-thiodiazole (Terrazole), from Olin Mathieson; (13) 3-methylpyrazole (3-MP); (14) 1-carbamoyle-3-methyl-pyrazole (CMP); (15) Neem; and (16) DMPP.

(404) Urease inhibitor that may be used herein entails: A substance that inhibits hydrolytic action on urea by the enzyme urease. Thousands of chemicals have been evaluated as soil urease inhibitors (Kiss and Simihaian, 2002). However, only a few of the many compounds tested meet the necessary requirements of being non toxic, effective at low concentration, stable, and compatible with urea (solid and solutions), degradable in the soil and inexpensive. They can be classified according to their structures and their assumed interaction with the enzyme urease (Watson, 2000, 2005). Four main classes of urease inhibitors have been proposed: (a) reagents which interact with the sulphydryl groups (sulphydryl reagents), (b) hydroxamates, (c) agricultural crop protection chemicals, and (d) structural analogues of urea and related compounds. N-(n-Butyl) thiophosphoric triamide (NBPT), phenylphosphorodiamidate (PPD/PPDA), and hydroquinone are probably the most thoroughly studied urease inhibitors (Kiss and Simihaian, 2002). Research and practical testing has also been carried out with N-(2-nitrophenyl) phosphoric acid triamide (2-NPT) and ammonium thiosulphate (ATS). The organo-phosphorus compounds are structural analogues of urea and are some of the most effective inhibitors of urease activity, blocking the active site of the enzyme (Watson, 2005).

(405) Insecticidal Seed Treatments (ISTs) for Corn

(406) Corn seed treatments normally target three spectrums of pests: nematodes, fungal seedling diseases, and insects.

(407) Insecticide seed treatments are usually the main component of a seed treatment package. Most corn seed available today comes with a base package that includes a fungicide and insecticide. In some aspects, the insecticide options for seed treatments include PONCHO (clothianidin), CRUISER/CRUISER EXTREME (thiamethoxam) and GAUCHO (Imidacloprid). All three of these products are neonicotinoid chemistries. CRUISER and PONCHO at the 250 (0.25 mg AI/seed) rate are some of the most common base options available for corn. In some aspects, the insecticide options for treatments include CRUISER 250 thiamethoxam, CRUISER 250 (thiamethoxam) plus LUMIVIA (chlorantraniliprole), CRUISER 500 (thiamethoxam), and PONCHO VOTIVO 1250 (Clothianidin & *Bacillus firmus* 1-1582).

(408) Pioneer's base insecticide seed treatment package consists of CRUISER 250 with PONCHO/VOTIVO 1250 also available. VOTIVO is a biological agent that protects against nematodes.

(409) Monsanto's products including corn, soybeans, and cotton fall under the ACCELERON treatment umbrella. Dekalb corn seed comes standard with PONCHO 250. Producers also have the option to upgrade to PONCHO/VOTIVO, with PONCHO applied at the 500 rate.

(410) Agrisure, Golden Harvest and Garst have a base package with a fungicide and CRUISER 250. AVICTA complete corn is also available; this includes CRUISER 500, fungicide, and nematode protection. CRUISER EXTREME is another option available as a seed treatment package, however; the amounts of CRUISER are the same as the conventional CRUISER seed treatment, i.e. 250, 500, or 1250.

(411) Another option is to buy the minimum insecticide treatment available, and have a dealer treat the seed downstream.

(412) Commercially available ISTs for corn are listed in the below Table 13 and can be combined with one or more of the microbes taught herein.

(413) TABLE-US-00013 TABLE 13 List of exemplary seed treatments, including ISTs, which can be combined with microbes of the disclosure Treatment Type Active Ingredient(s) Product Trade Name Crop F azoxystrobin DYNASTY Corn, Soybean PROTÉGÉ FL Corn F *Bacillus pumilus* YIELD SHIELD Corn, Soybean F *Bacillus subtilis* HISTICK N/T Soybean VAULT HP Corn, Soybean F Captan CAPTAN 400 Corn, Soybean CAPTAN 400-C Corny Soybean F Fludioxonil MAXIM 4FS Corn, Soybean F Hydrogen peroxide OXIDATE Soybean STOROX Soybean F ipconazole ACCELERON DC-509 Corn RANCONA 3.8 FS Corn, Soybean VORTEX Corn F mancozeb BONIDE MANCOZEB w/Zinc Corn Concentrate DITHANE 75DF RAINSHIELD Corn DITHANE DF RAINSHIELD Corn DITHANE F45 RAINSHIELD Corn DITHANE M45 Corn LESCO 4 FLOWABLE Corn MANCOZEB PENNCOZEB 4FL Corn FLOWABLE PENNCOZEB 75DF DRY Corn FLOWABLE PENNCOZEB 80WP Corn F mefenoxam APRON XL Corn, Soybean F metalaxyl ACCELERON DC-309 Corn ACCELERON DX-309 Corn, Soybean ACQUIRE Corn, Soybean AGRI STAR METALAXYL Corn, Soybean 265 ST ALLEGIANCE DRY Corn, Soybean ALLEGIANCE FL Corn, Soybean BELMONT 2.7 FS Corn, Soybean DYNA-SHIELD Corn, Soybean METALAXYL SEBRING 2.65 ST Corn, Soybean SEBRING 318 FS Corn, Soybean SEBRING 480 FS Corn, Soybean VIREO MEC Soybean F pyraclostrobin ACCELERON DX-109 Soybean STAMINA Corn F *Streptomyces* MYCOSTOP Corn, Soybean *griseoviridis* F *Streptomyces lydicus* ACTINOGROW ST Corn, Soybean F tebuconazole AMTIDE TEBU 3.6F Corn SATIVA 309 FS Corn SATIVA 318 FS Corn TEBUSHA 3.6FL Corn TEBUZOL 3.6F Corn F thiabendazole MERTECT 340-F Soybean F thiram 42-S THIRAM Corn, Soybean FLOWSAN Corn, Soybean SIGNET 480 FS Corn, Soybean F *Trichoderma* T-22 HC Corn, Soybean *harzianum Rifai* F trifloxystrobin ACCELERON DX-709 Corn TRILEX FLOWABLE Corn, soybean I chlorpyrifos LORSBAN 50W in water Corn soluble packets I clothianidin ACCELERON IC-609 Corn NIPSIT INSIDE Corn, Soybean PONCHO 600 Corn I imidacloprid ACCELERON IX-409 Corn AGRI STAR MACHO 600 ST Corn, Soybean AGRISOLUTIONS NITRO Corn, Soybean SHIELD Corn, Soybean ATTENDANT 600 Corn, Soybean AXCESS Soybean COURAZE 2F Corn, Soybean DYNA-SHIELD IMIDACLOPRID 5 Corn, Soybean GAUCHO 480 FLOWABLE Corn, Soybean GAUCHO 600 FLOWABLE Corn, Soybean GAUCHO SB FLOWABLE Soybean NUPRID 4.6F PRO Corn, Soybean SENATOR 600 FS I thiamethoxam CRUISER 5FS Corn, Soybean N abamectin AVICTA 500 FS Corn, Soybean N *Bacillus firmus* VOTIVO FS Soybean P cytokinin SOIL X-CYTO Soybean X-CYTE Soybean P harpin alpha beta ACCELERON HX-209 Corn, Soybean protein N-HIBIT GOLD CST Corn, Soybean N-HIBIT HX-209 Corn, Soybean P indole butyric acid KICKSTAND PGR Corn, Soybean I, N thiamethoxam, AVICTA DUO CORN Corn abamectin AVICTA DUO 250 I, F clothianidin, *Bacillus* PONCHO VOTIVO Corn, Soybean *firmus* F, F carboxin, captan ENHANCE Soybean I, F permethrin, carboxin KERNEL GUARD SUPREME Corn, Soybean F, F carboxin, thiram VITAFLO 280 Corn, Soybean F, F mefenoxam, MAXIM XL Corn, Soybean fludioxonil WARDEN RTA Soybean APRON MAXX RFC APRON MAXX RTA + MOLY APRON MAXX RTA I, F imidacloprid, AGRISOLUTIONS CONCUR Corn metalaxyl F, F metalaxyl, ipconazole RANCONA SUMMIT Soybean RANCONA XXTRA F, F thiram, metalaxyl PROTECTOR-L- Soybean ALLEGIANCE F, F trifloxystrobin, TRILEX AL Soybean metalaxyl TRILEX 2000 P, P, P cytokinin, gibberellic STIMULATE YIELD Corn, Soybean acid, indole butyric ENHANCER ASCEND acid F, F, I mefenoxam, CRUISERMAXX PLUS Soybean fludioxonil, thiamethoxam F, F, F captan, carboxin, BEAN GUARD/ Soybean metalaxyl ALLEGIANCE F, F, I captan, carboxin, ENHANCE AW Soybean imidacloprid F, F, I carboxin, LATITUDE Corn, Soybean metalaxyl, imidacloprid F, F, F metalaxyl, STAMINA F3 HL Corn pyraclostrobin, triticonazole F, F, F, I azoxystrobin, CRUISER EXTREME Corn fludioxonil, mefenoxam, thiamethoxam F, F, F, F, azoxystrobin, MAXIM QUATTRO Corn F fludioxonil, mefenoxam, thiabendazole I Chlorantraniliprole LUMIVIA Corn F = Fungicide; I = Insecticide; N = Nematicide; P = Plant Growth Regulator

Application of Bacterial Populations on Crops

(414) The composition of the bacteria or bacterial population described herein can be applied in furrow, in talc, or as seed treatment. The composition can be applied to a seed package in bulk, mini bulk, in a bag, or in talc.

(415) The planter can plant the treated seed and grows the crop according to conventional ways, twin row, or ways that do not require tilling. The seeds can be distributed using a control hopper or an individual hopper. Seeds can also be distributed using pressurized air or manually. Seed placement can be performed using variable rate technologies. Additionally, application of the bacteria or bacterial population described herein may be applied using variable rate technologies. In some examples, the bacteria can be applied to seeds of corn, soybean, canola, *sorghum*, potato, rice, vegetables, cereals, pseudocereals, and oilseeds. Examples of cereals may include barley, fonio, oats, palmer's grass, rye, pearl millet, *sorghum*, spelt, teff, triticale, and wheat. Examples of pseudocereals may include breadnut, buckwheat, cattail, chia, flax, grain amaranth, hanza, quinoa, and sesame. In some examples, seeds can be genetically modified organisms (GMO), non-GMO, organic or conventional.

(416) Additives such as micro-fertilizer, PGR, herbicide, insecticide, and fungicide can be used additionally to treat the crops. Examples of additives include crop protectants such as insecticides, nematicides, fungicide, enhancement agents such as colorants, polymers, pelleting, priming, and disinfectants, and other agents such as inoculant, PGR, softener, and micronutrients. PGRs can be natural or synthetic plant hormones that affect root growth, flowering, or stem elongation. PGRs can include auxins, gibberellins, cytokinins, ethylene, and abscisic acid (ABA).

(417) The composition can be applied in furrow in combination with liquid fertilizer. In some examples, the liquid fertilizer may be held in tanks. NPK fertilizers contain macronutrients of sodium, phosphorous, and potassium.

(418) The composition may improve plant traits, such as promoting plant growth, maintaining high chlorophyll content in leaves, increasing fruit or seed numbers, and increasing fruit or seed unit weight. Methods of the present disclosure may be employed to introduce or improve one or more of a variety of desirable traits. Examples of traits that may introduced or improved include: root biomass, root length, height, shoot length, leaf number, water use efficiency, overall biomass, yield, fruit size, grain size, photosynthesis rate, tolerance to drought, heat tolerance, salt tolerance, tolerance to low nitrogen stress, nitrogen use efficiency, resistance to nematode stress, resistance to a fungal pathogen, resistance to a bacterial pathogen, resistance to a viral pathogen, level of a metabolite, modulation in level of a metabolite, proteome expression. The desirable traits, including height, overall biomass, root and/or shoot biomass, seed germination, seedling survival, photosynthetic efficiency, transpiration rate, seed/fruit number or mass, plant grain or fruit yield, leaf chlorophyll content, photosynthetic rate, root length, or any combination thereof, can be used to measure growth, and compared with the growth rate of reference agricultural plants (e.g., plants without the introduced and/or improved traits) grown under identical conditions. In some examples, the desirable traits, including height, overall biomass, root and/or shoot biomass, seed germination, seedling survival, photosynthetic efficiency, transpiration rate, seed/fruit number or mass, plant grain or fruit yield, leaf chlorophyll content, photosynthetic rate, root length, or any combination thereof, can be used to measure growth, and compared with the growth rate of reference agricultural plants (e.g., plants without the introduced and/or improved traits) grown under similar conditions.

(419) An agronomic trait to a host plant may include, but is not limited to, the following: altered oil content, altered protein content, altered seed carbohydrate composition, altered seed oil composition, and altered seed protein composition, chemical tolerance, cold tolerance, delayed senescence, disease resistance, drought tolerance, ear weight, growth improvement, health enhancement, heat tolerance, herbicide tolerance, herbivore resistance improved nitrogen fixation, improved nitrogen utilization, improved root architecture, improved water use efficiency, increased

biomass, increased root length, increased seed weight, increased shoot length, increased yield, increased yield under water-limited conditions, kernel mass, kernel moisture content, metal tolerance, number of ears, number of kernels per ear, number of pods, nutrition enhancement, pathogen resistance, pest resistance, photosynthetic capability improvement, salinity tolerance, stay-green, vigor improvement, increased dry weight of mature seeds, increased fresh weight of mature seeds, increased number of mature seeds per plant, increased chlorophyll content, increased number of pods per plant, increased length of pods per plant, reduced number of wilted leaves per plant, reduced number of severely wilted leaves per plant, and increased number of non-wilted leaves per plant, a detectable modulation in the level of a metabolite, a detectable modulation in the level of a transcript, and a detectable modulation in the proteome, compared to an isoline plant grown from a seed without said seed treatment formulation.

(420) In some cases, plants are inoculated with bacteria or bacterial populations that are isolated from the same species of plant as the plant element of the inoculated plant. For example, an bacteria or bacterial population that is normally found in one variety of *Zea mays* (corn) is associated with a plant element of a plant of another variety of *Zea mays* that in its natural state lacks said bacteria and bacterial populations. In one embodiment, the bacteria and bacterial populations is derived from a plant of a related species of plant as the plant element of the inoculated plant. For example, an bacteria and bacterial populations that is normally found in *Zea diploperennis* Iltis et al., (diploperennial teosinte) is applied to a *Zea mays* (corn), or vice versa. In some cases, plants are inoculated with bacteria and bacterial populations that are heterologous to the plant element of the inoculated plant. In one embodiment, the bacteria and bacterial populations is derived from a plant of another species. For example, bacteria and bacterial populations that are normally found in dicots are applied to a monocot plant (e.g., inoculating corn with a soybean-derived bacteria and bacterial populations), or vice versa. In other cases, the bacteria and bacterial populations to be inoculated onto a plant is derived from a related species of the plant that is being inoculated. In one embodiment, the bacteria and bacterial populations is derived from a related taxon, for example, from a related species. The plant of another species can be an agricultural plant. In another embodiment, the bacteria and bacterial populations is part of a designed composition inoculated into any host plant element.

(421) In some examples, the bacteria or bacterial population is exogenous wherein the bacteria and bacterial population is isolated from a different plant than the inoculated plant. For example, in one embodiment, the bacteria or bacterial population can be isolated from a different plant of the same species as the inoculated plant. In some cases, the bacteria or bacterial population can be isolated from a species related to the inoculated plant.

(422) In some examples, the bacteria and bacterial populations described herein are capable of moving from one tissue type to another. For example, the present disclosure's detection and isolation of bacteria and bacterial populations within the mature tissues of plants after coating on the exterior of a seed demonstrates their ability to move from seed exterior into the vegetative tissues of a maturing plant. Therefore, in one embodiment, the population of bacteria and bacterial populations is capable of moving from the seed exterior into the vegetative tissues of a plant. In one embodiment, the bacteria and bacterial populations that is coated onto the seed of a plant is capable, upon germination of the seed into a vegetative state, of localizing to a different tissue of the plant. For example, bacteria and bacterial populations can be capable of localizing to any one of the tissues in the plant, including: the root, adventitious root, seminal 5 root, root hair, shoot, leaf, flower, bud, tassel, meristem, pollen, pistil, ovaries, stamen, fruit, stolon, rhizome, nodule, tuber, trichome, guard cells, hydathode, petal, sepal, glume, rachis, vascular cambium, phloem, and xylem. In one embodiment, the bacteria and bacterial populations is capable of localizing to the root and/or the root hair of the plant. In another embodiment, the bacteria and bacterial populations is capable of localizing to the photosynthetic tissues, for example, leaves and shoots of the plant. In other cases, the bacteria and bacterial populations is localized to the vascular tissues of the plant,

for example, in the xylem and phloem. In still another embodiment, the bacteria and bacterial populations is capable of localizing to the reproductive tissues (flower, pollen, pistil, ovaries, stamen, fruit) of the plant. In another embodiment, the bacteria and bacterial populations is capable of localizing to the root, shoots, leaves and reproductive tissues of the plant. In still another embodiment, the bacteria and bacterial populations colonizes a fruit or seed tissue of the plant. In still another embodiment, the bacteria and bacterial populations is able to colonize the plant such that it is present in the surface of the plant (i.e., its presence is detectably present on the plant exterior, or the episphere of the plant). In still other embodiments, the bacteria and bacterial populations is capable of localizing to substantially all, or all, tissues of the plant. In certain embodiments, the bacteria and bacterial populations is not localized to the root of a plant. In other cases, the bacteria and bacterial populations is not localized to the photosynthetic tissues of the plant.

(423) The effectiveness of the compositions can also be assessed by measuring the relative maturity of the crop or the crop heating unit (CHU). For example, the bacterial population can be applied to corn, and corn growth can be assessed according to the relative maturity of the corn kernel or the time at which the corn kernel is at maximum weight. The crop heating unit (CHU) can also be used to predict the maturation of the corn crop. The CHU determines the amount of heat accumulation by measuring the daily maximum temperatures on crop growth.

(424) In examples, bacterial may localize to any one of the tissues in the plant, including: the root, adventitious root, seminal root, root hair, shoot, leaf, flower, bud tassel, meristem, pollen, pistil, ovaries, stamen, fruit, stolon, rhizome, nodule, tuber, trichome, guard cells, hydathode, petal, sepal, glume, rachis, vascular cambium, phloem, and xylem. In another embodiment, the bacteria or bacterial population is capable of localizing to the photosynthetic tissues, for example, leaves and shoots of the plant. In other cases, the bacteria and bacterial populations is localized to the vascular tissues of the plant, for example, in the xylem and phloem. In another embodiment, the bacteria or bacterial population is capable of localizing to reproductive tissues (flower, pollen, pistil, ovaries, stamen, or fruit) of the plant. In another embodiment, the bacteria and bacterial populations is capable of localizing to the root, shoots, leaves and reproductive tissues of the plant. In another embodiment, the bacteria or bacterial population colonizes a fruit or seed tissue of the plant. In still another embodiment, the bacteria or bacterial population is able to colonize the plant such that it is present in the surface of the plant. In another embodiment, the bacteria or bacterial population is capable of localizing to substantially all, or all, tissues of the plant. In certain embodiments, the bacteria or bacterial population is not localized to the root of a plant. In other cases, the bacteria and bacterial populations is not localized to the photosynthetic tissues of the plant.

(425) The effectiveness of the bacterial compositions applied to crops can be assessed by measuring various features of crop growth including, but not limited to, planting rate, seeding vigor, root strength, drought tolerance, plant height, dry down, and test weight.

(426) Plant Species

(427) The methods and bacteria described herein are suitable for any of a variety of plants, such as plants in the genera *Hordeum*, *Oryza*, *Zea*, and Triticeae. Other non-limiting examples of suitable plants include mosses, lichens, and algae. In some cases, the plants have economic, social and/or environmental value, such as food crops, fiber crops, oil crops, plants in the forestry or pulp and paper industries, feedstock for biofuel production and/or ornamental plants. In some examples, plants may be used to produce economically valuable products such as a grain, a flour, a starch, a syrup, a meal, an oil, a film, a packaging, a nutraceutical product, a pulp, an animal feed, a fish fodder, a bulk material for industrial chemicals, a cereal product, a processed human-food product, a sugar, an alcohol, and/or a protein. Non-limiting examples of crop plants include maize, rice, wheat, barley, *sorghum*, millet, oats, rye triticale, buckwheat, sweet corn, sugar cane, onions, tomatoes, strawberries, and asparagus. In some embodiments, the methods and bacteria described herein are suitable for any of a variety of transgenic plants, non-transgenic plants, and hybrid plants

thereof.

(428) In some examples, plants that may be obtained or improved using the methods and composition disclosed herein may include plants that are important or interesting for agriculture, horticulture, biomass for the production of biofuel molecules and other chemicals, and/or forestry. Some examples of these plants may include pineapple, banana, coconut, lily, grasspeas and grass; and dicotyledonous plants, such as, for example, peas, alfalfa, tomatillo, melon, chickpea, chicory, clover, kale, lentil, soybean, tobacco, potato, sweet potato, radish, cabbage, rape, apple trees, grape, cotton, sunflower, thale cress, canola, citrus (including orange, mandarin, kumquat, lemon, lime, grapefruit, tangerine, tangelo, citron, and pomelo), pepper, bean, lettuce, *Panicum virgatum* (switch), *Sorghum bicolor* (*sorghum*, sudan), *Miscanthus giganteus* (*miscanthus*), *Saccharum* sp. (energycane), *Populus balsamifera* (poplar), *Zea mays* (corn), *Glycine max* (soybean), *Brassica napus* (canola), *Triticum aestivum* (wheat), *Gossypium hirsutum* (cotton), *Oryza sativa* (rice), *Helianthus annuus* (sunflower), *Medicago sativa* (alfalfa), *Beta vulgaris* (sugarbeet), *Pennisetum glaucum* (pearl millet), *Panicum* spp. *Sorghum* spp., *Miscanthus* spp., *Saccharum* spp., *Erianthus* spp., *Populus* spp., *Secale cereale* (rye), Salix spp. (willow), *Eucalyptus* spp. (*eucalyptus*), *Triticosecale* spp. (*triticum*-25 wheat X rye), Bamboo, *Carthamus tinctorius* (safflower), *Jatropha curcas* (*Jatropha*), *Ricinus communis* (castor), *Elaeis guineensis* (oil palm), *Phoenix dactylifera* (date palm), *Archontophoenix cunninghamiana* (king palm), *Syagrus romanzoffiana* (queen palm), *Linum usitatissimum* (flax), *Brassica juncea, Manihot esculenta* (cassaya), *Lycopersicon esculentum* (tomato), *Lactuca saliva* (lettuce), *Musa paradisiaca* (banana), *Solanum tuberosum* (potato), *Brassica oleracea* (broccoli, cauliflower, brussel sprouts), *Camellia sinensis* (tea), Fragaria *ananassa* (strawberry), *Theobroma cacao* (cocoa), *Coffea arabica* (coffee), *Vitis vinifera* (grape), *Ananas comosus* (pineapple), *Capsicum* annum (hot & sweet pepper), *Allium cepa* (onion), *Cucumis melo* (melon), *Cucumis sativus* (cucumber), *Cucurbita maxima* (squash), *Cucurbita moschata* (squash), *Spinacea oleracea* (spinach), *Citrullus lanatus* (watermelon), *Abelmoschus esculentus* (okra), *Solanum melongena* (eggplant), *Papaver somniferum* (opium poppy), *Papaver orientale, Taxus baccata, Taxus brevifolia, Artemisia annua, Cannabis saliva, Camptotheca acuminate, Catharanthus roseus, Vinca rosea, Cinchona officinalis, Coichicum autumnale, Veratrum californica, Digitalis lanata, Digitalis purpurea, Dioscorea* 5 spp., *Andrographis paniculata, Atropa belladonna, Datura stomonium, Berberis* spp., *Cephalotaxus* spp., *Ephedra sinica, Ephedra* spp., *Erythroxylum coca, Galanthus wornorii, Scopolia* spp., *Lycopodium serratum* (*Huperzia serrata*), *Lycopodium* spp., *Rauwolfia serpentina, Rauwolfia* spp., *Sanguinaria canadensis, Hyoscyamus* spp., *Calendula officinalis, Chrysanthemum parthenium, Coleus forskohlii, Tanacetum parthenium, Parthenium argentatum* (guayule), Hevea spp. (rubber), *Mentha spicata* (mint), *Mentha piperita* (mint), *Bixa orellana, Alstroemeria* spp., Rosa spp. (rose), *Dianthus caryophyllus* (carnation), *Petunia* spp. (*petunia*), *Poinsettia pulcherrima* (poinsettia), *Nicotiana tabacum* (tobacco), *Lupinus albus* (lupin), *Uniola paniculata* (oats), *Hordeum vulgare* (barley), and *Lolium* spp. (rye).

(429) In some examples, a monocotyledonous plant may be used. Monocotyledonous plants belong to the orders of the Alismatales, Arales, Arecales, Bromeliales, Commelinales, Cyclanthales, Cyperales, Eriocaulales, Hydrocharitales, Juncales, Lilliales, Najadales, Orchidales, Pandanales, Poales, Restionales, Triuridales, Typhales, and Zingiberales. Plants belonging to the class of the Gymnospermae are Cycadales, Ginkgoales, Gnetales, and Pinales. In some examples, the monocotyledonous plant can be selected from the group consisting of a maize, rice, wheat, barley, and sugarcane.

(430) In some examples, a dicotyledonous plant may be used, including those belonging to the orders of the Aristochiales, Asterales, Batales, Campanulales, Capparales, Caryophyllales, Casuarinales, Celastrales, Cornales, Diapensales, Dilleniales, Dipsacales, Ebenales, Ericales, Eucomiales, Euphorbiales, Fabales, Fagales, Gentianales, Geraniales, Haloragales, Hamamelidales, Middles, Juglandales, Lamiales, Laurales, Lecythidales, Leitneriales, Magniolales, Malvales,

Myricales, Myrtales, Nymphaeales, Papeverales, Piperales, Plantaginales, Plumb aginales, Podostemales, Polemoniales, Polygalales, Polygonales, Primulales, Proteales, Rafflesiales, Ranunculales, Rhamnales, Rosales, Rubiales, Salicales, Santales, Sapindales, Sarraceniaceae, Scrophulariales, Theales, Trochodendrales, Umbellales, Urticales, and Violates. In some examples, the dicotyledonous plant can be selected from the group consisting of cotton, soybean, pepper, and tomato.

(431) In some cases, the plant to be improved is not readily amenable to experimental conditions. For example, a crop plant may take too long to grow enough to practically assess an improved trait serially over multiple iterations. Accordingly, a first plant from which bacteria are initially isolated, and/or the plurality of plants to which genetically manipulated bacteria are applied may be a model plant, such as a plant more amenable to evaluation under desired conditions. Non-limiting examples of model plants include *Setaria, Brachypodium*, and *Arabidopsis*. Ability of bacteria isolated according to a method of the disclosure using a model plant may then be applied to a plant of another type (e.g. a crop plant) to confirm conferral of the improved trait.

(432) Traits that may be improved by the methods disclosed herein include any observable characteristic of the plant, including, for example, growth rate, height, weight, color, taste, smell, changes in the production of one or more compounds by the plant (including for example, metabolites, proteins, drugs, carbohydrates, oils, and any other compounds). Selecting plants based on genotypic information is also envisaged (for example, including the pattern of plant gene expression in response to the bacteria, or identifying the presence of genetic markers, such as those associated with increased nitrogen fixation). Plants may also be selected based on the absence, suppression or inhibition of a certain feature or trait (such as an undesirable feature or trait) as opposed to the presence of a certain feature or trait (such as a desirable feature or trait).

(433) Non-Genetically Modified Maize

(434) The methods and bacteria described herein are suitable for any of a variety of non-genetically modified maize plants or part thereof. And in some aspects, the corn is organic. Furthermore, the methods and bacteria described herein are suitable for any of the following non-genetically modified hybrids, varieties, lineages, etc. In some embodiments, corn varieties generally fall under six categories: sweet corn, flint corn, popcorn, dent corn, pod corn, and flour corn.

(435) Sweet Corn

(436) Yellow su varieties include Earlivee, Early Sunglow, Sundance, Early Golden Bantam, Iochief, Merit, Jubilee, and Golden Cross Bantam. White su varieties include True Platinum, Country Gentleman, Silver Queen, and Stowell's Evergreen. Bicolor su varieties include Sugar & Gold, Quickie, Double Standard, Butter & Sugar, Sugar Dots, Honey & Cream. Multicolor su varieties include Hookers, Triple Play, Painted Hill, Black Mexican/Aztec.

(437) Yellow se varieties include Buttergold, Precocious, Spring Treat, Sugar Buns, Colorow, Kandy King, Bodacious R/M, Tuxedo, Incredible, Merlin, Miracle, and Kandy Korn EH. White se varieties include Spring Snow, Sugar Pearl, Whiteout, Cloud Nine, Alpine, Silver King, and Argent. Bicolor se varieties include Sugar Baby, Fleet, Bon Jour, Trinity, Bi-Licious, Temptation, Luscious, Ambrosia, Accord, Brocade, Lancelot, Precious Gem, Peaches and Cream Mid EH, and Delectable R/M. Multicolor se varieties include Ruby Queen.

(438) Yellow sh2 varieties include Extra Early Super Sweet, Takeoff, Early Xtra Sweet, Raveline, Summer Sweet Yellow, Krispy King, Garrison, Illini Gold, Challenger, Passion, Excel, Jubilee SuperSweet, Illini Xtra Sweet, and Crisp 'N Sweet. White sh2 varieties include Summer Sweet White, Tahoe, Aspen, Treasure, How Sweet It Is, and Camelot. Bicolor sh2 varieties include Summer Sweet Bicolor, Radiance, Honey 'N Pearl, Aloha, Dazzle, Hudson, and Phenomenal.

(439) Yellow sy varieties include Applause, Inferno, Honeytreat, and Honey Select. White sy varieties include Silver Duchess, *Cinderella*, Mattapoisett, Avalon, and Captivate. Bicolor sy varieties include Pay Dirt, Revelation, Renaissance, *Charisma*, Synergy, Montauk, Kristine, Serendipity/Providence, and Cameo.

(440) Yellow augmented supersweet varieties include Xtra-Tender 1 ddA, Xtra-Tender 11dd, Mirai 131Y, Mirth 130Y, Vision, and Mirai 002. White augmented supersweet varieties include Xtra-Tender 3dda, Xtra-Tender 31dd, Mirai 421W, XTH 3673, and Devotion. Bicolor augmented supersweet varieties include Xtra-Tender 2dda, Xtra-Tender 21dd, Kickoff XR, Mirth 308BC, Anthem XR, Mirai 336BC, Fantastic XR, Triumph, Mirai 301BC, Stellar, American Dream, Mirai 350BC, and Obsession.
(441) Flint Corn
(442) Flint corn varieties include Bronze-Orange, Candy Red Flint, Floriani Red Flint, Glass Gem, Indian Ornamental (Rainbow), Mandan Red Flour, Painted Mountain, Petmecky, Cherokee White Flour,
(443) PopCorn
(444) Pop corn varieties include Monarch Butterfly, Yellow Butterfly, Midnight Blue, Ruby Red, Mixed Baby Rice, Queen Mauve, Mushroom Flake, Japanese Hull-less, Strawberry, Blue Shaman, Miniature Colored, Miniature Pink, Pennsylvania Dutch Butter Flavor, and Red Strawberry.
(445) Dent Corn
(446) Dent corn varieties include Bloody Butcher, Blue Clarage, Ohio Blue Clarage, Cherokee White Eagle, Hickory Cane, Hickory King, Jellicorse Twin, Kentucky Rainbow, Daymon Morgan's Knt. Butcher, Learning, Leaming's Yellow, McCormack's Blue Giant, Neal Paymaster, Pungo Creek Butcher, Reid's Yellow Dent, Rotten Clarage, and Tennessee Red Cob.
(447) In some embodiments, corn varieties include P1618W, P1306W, P1345, P1151, P1197, P0574, P0589, and P0157. W=white corn.
(448) In some embodiments, the methods and bacteria described herein are suitable for any hybrid of the maize varieties setforth herein.
(449) Genetically Modified Maize
(450) The methods and bacteria described herein are suitable for any of a hybrid, variety, lineage, etc. of genetically modified maize plants or part thereof.
(451) Furthermore, the methods and bacteria described herein are suitable for any of the following genetically modified maize events, which have been approved in one or more countries: 32138 (32138 SPT Maintainer), 3272 (ENOGEN), 3272 x Bt11, 3272 x bt11 x GA21, 3272 x Bt11 x MIR604, 3272 x Bt11 x MIR604 x GA21, 3272 x Bt11 x MIR604 x TC1507×5307 x GA21, 3272 x GA21, 3272 x MIR604, 3272 x MIR604 x GA21, 4114, 5307 (AGRISURE Duracade), 5307 x GA21, 5307 x MIR604 x Bt11 x TC1507 x GA21 (AGRISURE Duracade 5122), 5307 x MIR604 x Bt11 x TC1507 x GA21 x MIR162 (AGRISURE Duracade 5222), 59122 (HERCULEX RW), 59122 x DAS40278, 59122 x GA21, 59122 x MIR604, 59122 x MIR604 x GA21, 59122 x MIR604 x TC1507, 59122 x MIR604 x TC1507 x GA21, 59122 x MON810, 59122 x MON810 x MIR604, 59122 x MON810 x NK603, 59122 x MON810 x NK603 x MIR604, 59122 x MON88017, 59122 x MON88017 x DAS40278, 59122 x NK603 (Herculex RW ROUNDUP READY 2), 59122 x NK603 x MIR604, 59122 x TC1507 x GA21, 676, 678, 680, 3751 IR, 98140, 98140×59122, 98140 x TC1507, 98140 x TC1507×59122, Bt10 (Bt10), Bt11 [X4334CBR, X4734CBR] (AGRISURE CB/LL), Bt11×5307, Bt11×5307 x GA21, Bt11×59122 x MIR604, Br11×59122 x MIR604 x GA21, Bt11×59122 x MIR604 x TC1507, M53, M56, DAS-59122-7, Bt11×59122 x MIR604 x TC1507 x GA21, Bt11×59122 x TC1507, TC1507 x DAS-59122-7, Bt11×59122 x TC1507 x GA21, Bt11 x GA21 (AGRISURE GT/CB/LL), Bt11 x MIR162 (AGRISURE Viptera 2100), BT11 x MIR162×5307, Bt11 x MIR162×5307 x GA21, Bt11 x MIR162 x GA21 (AGRISURE Viptera 3110), Bt11 x MIR162 x MIR604 (AGRISURE Viptera 3100), Bt11 x MIR162 x MIR604×5307, Bt11 x MIR162 x MIR604×5307 x GA21, Bt11 x MIR162 x MIR604 x GA21 (AGRISURE Viptera 3111/AGRISURE Viptera 4), Bt11, MIR162 x MIR604 x MON89034×5307 x GA21, Bt11 x MIR162 x MIR604 x TC1507, Bt11 x MIR162 x MIR604 x TC1507×5307, Bt11 x MIR162 x MIR604 x TC1507 x GA21, Bt11 x MIR162 x MON89034, Bt11 x MIR162 x MON89034 x GA21, Bt11 x MIR162 x TC1507, Bt11 x MIR162 x

TC1507×5307, Bt11 x MIR162 x TC1507×5307 x GA21, Bt11 x MR162 x TC1507 x GA21 (AGRISURE Viptera 3220), BT11 x MIR604 (Agrisure BC/LL/RW), Bt11 x MIR604×5307, Bt11 x MIR604×5307 x GA21, Bt11 x MIR604 x GA21, Bt11 x MIR604 x TC1507, Bt11 x MIR604 x TC1507×5307, Bt11 x MIR604 x TC1507 x GA21, Bt11 x MON89034 x GA21, Bt11 x TC1507, Bt11 x TC1507×5307, Bt11 x TC1507 x GA21, Bt176 (NaturGard KnockOut/Maximizer), BVLA430101, CBH-351 (STARLINK Maize), DAS40278 (ENLIST Maize), DAS40278 x NK603, DBT418 (Bt Xtra Maize), DLL25 [B16], GA21 (ROUNDUP READY Maize/AGRISURE GT), GA21 x MON810 (ROUNDUP READY Yieldgard Maize), GA21 x T25, HCEM485, LY038 (MAVERA Maize), LY038 x MON810 (MAVERA Yieldgard Maize), MIR162 (AGRISURE Viptera), MIR162×5307, MIR162×5307 x GA21, MIR162 x GA21, MIR162 x MIR604, MIR162 x MIR604×5307, MIR162 x MIR604×5307 x GA21, MIR162 x MIR604 x GA21, MIR162 x MIR604 x TC1507 x 5307, MIR162 x MIR604 x TC1507×5307 x GA21, MIR162 x MIR604 x TC1507 x GA21, MIR162 x MON89034, MIR162 x NK603, MIR162 x TC1507, MIR162 x TC1507×5307, MIR162 x TC1507×5307 x GA21, MIR162 x TC1507 x GA21, MIR604 (AGRISURE RW), MIR604×5307, MIR604×5307 x GA21, MIR604 x GA21 (AGRISURE GT/RW), MIR604 x NK603, MIR604 x TC1507, MIR604 x TC1507×5307, MIR604 x TC1507×5307 xGA21, MIR604 x TC1507 x GA21, MON801 [MON80100], MON802, MON809, MON810 (YIELDGARD, MAIZEGARD), MON810 x MIR162, MON810 x MIR162 x NK603, MON810 x MIR604, MON810 x MON88017 (YIELDGARD VT Triple), MON810 x NK603 x MIR604, MON832 (ROUNDUP READY Maize), MON863 (YIELDGARD Rootworm RW, MAXGARD), MON863 x MON810 (YIELDGARD Plus), MON863 x MON810 x NK603 (YIELDGARD Plus with RR), MON863 x NK603 (YIELDGARD RW+RR), MON87403, MON87411, MON87419, MON87427 (ROUNDUP READY Maize), MON87427×59122, MON87427 x MON88017, MON87427 x MON88017×59122, MON87427 x MON89034, MON87427 x MON89034×59122, MON87427 x MON89034 x MIR162 x MON87411, MON87427 x MON89034 x MON88017, MON87427 x MON89034 x MON88017×59122, MON87427 x MON89034 x NK603, MON87427 x MON89034 x TC1507, MON87427 x MON89034 x TC1507×59122, MON87427 x MON89034 x TC1507 x MON87411×59122, MON87427 x MON89034 x TC1507 x MON87411×59122 x DAS40278, MON87427 x MON89034 x TC1507 x MON88017, MON87427 x MON89034 x MIR162 x NK603, MON87427 x MON89034 x TC1507 x MON88017×59122, MON87427 x TC1507, MON87427 x TC1507×59122, MON87427 x TC1507 x MON88017, MON87427 x TC1507 x MON88017×59122, MON87460 (GENUITY DROUGHTGARD), MON87460 x MON88017, MON87460 x MON89034 x MON88017, MON87460 x MON89034 x NK603, MON87460 x NK603, MON88017, MON88017 x DAS40278, MON89034, MON89034 x 59122, MON89034×59122 x DAS40278, MON89034×59122 x MON88017, MON89034 x 59122 x MON88017 x DAS40278, MON89034 x DAS40278, MON89034 x MON87460, MON89034 x MON88017 (GENUITY VT Triple Pro), MON89034 x MON88017 x DAS40278, MON89034 x NK603 (GENUITY VT Double Pro), MON89034 x NK603 x DAS40278, MON89034 x TC1507, MON89034 x TC1507×59122, MON89034 x TC1507 x 59122 x DAS40278, MON89034 x TC1507 x DAS40278, MON89034 x TC1507 x MON88017, MON89034 x TC1507 x MON88017×59122 (GENUITY SMARTSTAX), MON89034 x TC1507 x MON88017×59122 x DAS40278, MON89034 x TC1507 x MON88017 x DAS40278, MON89034 x TC1507 x NK603 (POWER CORE), MON89034 x TC1507 x NK603 x DAS40278, MON89034 x TC1507 x NK603 x MIR162, MON89034 x TC1507 x NK603 x MIR162 x DAS40278, MON89034 x GA21, MS3 (INVIGOR Maize), MS6 (INVIGOR Maize), MZHGOJG, MZIR098, NK603 (ROUNDUP READY 2 Maize), NK603 x MON810×4114 x MIR604, NK603 x MON810 (YIELDGARD CB+RR), NK603 x T25 (ROUNDUP READY LIBERTY LINK Maize), T14 (LIBERTY LINK Maize), T25 (LIBERTY LINK Maize), T25 x MON810 (LIBERTY LINK YIELDGARD Maize), TC1507 (HERCULEX I, HERCULEX CB), TC1507×59122 x MON810 x MIR604 x NK603

(OPTIMUM INTRASECT XTREME), TC1507 x MON810 x MIR604 x NK603, TC1507 x 5307, TC1507×5307 x GA21, TC1507×59122 (HERCULEX XTRA), TC1507×59122 x DAS40278, TC1507×59122 x MON810, TC1507×59122 x MON810 x MIR604, TC1507 x 59122 x MON810 x NK603 (OPTIMUM INTRASECT XTRA), TC1507×59122 x MON88017, TC1507×59122 x MON88017 x DAS40278, TC1507×59122 x NK603 (HERCULEX XTRA RR), TC1507×59122 x NK603 x MIR604, TC1507 x DAS40278, TC1507 x GA21, TC1507 x MIR162 x NK603, TC1507 x MIR604 x NK603 (OPTIMUM TRISECT), TC1507 x MON810, TC1507 x MON810 x MIR162, TC1507 x MON810 x MIR162 x NK603, TC1507 x MON810 x MIR604, TC1507 x MON810 x NK603 (OPTIMUM INTRASECT), TC1507 x MON810 x NK603 x MIR604, TC1507 x MON88017, TC1507 x MON88017 x DAS40278, TC1507 x NK603 (HERCULEX I RR), TC1507 x NK603 x DAS40278, TC6275, and VCO-01981-5.

(452) Additional Genetically Modified Plants

(453) The methods and bacteria described herein are suitable for any of a variety of genetically modified plants or part thereof.

(454) Furthermore, the methods and bacteria described herein are suitable for any of the following genetically modified plant events which have been approved in one or more countries.

(455) TABLE-US-00014 TABLE 14 Rice Traits, which can be combined with microbes of the disclosure *Oryza sativa* Rice Event Company Description CL121, CL141, BASF Inc. Tolerance to the imidazolinone CFX51 herbicide, imazethapyr, induced by chemical mutagenesis of the acetolactate synthase (ALS) enzyme using ethyl methanesulfonate (EMS). IMINTA-1, IMINTA-4 BASF Inc. Tolerance to imidazolinone herbicides induced by chemical mutagenesis of the acetolactate synthase (ALS) enzyme using sodium azide. LLRICE06, Aventis CropScience Glufosinate ammonium herbicide LLRICE62 tolerant rice produced by inserting a modified phosphinothricin acetyltransferase (PAT) encoding gene from the soil bacterium *Streptomyces hygroscopicus*). LLRICE601 Bayer CropScience (Aventis Glufosinate ammonium herbicide CropScience(AgrEvo)) tolerant rice produced by inserting a modified phosphinothricin acetyltransferase (PAT) encoding gene from the soil bacterium *Streptomyces hygroscopicus*). PWC16 BASF Inc. Tolerance to the imidazolinone herbicide, imazethapyr, induced by chemical mutagenesis of the acetolactate synthase (ALS) enzyme using ethyl methanesulfonate (EMS).

(456) TABLE-US-00015 TABLE 15 Alfalfa Traits, which can be combined with microbes of the disclosure *Medicago sativa* Alfalfa Event Company Description J101, J163 Monsanto Company and Glyphosate herbicide tolerant Forage Genetics alfalfa (lucerne) produced by International inserting a gene encoding the enzyme 5-enolypyruvylshikimate- 3-phosphate synthase (EPSPS) from the CP4 strain of *Agrobacterium tumefaciens*.

(457) TABLE-US-00016 TABLE 16 Wheat Traits, which can be combined with microbes of the disclosure *Triticum aestivum* Wheat Event Company Description AP205CL BASF Inc. Selection for a mutagenized version of the enzyme acetohydroxyacid synthase (AHAS), also known as acetolactate synthase (ALS) or acetolactate pyruvate-lyase. AP602CL BASF Inc. Selection for a mutagenized version of the enzyme acetohydroxyacid synthase (AHAS), also known as acetolactate synthase (ALS) or acetolactate pyruvate-lyase. BW255-2, BW238-3 BASF Inc. Selection for a mutagenized version of the enzyme acetohydroxyacid synthase (AHAS), also known as acetolactate synthase (ALS) or acetolactate pyruvate-lyase. BW7 BASF Inc. Tolerance to imidazolinone herbicides induced by chemical mutagenesis of the acetohydroxyacid synthase (AHAS) gene using sodium azide. MON71800 Monsanto Company Glyphosate tolerant wheat variety produced by inserting a modified 5-enolpyruvylshikimate-3- phosphate synthase (EPSPS) encoding gene from the soil bacterium *Agrobacterium tumefaciens*, strain CP4. SWP965001 Cyanamid Crop Selection for a mutagenized Protection version of the enzyme acetohydroxyacid synthase (AHAS), also known as acetolactate synthase (ALS) or acetolactate pyruvate-lyase. Teal 11A BASF Inc. Selection for a mutagenized version of the enzyme acetohydroxyacid synthase (AHAS), also known as acetolactate synthase (ALS) or acetolactate pyruvate-lyase.

(458) TABLE-US-00017 TABLE 17 Sunflower Traits, which can be combined with microbes of the disclosure *Helianthus annuus* Sunflower Event Company Description X81359 BASF Inc. Tolerance to imidazolinone herbicides by selection of a naturally occurring mutant.
(459) TABLE-US-00018 TABLE 18 Soybean Traits, which can be combined with microbes of the disclosure *Glycine max* L. Soybean Event Company Description A2704-12, A2704-21, Bayer CropScience Glufosinate ammonium herbicide A5547-35 (Aventis CropScience tolerant soybean produced by (AgrEvo)) inserting a modified phosphinothricin acetyltransferase (PAT) encoding gene from the soil bacterium *Streptomyces viridochromogenes*. A5547-127 Bayer CropScience Glufosinate ammonium herbicide (Aventis CropScience tolerant soybean produced by (AgrEvo)) inserting a modified phosphinothricin acetyltransferase (PAT) encoding gene from the soil bacterium *Streptomyces viridochromogenes*. BPS-CV127-9 BASF Inc. The introduced csrl-2 gene from *Arabidopsis thaliana* encodes an acetohydroxyacid synthase protein that confers tolerance to imidazolinone herbicides due to a point mutation that results in a single amino acid substitution in which the serine residue at position 653 is replaced by asparagine (S653N). DP-305423 Pioneer Hi-Bred High oleic acid soybean produced International Inc. by inserting additional copies of a portion of the omega 6 desaturase encoding gene, gm-fad2-1 resulting in silencing of the endogenous omega-6 desaturase gene (FAD2-1). DP356043 Pioneer Hi-Bred Soybean event with two herbicide International Inc. tolerance genes: glyphosate N- acetlytransferase, which detoxifies glyphosate, and a modified acetolactate synthase (ALS) gene which is tolerant to ALS-inhibiting herbicides. G94-1, G94-19, G168 DuPont Canada High oleic acid soybean produced Agricultural Products by inserting a second copy of the fatty acid desaturase (Gm Fad2-1) encoding gene from soybean, which resulted in “silencing” of the endogenous host gene. GTS 40-3-2 Monsanto Company Glyphosate tolerant soybean variety produced by inserting a modified 5-enolpyruvylshikimate- 3-phosphate synthase (EPSPS) encoding gene from the soil bacterium *Agrobacterium tumefaciens*. GU262 Bayer CropScience Glufosinate ammonium herbicide (Aventis tolerant soybean produced by CropScience(AgrEvo)) inserting a modified phosphinothricin acetyltransferase (PAT) encoding gene from the soil bacterium *Streptomyces viridochromogenes*. MON87701 Monsanto Company Resistance to Lepidopteran pests of soybean including velvetbean caterpillar (*Anticarsia gemmatalis*) and soybean looper (*Pseudoplusia includens*). MON87701 x Monsanto Company Glyphosate herbicide tolerance MON89788 through expression of the EPSPS encoding gene from *A. tumefaciens* strain CP4, and resistance to Lepidopteran pests of soybean including velvetbean caterpillar (*Anticarsia gemmaialis*) and soybean looper (*Pseudoplusia includens*) via expression of the Cry1Ac encoding gene from *B. thuringiensis*. MON89788 Monsanto Company Glyphosate-tolerant soybean produced by inserting a modified 5-enolpyruvylshikimate-3- phosphate synthase (EPSPS) encoding aroA (epsps) gene from *Agrobacterium tumefaciens* CP4. OT96-15 Agriculture & Agri-Food Low linolenic acid soybean Canada produced through traditional cross- breeding to incorporate the novel trait from a naturally occurring fan1 gene mutant that was selected for low linolenic acid. W62, W98 Bayer CropScience Glufosinate ammonium herbicide (Aventis tolerant soybean produced by CropScience(AgrEvo)) inserting a modified phosphinothricin acetyltransferase (PAT) encoding gene from the soil bacterium *Streptomyces hygroscopicus*.
(460) TABLE-US-00019 TABLE 19 Corn Traits, which can be combined with microbes of the disclosure *Zea mays* L. Maize Event Company Description 176 Syngenta Seeds, Inc. Insect-resistant maize produced by inserting the Cry1Ab gene from *Bacillus thuringiensis* subsp. *kurstaki*. The genetic modification affords resistance to attack by the European corn borer (ECB). 3751 IR Pioneer Hi-Bred Selection of somaclonal variants 676, 678, 680 International Inc. by culture of embryos on Pioneer Hi-Bred imidazolinone containing media. International Inc. Male-sterile and glufosinate ammonium herbicide tolerant maize produced by inserting genes encoding DNA adenine methylase and phosphinothricin acetyltransferase (PAT) from *Escherichia coli* and *Streptomyces viridochromogenes*, respectively. B16 (DLL25) Dekalb Genetics Glufosinate ammonium herbicide Corporation tolerant maize produced by inserting the gene encoding

phosphinothricin acetyltransferase (PAT) from *Streptomyces hygroscopicus*. BT11 (X4334CBR, Syngenta Seeds, Inc. Insect-resistant and herbicide X4734CBR) tolerant maize produced by inserting the Cry1Ab gene from *Bacillus thuringiensis* subsp. *kurstaki*, and the phosphinothricin N-acetyltransferase (PAT) encoding gene from *S. viridochromogenes*. BT11 x GA21 Syngenta Seeds, Inc. Stacked insect resistant and herbicide tolerant maize produced by conventional cross breeding of parental lines BT11 (OECD unique identifier: SYN-BTO11-1) and GA21 (OECD unique identifier: MON-OOO21-9). BT11 x MIR162 x Syngenta Seeds, Inc. Resistance to Coleopteran pests, MIR604 x GA21 particularly corn rootworm pests (*Diabrotica* spp.) and several Lepidopteran pests of corn, including European corn borer (ECB, *Ostrinia nubilalis*), corn earworm (CEW, *Helicoverpa zea*), fall army worm (FAW, *Spodoptera frugiperda*), and black cutworm (BCW, *Agrotis ipsilon*); tolerance to glyphosate and glufosinate- ammonium containing herbicides. BT11 XMIR162 Syngenta Seeds, Inc. Stacked insect resistant and herbicide tolerant maize produced by conventional cross breeding of parental lines BT11 (OECD unique identifier: SYN-BTO11-1) and MIR162 (OECD unique identifier: SYN-1R162-4). Resistance to the European Corn Borer and tolerance to the herbicide glufosinate ammonium (Liberty) is derived from BT11, which contains the Cry1Ab gene from *Bacillus thuringiensis* subsp. *kurstaki*, and the phosphinothricin N-acetyltransferase (PAT) encoding gene from *S. viridochromogenes*. Resistance to other Lepidopteran pests, including *H. zea, S. frugiperda, A. ipsilon*, and *S. albicosta*, is derived from MIR162, which contains the vip3Aa gene from *Bacillus thuringiensis* strain AB88. BT11 x MIR162 x Syngenta Seeds, Inc. *Bacillus thuringiensis* Cry1Ab MIR604 delta-endotoxin protein and the genetic material necessary for its production (via elements of vector pZO1502) in Event Bill corn (OECD Unique Identifier: SYNBTO11-1) x *Bacillus thuringiensis* Vip3Aa20 insecticidal protein and the genetic material necessary for its production (via elements of vector pNOV1300) in Event MIR162 maize (OECD Unique Identifier: SYN-IR162-4) x modified Cry3A protein and the genetic material necessary for its production (via elements of vector pZM26) in Event MIR604 corn (OECD Unique Identifier: SYN-1R604-5). CBH-351 Aventis CropScience Insect-resistant and glufosinate ammonium herbicide tolerant maize developed by inserting genes encoding Cry9C protein from *Bacillus thuringiensis* subsp *tolworthi* and phosphinothricin acetyltransferase (PAT) from *Streptomyces hygroscopicus*. DAS-06275-8 DOW AgroSciences LLC Lepidopteran insect resistant and glufosinate ammonium herbicide- tolerant maize variety produced by inserting the Cry1F gene from *Bacillus thuringiensis* var *aizawai* and the phosphinothricin acetyltransferase (PAT) from *Streptomyces hygroscopicus*. BT11 x MIR604 Syngenta Seeds, Inc. Stacked insect resistant and herbicide tolerant maize produced by conventional cross breeding of parental lines BT11 (OECD unique identifier: SYN-BTO11-1) and MIR604 (OECD unique identifier: SYN-1R6O5-5). Resistance to the European Corn Borer and tolerance to the herbicide glufosinate ammonium (Liberty) is derived from BT11, which contains the Cry1Ab gene from *Bacillus thuringiensis* subsp. *kurstaki*, and the phosphinothricin N-acetyltransferase (PAT) encoding gene from *S. viridochromogenes*. Corn rootworm-resistance is derived from MIR604 which contains the mCry3A gene from *Bacillus thuringiensis*. BT11 x MIR604 x Syngenta Seeds, Inc. Stacked insect resistant and GA2I herbicide tolerant maize produced by conventional cross breeding of parental lines BT11 (OECD unique identifier: SYN-BTO11-1), MIR604 (OECD unique identifier: SYN-1R6O5-5) and GA21 (OECD unique identifier: MON- OOO21-9). Resistance to the European Corn Borer and tolerance to the herbicide glufosinate ammonium (Liberty) is derived from BT11, which contains the Cry1Ab gene from *Bacillus thuringiensis* subsp. *kurstaki*, and the phosphinothricin N-acetyltransferase (PAT) encoding gene from *S. viridochromogenes*. Corn rootworm-resistance is derived from MIR604 which contains the mCry3A gene from *Bacillus thuringiensis*. Tolerance to glyphosate herbicide is derived from GA21 which contains a a modified EPSPS gene from maize. DAS-59122-7 DOW AgroSciences LLC Corn rootworm-resistant maize and Pioneer Hi-Bred produced by inserting the International Inc. Cry34Ab1 and Cry35Ab1 genes from *Bacillus thuringiensis* strain PS149B1. The PAT encoding gene from *Streptomyces*

viridochromogenes was introduced as a selectable marker. DAS-59122-7 x TC1507 DOW AgroSciences LLC Stacked insect resistant and x NK603 and Pioneer Hi-Bred herbicide tolerant maize produced International Inc. by conventional cross breeding of parental lines DAS-59122-7 (OECD unique identifier: DAS- 59122-7) and TC1507 (OECD unique identifier: DAS-01507-1) with NK603 (OECD unique identifier: MON-00603-6). Corn rootworm-resistance is derived from DAS-59122- 7 which contains the Cry34Ab1 and Cry35Ab1 genes from *Bacillus thuringiensis* strain P5149B1. Lepidopteran resistance and tolerance to glufosinate ammonium herbicide is derived from TC1507. Tolerance to glyphosate herbicide is derived from NK603. DBT418 Dekalb Genetics Insect-resistant and glufosinate Corporation ammonium herbicide tolerant maize developed by inserting genes encoding Cry1AC protein from *Bacillus thuringiensis* subsp *kurstaki* and phosphinothricin acetyltransferase (PAT) from *Streptomyces hygroscopicus*. MIR604xGA21 Syngenta Seeds, Inc. Stacked insect resistant and herbicide tolerant maize produced by conventional cross breeding of parental lines MIR604 (OECD unique identifier: SYN-1R605-5) and GA21 (OECD unique identifier: MON-00021-9). Corn rootworm-resistance is derived from MIR604 which contains the mCry3 A gene from *Bacillus thuringiensis*. Tolerance to glyphosate herbicide is derived from GA21. MON80100 Monsanto Company Insect-resistant maize produced by inserting the Cry1Ab gene from *Bacillus thuringiensis* subsp. *kurstaki*. The genetic modification affords resistance to attack by the European corn borer (ECB). MON802 Monsanto Company Insect-resistant and glyphosate herbicide tolerant maize produced by inserting the genes encoding the Cry1Ab protein from *Bacillus thuringiensis* and the 5- enolpyruvylshikimate-3-phosphate synthase (EPSPS) from *A. tumefaciens* strain CP4. MON809 Pioneer Hi-Bred Resistance to European corn borer International Inc. (*Ostrinia nubilalis*) by introduction of a synthetic Cry1Ab gene. Glyphosate resistance via introduction of the bacterial version of a plant enzyme, 5-enolpynivyl shikimate-3- phosphate synthase (EPSPS). MON810 Monsanto Company Insect-resistant maize produced by inserting a truncated form of the Cry1Ab gene from *Bacillus thuringiensis* subsp. *kurstaki* HD- 1. The genetic modification affords resistance to attack by the European corn borer (ECB). MON810 x LY038 Monsanto Company Stacked insect resistant and enhanced lysine content maize derived from conventional crossbreeding of the parental lines MON810 (OECD identifier: MON-OO81O-6) and LY038 (OECD identifier: REN-OOO38- 3). MON810 x MON88017 Monsanto Company Stacked insect resistant and glyphosate tolerant maize derived from conventional cross-breeding of the parental lines MON810 (OECD identifier: M0N-OO81O- 6) and MON88017 (OECD identifier: MON-88017-3). European corn borer (ECB) resistance is derived from a truncated form of the Cry1Ab gene from *Bacillus thuringiensis* subsp. *kurstaki* HD-1 present in MON810. Corn rootworm resistance is derived from the Cry3Bbl gene from *Bacillus thuringiensis* subspecies *kumamotoensis* strain EG4691 present in MON88017. Glyphosate tolerance is derived from a 5- enolpyruvylshikimate-3-phosphate synthase (EPSPS) encoding gene from *Agrobacterium tumefaciens* strain CP4 present in MON88017. MON832 Monsanto Company Introduction, by particle bombardment, of glyphosate oxidase (GOX) and a modified 5- enolpyruvyl shikimate-3 -phosphate synthase (EPSPS), an enzyme involved in the shikimate biochemical pathway for the production of the aromatic amino acids. MON863 Monsanto Company Corn rootworm resistant maize produced by inserting the Cry3Bbl gene from *Bacillus thuringiensis* subsp. *kumamotoensis*. MON863 x MON810 Monsanto Company Stacked insect resistant corn hybrid derived from conventional cross-breeding of the parental lines MON863 (OECD identifier: MON-00863-5) and MON810 (OECD identifier: MON-00810-6) MON863 x MON810 x Monsanto Company Stacked insect resistant and Monsanto NK603 herbicide tolerant corn hybrid derived from conventional crossbreeding of the stacked hybrid MON-00863-5 x MON- 00810-6 andNK603 (OECD identifier: MON-00603-6). MON863 x NK603 Monsanto Company Stacked insect resistant and herbicide tolerant corn hybrid derived from conventional crossbreeding of the parental lines MON863 (OECD identifier: MON-OO863-5) and NK603 (OECD identifier: MON-OO6O3- 6). MON87460 Monsanto Company MON 87460

was developed to provide reduced yield loss under water-limited conditions compared to conventional maize. Efficacy in MON 87460 is derived by expression of the inserted *Bacillus subtilis* cold shock protein B (CspB). MON88017 Monsanto Company Corn rootworm-resistant maize produced by inserting the Cry3Bbl gene from *Bacillus thuringiensis* subspecies *kumamotoensis* strain EG4691. Glyphosate tolerance derived by inserting a 5-enolpyruvylshikimate-3-phosphate synthase (EPSPS) encoding gene from *Agrobacterium tumefaciens* strain CP4. MON89034 Monsanto Company Maize event expressing two different insecticidal proteins from *Bacillus thuringiensis* providing resistance to number of Lepidopteran pests. MON89034 x Monsanto Company Stacked insect resistant and MON88017 glyphosate tolerant maize derived from conventional cross-breeding of the parental lines MON89034 (OECD identifier: MON-89O34-3) and MON88017 (OECD identifier: MON-88O17-3). Resistance to Lepidopteran insects is derived from two Cry genes present in MON89043. Corn rootworm resistance is derived from a single Cry genes and glyphosate tolerance is derived from the 5-enolpyruvylshikimate-3- phosphate synthase (EPSPS) encoding gene from *Agrobacterium tumefaciens* present in MON88017. MON89034 x NK603 Monsanto Company Stacked insect resistant and herbicide tolerant maize produced by conventional cross breeding of parental lines MON89034 (OECD identifier: MON-89034-3) with NK603 (OECD unique identifier: MON-00603-6). Resistance to Lepidopteran insects is derived from two Cry genes present in MON89043. Tolerance to glyphosate herbicide is derived from NK603. NK603 X MON810 Monsanto Company Stacked insect resistant and herbicide tolerant corn hybrid derived from conventional crossbreeding of the parental lines NK603 (OECD identifier: MON- 00603-6) and MON810 (OECD identifier: MON-00810-6). MON89034 x TC1507 x Monsanto Company and Stacked insect resistant and MON88017 x DAS- My cogen Seeds c/o Dow herbicide tolerant maize produced 59122-7 AgroSciences LLC by conventional cross breeding of parental lines: MON89034, TC1507, MON88017, and DAS-59 122. Resistance to the above- ground and below-ground insect pests and tolerance to glyphosate and glufosinate-ammonium containing herbicides. M53 Bayer CropScience Male sterility caused by expression (Aventis of the barnase ribonuclease gene CropScience(AgrEvo)) from *Bacillus amyloliquefaciens*; PPT resistance was via PPT-acetyltransferase (PAT). M56 Bayer CropScience Male sterility caused by expression (Aventis of the barnase ribonuclease gene CropScience(AgrEvo) from *Bacillus amyloliquefaciens*; PPT resistance was via PPT- acetyltransferase (PAT). NK603 Monsanto Company Introduction, by particle bombardment, of a modified 5- enolpyruvyl shikimate-3 -phosphate synthase (EPSPS), an enzyme involved in the shikimate biochemical pathway for the production of the aromatic amino acids. NK603 x T25 Monsanto Company Stacked glufosinate ammonium and glyphosate herbicide tolerant maize hybrid derived from conventional cross-breeding of the parental lines NK603 (OECD identifier: MON-00603-6) and T25 (OECD identifier: ACS-ZM003- 2). T25 x MON810 Bayer CropScience Stacked insect resistant and (Aventis herbicide tolerant corn hybrid CropScience(AgrEvo)) derived from conventional crossbreeding of the parental lines T25 (OECD identifier: ACS- ZMOO3-2) and MON810 (OECD identifier: MON-OO81O-6). TC1507 Mycogen (c/o Dow Insect-resistant and glufosinate AgroSciences); Pioneer ammonium herbicide tolerant (c/o DuPont) maize produced by inserting the Cry1F gene from *Bacillus thuringiensis* var. *aizawai* and the phosphinothricin N-acetyltransferase encoding gene from *Streptomyces viridochromogenes*. TC1507 x NK603 DOW AgroSciences LLC Stacked insect resistant and herbicide tolerant corn hybrid derived from conventional crossbreeding of the parental lines 1507 (OECD identifier: DAS- O15O7-1) and NK603 (OECD identifier: MON-OO6O3-6). TC1507 x DAS-59122-7 DOW AgroSciences LLC Stacked insect resistant and and Pioneer Hi-Bred herbicide tolerant maize produced International Inc. by conventional cross breeding of parental lines TCI507 (OECD unique identifier: DAS-O15O7-1) with DAS-59122-7 (OECD unique identifier: DAS-59122-7). Resistance to Lepidopteran insects is derived from TCI507 due the presence of the Cry1F gene from *Bacillus thuringiensis* var. *aizawai*. Corn rootworm-resistance is

derived from DAS-59122-7 which contains the Cry34Ab1 and Cry35Ab1 genes from *Bacillus thuringiensis* strain P5149B1. Tolerance to glufosinate ammonium herbicide is derived from TC1507 from the phosphinothricin N-acetyltransferase encoding gene from *Streptomyces viridochromogenes*. Event Company Description Hybrid Family P0157 Dupont Pioneer P0157 P0157AM Dupont Pioneer AM LL RR2 P0157 P0157AMXT Dupont Pioneer AMXT LL RR2 P0157 P0157R Dupont Pioneer RR2 P0157 P0339AM Dupont Pioneer AM LL RR2 P0339 P0339AMXT Dupont Pioneer AMXT LL RR2 P0339 P0306AM Dupont Pioneer AM LL RR2 P0306 P0589 Dupont Pioneer P0589 P0589AM Dupont Pioneer AM LL RR2 P0589 P0589AMXT Dupont Pioneer AMXT LL RR2 P0589 P0589R Dupont Pioneer RR2 P0589 P0574 Dupont Pioneer P0574 P0574AM Dupont Pioneer AM LL RR2 P0574 P0574AMXT Dupont Pioneer AMXT LL RR2 P0574 P0533EXR Dupont Pioneer HXX LL RR2 P0533 P0506AM Dupont Pioneer AM LL RR2 P0566 P0760AMXT Dupont Pioneer AMXT LL RR2 P0760 P0707AM Dupont Pioneer AM LL RR2 P0707 P0707AMXT Dupont Pioneer AMXT LL RR2 P0707 P0825AM Dupont Pioneer AM LL RR2 P0825 P0825AMXT Dupont Pioneer AMXT LL RR2 P0825 P0969AM Dupont Pioneer AM LL RR2 P0969 P0969AMXT Dupont Pioneer AMXT LL RR2 P0969 P0937AM Dupont Pioneer AM LL RR2 P0937 P0919AM Dupont Pioneer AM LL RR2 P0919 P0905EXR Dupont Pioneer HXX LL RR2 P0905 P1197 Dupont Pioneer P1197 P1197AM Dupont Pioneer AM LL RR2 P1197 P1197AMXT Dupont Pioneer AMXT LL RR2 P1197 P1197R Dupont Pioneer RR2 P1197 P1151 Dupont Pioneer P1151 P1151AM Dupont Pioneer AM LL RR2 P1151 P1151R Dupont Pioneer RR2 P1151 P1138AM Dupont Pioneer AM LL RR2 P1138 P1366AM Dupont Pioneer AM LL RR2 P1366 P1366AMXT Dupont Pioneer AMXT LL RR2 P1366 P1365AMX Dupont Pioneer AMX LL RR2 P1365 P1353AM Dupont Pioneer AM LL RR2 P1353 P1345 Dupont Pioneer P1345 P1311AMXT Dupont Pioneer AMXT LL RR2 P1311 P1498EHR Dupont Pioneer HX1 LL RR2 P1498 P1498R Dupont Pioneer RR2 P1498 P1443AM Dupont Pioneer AM LL RR2 Pl 443 P1555CHR Dupont Pioneer RW HX1 LL P1555 RR2 P1751AMT Dupont Pioneer AMT LL RR2 P1751 P2089AM Dupont Pioneer AM LL RR2 P2089 QROME Dupont Pioneer Q LL RR2

(461) The following are the definitions for the shorthand occurring in Table 19. AM—OPTIMUM ACREMAX Insect Protection system with YGCB, HX1, LL, RR2. AMT—OPTIMUM ACREMAX TRISECT Insect Protection System with RW,YGCB,HX1,LL,RR2. AMXT—(OPTIMUM ACREMAX XTreme). HXX—HERCULEX XTRA contains the Herculex I and Herculex RW genes. HX1—Contains the HERCULEX I Insect Protection gene which provides protection against European corn borer, southwestern corn borer, black cutworm, fall armyworm, western bean cutworm, lesser corn stalk borer, southern corn stalk borer, and sugarcane borer; and suppresses corn earworm. LL—Contains the LIBERTYLINK gene for resistance to LIBERTY herbicide. RR2—Contains the ROUNDUP READY Corn 2 trait that provides crop safety for over-the-top applications of labeled glyphosate herbicides when applied according to label directions. YGCB—contains the YIELDGARD Corn Borer gene offers a high level of resistance to European corn borer, southwestern corn borer, and southern cornstalk borer; moderate resistance to corn earworm and common stalk borer; and above average resistance to fall armyworm. RW—contains the AGRISURE root worm resistance trait. Q—provides protection or suppression against susceptible European corn borer, southwestern corn borer, black cutworm, fall armyworm, lesser corn stalk borer, southern corn stalk borer, stalk borer, sugarcane borer, and corn earworm; and also provides protection from larval injury caused by susceptible western corn rootworm, northern corn rootworm, and Mexican corn rootworm; contains (1) HERCULEX XTRA Insect Protection genes that produce Cry1F and Cry34ab1 and Cry35ab1 proteins, (2) AGRISURE RW trait that includes a gene that produces mCry3A protein, and (3) YIELDGARD Corn Borer gene which produces Cry1Ab protein.

(462) Concentrations and Rates of Application of Agricultural Compositions

(463) As aforementioned, the agricultural compositions of the present disclosure, which comprise a

taught microbe, can be applied to plants in a multitude of ways. In two particular aspects, the disclosure contemplates an in-furrow treatment or a seed treatment

(464) For seed treatment embodiments, the microbes of the disclosure can be present on the seed in a variety of concentrations. For example, the microbes can be found in a seed treatment at a cfu concentration, per seed of: 1×10.sup.1, 1×10.sup.2, 1×10.sup.3, 1×10.sup.4, 1×10.sup.5, 1×10.sup.6, 1×10.sup.7, 1×10.sup.8, 1×10.sup.9, 1×10.sup.10, or more. In particular aspects, the seed treatment compositions comprise about 1×10.sup.4 to about 1×10.sup.8 cfu per seed. In other particular aspects, the seed treatment compositions comprise about 1×10.sup.5 to about 1×10.sup.7 cfu per seed. In other aspects, the seed treatment compositions comprise about 1×10.sup.6 cfu per seed.

(465) In the United States, about 10% of corn acreage is planted at a seed density of above about 36,000 seeds per acre; ⅓ of the corn acreage is planted at a seed density of between about 33,000 to 36,000 seeds per acre; ⅓ of the corn acreage is planted at a seed density of between about 30,000 to 33,000 seeds per acre, and the remainder of the acreage is variable. See, “Corn Seeding Rate Considerations,” written by Steve Butzen, available at: www.pioneer.com/home/site/us/agronomy/library/corn-seeding-rate-considerations/

(466) Table 20 below utilizes various cfu concentrations per seed in a contemplated seed treatment embodiment (rows across) and various seed acreage planting densities (1.sup.st column: 15K-41K) to calculate the total amount of cfu per acre, which would be utilized in various agricultural scenarios (i.e. seed treatment concentration per seed×seed density planted per acre). Thus, if one were to utilize a seed treatment with 1×10.sup.6 cfu per seed and plant 30,000 seeds per acre, then the total cfu content per acre would be 3×10.sup.10 (i.e. 30K*1×10.sup.6).

(467) TABLE-US-00020 TABLE 20 Total CFU Per Acre Calculation for Seed Treatment Embodiments Corn Population (i.e. seeds per acre) 1.00E+02 1.00E+03 1.00E+04 1.00E+05 1.00E+06 1.00E+07 1.00E+08 1.00E+09 15,000 1.50E+06 1.50E+07 1.50E+08 1.50E+09 1.50E+10 1.50E+11 1.50E+12 1.50E+13 16,000 1.60E+06 1.60E+07 1.60E+08 1.60E+09 1.60E+10 1.60E+11 1.60E+12 1.60E+13 17,000 1.70E+06 1.70E+07 1.70E+08 1.70E+09 1.70E+10 1.70E+11 1.70E+12 1.70E+13 18,000 1.80E+06 1.80E+07 1.80E+08 1.80E+09 1.80E+10 1.80E+11 1.80E+12 1.80E+13 19,000 1.90E+06 1.90E+07 1.90E+08 1.90E+09 1.90E+10 1.90E+11 1.90E+12 1.90E+13 20,000 2.00E+06 2.00E+07 2.00E+08 2.00E+09 2.00E+10 2.00E+11 2.00E+12 2.00E+13 21,000 2.10E+06 2.10E+07 2.10E+08 2.10E+09 2.10E+10 2.10E+11 2.10E+12 2.10E+13 22,000 2.20E+06 2.20E+07 2.20E+08 2.20E+09 2.20E+10 2.20E+11 2.20E+12 2.20E+13 23,000 2.30E+06 2.30E+07 2.30E+08 2.30E+09 2.30E+10 2.30E+11 2.30E+12 2.30E+13 24,000 2.40E+06 2.40E+07 2.40E+08 2.40E+09 2.40E+10 2.40E+11 2.40E+12 2.40E+13 25,000 2.50E+06 2.50E+07 2.50E+08 2.50E+09 2.50E+10 2.50E+11 2.50E+12 2.50E+13 26,000 2.60E+06 2.60E+07 2.60E+08 2.60E+09 2.60E+10 2.60E+11 2.60E+12 2.60E+13 27,000 2.70E+06 2.70E+07 2.70E+08 2.70E+09 2.70E+10 2.70E+11 2.70E+12 2.70E+13 28,000 2.80E+06 2.80E+07 2.80E+08 2.80E+09 2.80E+10 2.80E+11 2.80E+12 2.80E+13 29,000 2.90E+06 2.90E+07 2.90E+08 2.90E+09 2.90E+10 2.90E+11 2.90E+12 2.90E+13 30,000 3.00E+06 3.00E+07 3.00E+08 3.00E+09 3.00E+10 3.00E+11 3.00E+12 3.OOE+13 31,000 3.10E+06 3.10E+07 3.10E+08 3.10E+09 3.10E+10 3.10E+11 3.10E+12 3.1OE+13 32,000 3.20E+06 3.20E+07 3.20E+08 3.20E+09 3.20E+10 3.20E+11 3.20E+12 3.20E+13 33,000 3.30E+06 3.30E+07 3.30E+08 3.30E+09 3.3OE+1O 3.3OE+11 3.30E+12 3.3OE+13 34,000 3.40E+06 3.40E+07 3.40E+08 3.40E+09 3.40E+10 3.40E+11 3.40E+12 3.40E+13 35,000 3.50E+06 3.50E+07 3.50E+08 3.50E+09 3.50E+10 3.50E+11 3.50E+12 3.5OE+13 36,000 3.60E+06 3.60E+07 3.60E+08 3.60E+09 3.60E+10 3.60E+11 3.60E+12 3.60E+13 37,000 3.70E+06 3.70E+07 3.70E+08 3.70E+09 3.70E+10 3.70E+11 3.70E+12 3.70E+13 38,000 3.80E+06 3.80E+07 3.80E+08 3.80E+09 3.80E+10 3.80E+11 3.80E+12 3.80E+13 39,000 3.90E+06 3.90E+07 3.90E+08 3.90E+09 3.90E+10 3.90E+11 3.90E+12 3.90E+13 40,000 4.00E+06 4.00E+07 4.00E+08 4.00E+09

4.00E+10 4.00E+11 4.00E+12 4.00E+13 41,000 4.10E+06 4.10E+07 4.10E+08 4.10E+09 4.10E+10 4.10E+11 4.10E+12 4.10E+13

(468) For in-furrow embodiments, the microbes of the disclosure can be applied at a cfu concentration per acre of: 1×10.sup.6, 3.20×10.sup.10, 1.60×10.sup.11, 3.20×10.sup.11, 8.0×10.sup.11, 1.6×10.sup.12, 3.20×10.sup.12, or more. Therefore, in aspects, the liquid in-furrow compositions can be applied at a concentration of between about 1×10.sup.6 to about 3×10.sup.12 cfu per acre.

(469) In some aspects, the in-furrow compositions are contained in a liquid formulation. In the liquid in-furrow embodiments, the microbes can be present at a cfu concentration per milliliter of: 1×10.sup.1, 1×10.sup.2, 1×10.sup.3, 1×10.sup.4, 1×10.sup.5, 1×10.sup.6, 1×10.sup.7, 1×10.sup.8, 1×10.sup.9, 1×10.sup.10, 1×10.sup.11, 1×10.sup.12, 1×10.sup.13, or more. In certain aspects, the liquid in-furrow compositions comprise microbes at a concentration of about 1×10.sup.6 to about 1×10.sup.11 cfu per milliliter. In other aspects, the liquid in-furrow compositions comprise microbes at a concentration of about 1×10.sup.7 to about 1×10.sup.10 cfu per milliliter. In other aspects, the liquid in-furrow compositions comprise microbes at a concentration of about 1×10.sup.8 to about 1×10.sup.9 cfu per milliliter. In other aspects, the liquid in-furrow compositions comprise microbes at a concentration of up to about 1×10.sup.13 cfu per milliliter.

(470) Transcriptomic Profiling of Candidate Microbes

(471) Previous work by the inventors entailed transcriptomic profiling of strain CI010 to identify promoters that are active in the presence of environmental nitrogen. Strain CI010 was cultured in a defined, nitrogen-free media supplemented with 10 mM glutamine. Total RNA was extracted from these cultures (QIAGEN RNeasy kit) and subjected to RNAseq sequencing via Illumina HiSeq (SeqMatic, Fremont CA). Sequencing reads were mapped to the CI010 genome data using Geneious, and highly expressed genes under control of proximal transcriptional promoters were identified.

(472) Tables 21-23 list genes and their relative expression level as measured through RNASeq sequencing of total RNA. Sequences of the proximal promoters were recorded for use in mutagenesis of nif pathways, nitrogen utilization related pathways, or other genes with a desired expression level.

(473) TABLE-US-00021 TABLE 21 Name Minimum Maximum Length Direction murein lipoprotein CDS 2,929,898 2,930,134 237 forward membrane protein CDS 5,217,517 5,217,843 327 forward zinc/cadmium-binding 3,479,979 3,480,626 648 forward protein CDS acyl carrier protein CDS 4,563,344 4,563,580 237 reverse ompX CDS 4,251,002 4,251,514 513 forward DNA-binding protein 375,156 375,428 273 forward HU-beta CDS sspA CDS 629,998 630,636 639 reverse tatE CDS 3,199,435 3,199,638 204 reverse LexA repressor CDS 1,850,457 1,851,065 609 forward hisS CDS <3999979 4,001,223 >1245 forward

(474) TABLE-US-00022 TABLE 22 Differential RNASeq_nifL - RNASeq_WT - Expression Differential RNASeq_nifL - Raw RNASeq_WT- Raw Absolute Expression Raw Read Transcript Raw Read Transcript Name Confidence Ratio Count Count Count Count murein 1000 −1.8 12950.5 10078.9 5151.5 4106.8 lipoprotein CDS membrane 1000 −1.3 9522.5 5371.3 5400 3120 protein CDS zinc/cadmium- 3.3 1.1 6461 1839.1 5318 1550.6 binding protein CDS acyl carrier 25.6 1.6 1230.5 957.6 1473.5 1174.7 protein CDS ompX CDS 1.7 1.1 2042 734.2 1687.5 621.5 DNA-binding 6.9 −1.3 1305 881.7 725 501.8 protein HU- beta CDS sspA CDS 0.2 1 654 188.8 504.5 149.2 tatE CDS 1.4 1.3 131 118.4 125 115.8 LexA 0.1 −1.1 248 75.1 164 50.9 repressor CDS hisS CDS 0 −1.1 467 69.2 325 49.3

(475) TABLE-US-00023 TABLE 23 Prm (In Forward direction, −250 Expressed Neighbor to +10 region) Sequence Sequence Name SEQ ID NO: SEQ ID NO: SEQ ID NO: murein lipoprotein CDS SEQ ID NO: 3 SEQ ID NO: 13 SEQ ID NO: 23 membrane protein CDS SEQ ID NO: 4 SEQ ID NO: 14 SEQ ID NO: 24 zinc/cadmium-binding SEQ ID NO: 5 SEQ ID NO: 15 SEQ ID NO: 25 protein CDS acyl carrier protein CDS SEQ ID NO: 6 SEQ ID NO: 16 SEQ ID NO: 26 ompX CDS

SEQ ID NO: 7 SEQ ID NO: 17 SEQ ID NO: 27 DNA-binding protein SEQ ID NO: 8 SEQ ID NO: 18 SEQ ID NO: 28 HU-beta CDS sspA CDS SEQ ID NO: 9 SEQ ID NO: 19 SEQ ID NO: 29 tatE CDS SEQ ID NO: 10 SEQ ID NO: 20 SEQ ID NO: 30 LexA repressor CDS SEQ ID NO: 11 SEQ ID NO: 21 SEQ ID NO: 31 hisS CDS SEQ ID NO: 12 SEQ ID NO: 22 SEQ ID NO: 32

(476) TABLE-US-00024 TABLE 24 Table of Strains Mutagenic DNA Gene 1 Gene 2 Name Lineage Description Genotype mutation mutation CI006 Isolated strain None WT from *Enterobacter* (now *Kosakonia*) genera CI008 Isolated strain None WT from *Burkholderia* genera CI010 Isolated strain None WT from *Klebsiella* genera CI019 Isolated strain None WT from *Rahnella* genera CI028 Isolated strain None WT from *Enterobacter* genera CI050 Isolated strain None WT from *Klebsiella* genera CM002 Mutant of CI050 Disruption of nifL gene ΔnifL::KanR SEQ ID with a kanamycin NO: 33 resistance expression cassette (KanR) encoding the aminoglycoside O- phosphotransferase gene aph1 inserted. CM011 Mutant of CI019 Disruption of nifL gene ΔnifL::SpecR SEQ ID with a spectinomycin NO: 34 resistance expression cassette (SpecR) encoding the streptomycin 3″-O- adenylyltransferase gene aadA inserted. CM013 Mutant of CI006 Disruption of nifL gene ΔnifL::KanR SEQ ID with a kanamycin NO: 35 resistance expression cassette (KanR) encoding the aminoglycoside O- phosphotransferase gene aph1 inserted. CM004 Mutant of CI010 Disruption of amtB gene ΔamtB::KanR SEQ ID with a kanamycin NO: 36 resistance expression cassette (KanR) encoding the aminoglycoside O- phosphotransferase gene aph1 inserted. CM005 Mutant of CI010 Disruption of nifL gene ΔnifL::KanR SEQ ID with a kanamycin NO: 37 resistance expression cassette (KanR) encoding the aminoglycoside O- phosphotransferase gene aph1 inserted. CM015 Mutant of CI006 Disruption of nifL gene ΔnifL::Prm5 SEQ ID with a fragment of the NO: 38 region upstream of the ompX gene inserted (Prm5). CM021 Mutant of CI006 Disruption of nifL gene ΔnifL::Prm2 SEQ ID with a fragment of the NO: 39 region upstream of an unanotated gene and the first 73 bp of that gene inserted (Prm2). CM023 Mutant of CI006 Disruption of nifL gene ΔnifL::Prm4 SEQ ID with a fragment of the NO: 40 region upstream of the acpP gene and the first 121 bp of the acpP gene inserted (Prm4). CM014 Mutant of CI006 Disruption of nifL gene ΔnifL::Prm1 SEQ ID with a fragment of the NO: 41 region upstream of the lpp gene and the first 29 bp of the lpp gene inserted (Prm1). CM016 Mutant of CI006 Disruption of nifL gene ΔnifL::Prm9 SEQ ID with a fragment of the NO: 42 region upstream of the lexA 3 gene and the first 21 bp of the lexA 3 gene inserted (Prm9). CM022 Mutant of CI006 Disruption of nifL gene ΔnifL::Prm3 SEQ ID with a fragment of the NO: 43 region upstream of the mntP 1 gene and the first 53 bp of the mntP 1 gene inserted (Prm3). CM024 Mutant of CI006 Disruption of nifL gene ΔnifL::Prm7 SEQ ID with a fragment of the NO: 44 region upstream of the sspA gene inserted (Prm7). CM025 Mutant of CI006 Disruption of nifL gene ΔnifL::Prm10 SEQ ID with a fragment of the NO: 45 region upstream of the hisS gene and the first 52 bp of the hisS gene inserted (Prm10). CM006 Mutant of CI010 Disruption of glnB gene ΔglnB::KanR SEQ ID with a kanamycin NO: 46 resistance expression cassette (KanR) encoding the aminoglycoside O- phosphotransferase gene aph1 inserted. CM017 Mutant of CI028 Disruption of nifL gene ΔnifL::KanR SEQ ID with a kanamycin NO: 47 resistance expression cassette (KanR) encoding the aminoglycoside O- phosphotransferase gene aph1 inserted. CM011 Mutant of CI019 Disruption of nifL gene ΔnifL::SpecR SEQ ID with a spectinomycin NO: 48 resistance expression cassette (SpecR) encoding the streptomycin 3″-O- adenylyltransferase gene aadA inserted. CM013 Mutant of CI006 Disruption of nifL gene ΔnifL::KanR SEQ ID with a kanamycin NO: 49 resistance expression cassette (KanR) encoding the aminoglycoside O- phosphotransferase gene aph1 inserted. CM005 Mutant of CI010 Disruption of nifL gene ΔnifL::KanR SEQ ID with a kanamycin NO: 50 resistance expression cassette (KanR) encoding the aminoglycoside O- phosphotransferase gene aph1 inserted. CM014 Mutant of CI006 Disruption of nifL gene ΔnifL::Prm1 SEQ ID with a fragment of the NO: 51 region upstream of the lpp gene and the first 29 bp of the lpp gene inserted (Prm1). CM015 Mutant of CI006 Disruption of nifL gene ΔnifL::Prm5 SEQ ID with a fragment of the NO: 52 region upstream of the ompX gene

inserted (Prm5). CM023 Mutant of CI006 Disruption of nifL gene ΔnifL::Prm4 SEQ ID with a fragment of the NO: 53 region upstream of the acpP gene and the first 121 bp of the acpP gene inserted (Prm4). CM029 Mutant of CI006 Disruption of nifL gene ΔnifL::Prm5 SEQ ID SEQ ID with a fragment of the ΔglnE- NO: 54 NO: 61 region upstream of the AR_KO1 ompX gene inserted (Prm5) and deletion of the 1287 bp after the start codon of the glnE gene containing the adenylyl- removing domain of glutamate-ammonia-ligase adenylyltransferase (AglnE-AR_KOl). CM014 Mutant of CI006 Disruption of nifL gene ΔnifL::Prm1 SEQ ID with a fragment of the NO: 55 region upstream of the lpp gene and the first 29 bp of the lpp gene inserted (Prm1). CM011 Mutant of CI019 Disruption of nifL gene ΔnifL::SpecR SEQ ID with a spectinomycin NO: 56 resistance expression cassette (SpecR) encoding the streptomycin 3″-O- adenylyltransferase gene aadA inserted. CM011 Mutant of CI019 Disruption of nifL gene ΔnifL::SpecR SEQ ID with a spectinomycin NO: 57 resistance expression cassette (SpecR) encoding the streptomycin 3″-O- adenylyltransferase gene aadA inserted. CM013 Mutant of CI006 Disruption of nifL gene ΔnifL::KanR SEQ ID with a kanamycin NO: 58 resistance expression cassette (KanR) encoding the aminoglycoside O- phosphotransferase gene aph1 inserted. CM011 Mutant of CI019 Disruption of nifL gene ΔnifL::SpecR SEQ ID with a spectinomycin NO: 59 resistance expression cassette (SpecR) encoding the streptomycin 3″-O- adenylyltransferase gene aadA inserted. CM011 Mutant of CI019 Disruption of nifL gene ΔnifL:: SpecR SEQ ID with a spectinomycin NO: 60 resistance expression cassette (SpecR) encoding the streptomycin 3″-O- adenylyltransferase gene aadA inserted.

EXAMPLES

(477) The following examples are given for the purpose of illustrating various embodiments of the disclosure and are not meant to limit the present disclosure in any fashion. Changes therein and other uses which are encompassed within the spirit of the disclosure, as defined by the scope of the claims, will be recognized by those skilled in the art.

Example 1: Guided Microbial Remodeling—A Platform for the Rational Improvement of Microbial Species for Agriculture

(478) An example overview of an embodiment of the Guided Microbial Remodeling (GMR) platform can be summarized in the schematic of FIG. **1**A.

(479) FIG. **1**A illustrates that the composition of the microbiome can first be characterized and a species of interest is identified (e.g. to find a microbe with the appropriate colonization characteristics).

(480) The metabolism of the species of interest can be mapped and linked to genetics. For example, the nitrogen fixation pathway of the microbe can be characterized. The pathway that is being characterized can be examined under a range of environmental conditions. For example, the microbe's ability to fix atmospheric nitrogen in the presence of various levels of exogenous nitrogen in its environment can be examined. The metabolism of nitrogen can involve the entrance of ammonia (NH.sub.4.sup.+) from the rhizosphere into the cytosol of the bacteria via the AmtB transporter. Ammonia and L-glutamate (L-Glu) are catalyzed by glutamine synthetase and ATP into glutamine. Glutamine can lead to the formation of bacterial biomass and it can also inhibit expression of the nif operon, i.e. it can be a competing force when one desires the microbe to fix atmospheric nitrogen and excrete ammonia. The nitrogen fixation pathway is characterized in great detail in earlier sections of the specification.

(481) Afterwards, a targeted non-intergeneric genomic alteration can be introduced to the microbe's genome, using methods including, but not limited to: conjugation and recombination, chemical mutagenesis, adaptive evolution, and gene editing. The targeted non-intergeneric genomic alteration can include an insertion, disruption, deletion, alteration, perturbation, modification, etc. of the genome.

(482) Derivative remodeled microbes, which comprise the desired phenotype resulting from the remodeled underlying genotype, are then used to inoculate crops.

(483) The present disclosure provides, in certain embodiments, non-intergeneric remodeled microbes that are able to fix atmospheric nitrogen and supply such nitrogen to a plant. In aspects, these non-intergeneric remodeled microbes are able to fix atmospheric nitrogen, even in the presence of exogenous nitrogen.
(484) FIG. **1**B depicts an expanded view of the measurement of the microbiome step.
(485) In some embodiments, the present disclosure finds microbial species that have desired colonization characteristics, and then utilizes those species in the subsequent remodeling process.
(486) The aforementioned Guided Microbial Remodeling (GMR) platform will now be described with more specificity.
(487) In aspects, the GMR platform comprises the following steps: A. Isolation—Obtain microbes from the soil, rhizosphere, surface, etc. of a crop plant of interest; B. Characterization—Involves characterizing the isolated microbes for genotype/phenotypes of interest (e.g. genome sequence, colonization ability, nitrogen fixation activity, solubilization of P ability, excretion of a metabolite of interest, excretion of a plant promoting compound, etc.) C. Domestication—Development of a molecular protocol for non-intergeneric genetic modification of the microbe; D. Non-Intergeneric Engineering Campaign and Optimization—Generation of derivative non-intergeneric microbial strains with genetic modifications in key pathways (e.g. colonization associated genes, nitrogen fixation/assimilation genes, P solubilization genes); E. Analytics—Evaluation of derived non-intergeneric strains for phenotypes of interest both in vitro (e.g. ARA assays) and in planta (e.g. colonization assays). F. Iterate Engineering Campaign/Analytics—Iteration of steps D and E for further improvement of microbial strain.
(488) Each of the GMR platform process steps will now be elaborated upon below.
(489) A. Isolation of Microbes
(490) 1. Obtain a Soil Sample
(491) Microbes will be isolated from soil and/or roots of a plant. In one example, plants will be grown in a laboratory or a greenhouse in small pots. Soil samples will be obtained from various agricultural areas. For example, soils with diverse texture characteristics can be collected, including loam (e.g. peaty clay loam, sandy loam), clay soil (e.g. heavy clay, silty clay), sandy soil, silty soil, peaty soil, chalky soil, and the like.
(492) 2. Grow Bait Plants
(493) Seeds of a bait plant (a plant of interest) (e.g. corn, wheat, rice, *sorghum*, millet, soybean, vegetables, fruits, etc.) will be planted into each soil type. In one example, different varieties of a bait plant will be planted in various soil types. For example, if the plant of interest is corn, seeds of different varieties of corn such as field corn, sweet corn, heritage corn, etc. will be planted in various soil types described above.
(494) 3. Harvest Soil and/or Root Samples and Plate on Appropriate Medium
(495) Plants will be harvested by uprooting them after a few weeks (e.g. 2-4 weeks) of growth. Alternative to growing plants in a laboratory/greenhouse, soil and/or roots of the plant of interest can be collected directly from the fields with different soil types.
(496) To isolate rhizosphere microbes and epiphytes, plants will be removed gently by saturating the soil with distilled water or gently loosening the soil by hand to avoid damage to the roots. If larger soil particles are present, these particles will be removed by submerging the roots in a still pool of distilled water and/or by gently shaking the roots. The root will be cut and a slurry of the soil sticking to the root will be prepared by placing the root in a plate or tube with small amount of distilled water and gently shaking the plate/tube on a shaker or centrifuging the tube at low speed. This slurry will be processed as described below.
(497) To isolate endophytes, excess soil on root surfaces will be removed with deionized water. Following soil removal, plants will be surface sterilized and rinsed vigorously in sterile water. A cleaned, 1 cm section of root will be excised from the plant and placed in a phosphate buffered saline solution containing 3 mm steel beads. A slurry will be generated by vigorous shaking of the

solution with a Qiagen TissueLyser II.
(498) The soil and/or root slurry can be processed in various ways depending on the desired plant-beneficial trait of microbes to be isolated. For example, the soil and root slurry can be diluted and inoculated onto various types of screening media to isolate rhizospheric, endophytic, epiphytic, and other plant-associated microbes. For example, if the desired plant-beneficial trait is nitrogen fixation, then the soil/root slurry will be plated on a nitrogen free media (e.g. Nfb agar media) to isolate nitrogen fixing microbes. Similarly, to isolate phosphate solubilizing bacteria (PSB), media containing calcium phosphate as the sole source of phosphorus can be used. PSB can solubilize calcium phosphate and assimilate and release phosphorus in higher amounts. This reaction is manifested as a halo or a clear zone on the plate and can be used as an initial step for isolating PSB.
(499) 4. Pick Colonies, Purify Cultures, and Screen for the Presence of Genes of Interest
(500) Populations of microbes obtained in step A3 are streaked to obtain single colonies (pure cultures). A part of the pure culture is resuspended in a suitable medium (e.g. a mixture of R2A and glycerol) and subjected to PCR analysis to screen for the presence of one or more genes of interest. For example, to identify nitrogen fixing bacteria (diazotrophs), purified cultures of isolated microbes can be subjected to a PCR analysis to detect the presence of nif genes that encode enzymes involved in the fixation of atmospheric nitrogen into a form of nitrogen available to living organisms.
(501) 5. Bank a Purified Culture
(502) Purified cultures of isolated strains will be stored, for example at −80° C., for future reference and analysis.
(503) B. Characterization of Isolated Microbes
(504) 1. Phylogenetic Characterization and Whole Genome Sequencing
(505) Isolated microbes will be analyzed for phylogenetic characterization (assignment of genus and species) and the whole genome of the microbes will be sequenced.
(506) For phylogenetic characterization, 16S rDNA of the isolated microbe will be sequenced using degenerate 16S rDNA primers to generate phylogenetic identity. The 16S rDNA sequence reads will be mapped to a database to initially assign the genus, species and strain name for isolated microbes. Whole genome sequencing is used as the final step to assign phylogenetic genus/species to the microbes.
(507) The whole genome of the isolated microbes will be sequenced to identify key pathways. For the whole genome sequencing, the genomic DNA will be isolated using a genomic DNA isolation kit (e.g. QIAmp DNA mini kit from QIAGEN) and a total DNA library will be prepared using the methods known in the art. The whole genome will be sequenced using high throughput sequencing (also called Next Generation Sequencing) methods known in the art. For example, Illumina, Inc., Roche, and Pacific Biosciences provide whole genome sequencing tools that can be used to prepare total DNA libraries and perform whole genome sequencing.
(508) The whole genome sequence for each isolated strain will be assembled; genes of interest will be identified; annotated; and noted as potential targets for remodeling. The whole genome sequences will be stored in a database.
(509) 2. Assay the Microbe for Colonization of a Host Plant in a Greenhouse
(510) Isolated microbes will be characterized for the colonization of host plants in a greenhouse. For this, seeds of the desired host plant (e.g., corn, wheat, rice, *sorghum*, soybean) will be inoculated with cultures of isolated microbes individually or in combination and planted into soil. Alternatively, cultures of isolated microbes, individually or in combination, can be applied to the roots of the host plant by inoculating the soil directly over the roots. The colonization potential of the microbes will be assayed, for example, using a quantitative PCR (qPCR) method described in a greater detail below.
(511) 3. Assay the Microbe for Colonization of the Host Plant in Small-Scale Field Trials and Isolate RNA from Colonized Root Samples (CAT Trials)

(512) Isolated microbes will be assessed for colonization of the desired host plant in small-scale field trials. Additionally, RNA will be isolated from colonized root samples to obtain transcriptome data for the strain in a field environment. These small-scale field trials are referred to herein as CAT (Colonization and Transcript) trials, as these trials provide Colonization and Transcript data for the strain in a field environment.
(513) For these trials, seeds of the host plant (e.g., corn, wheat, rice, *sorghum*, soybean) will be inoculated using cultures of isolated microbes individually or in combination and planted into soil. Alternatively, cultures of isolated microbes, individually or in combination, can be applied to the roots of the host plant by inoculating the soil directly over the roots. The CAT trials can be conducted in a variety of soils and/or under various temperature and/or moisture conditions to assess the colonization potential and obtain transcriptome profile of the microbe in various soil types and environmental conditions.
(514) Colonization of roots of the host plant by the inoculated microbe(s) will be assessed, for example, using a qPCR method as described below.
(515) In one protocol, the colonization potential of isolated microbes was assessed as follows. One day after planting of corn seeds, 1 ml of microbial overnight culture (SOB media) was drenched right at the spot of where the seed was located. 1 mL of this overnight culture was roughly equivalent to about 10{circumflex over ( )}9 cfu, varying within 3-fold of each other, depending on which strain is being used. Each seedling was fertilized 3× weekly with 50 mL modified Hoagland's solution supplemented with either 2.5 mM or 0.25 mM ammonium nitrate. At four weeks after planting, root samples were collected for DNA extraction. Soil debris were washed away using pressurized water spray. These tissue samples were then homogenized using QIAGEN Tissuelyzer and the DNA was then extracted using QIAmp DNA Mini Kit (QIAGEN) according to the recommended protocol. qPCR assay was performed using Stratagene Mx3005P RT-PCR on these DNA extracts using primers that were designed (using NCBI's Primer BLAST) to be specific to a loci in each of the microbe's genome.
(516) The presence of the genome copies of the microbe was quantified, which reflected the colonization potential of the microbe. Identity of the microbial species was confirmed by sequencing the PCR amplification products.
(517) Additionally, RNA will be isolated from colonized root and/or soil samples and sequenced.
(518) Unlike the DNA profile, an RNA profile varies depending on the environmental conditions. Therefore, sequencing of RNA isolated from colonized roots and/or soil will reflect the transcriptional activity of genes in planta in the rhizosphere.
(519) RNA can be isolated from colonized root and/or soil samples at different time points to analyze the changes in the RNA profile of the colonized microbe at these time points.
(520) For example, RNA can be isolated from colonized root and/or soil samples right after fertilization of the field and a few weeks after fertilization of the field and sequenced to generate corresponding transcriptional profile.
(521) Similarly, RNA sequencing can be carried out under high phosphate and low phosphate conditions to understand which genes are transcriptionally active or repressed under these conditions.
(522) Methods for transcriptomic/RNA sequencing are known in the art. Briefly, total RNA will be isolated from the purified culture of the isolated microbe; cDNA will be prepared using reverse transcriptase; and the cDNA will be sequenced using high throughput sequencing tools described above.
(523) Sequencing reads from the transcriptome analysis can be mapped to the genomic sequence and transcriptional promoters for the genes of interest can be identified.
(524) 4. Assay the Plant-Beneficial Activity of Isolated Microbes
(525) The plant-beneficial activity of isolated microbes will be assessed.
(526) For example, nitrogen fixing microbes will be assayed for nitrogen fixation activity using an

acetylene reduction assay (ARA) or phosphate solubilizing microbes will be assayed for phosphate solubilization. Any parameter of interest can be utilized and an appropriate assay developed for such. For instance, assays could include growth curves for colonization metrics and assays for production of phytohormones like indole acetic acid (IAA) or gibberellins. An assay for any plant-beneficial activity that is of interest can be developed.
(527) This step will confirm the phenotype of interest and eliminate any false positives.
(528) 5. Selection of Potential Candidates from Isolated Microbes
(529) The data generated in the above steps will be used to select microbes for further development. For example, microbes showing a desired combination of colonization potential, plant-beneficial activity, and/or relevant DNA and RNA profile will be selected for domestication and remodeling.
(530) C. Domestication of Selected Microbes
(531) The selected microbes will be domesticated; wherein, the microbes will be converted to a form that is genetically tractable and identifiable.
(532) 1. Test for Antibiotic Sensitivity
(533) One way to domesticate the microbes is to engineer them with antibiotic resistance. For this, the wild type microbial strain will be tested for sensitivity to various antibiotics. If the strain is sensitive to the antibiotic, then the antibiotic can be a good candidate for use in genetic tools/vectors for remodeling the strain.
(534) 2. Design and Build a Vector
(535) Vectors that are conditional for their replication (e.g. a suicide plasmid) will be constructed to domesticate the selected microbes (host microbes). For example, a suicide plasmid containing an appropriate antibiotic resistance marker, a counter selectable marker, an origin of replication for maintenance in a donor microbe (e.g. *E. coli*), a gene encoding a fluorescent protein (GFP, RFP, YFP, CFP, and the like) to screen for insertion through fluorescence, an origin of transfer for conjugation into the host microbe, and a polynucleotide sequence comprising homology arms to the host genome with a desired genetic variation will be constructed. The vector may comprise a SceI site and other additional elements.
(536) Exemplary antibiotic resistance markers include ampicillin resistance marker, kanamycin resistance marker, tetracycline resistance marker, chloramphenicol resistance marker, erythromycin resistance marker, streptomycin resistance marker, spectinomycin resistance marker, etc. Exemplary counter selectable markers include sacB, rpsL, tetAR, pheS, thyA, lacY, gata-1, ccdB, etc.
(537) 3. Generation of Donor Microbes
(538) In one protocol, a suicide plasmid containing an appropriate antibiotic resistance marker, a counter selectable marker, the λpir origin of replication for maintenance in *E. coli* ST18 containing the pir replication initiator gene, a gene encoding green fluorescent protein (GFP) to screen for insertion through fluorescence, an origin of transfer for conjugation into the host microbe, and a polynucleotide sequence comprising homology arms to the host genome with a desired genetic variation (e.g. a promoter from within the microbe's own genome for insertion into a heterologous location) will be transformed into *E. coli* ST18 (an auxotroph for aminolevulinic acid, ALA) to generate donor microbes.
(539) 4. Mix Donor Microbes with Host Microbes
(540) Donor microbes will be mixed with host microbes (selected candidate microbes from step B5) to allow conjugative integration of the plasmid into the host genome. The mixture of donor and host microbes will be plated on a medium containing the antibiotic and not containing ALA. The suicide plasmid is able to replicate in donor microbes (*E. coli* ST18), but not in the host. Therefore, when the mixture containing donor and host microbes is plated on a medium containing the antibiotic and not containing ALA, only host cells that integrated the plasmid into its genome will be able to grow and form colonies on the medium. The donor microbes will not grow due to the

absence of ALA.
(541) 5. Confirm Integration of the Vector
(542) A proper integration of the suicide plasmid containing the fluorescent protein marker, the antibiotic resistance marker, the counter selectable marker, etc. at the intended locus of the host microbe will be confirmed through fluorescence of colonies on the plate and using colony PCR.
(543) 6. Streak Confirm Integration Colony
(544) A second round of homologous recombination in the host microbes will loop out (remove) the plasmid backbone leaving the desired genetic variation (e.g. a promoter from within the microbe's own genome for insertion into a heterologous location) integrated into the host genome of a certain percentage of host microbes, while reverting a certain percentage back to wild type.
(545) Colonies of host microbes that have looped out the plasmid backbone (and therefore, looped out the counter selectable marker) can be selected by growing them on an appropriate medium.
(546) For example, if sacB is used as a counter selectable marker, loss of this marker due to the loss of the plasmid backbone will be tested by growing the colonies on a medium containing sucrose (sacB confers sensitivity to sucrose). Colonies that grow on this medium would have lost the sacB marker and the plasmid backbone and would either contain the desired genetic variation or be reverted to wild type. Also, these colonies will not fluoresce on the plate due to the loss of the fluorescent protein marker.
(547) In some isolates, the sacB or other counterselectable markers do not confer full sensitivity to sucrose or other counterselection mechanisms, which necessitates screening large numbers of colonies to isolate a successful loop-out. In those cases, loop-out may be aided by use of a “helper plasmid” that replicates independently in the host cell and expresses a restriction endonuclease, e.g. SceI, which recognizes a site in the integrated suicide plasmid backbone. The strain with the integrated suicide plasmid is transformed with the helper plasmid containing an antibiotic resistance marker, an origin of replication compatible with the host strain, and a gene encoding a restriction endonuclease controlled by a constitutive or inducible promoter. The double-strand break induced in the integrated plasmid backbone by the restriction endonuclease promotes homologous recombination to loop-out the suicide plasmid. This increases the number of looped-out colonies on the counterselection plate and decreases the number of colonies that need to be screened to find a colony containing the desired mutation. The helper plasmid is then removed from the strain by culture and serial passaging in the absence of antibiotic selection for the plasmid. The passaged cultures are streaked for single colonies, colonies are picked and screened for sensitivity to the antibiotic used for selection of the helper plasmid, as well as absence of the plasmid confirmed by colony PCR. Finally, the genome is sequenced and the absence of helper plasmid DNA is confirmed as described in D6.
(548) 7. Confirm Integration of the Genetic Variation Through Colony PCR
(549) The colonies that grew better on the sucrose-containing medium (or other appropriate media depending on the counter selectable marked used) will be picked and the presence of the genetic variation at the intended locus will be confirmed by screening the colonies using colony PCR.
(550) Although this example describes one protocol for domesticating the microbe and introducing genetic variation into the microbe, one of ordinary skill in the art would understand that the genetic variation can be introduced into the selected microbes using a variety of other techniques known in the art such as: polymerase chain reaction mutagenesis, oligonucleotide-directed mutagenesis, saturation mutagenesis, fragment shuffling mutagenesis, homologous recombination, ZFN, TALENS, CRISPR systems (Cas9, Cpf1, etc.), chemical mutagenesis, and combinations thereof.
(551) 8. Iterate Upon Steps C2-C7
(552) If any of the steps C2-C7 fail to provide the intended outcome, the steps will be repeated to design an alternative vector that may comprise different elements for facilitating incorporation of desired genetic variations and markers into the host microbe.
(553) 9. Develop a Standard Operating Procedure (SOP)

(554) Once the steps C2-C7 can be reproduced consistently for a given strain, the steps will be used to develop a standard operating procedure (SOP) for that strain and vector. This SOP can be used to improve other plant-beneficial traits of the microbe.
(555) D. Non-Intergeneric Engineering Campaign and Optimization
(556) 1. Identify Gene Targets for Optimization
(557) Selected microbes will be engineered/remodeled to improve performance of the plant-beneficial activity. For this, gene targets for improving the plant-beneficial activity will be identified.
(558) Gene targets can be identified in various ways. For example, genes of interest can be identified while annotating the genes from the whole genome sequencing of isolated microbes. They can be identified through a literature search. For example, genes involved in nitrogen fixation are known in the literature. These known genes can be used as targets for introducing genetic variations. Gene targets can also be identified based on the RNA sequencing data obtained in the step B3 (small-scale field trials for colonization) or by performing RNA sequencing described in the step below.
(559) 2. Select Promoters for Promoter Swaps
(560) A desired genetic variation for improving the plant-beneficial activity can comprise promoter swapping, in which the native promoter for a target gene is replaced with a stronger or weaker promoter (when compared to the native promoter) from within the microbe's genome, or differently regulated promoter (e.g. a N-independent). If the expression of a target gene increases the plant-beneficial activity (e.g., nifA, the expression of which enhances nitrogen fixation in microbes), the desired promoter for promoter swapping is a stronger promoter (compared to the native promoter of the target gene) that would further increase the expression level of the target gene compared to the native promoter. If the expression of a target gene decreases the plant-beneficial activity (e.g., nifL that downregulates nitrogen fixation), the desired promoter for promoter swapping is a weak promoter (compared to the native promoter of the target gene) that would substantially decrease the expression level of the target gene compared to the native promoter. Promoters can be inserted into genes to “knock-out” a gene's expression, while at the same time upregulating the expression of a downstream gene.
(561) Promoters for promoter swapping can be selected based on the RNA sequencing data. For example, the RNA sequencing data can be used to identify strong and weak promoters, or constitutively active vs. inducible promoters.
(562) For example, to identify strong and weak promoters, or constitutively active vs. inducible promoters, in the nitrogen fixation pathway, selected microbes will be cultured in vitro under nitrogen-depleted and nitrogen-replete conditions; RNA of the microbe will be isolated from these cultures; and sequenced.
(563) In one protocol, the RNA profile of the microbe under nitrogen-depleted and nitrogen-replete conditions will be compared and active promoters with a desired transcription level will be identified. These promoters can be selected to swap a weak promoter.
(564) Promoters can also be selected using the RNA sequencing data obtained in the step B3 that reflects the RNA profile of the microbe in planta in the host plant rhizosphere.
(565) RNA sequencing under various conditions allows for selection of promoters that: a) are active in the rhizosphere during the host plant growth cycle in fertilized field conditions, and b) are also active in relevant in vitro conditions so they can be rapidly screened.
(566) In an exemplary protocol, in planta RNA sequencing data from colonization assays (e.g. step B3) is used to measure the expression levels of genes in isolated microbes. In one embodiment, the level of gene expression is calculated as reads per kilobase per million mapped reads (RPKM). The expression level of various genes is compared to the expression level of a target gene and at least the top 10, 20, 30, 40, 50, 60, or 70 promoters, associated with the various genes, that show the highest or lowest level of expression compared to the target gene are selected as possible

candidates for promoter swapping. Thus, one looks at expression levels of various genes relative to a target gene and then selects genes that demonstrate increased expression relative to a target (or standard) gene and then find the promoters associated with said genes.
(567) For example, if the target gene is upregulation of nifA, the first 10, 20, 30, 40, 50, or 60 promoters for genes that show the highest level of expression compared to nifA are selected as possible candidates for promoter swapping.
(568) These candidates can be further short-listed based on in vitro RNA sequencing data. For example, for nifA as the target gene, possible promoter candidates selected based on the in planta RNA sequencing data are further selected by choosing promoters with similar or increased gene expression levels compared to nifA under in vitro nitrogen-deplete vs. nitrogen-replete conditions.
(569) The set of promoters selected in this step are used to swap the native promoter of the target gene (e.g. nifA). Remodeled strains with swapped promoters are tested in in vitro assays; strains with lower than expected activity are eliminated; and strains with expected or higher than expected activity are tested in field. The cycle of promoter selection may be repeated on remodeled strains to further improve their plant-beneficial activity.
(570) Described here is an exemplary promoter swap experiment that was carried out based on in planta and in vitro RNA sequencing data from *Klebsiella variicola* strain, CI137 to improve the nitrogen fixation trait. CI137 was analyzed in ARA assays at 0 mM and 5 mM glutamine concentration and RNA was extracted from these ARA samples. The RNA was sequenced via NextSeq and a subset of reads from one sample was mapped to the CI137 genome (in vitro RNA sequencing data). RNA was extracted from the roots of corn plants at V5 stage in the colonization and activity assay (e.g. step B3) for CI137. Samples from 6 plants were pooled; the RNA from the pooled sample was sequenced using NextSeq, and reads were mapped to the CI137 genome (in planta RNA sequencing data). Out of 2×10.sup.8 total reads, 7×10.sup.4 reads mapped to CI137. In planta RNA sequencing data was used to rank genes in order of in planta expression levels and the expression levels were compared to the native nifA expression level. The first 40 promoters that showed the highest expression level (based on gene expression) compared to the native nifA expression level were selected. These 40 promoters were further short-listed based on the in vitro RNA sequencing data, where promoters with increased or similar in vitro expression levels compared to nifA were selected. The final list of promoters included 17 promoters and 2 versions of most promoters were used to generate promoter swap mutants; thus a total of 30 promoters were tested. Generation of a suite of CI137 mutants where nifL was deleted partially or completely and the 30 promoters inserted (ΔnifL::Prm) was attempted. 28 out of 30 mutants were generated successfully. The ΔnifL::Prm mutants were analyzed in ARA assays at 0 mM and 5 mM glutamine concentration and RNA was extracted from these ARA samples. Several mutants showed lower than expected or decreased ARA activity compared to the WT CI137 strain. A few mutants showed higher than expected ARA activity.
(571) A person of ordinary skill in the art would appreciate from the above example that while in planta and/or in vitro RNA sequencing data can be used to select promoters for promoter swapping, the step of promoter selection is highly unpredictable and involves many challenges.
(572) For example, in planta RNA sequencing mainly reveals the genes that are highly expressed; however, it is difficult to detect fine differences in gene expression and/or genes with low expression levels. For instance, in some in planta RNA sequencing experiments, only about 40 out of about 5000 genes from a microbial genome were detected. Thus, in planta RNA sequencing technique is useful to identify abundantly expressed genes and their corresponding promoters; however, the technique has difficulty in identifying low expression genes and corresponding promoters and small differences between gene expression.
(573) Furthermore, in planta RNA profile reflects the status of the genes at the time the microbes were isolated; however, a slight change in the field conditions can substantially change the RNA profile of rhizosphere/epiphytic/endophytic microbes. Therefore, it is difficult to predict in advance

whether the promoters selected based on one field trial RNA sequencing data would provide desirable expression levels of the target gene when remodeled strains are tested in vitro and in field.
(574) Additionally, in planta evaluation is time and resource-consuming; therefore, in planta experiments cannot be conducted often and/or repeated quickly or easily. On the other hand, while in vitro RNA sequencing can be conducted relatively quickly and easily, the in vitro conditions do not mimic the field conditions and promoters that may show high activity in vitro may not show comparable activity in planta.
(575) Moreover, promoters often don't behave as predicted in a new context. Therefore, in planta and in vitro RNA sequencing data can at best serve as a starting point in the step of promoter selection; however, arriving at any particular promoter that would provide desirable expression levels of the target gene in the field is, in some instances, unpredictable.
(576) Another limitation in the step of promoter selection is the number of available promoters. Because one of the goals of the present invention is to provide non-transgenic microbes; promoters for promoter swapping need to be selected from within the microbe's genome, or genus. Thus, unlike a transgenic approach, the present process can not merely go out into the literature and find/use a well characterized transgenic promoter from a different host organism.
(577) Another constraint is that the promoter must be active in planta during a desired growth phase. For example, the highest requirement for nitrogen in plants is generally late in the growing season, e.g. late vegetative and early reproductive phases. For example, in corn, nitrogen uptake is the highest during V6 (6 leaves) through R1 (reproductive stage 1) stages. Therefore, to increase the availability of nitrogen during V6 through R1 stages of corn, remodeled microbes must show highest nitrogen fixation activity during these stages of the corn lifecycle. Accordingly, promoters that are active in planta during the late vegetative and early reproductive stages of corn need to be selected. This constraint not only reduces the number of promoters that may be tested in promoter swapping, but also make the step of promoter selection unpredictable. As discussed above, unpredictability arises, in part, because although the RNA sequencing data from small scale field trials (e.g. step B3) may be used to identify promoters that are active in planta during a desired growth stage, the RNA data is based on the field conditions (e.g., type of soil, level of water in the soil, level of available nitrogen, etc.) at the time of sample collection. As one of ordinary skill in the art would understand, the field conditions may change over the period of time within the same field and also change substantially across various fields. Thus, the promoters selected under one field condition may not behave as expected under other field conditions. Similarly, selected promoters may not behave as expected after swapping. Therefore, it is difficult to anticipate in advance whether the selected promoters would be active in planta during a desired growth phase of a plant of interest.
(578) 3. Design Non-Intergeneric Genetic Variations
(579) Based on steps D1 (identification of gene targets) and D2 (identification of promoters for promoter swaps), non-intergeneric genetic variations will be designed.
(580) The term “non-intergeneric” indicates that the genetic variation to be introduced into the host does not contain a nucleic acid sequence from outside the host genus (i.e., no transgenic DNA). Although vectors and/or other genetic tools will be used to introduce the genetic variation into the host microbe, the methods of the present disclosure include steps to loop-out (remove) the backbone vector sequences or other genetic tools introduced into the host microbe leaving only the desired genetic variation into the host genome. Thus, the resulting microbe is non-transgenic.
(581) Exemplary non-intergeneric genetic variations include a mutation in the gene of interest that may improve the function of the protein encoded by the gene; a constitutionally active promoter that can replace the endogenous promoter of the gene of interest to increase the expression of the gene; a mutation that will inactivate the gene of interest; the insertion of a promoter from within the host's genome into a heterologous location, e.g. insertion of the promoter into a gene that results in

inactivation of said gene and upregulation of a downstream gene; and the like. The mutations can be point mutations, insertions, and/or deletions (full or partial deletion of the gene). For example, in one protocol, to improve the nitrogen fixation activity of the host microbe, a desired genetic variation may comprise an inactivating mutation of the nifL gene (negative regulator of nitrogen fixation pathway) and/or comprise replacing the endogenous promoter of the nifH gene (nitrogenase iron protein that catalyzes a key reaction to fix atmospheric nitrogen) with a constitutionally active promoter that will drive the expression of the nifH gene constitutively.
(582) 4. Generate Non-Intergeneric Derivative Strains
(583) After designing the non-intergeneric genetic variations, steps C2-C7 will be carried out to generate non-intergeneric derivative strains (i.e. remodeled microbes).
(584) 5. Bank a Purified Culture of the Remodeled Microbe
(585) A purified culture of the remodeled microbe will be preserved in a bank, so that gDNA can be extracted for whole genome sequencing described below.
(586) 6. Confirm Presence of the Desired Genetic Variation
(587) The genomic DNA of the remodeled microbe will be extracted and the whole genome sequencing will be performed on the genomic DNA using methods described previously. The resulting reads will be mapped to the reads previously stored in LIMS to confirm: a) presence of the desired genetic variation, and b) complete absence of reads mapping to vector sequences (e.g. plasmid backbone or helper plasmid sequence) that were used to generate the remodeled microbe.
(588) This step allows sensitive detection of non-host genus DNA (transgenic DNA) that may remain in the strain after looping out of the vector backbone (e.g. suicide plasmid) method and could provide a control for accidental off-target insertion of the genetic variation, etc.
(589) E. Analytics Upon Remodeled Microbes
(590) 1. Analysis of the Plant-Beneficial Activity
(591) The plant-beneficial activity and growth kinetics of the remodeled microbes will be assessed in vitro.
(592) For example, strains remodeled for improving nitrogen fixation function will be assessed for nitrogen fixation activity and fitness through acetylene reduction assays, ammonium excretion assays, etc.
(593) Strains remodeled for improved phosphate solubilization will be assessed for the phosphate solubilization activity.
(594) This step allows rapid, medium to high throughput screening of remodeled strains for the phenotypes of interest.
(595) 2. Analysis of Colonization and Transcription of the Altered Genes
(596) Remodeled strains will be assessed for colonization of the host plant either in the greenhouse or in the field using the steps described in B3. Additionally, RNA will be isolated from colonized root and/or soil samples and sequenced to analyze the transcriptional activity of target genes. Target genes comprise the genes containing the genetic variation introduced and may also comprise other genes that play a role in the plant-beneficial trait of the microbe.
(597) For example, a cluster of genes, the nif genes, controls the nitrogen fixation activity of microbes. Using the protocol described above, a genetic variation may be introduced into one of the nif genes (e.g. a promoter insertion), whereas the other genes in the nif cluster are in their endogenous form (i.e. their gene sequence and/or the promoter region is not altered). The RNA sequencing data will be analyzed for the transcriptional activity of the nif gene containing the genetic variation and may also be analyzed for other nif genes that are not altered directly, by the inserted genetic change, but nonetheless may be influenced by the introduced genetic change.
(598) This step allows determination of the fitness of top in vitro performing strains in the rhizosphere and allows measurement of the transcriptional activity of altered genes in planta.
(599) F. Iterate Engineering Campaign/Analytics
(600) The data from in vitro and in planta analytics (steps E1 and E2) will be used to iteratively

stack beneficial mutations.

(601) Furthermore, steps A-E described above may be repeated to fine tune the plant-beneficial traits of the microbes. For example, plants will be inoculated using microbial strains remodeled in the first round; harvested after a few weeks of growth; and microbes from the soil and/or roots of the plants will be isolated. The functional activity (plant-beneficial trait and colonization potential) and the DNA and RNA profile of isolated microbes will be characterized, in order to select microbes with improved plant-beneficial activity and colonization potential. The selected microbes will be remodeled to further improve the plant-beneficial activity. Remodeled microbes will be screened for the functional activity (plant-beneficial trait and colonization potential) and RNA profile in vitro and in planta and the top performing strains will be selected. If desired, steps A-E can be repeated to further improve the plant-beneficial activity of the remodeled microbes from the second round. The process can be repeated for 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, or more rounds.

(602) The exemplary steps described above are summarized in Table A below.

(603) TABLE-US-00025 TABLE A An Overview of an Embodiment of the Guided Microbial Remodeling Platform Steps Contribution Alternate Forms A Isolation 1 Obtain a soil sample Provides WT soil microbes to be isolated 2 Grow corn “bait Allows selection of plant- Wheat, sorghum, rice, millet, plants” in soil sample beneficial microbes by soybean, etc. rhizosphere 3 Harvest, clean and Down-select soil microbes Other nitrogen-free media, other extract root sample to those that a) colonize selective or screening media and plate on nitrogen- the root and b) fix (e.g. for phosphate free (specifically atmospheric nitrogen solubilization) NfB) media 4 Pick colonies, purify Down-select microbes to Degenerate primers for other cultures and screen those containing the nifH genes of interest, e.g. ipdC for presence of nifH gene (eliminate false- (phytohormone biosynthesis) using degenerate positives from media primers screen) 5 Bank a purified culture of the strain B Characterization 1 Sequence and Characterize genome for assemble the genome key pathways of the strain using Illumina and/or PacBio platform 2 Assay the microbe for Down-select for microbes Wheat, sorghum, rice, millet, colonization of corn that colonize the plant well soybean, etc., other methods for roots in the assaying colonization (e.g. greenhouse (qPCR- plating) based method) 3 Assay the microbe for Known internally as Larger field trials, other crops, colonization of corn “CAT” trials, these other methods for assaying roots in a small-scale provide Colonization And colonization (e.g. plating) field trials (qPCR- Transcript data for the based method) and strain in a field isolate RNA from environment colonized root samples 4 Assay the microbe for Confirm N-fixation nitrogen fixation phenotype of strain activity in an acetylene reduction assay (ARA) 5 Use the above data to Allows selection of select candidate greatest-potential microbe for further candidates domestication and optimization C Domestication 1 Test microbes for Determine which sensitivity to various antibiotic selection antibiotics markers can be used to transform genetic tools 2 Design and build a These are the “parts” Plasmid could contain a SceI site suicide plasmid necessary to maintain the or other counter-selectable containing an plasmid and carry out marker, alternate fluorescent appropriate antibiotic conjugation, insertion and reporters, additional elements resistance marker, “loop-out” of the hose sacB counter- genome selectable marker, origin of replication for maintenance in *E. coli*, GFP to screen for insertion through fluorescence, origin of transfer for conjugation into the host, homology arms to the host genome, and the desired mutation. 3 Transform suicide Preparation for Could use a different donor plasmid into *E. coli* conjugation into host; strain of *E. coli* or other ST18 (an auxotroph plasmid maintenance microbe; different auxotrophic for aminolevulinic marker acid, ALA) to generate donor cells 4 Mix donor cells with The suicide plasmid is able Could use a different donor recipient host cells to to replicate in *E. coli* but strain of *E. coli* or other conjugate, and plate not in the host. Therefore, microbe; different auxotrophic on media selecting for plating of the mixture on marker the antibiotic such plates means that resistance marker and only host cells that NOT containing ALA received the plasmid and experience plasmid integration into the chromosome will be able to grow and form colonies. The *E coli* ST18 is unable to grow due to the absence of ALA. 5 Confirm

integration Confirms proper of the plasmid integration of the suicide through GFP plasmid backbone fluorescence, and containing GFP, the integration at the antibiotic resistance intended locus cassette, the sacB marker, through colony PCR etc. 6 Streak confirmed The sacB marker confers Different counter selectable integration colony on sensitivity to sucrose; marker, SceI-mediated loop-out, a plate containing colonies which have etc. sucrose and screen for undergone a second round non-fluorescent of homologous colonies recombination and “looped-out” the plasmid will grow better and not fluoresce on the plate. 7 Screen looped-out Upon the second colonies for the homologous intended mutation recombination event only using colony PCR 50% of looped out colonies should contain the mutation, the other 50% will be WT 8 If any of the steps 2-7 Allows iterative fail, go back to step 2 troubleshooting of suicide and re-design with plasmid to develop a alternate plasmid working protocol parts 9 Once steps 2-7 can be reliably performed, develop an SOP for that strain/plasmid to be used for Optimization D Non-Intergeneric Engineering Campaign and Optimization 1 Identify gene targets for optimizing a pathway, e.g. nif genes through literature search 2 Select promoters for Allows for selection of Alternate crops; alternate promoter swaps using promoters that a) are RNAseq data conditions RNAseq data active in the rhizosphere (greenhouse, field, in vitro, collected both in vitro during the corn growth whatever's relevant for the in N-depleted and N- cycle in fertilized field phenotype targeted) replete conditions, conditions b) are also and in planta from active in in vitro N-replete the corn rhizosphere conditions so they can be (Collected in step B3) rapidly screened. 3 Design non- No DNA from outside the Alter regulatory sequences (e.g. intergeneric host chromosome is added, RBS), non-coding RNAs, etc. mutations in key therefore the resulting genes: deletions (full microbe is non-transgenic or partial gene), promoter swaps, or single base pair changes; store these designs in our LIMS 4 Using the established We perform this in higher protocol, carry out throughput than the steps C2-7 to generate domestication step - up to non-intergeneric 20 or so strains at once per derivative strains person. (mutants) 5 Bank a purified culture of the strain, extract gDNA and conduct WGS via Illumina 6 Map the resulting Allows very sensitive Suicide plasmid removal is fairly reads to the designs detection of non- reliable; however use of other stored in LIMS to intergeneric DNA that stable plasmids in alternate confirm a) presence may remain in the strain methods necessitates this extra of the desire mutation after the suicide plasmid step to ensure with complete and b) complete method; confirm absence confidence that no transgenic absence of reads of transgenic DNA, DNA that was previously mapping to any controls for accidental off- transformed in remains in the suicide plasmid or target insertion of the strain. other plasmid suicide plasmid, etc. sequences used to generate the strains E Analytics 1 Analyze the strains Allow rapid, med- to high- Any other in vitro assay, e.g. for in vitro nitrogen throughput screening of phosphate solubilization, qPCR fixation activity and mutants for phenotypes of for transcription of specific fitness through ARA, interest genes, etc. ammonium excretion assays, and growth curves 2 Analyze the strains Measure fitness of top in for colonization vitro performing strains in (qPCR) and the rhizosphere; measure transcription of target transcription of promoter- and promoter- swapped genes in planta swapped genes (Nanostring) in the plant (greenhouse or field) F Iterate Engineering Campaign/Analytics 1 Use data from in vitro and in planta analytics to iteratively stack beneficial mutations.

Traditional Approaches to Creating Biologicals for Agriculture Suffer from Drawbacks Inherent in their Methodology

(604) Unlike pure bioprospecting of wild-type (WT) microbes or transgenic approaches, GMR allows for non-intergeneric genetic optimization of key regulatory networks within the microbe, which improves plant-beneficial phenotypes over WT microbes, but doesn't have the risks associated with transgenic approaches (e.g. unpredictable gene function, public concerns). See, FIG. **1**C for a depiction of a problematic “traditional bioprospecting” approach, which has several drawbacks compared to the taught GMR platform.

(605) Other methods for developing microbials for agriculture are focused on either extensive lab development, which often fails at the field scale, or extensive greenhouse or “field-first” testing

without an understanding of the underlying mechanisms/plant-microbe interactions. See, FIG. **1**D for a depiction of a problematic “field-first approach to bioprospecting” system, which has several drawbacks compared to the taught GMR platform.

(606) The GMR Platform Solves These Problems in Numerous Ways

(607) One strength of the GMR platform is the identification of active promoters, which are active at key physiologically important times for a target crop, and which are also active under particular, agriculturally relevant, environmental conditions.

(608) As has been explained, within the context of nitrogen fixation, the GMR platform is able to identify microbial promoter sequences, which are active under environmental conditions of elevated exogenous nitrogen, which thereby allows the remodeled microbe to fix atmospheric nitrogen and deliver it to a target crop plant, under modern agricultural row crop conditions, and at a time when a plant needs the fixed nitrogen the most. See, FIG. **1**E for a depiction of the time period in the corn growth cycle, at which nitrogen is needed most by the plant. The taught GMR platform is able to create remodeled microbes that supply nitrogen to a corn plant at the time period in which the nitrogen is needed, and also deliver such nitrogen even in the presence of exogenous nitrogen in the soil environment.

(609) These promoters can be identified by rhizosphere RNA sequencing and read mapping to the microbe's genome sequence, and key pathways can be “reprogrammed” to be turned on or off during key stages of the plant growth cycle. Additionally, through whole genome sequencing of optimized microbes and mapping to previously-transformed sequences, the method has the ability to ensure that no transgenic sequences are accidentally released into the field through off-target insertion of plasmid DNA, low-level retention of plasmids not detected through PCR or antibiotic resistance, etc.

(610) The GMR platform combines these approaches by evaluating microbes iteratively in the lab and plant environment, leading to microbes that are robust in greenhouse and field conditions rather than just in lab conditions.

(611) Various aspects and embodiments of the taught GMR platform can be found in FIGS. **1**F-**1**I. The GMR platform culminates in the derivation/creation/production of remodeled microbes that possess a plant-beneficial property, e.g. nitrogen fixation.

(612) The traditional bioprospecting methods are not able to produce microbes having the aforementioned properties.

(613) Properties of a Microbe Remodeled for Nitrogen Fixation

(614) In the context of remodeling microbes for nitrogen fixation, there are several properties that the remodeled microbe may possess. For instance, FIG. **1**J depicts 5 properties that can be possessed by remodeled microbes of the present disclosure.

(615) Furthermore, as can be seen in Example 2, the present inventors have utilized the GMR platform to produce remodeled non-intergeneric bacteria (i.e. *Kosakonia sacchari*) capable of fixing atmospheric nitrogen and delivering said nitrogen to a corn plant, even under conditions in which exogenous nitrogen is present in the environment. See, FIG. **1**K-M, which illustrate that the remodeling process successfully: (1) decoupled nifA expression from endogenous nitrogen regulation; and (2) improved the assimilation and excretion of fixed nitrogen.

(616) These remodeled microbes ultimately result in corn yield improvement, when applied to corn crops. See, FIG. **1**N.

(617) The GMR Platform Provides an Approach to Nitrogen Fixation and Delivery That Solves Pressing Environmental Concerns

(618) As explained previously, the nitrogen fertilizer produced by the industrial Haber-Bosch process is not well utilized by the target crop. Rain, runoff, heat, volatilization, and the soil microbiome degrade the applied chemical fertilizer. This equates to not only wasted money, but also adds to increased pollution instead of harvested yield. To this end, the United Nations has calculated that nearly 80% of fertilizer is lost before a crop can utilize it. Consequently, modern

agricultural fertilizer production and delivery is not only deleterious to the environment, but it is extremely inefficient. See, FIG. **1**O, illustrating the inefficiency of current nitrogen delivery systems, which result in underfertilized fields, over fertilized fields, and environmentally deleterious nitrogen runoff.

(619) The current GMR platform, and resulting remodeled microbes, provide a better approach to nitrogen fixation and delivery to plants. As will be seen in the below Examples, the non-intergeneric remodeled microbes of the disclosure are able to colonize the roots of a corn plant and spoon feed said corn plants with fixed atmospheric nitrogen, even in the presence of exogenous nitrogen. This system of nitrogen fixation and delivery—enabled by the taught GMR platform—will help transform modern agricultural to a more environmentally sustainable system.

Example 2: Guided Microbial Remodeling—an Example Embodiment for the Rational Improvement of Nitrogen Fixation

(620) A diversity of nitrogen fixing bacteria can be found in nature, including in agricultural soils. However, the potential of a microbe to provide sufficient nitrogen to crops to allow decreased fertilizer use may be limited by repression of nitrogenase genes in fertilized soils as well as low abundance in close association with crop roots. Identification, isolation and breeding of microbes that closely associate with key commercial crops might disrupt and improve the regulatory networks linking nitrogen sensing and nitrogen fixation and unlock significant nitrogen contributions by crop-associated microbes. To this end, nitrogen fixing microbes that associate with and colonize the root system of corn were identified. This step corresponds to the “Measure the Microbiome Composition” depicted in FIG. **1**A and FIG. **1**B.

(621) Root samples from corn plants grown in agronomically relevant soils were collected, and microbial populations extracted from the rhizosphere and endosphere. Genomic DNA from these samples was extracted, followed by 16S amplicon sequencing to profile the community composition.

(622) A *Kosakonia sacchari* microbe (strain PBC6.1) was isolated and classified through 16S rRNA and whole genome sequencing. This is a particularly interesting nitrogen fixer capable of colonizing to nearly 21% abundance of the root-associated microbiota (FIG. **2**). To assess strain sensitivity to exogenous nitrogen, nitrogen fixation rates in pure culture were measured with the classical acetylene reduction assay (ARA) and varying levels of glutamine supplementation. The species exhibited a high level of nitrogen fixing activity in nitrogen-free media, yet exogenous fixed nitrogen repressed nif gene expression and nitrogenase activity (Strain PBC6.1, FIG. **3**C, FIG. **3**D). Additionally, when released ammonia was measured in the supernatant of PBC6.1 grown in nitrogen-fixing conditions, very little release of fixed nitrogen could be detected (FIG. **3**E).

(623) We hypothesized that PBC6.1 could be a significant contributor of fixed nitrogen in fertilized fields if regulatory networks controlling nitrogen metabolism were remodeled to allow optimal nitrogenase expression and ammonia release in the presence of fixed nitrogen.

(624) Sufficient genetic diversity should exist within the PBC6.1 genome to enable broad phenotypic remodeling (as a result of remodeling the underlying genetic architecture in a non-intergeneric manner) without the insertion of transgenes or synthetic regulatory elements. The isolated strain has a genome of at least 5.4 Mbp and a canonical nitrogen fixation gene cluster. Related nitrogen metabolism pathways in PBC6.1 are similar to those of the model organism for nitrogen fixation, *Klebsiella oxytoca* m5al.

(625) Several gene regulatory network nodes were identified which may augment nitrogen fixation and subsequent transfer to a host plant, particularly in high exogenous concentrations of fixed nitrogen (FIG. **3**A). The nifLA operon directly regulates the rest of the nif cluster through transcriptional activation by NifA and nitrogen- and oxygen-dependent repression of NifA by NifL. Disruption of nifL can abolish inhibition of NifA and improve nif expression in the presence of both oxygen and exogenous fixed nitrogen. Furthermore, expressing nifA under the control of a nitrogen-independent promoter may decouple nitrogenase biosynthesis from regulation by the

NtrB/NtrC nitrogen sensing complex.
(626) The assimilation of fixed nitrogen by the microbe to glutamine by glutamine synthetase (GS) is reversibly regulated by the two-domain adenylyltransferase (ATase) enzyme GlnE through the adenylylation and deadenylylation of GS to attenuate and restore activity, respectively. Truncation of the GlnE protein to delete its adenylyl-removing (AR) domain may lead to constitutively adenylylated glutamine synthetase, limiting ammonia assimilation by the microbe and increasing intra- and extracellular ammonia.
(627) Finally, reducing expression of AmtB, the transporter responsible for uptake of ammonia, could lead to greater extracellular ammonia.
(628) To generate rationally designed microbial phenotypes without the use of transgenes, two approaches were employed to remodel the underlying genetic architecture of the microbe: (1) creating markerless deletions of genomic sequences encoding protein domains or whole genes, and (2) rewiring regulatory networks by intragenomic promoter rearrangement.
(629) Through an iterative remodeling process, several non-transgenic derivative strains of PBC6.1 were generated (Table 25).
(630) TABLE-US-00026 TABLE 25 List of isolated and derivative *K. sacchari* strains used in this work. Prm, promoter sequence derived from the PBC6.1 genome; Δ glnE.sub.AR1 and ΔglnE.sub.AR2. different truncated versions of glnE gene removing the adenylyl-removing domain sequence. Strain ID Genotype PBC6.1 WT PBC6.14 ΔnifL::Prm1 PBC6.15 ΔnifL::Prm5 PBC6.22 ΔnifL::Prm3 PBC6.37 ΔnifL::Prm1 ΔglnE.sub.AR2 PBC6.38 ΔnifL::Prm1 ΔglnE.sub.AR1 PBC6.93 ΔnifL::Prm1 ΔglnE.sub.AR2 ΔamtB PBC6.94 ΔnifL::Prm1 ΔglnE.sub.AR1 ΔamtB
(631) Several in vitro assays were performed to characterize specific phenotypes of the derivative strains. The ARA was used to assess strain sensitivity to exogenous nitrogen, in which PBC6.1 exhibited repression of nitrogenase activity at high glutamine concentrations (FIG. **3**D). In contrast, most derivative strains showed a derepressed phenotype with varying levels of acetylene reduction observed at high glutamine concentrations. Transcriptional rates of nifA in samples analyzed by qPCR correlated well with acetylene reduction rates (FIG. **4**), supporting the hypothesis that nifL disruption and insertion of a nitrogen-independent promoter to drive nifA can lead to nif cluster derepression.
(632) Strains with altered GlnE or AmtB activity showed markedly increased ammonium excretion rates compared to wild type or derivative strains without these mutations (FIG. **3**E), illustrating the effect of these genotypes on ammonia assimilation and reuptake.
(633) Two experiments were performed to study the interaction of PBC6.1 derivatives (remodeled microbes) with corn plants and quantify incorporation of fixed nitrogen into plant tissues. First, rates of microbial nitrogen fixation were quantified in a greenhouse study using isotopic tracers. Briefly, plants are grown with 15N labeled fertilizer, and diluted concentrations of 15N in plant tissues indicate contributions of fixed nitrogen from microbes. Corn seedlings were inoculated with selected microbial strains, and plants were grown to the V6 growth stage. Plants were subsequently deconstructed to enable measurement of microbial colonization and gene expression as well as measurement of 15N/14N ratios in plant tissues by isotope ratio mass spectrometry (IRMS). Analysis of the aerial tissue showed a small, nonsignificant contribution by PBC6.38 to plant nitrogen levels, and a significant contribution by PBC6.94 ($p=0.011$). Approximately 20% of the nitrogen found in above-ground corn leaves was produced by PBC6.94, with the remainder coming from the seed, potting mix, or “background” fixation by other soilborne microbes (FIG. **5**C). This illustrates that our microbial breeding and remodeling pipeline can generate remodeled strains capable of making significant nitrogen contributions to plants in the presence of nitrogen fertilizer. Microbial transcription within plant tissues was measured, and expression of the nif gene cluster was observed in derivative remodeled strains, but not the wild type strain (FIG. **5**B), showing the importance of nif derepression for contribution of BNF to crops in fertilized conditions. Root colonization measured by qPCR demonstrated that colonization density is different for each of the

strains tested (FIG. **5**A). A 50 fold difference in colonization was observed between PBC6.38 and PBC6.94. This difference could be an indication that PBC6.94 has reduced fitness in the rhizosphere relative to PBC6.38 as a result of high levels of fixation and excretion.

Methods

(634) Media

(635) Minimal medium contains (per liter) 25 g Na.sub.2HPO.sub.4, 0.1 g CaCL.sub.2-2H.sub.2O, 3 g KH.sub.2PO.sub.4, 0.25 g MgSO.sub.4.Math.7H.sub.2O, 1 g NaCl, 2.9 mg FeCl.sub.3, 0.25 mg Na.sub.2MoO.sub.4.Math.2H.sub.2O, and 20 g sucrose. Growth medium is defined as minimal medium supplemented with 50 ml of 200 mM glutamine per liter.

(636) Isolation of Diazotrophs

(637) Corn seedlings were grown from seed (DKC 66-40, DeKalb, IL) for two weeks in a greenhouse environment controlled from 22° C. (night) to 26° C. (day) and exposed to 16 hour light cycles in soil collected from San Joaquin County, CA. Roots were harvested and washed with sterile deionized water to remove bulk soil. Root tissues were homogenized with 2 mm stainless steel beads in a tissue lyser (TissueLyser II, Qiagen P/N 85300) for three minutes at setting 30, and the samples were centrifuged for 1 minute at 13,000 rpm to separate tissue from root-associated bacteria. Supernatants were split into two fractions, and one was used to characterize the microbiome through 16S rRNA amplicon sequencing and the remaining fraction was diluted and plated on Nitrogen-free Broth (NfB) media supplemented with 1.5% agar. Plates were incubated at 30° C. for 5-7 days. Colonies that emerged were tested for the presence of the nifH gene by colony PCR with primers Ueda19f and Ueda406r. Genomic DNA from strains with a positive nifH colony PCR was isolated (QIAamp DNA Mini Kit, Cat No. 51306, QIAGEN, Germany) and sequenced (Illumina MiSeq v3, SeqMatic, Fremont, CA). Following sequence assembly and annotation, the isolates containing nitrogen fixation gene clusters were utilized in downstream research.

(638) Microbiome Profiling of Isolation Seedlings

(639) Genomic DNA was isolated from root-associated bacteria using the ZR-96 Genomic DNA I Kit (Zymo Research P/N D3011), and 16S rRNA amplicons were generated using nextera-barcoded primers targeting 799f and 1114r. The amplicon libraries were purified and sequenced with the Illumina MiSeq v3 platform (SeqMatic, Fremont, CA). Reads were taxonomically classified using Kraken using the minikraken database (FIG. **2**).

(640) Acetylene Reduction Assay (ARA)

(641) A modified version of the Acetylene Reduction Assay was used to measure nitrogenase activity in pure culture conditions. Strains were propagated from single colony in SOB (RPI, P/N S25040-1000) at 30° C. with shaking at 200 RPM for 24 hours and then subcultured 1:25 into growth medium and grown aerobically for 24 hours (30° C., 200 RPM). 1 ml of the minimal media culture was then added to 4 ml of minimal media supplemented with 0 to 10 mM glutamine in air-tight Hungate tubes and grown anaerobically for 4 hours (30° C., 200 RPM). 10% headspace was removed then replaced by an equal volume of acetylene by injection, and incubation continued for 1 hr. Subsequently, 2 ml of headspace was removed via gas tight syringe for quantification of ethylene production using an Agilent 6850 gas chromatograph equipped with a flame ionization detector (FID).

(642) Ammonium Excretion Assay

(643) Excretion of fixed nitrogen in the form of ammonia was measured using batch fermentation in anaerobic bioreactors. Strains were propagated from single colony in 1 ml/well of SOB in a 96 well DeepWell plate. The plate was incubated at 30° C. with shaking at 200 RPM for 24 hours and then diluted 1:25 into a fresh plate containing 1 ml/well of growth medium. Cells were incubated for 24 hours (30° C., 200 RPM) and then diluted 1:10 into a fresh plate containing minimal medium. The plate was transferred to an anaerobic chamber with a gas mixture of >98.5% nitrogen, 1.2-1.5% hydrogen and <30 ppM oxygen and incubated at 1350 RPM, room temperature for 66-70 hrs. Initial culture biomass was compared to ending biomass by measuring optical density at 590

nm. Cells were then separated by centrifugation, and supernatant from the reactor broth was assayed for free ammonia using the Megazyme Ammonia Assay kit (P/N K-AMIAR) normalized to biomass at each timepoint.

(644) Extraction of Root-Associated Microbiome

(645) Roots were shaken gently to remove loose particles, and root systems were separated and soaked in a RNA stabilization solution (Thermo Fisher P/N AM7021) for 30 minutes. The roots were then briefly rinsed with sterile deionized water. Samples were homogenized using bead beating with ½-inch stainless steel ball bearings in a tissue lyser (TissueLyser II, Qiagen P/N 85300) in 2 ml of lysis buffer (Qiagen P/N 79216). Genomic DNA extraction was performed with ZR-96 Quick-gDNA kit (Zymo Research P/N D3010), and RNA extraction using the RNeasy kit (Qiagen P/N 74104).

(646) Root Colonization Assay

(647) Four days after planting, 1 ml of a bacterial overnight culture (approximately 10.sup.9 cfu) was applied to the soil above the planted seed. Seedlings were fertilized three times weekly with 25 ml modified Hoagland's solution supplemented with 0.5 mM ammonium nitrate. Four weeks after planting, root samples were collected and the total genomic DNA (gDNA) was extracted. Root colonization was quantified using qPCR with primers designed to amplify unique regions of either the wild type or derivative strain genome. QPCR reaction efficiency was measured using a standard curve generated from a known quantity of gDNA from the target genome. Data was normalized to genome copies per g fresh weight using the tissue weight and extraction volume. For each experiment, the colonization numbers were compared to untreated control seedlings.

(648) In Planta Transcriptomics

(649) Transcriptional profiling of root-associated microbes was measured in seedlings grown and processed as described in the Root Colonization Assay. Purified RNA was sequenced using the Illumina NextSeq platform (SeqMatic, Fremont, CA). Reads were mapped to the genome of the inoculated strain using bowtie2 using '—very-sensitive-local' parameters and a minimum alignment score of 30. Coverage across the genome was calculated using samtools. Differential coverage was normalized to housekeeping gene expression and visualized across the genome using Circos and across the nif gene cluster using DNAplotlib. Additionally, the in planta transcriptional profile was quantified via targeted Nanostring analysis. Purified RNA was processed on an nCounter Sprint (Core Diagnostics, Hayward, CA).

(650) 15N Dilution Greenhouse Study

(651) A 15N fertilizer dilution experiment was performed to assess optimized strain activity in planta. A planting medium containing minimal background N was prepared using a mixture of vermiculite and washed sand (5 rinses in DI H.sub.2O). The sand mixture was autoclaved for 1 hour at 122° C. and approximately 600 g measured out into 40 cubic inch (656 mL) pots, which were saturated with sterile DI H.sub.2O and allowed to drain 24 hours before planting. Corn seeds (DKC 66-40) were surface sterilized in 0.625% sodium hypochlorite for 10 minutes, then rinsed five times in sterile distilled water and planted 1 cm deep. The plants were maintained under fluorescent lamps for four weeks with 16-hour day length at room temperatures averaging 22° C. (night) to 26° C. (day).

(652) Five days after planting, seedlings were inoculated with a 1 ml suspension of cells drenched directly over the emerging coleoptile. Inoculum was prepared from 5 ml overnight cultures in SOB, which were spun down and resuspended twice in 5 ml PBS to remove residual SOB before final dilution to OD of 1.0 (approximately 10.sup.9 CFU/ml). Control plants were treated with sterile PBS, and each treatment was applied to ten replicate plants.

(653) Plants were fertilized with 25 ml fertilizer solution containing 2% 15N-enriched 2 mM KNO.sub.3 on 5, 9, 14, and 19 days after planting, and the same solution without KNO.sub.3 on 7, 12, 16, and 18 days after planting. The fertilizer solution contained (per liter) 3 mmol CaCl.sub.2, 0.5 mmol KH.sub.2PO.sub.4, 2 mmol MgSO.sub.4, 17.9 μmol FeSO.sub.4, 2.86 mg

H.sub.3BO.sub.3, 1.81 mg MnCl.sub.2.Math.4H.sub.2O, 0.22 mg ZnSO.sub.4.Math.7H.sub.2O, 51 μg CuSO.sub.4.Math.5H.sub.2O, 0.12 mg Na.sub.2Mo.sub.4.Math.2H.sub.2O, and 0.14 nmol NiCl.sub.2. All pots were watered with sterile DI H.sub.2O as needed to maintain consistent soil moisture without runoff.

(654) At four weeks, plants were harvested and separated at the lowest node into samples for root gDNA and RNA extraction and aerial tissue for IRMS. Aerial tissues were wiped as needed to remove sand, placed whole into paper bags and dried for at least 72 hours at 60° C. Once completely dry, total aerial tissue was homogenized by bead beating and 5-7 mg samples were analyzed by isotope ratio mass spectrometry (IRMS) for δ15N by the MBL Stable Isotope Laboratory (The Ecosystems Center, Woods Hole, MA). Percent NDFA was calculated using the following formula: % NDFA=(δ15N of UTC average—δ15N of sample)/(δ15N of UTC average)×100.

Example 3: Field Trials with Remodeled Microbes of the Disclosure—Summer 2016

(655) In order to evaluate the efficacy of remodeled strains of the present disclosure on corn growth and productivity under varying nitrogen regimes, field trials were conducted.

(656) Trials were conducted with (1) seven subplot treatments of six strains plus the control—four main plots comprised 0, 15, 85, and 100% of maximum return to nitrogen (MRTN) with local verification. The control (UTC only) was conducted with 10 100% MRTN plus, 5, 10, or 15 pounds. Treatments had four replications.

(657) Plots of corn (minimum) were 4 rows of 30 feet in length, with 124 plots per location. All observations were taken from the center two rows of the plots, and all destructive sampling was taken from the outside rows. Seed samples were refrigerated until 1.5 to 2 hours prior to use.

(658) Local Agricultural Practice: The seed was a commercial corn without conventional fungicide and insecticide treatment. All seed treatments were applied by a single seed treatment specialist to assure uniformity. Planting date, seeding rate, weed/insect management, etc. were left to local agricultural practices. With the exception of fungicide applications, standard management practices were followed.

(659) Soil Characterization: Soil texture and soil fertility were evaluated. Soil samples were pre-planted for each replicate to insure residual nitrate levels lower than 50 lbs/Ac. Soil cores were taken from 0 cm to 30 cm. The soil was further characterized for pH, CEC, total K and P.

(660) Assessments: The initial plant population was assessed 14 days after planting (DAP)/acre, and were further assessed for: (1) vigor (1 to 10 scale, w/10=excellent) 14 DAP & V10; (2) recordation of disease ratings any time symptoms are evident in the plots; (3) record any differences in lodging if lodging occurs in the plots; (4) yield (Bu/acre), adjusted to standard moisture pct; (5) test weight; and (6) grain moisture percentage.

(661) Sampling Requirements: The soil was sampled at three timepoints (prior to trial initiation, V10-VT, 1 week post-harvest). All six locations and all plots were sampled at 10 grams per sample (124 plots×3 timepoints×6 locations).

(662) Colonization Sampling: Colonization samples were collected at two timepoints (V10 and VT) for five locations and six timepoints (V4, V8, V10, VT, R5, and Post-Harvest). Samples were collected as follows: (1) from 0% and 100% MRTN, 60 plots per location; (2) 4 plants per plot randomly selected from the outside rows; (3) 5 grams of root, 8 inches of stalk, and top three leaves-bagged and IDed each separately—12/bags per plot; (4) five locations (60 plots×2 timepoints×12 bags/plot); and one location (60 plots×6 timepoints×12 bags/plot.

(663) Normalized difference vegetation index (NDVI) determination was made using a Greenseeker instrument at two timepoints (V4-V6 and VT). Assessed each plot at all six locations (124 plots×2 timepoints×6 locations).

(664) Root analysis was performed with Win Rhizo from one location that best illustrated treatment differentiation. Ten plants per plot were randomly sampled (5 adjacent from each outside row; V3-V4 stage plants were preferred) and gently washed to remove as much dirt as reasonable. Ten roots

were placed in a plastic bag and labelled. Analyzed with WinRhizo Root Analysis.
(665) Stalk Characteristics were measured at all six locations between R2 and R5. The stalk diameter of ten plants per plot at the 6″ height were recorded, as was the length of the first internode above the 6″ mark. Ten plants were monitored; five consecutive plants from the center of the two inside rows. Six locations were evaluated (124 plots×2 measures×6 locations).
(666) The tissue nitrates were analyzed from all plots and all locations. An 8″ segment of stalk beginning 6″ above the soil when the corn is between one and three weeks after black layer formation; leaf sheaths were removed. All locations and plots were evaluated (6 locations×124 plots).
(667) The following weather data was recorded for all locations from planting to harvest: daily maximum and minimum temperatures, soil temperature at seeding, daily rainfall plus irrigation (if applied), and any unusual weather events such as excessive rain, wind, cold, or heat.
(668) Yield data across all six locations is presented in Table 26. Nitrogen rate had a significant impact on yield, but strains across nitrogen rates did not. However, at the lowest nitrogen rate, strains CI006, CM029, and CI019 numerically out-yielded the UTC by 4 to 6 bu/acre. Yield was also numerically increased 2 to 4 bu/acre by strains CM029, CI019, and CM081 at 15% MRTN.
(669) TABLE-US-00027 TABLE 26 Yield data across all six locations Stalk Diameter Internode YLD (bu) Vigor_E Vigor_L (mm) Length (in) NDVI_Veg NDVI_Rep MRTN % 0 143.9 7.0 5.7 18.87 7.18 64.0 70.6 15 165.9 7.2 6.3 19.27 7.28 65.8 72.5 85 196.6 7.1 7.1 20.00 7.31 67.1 74.3 100 197.3 7.2 7.2 20.23 7.37 66.3 72.4 Strain CI006 (1) 176.6 7.2 6.6 19.56 18.78 66.1 72.3 CM029 (2) 176.5 7.1 6.5 19.54 18.61 65.4 71.9 CM038 (3) 175.5 7.2 6.5 19.58 18.69 65.7 72.8 CI019 (4) 176.0 7.1 6.6 19.51 18.69 65.5 72.9 CM081 (5) 176.2 7.1 6.6 19.57 18.69 65.8 73.1 CM029/CM081 (6) 174.3 7.1 6.6 19.83 18.79 66.2 72.5 UTC (7) 176.4 7.1 6.6 19.54 18.71 65.9 71.7 MRTN/Strain 0 1 145.6 7.0 5.6 19.07 7.12 63.5 70.3 0 2 147.0 7.0 5.5 18.74 7.16 64.4 70.4 0 3 143.9 7.0 5.5 18.83 7.37 64.6 70.5 0 4 146.0 6.9 5.7 18.86 7.15 63.4 70.7 0 5 141.7 7.0 5.8 18.82 7.05 63.6 70.9 0 6 142.2 7.2 5.8 19.12 7.09 64.7 69.9 0 7 141.2 7.0 5.8 18.64 7.32 64.0 71.4 15 1 164.2 7.3 6.1 19.09 7.21 66.1 71.5 15 2 167.3 7.2 6.3 19.32 7.29 65.5 72.7 15 3 165.6 7.3 6.3 19.36 7.23 64.8 72.5 15 4 167.9 7.3 6.4 19.31 7.51 66.1 72.3 15 5 169.3 7.2 6.2 19.05 7.32 66.0 72.8 15 6 161.9 7.1 6.3 19.45 7.20 66.2 72.2 15 7 165.1 7.3 6.4 19.30 7.18 66.0 73.3 85 1 199.4 7.3 7.2 19.70 7.32 67.2 74.0 85 2 195.1 7.1 7.2 19.99 7.09 66.5 74.4 85 3 195.0 7.0 7.0 20.05 7.26 67.3 74.6 85 4 195.6 7.2 7.1 20.04 7.29 66.4 74.4 85 5 196.4 7.2 7.0 19.87 7.39 67.3 74.5 85 6 195.1 7.0 6.9 20.35 7.34 67.4 74.4 85 7 199.5 6.9 7.2 19.97 7.48 67.4 74.1 100 1 197.1 7.2 7.3 20.38 7.68 67.5 73.4 100 2 196.5 7.0 7.1 20.11 7.21 65.3 70.2 100 3 197.6 7.5 7.3 20.08 7.42 66.3 73.4 100 4 194.6 7.1 7.1 19.83 7.40 66.1 74.1 100 5 197.4 7.2 7.3 20.53 7.36 66.2 74.3 100 6 198.1 7.2 7.4 20.40 7.16 66.6 73.6 100 7 199.9 7.2 7.2 20.26 7.32 66.2 68.1
(670) Another analysis approach is presented in Table 27. The table comprises the four locations where the response to nitrogen was the greatest which suggests that available residual nitrogen was lowest. This approach does not alter the assessment that the nitrogen rate significantly impacted yield, which strains did not when averaged across all nitrogen rates. However, the numerical yield advantage at the lowest N rate is more pronounced for all strains, particularly CI006, CM029, and CM029/CM081 where yields were increased from 8 to 10 bu/acre. At 15% MRTN, strain CM081 outyielded UTC by 5 bu.
(671) TABLE-US-00028 TABLE 27 Yield data across four locations 4 Location Average - SGS, AgIdea, Bennett, RFR Stalk Diameter Internode YLD (bu) Vigor_E Vigor_L (mm) Length (in) MRTN % 0 137.8 7.3 5.84 18.10 5.36 15 162.1 7.5 6.63 18.75 5.40 85 199.2 7.4 7.93 19.58 5.62 100 203.5 7.5 8.14 19.83 5.65 Strain CI006 (1) 175.4 7.5 7.08 19.03 5.59 CM029 (2) 176.1 7.4 7.08 19.09 5.39 CM038 (3) 175.3 7.5 7.05 19.01 5.59 CI019 (4) 174.8 7.5 7.16 19.02 5.45 CM081 (5) 176.7 7.4 7.16 19.00 5.53 CM029/CM081 (6) 175.1 7.4 7.17 19.33 5.46 UTC (7) 176.0 7.3 7.27 18.98 5.55 MRTN/Strain 0 1 140.0 7.3 5.69 18.32 5.28 0 2 140.7 7.4 5.69 18.19 5.23 0 3 135.5 7.3 5.63 17.95 5.50 0 4 138.8 7.3 5.81 17.99 5.36 0 5 136.3 7.3 6.06 18.05 5.34 0 6 141.4 7.5

6.00 18.43 5.30 0 7 131.9 7.3 6.00 17.75 5.48 15 1 158.0 7.6 6.44 18.53 5.34 15 2 164.1 7.5 6.56 19.13 5.42 15 3 164.3 7.6 6.63 18.68 5.51 15 4 163.5 7.6 6.81 18.84 5.34 15 5 166.8 7.5 6.63 18.60 5.39 15 6 156.6 7.4 6.56 18.86 5.41 15 7 161.3 7.5 6.81 18.62 5.42 85 1 199.4 7.6 8.00 19.15 5.63 85 2 199.0 7.4 8.09 19.49 5.46 85 3 198.2 7.4 7.75 19.88 5.69 85 4 196.8 7.4 8.00 19.65 5.60 85 5 199.5 7.4 7.75 19.26 5.70 85 6 198.7 7.3 7.81 19.99 5.61 85 7 202.8 7.2 8.13 19.66 5.65 100 1 204.3 7.4 8.19 20.11 6.10 100 2 200.6 7.3 8.00 19.53 5.46 100 3 203.3 7.7 8.19 19.55 5.67 100 4 200.2 7.6 8.00 19.59 5.49 100 5 203.9 7.4 8.19 20.08 5.68 100 6 203.8 7.5 8.31 20.05 5.52 100 7 208.1 7.4 8.13 19.90 5.63

(672) The results from the field trial are also illustrated in FIGS. **9-15**. The results indicate that the microbes of the disclosure are able to increase plant yield, which points to the ability of the taught microbes to increase nitrogen fixation in an important agricultural crop, i.e. corn.

(673) The field based results further validate the disclosed methods of non-intergenerically modifying the genome of selected microbial strains, in order to bring about agriculturally relevant results in a field setting when applying said engineered strains to a crop.

(674) FIG. **6** depicts the lineage of modified remodeled strains that were derived from strain CI006 (WT *Kosakonia sacchari*). The field data demonstrates that an engineered derivative of the CI006 WT strain, i.e. CM029, is able to bring about numerically relevant results in a field setting. For example, Table 26 illustrates that at 0% MRTN CM029 yielded 147.0 bu/acre compared to untreated control at 141.2 bu/acre (an increase of 5.8 bu/acre). Table 26 also illustrates that at 15% MRTN CM029 yielded 167.3 bu/acre compared to untreated control at 165.1 bu/acre (an increase of 2.2 bu/acre). Table 27 is supportive of these conclusions and illustrates that at 0% MRTN CM029 yielded 140.7 bu/acre compared to untreated control at 131.9 bu/acre (an increase of 8.8 bu/acre). Table 27 also illustrates that at 15% MRTN CM029 yielded 164.1 bu/acre compared to untreated control at 161.3 bu/acre (an increase of 2.8 bu/acre).

(675) FIG. **7** depicts the lineage of modified remodeled strains that were derived from strain CI019 (WT *Rahnella aquatilis*). The field data demonstrates that an engineered derivative of the CI019 WT strain, i.e. CM081, is able to bring about numerically relevant results in a field setting. For example, Table 26 illustrates that at 15% MRTN CM081 yielded 169.3 bu/acre compared to untreated control at 165.1 bu/acre (an increase of 4.2 bu/acre). Table 27 is supportive of these conclusions and illustrates that at 0% MRTN CM081 yielded 136.3 bu/acre compared to untreated control at 131.9 bu/acre (an increase of 4.4 bu/acre). Table 27 also illustrates that at 15% MRTN CM081 yielded 166.8 bu/acre compared to untreated control at 161.3 bu/acre (an increase of 5.5 bu/acre).

(676) Further, one can see in Table 27 that the combination of CM029/CM081 at 0% MRTN yielded 141.4 bu/acre compared to untreated control at 131.9 bu/acre (an increase of 9.5 bu/acre).

Example 4: Field Trials with Remodeled Microbes of the Disclosure—Summer 2017

(677) In order to evaluate the efficacy of remodeled strains of the present disclosure on corn growth and productivity under varying nitrogen regimes, field trials were conducted. The below field data demonstrates that the non-intergeneric microbes of the disclosure are able to fix atmospheric nitrogen and deliver said nitrogen to a plant—resulting in increased yields—in both a nitrogen limiting environment, as well as a non-nitrogen limiting environment.

(678) Trials were conducted at seven locations across the United States with six geographically diverse Midwestern locations. Five nitrogen regimes were used for fertilizer treatments: 100% of standard agricultural practice of the site/region, 100% minus 25 pounds, 100% minus 50 pounds, 100% minus 75 pounds, and 0%; all per acre. The pounds of nitrogen per acre for the 100% regime depended upon the standard agricultural practices of the site/region. The aforementioned nitrogen regimes ranged from about 153 pounds per acre to about 180 pounds per acre, with an average of about 164 pounds of nitrogen per acre.

(679) Within each fertilizer regime there were 14 treatments. Each regime had six replications, and a split plot design was utilized. The 14 treatments included: 12 different microbes, 1 UTC with the

same fertilizer rate as the main plot, and 1 UTC with 100% nitrogen. In the 100% nitrogen regime the 2.sup.nd UTC is 100 plus 25 pounds.
(680) Plots of corn, at a minimum, were 4 rows of 30 feet in length (30 inches between rows) with 420 plots per location. All observations, unless otherwise noted, were taken from the center two rows of the plants, and all destructive sampling was taken from the outside rows. Seed samples were refrigerated until 1.5 to 2 hours prior to use.
(681) Local Agricultural Practice: The seed was a commercial corn applied with a commercial seed treatment with no biological co-application. The seeding rate, planting date, weed/insect management, harvest times, and other standard management practices were left to the norms of local agricultural practices for the regions, with the exception of fungicide application (if required).
(682) Microbe Application: The microbes were applied to the seed in a seed treatment over seeds that had already received a normal chemical treatment. The seed were coated with fermentation broth comprising the microbes.
(683) Soil Characterization: Soil texture and soil fertility were evaluated. Standard soil sampling procedures were utilized, which included soil cores of depths from 0-30 cm and 30-60 cm. The standard soil sampling included a determination of nitrate nitrogen, ammonium nitrogen, total nitrogen, organic matter, and CEC. Standard soil sampling further included a determination of pH, total potassium, and total phosphorous. To determine the nitrogen fertilizer levels, preplant soil samples from each location were taken to ensure that the 0-12″ and potentially the 12″ to 24″ soil regions for nitrate nitrogen.
(684) Prior to planting and fertilization, 2 ml soil samples were collected from 0 to 6-12″ from the UTC. One sample per replicate per nitrogen region was collected using the middle of the row. (5 fertilizer regimes×6 replicates=thirty soil samples).
(685) Post-planting (V4-V6), 2 ml soil samples were collected from 0 to 6-12″ from the UTC. One sample per replicate per nitrogen region was collected using the middle of the row. (5 fertilizer regimes×6 replicates=thirty soil samples).
(686) Post-harvest (V4-V6), 2 ml soil samples were collected from 0 to 6-12″ from the UTC. One sample per replicate per nitrogen region was collected using the middle of the row. Additional post-harvest soil sample collected at 0-12″ from the UTC and potentially 12-24″ from the UTC (5 fertilizer regimes×6 replicates=thirty soil samples).
(687) A V6-V10 soil sample from each fertilizer regime (excluding the treatment of 100% and 100%+25 lbs [in the 100% block] for all fertilizer regimes at 0-12″ and 12-24″. (5 fertilizer regimes×2 depths=10 samples per location).
(688) Post-harvest soil sample from each fertilizer regime (excluding the treatment of 100% and 100%+25 lbs [in the 100% block] for all fertilizer regimes at 0-12″ and 12-24″. (5 fertilizer regimes×2 depths=10 samples per location).
(689) Assessments: The initial plant population was assessed at ˜50% UTC and the final plant population was assessed prior to harvest. Assessment included (1) potentially temperature (temperature probe); (2) vigor (1-10 scale with 10=excellent) at V4 and V8-V10; (3) plant height at V8-V10 and V14; (4) yield (bushels/acre) adjusted to standard moisture percentage; (5) test weight; (6) grain moisture percentage; (7) stalk nitrate tests at black layer (420 plots×7 locations); (8) colonization with 1 plant per plot in zip lock bag at 0% and 100% fertilizer at V4-V6 (1 plant×14 treatments×6 replicates×2 fertilizer regimes=168 plants); (9) transcriptomics with 1 plant per plot in zip lock bag at 0% and 100% fertilizer at V4-V6 (1 plant×14 treatments×6 replicates×2 fertilizer regimes=168 plants); (10) Normalized difference vegetative index (NDVI) or normalized difference red edge (NDRE) determination using a Greenseeker instrument at two time points (V4-V6 and VT) to assess each plot at all 7 locations (420 plots×2 time points×7 locations=5,880 data points); (11) stalk characteristics measured at all 7 locations between R2 and R5 by recording the stalk diameter of 10 plants/plot at 6″ height, record length of first internode above the 6″ mark, 10 plants monitored (5 consecutive plants from center of two inside rows) (420 plots×10 plants×7

locations=29,400 data points).
(690) Monitoring Schedule: Practitioners visited all trials at V3-V4 stage to assess early-season response to treatments and during reproductive growth stage to monitor maturity. Local cooperator visited research trial on an on-going basis.
(691) Weather Information: Weather data spanning from planting to harvest was collected and consisted of daily minimum and maximum temperatures, soil temperature at seeding, daily rainfall plus irrigation (if applied), and unusual weather events such as excessive wind, rain, cold, heat.
(692) Data Reporting: Including the data indicated above, the field trials generated data points including soil textures; row spacing; plot sizes; irrigation; tillage; previous crop; seeding rate; plant population; seasonal fertilizer inputs including source, rate, timing, and placement; harvest area dimensions, method of harvest, such as by hand or machine and measurement tools used (scales, yield monitor, etc.)
(693) Results: Select results from the aforementioned field trial are reported in FIG. **16** and FIG. **17**.
(694) In FIG. **16**, it can be seen that a remodeled microbe of the disclosure (i.e. 6-403) resulted in a higher yield than the wild type strain (WT) and a higher yield than the untreated control (UTC). The "−25 lbs N" treatment utilizes 25 lbs less N per acre than standard agricultural practices of the region. The "100% N" UTC treatment is meant to depict standard agricultural practices of the region, in which 100% of the standard utilization of N is deployed by the farmer. The microbe "6-403" was deposited as NCMA 201708004 and can be found in Table 1. This is a mutant *Kosakonia sacchari* (also called CM037) and is a progeny mutant strain from CI006 WT.
(695) In FIG. **17**, the yield results obtained demonstrate that the remodeled microbes of the disclosure perform consistently across locations. Furthermore, the yield results demonstrate that the microbes of the disclosure perform well in both a nitrogen stressed environment (i.e. a nitrogen limiting environment), as well as an environment that has sufficient supplies of nitrogen (i.e. a non-nitrogen-limiting condition). The microbe "6-881" (also known as CM094, PBC6.94), and which is a progeny mutant *Kosakonia sacchari* strain from CI006 WT, was deposited as NCMA 201708002 and can be found in Table 1. The microbe "137-1034," which is a progeny mutant *Klebsiella variicola* strain from CI137 WT, was deposited as NCMA 201712001 and can be found in Table 1. The microbe "137-1036," which is a progeny mutant *Klebsiella variicola* strain from CI137 WT, was deposited as NCMA 201712002 and can be found in Table 1. The microbe "6-404" (also known as CM38, PBC6.38), and which is a progeny mutant *Kosakonia sacchari* strain from CI006 WT, was deposited as NCMA 201708003 and can be found in Table 1.
Example 5: Genus of Non-Intergeneric Remodeled Microbes Beneficial for Agricultural Systems
(696) The remodeled microbes of the present disclosure were evaluated and compared against one another for the production of nitrogen produced in an acre across a season. See FIG. **8**, FIG. **24**, and FIG. **25**.
(697) It is hypothesized by the inventors that in order for a population of engineered non-intergeneric microbes to be beneficial in a modern row crop agricultural system, then the population of microbes needs to produce at least one pound or more of nitrogen per acre per season.
(698) To that end, the inventors have surprisingly discovered a functional genus of microbes that are able to contribute, inter alia, to: increasing yields in non-leguminous crops; and/or lessening a farmer's dependence upon exogenous nitrogen application; and/or the ability to produce at least one pound of nitrogen per acre per season, even in non-nitrogen-limiting environments, said genus being defined by the product of colonization ability×mmol of N produced per microbe per hour (i.e. the line partitioning FIGS. **8**, **24**, and **25**).
(699) With respect to FIGS. **8**, **24**, and **25**, certain data utilizing microbes of the disclosure was aggregated, in order to depict a heatmap of the pounds of nitrogen delivered per acre-season by microbes of the disclosure, which are recorded as a function of microbes per g-fresh weight by

mmol of nitrogen/microbe-hr. Below the thin line that transects the larger images are the microbes that deliver less than one pound of nitrogen per acre-season, and above the line are the microbes that deliver greater than one pound of nitrogen per acre-season.

(700) Field Data & Wild Type Colonization Heatmap: The microbes utilized in the FIG. **8** heatmap were assayed for N production in corn. For the WT strains CI006 and CI019, corn root colonization data was taken from a single field site. For the remaining strains, colonization was assumed to be the same as the WT field level. N-fixation activity was determined using an in vitro ARA assay at 5 mM glutamine. The table below the heatmap in FIG. **8** gives the precise value of mmol N produced per microbe per hour (mmol N/Microbe hr) along with the precise CFU per gram of fresh weight (CFU/g fw) for each microbe shown in the heatmap.

(701) Field Data Heatmap: The data utilized in the FIG. **24** heatmap is derived from microbial strains assayed for N production in corn in field conditions. Each point represents lb N/acre produced by a microbe using corn root colonization data from a single field site. N-fixation activity was determined using in vitro ARA assay at 5 mM N in the form of glutamine or ammonium phosphate. The below Table 28 gives the precise value of mmol N produced per microbe per hour (mmol N/Microbe hr) along with the precise CFU per gram of fresh weight (CFU/g fw) for each microbe shown in the heatmap of FIG. **24**.

(702) Greenhouse & Laboratory Data Heatmap: The data utilized in the FIG. **25** heatmap is derived from microbial strains assayed for N production in corn in laboratory and greenhouse conditions. Each point represents lb N/acre produced by a single strain. White points represent strains in which corn root colonization data was gathered in greenhouse conditions. Black points represent mutant strains for which corn root colonization levels are derived from average field corn root colonization levels of the wild-type parent strain. Hatched points represent the wild type parent strains at their average field corn root colonization levels. In all cases, N-fixation activity was determined by in vitro ARA assay at 5 mM N in the form of glutamine or ammonium phosphate. The below Table 29 gives the precise value of mmol N produced per microbe per hour (mmol N/Microbe hr) along with the precise CFU per gram of fresh weight (CFU/g fw) for each microbe shown in the heatmap of FIG. **25**.

(703) TABLE-US-00029 TABLE 28 FIG. 24 - Field Data Heatmap Activity Peak (mmol N/ Colonization N Produced/ Strain Name Microbe hr) (CFU/g fw) acre season Taxonomic Designation CI006 3.88E−16 1.50E+07 0.24 *Kosakonia sacchari* 6-404 1.61E−13 3.50E+05 2.28 *Kosakonia sacchari* 6-848 1.80E−13 2.70E+05 1.97 *Kosakonia sacchari* 6-881 1.58E−13 5.00E+05 3.20 *Kosakonia sacchari* 6-412 4.80E−14 1.30E+06 2.53 *Kosakonia sacchari* 6-403 1.90E−13 1.30E+06 10.00 *Kosakonia sacchari* CI019 5.33E−17 2.40E+06 0.01 *Rahnella aquatilis* 19-806 6.65E−14 2.90E+06 7.80 *Rahnella aquatilis* 19-750 8.90E−14 2.60E+05 0.94 *Rahnella aquatilis* 19-804 1.72E−14 4.10E+05 0.29 *Rahnella aquatilis* CI137 3.24E−15 6.50E+06 0.85 *Klebsiella variicola* 137-1034 1.16E−14 6.30E+06 2.96 *Klebsiella variicola* 137-1036 3.47E−13 1.30E+07 182.56 *Klebsiella variicola* 137-1314 1.70E−13 1.99E+04 0.14 *Klebsiella variicola* 137-1329 1.65E−13 7.25E+04 0.48 *Klebsiella variicola* 63 3.60E−17 3.11E+05 0.00 *Rahnella aquatilis* 63-1146 1.90E−14 5.10E+05 0.39 *Rahnella aquatilis* 1021 1.77E−14 2.69E+07 19.25 *Kosakonia pseudosacchari* 728 5.56E−14 1445240.09 3.25 *Klebsiella variicola*

(704) TABLE-US-00030 TABLE 29 FIG. 25 - Greenhouse & Laboratory Data Heatmap Activity Peak (mmol N/ Colonization N Produced/ Strain Name Microbe hr) (CFU/g fw) acre season Taxonomic Designation CI006 3.88E−16 1.50E+07 0.24 *Kosakonia sacchari* 6-400 2.72E−13 1.79E+05 1.97 *Kosakonia sacchari* 6-397 1.14E−14 1.79E+05 0.08 *Kosakonia sacchari* CI137 3.24E−15 6.50E+06 0.85 *Klebsiella variicola* 137-1586 1.10E−13 1.82E+06 8.10 *Klebsiella variicola* 137-1382 4.81E−12 1.82E+06 354.60 *Klebsiella variicola* 1021 1.77E−14 2.69E+07 19.25 *Kosakonia pseudosacchari* 1021-1615 1.20E−13 2.69E+07 130.75 *Kosakonia pseudosacchari* 1021-1619 3.93E−14 2.69E+07 42.86 *Kosakonia pseudosacchari* 1021-1612 1.20E−13 2.69E+07 130.75 *Kosakonia pseudosacchari* 1021-1623 4.73E−17 2.69E+07 0.05

Kosakonia pseudosacchari 1293 5.44E−17 8.70E+08 1.92 *Azospirillum lipoferum* 1116 1.05E−14 1.37E+07 5.79 *Enterobacter* sp. 1113 8.05E−15 4.13E+07 13.45 *Enterobacter* sp. 910 1.19E−13 1.34E+06 6.46 *Kluyvera intermedia* 910-1246 2.16E−13 1.34E+06 11.69 *Kluyvera intermedia* 850 7.2301E−16 1.17E+06 0.03 *Achromobacter spiritinus* 852 5.96E−16 1.07E+06 0.03 *Achromobacter marplatensis* 853 6.42E−16 2.55E+06 0.07 *Microbacterium murale*

(705) Conclusions: The data in FIGS. **8**, **24**, **25**, and Tables 28 and 29, illustrates more than a dozen representative members of the described genus (i.e. microbes to the right of the line in the figures). Further, these numerous representative members come from a diverse array of taxonomic genera, which can be found in the above Tables 28 and 29. Further still, the inventors have discovered numerous genetic attributes that depict a structure/function relationship that is found in many of the microbes. These genetic relationships can be found in the numerous tables of the disclosure setting forth the genetic modifications introduced by the inventors, which include introducing at least one genetic variation into at least one gene, or non-coding polynucleotide, of the nitrogen fixation or assimilation genetic regulatory network.

(706) Consequently, the newly discovered genus is supported by: (1) a robust dataset, (2) over a dozen representative members, (3) members from diverse taxonomic genera, and (4) classes of genetic modifications that define a structure/function relationship, in the underlying genetic architecture of the genus members.

Example 6: Methods and Assays for Detection of Non-Intergeneric Remodeled Microbes

(707) The present disclosure teaches primers, probes, and assays that are useful for detecting the microbes utilized in the various aforementioned Examples. The assays are able to detect the non-natural nucleotide “junction” sequences in the derived/mutant non-intergeneric remodeled microbes. These non-naturally occurring nucleotide junctions can be used as a type of diagnostic that is indicative of the presence of a particular genetic alteration in a microbe.

(708) The present techniques are able to detect these non-naturally occurring nucleotide junctions via the utilization of specialized quantitative PCR methods, including uniquely designed primers and probes. The probes can bind to the non-naturally occurring nucleotide junction sequences. That is, sequence-specific DNA probes consisting of oligonucleotides that are labelled with a fluorescent reporter, which permits detection only after hybridization of the probe with its complementary sequence can be used. The quantitative methods can ensure that only the non-naturally occurring nucleotide junction will be amplified via the taught primers, and consequently can be detected either via a non-specific dye, or via the utilization of a specific hybridization probe. Another aspect of the method is to choose primers such that the primers flank either side of a junction sequence, such that if an amplification reaction occurs, then said junction sequence is present.

(709) Consequently, genomic DNA can be extracted from samples and used to quantify the presence of microbes of the disclosure by using qPCR. The primers utilized in the qPCR reaction can be primers designed by Primer Blast (www.ncbi.nlm.nih.gov/tools/primer-blast/) to amplify unique regions of the wild-type genome or unique regions of the engineered non-intergeneric mutant strains. The qPCR reaction can be carried out using the SYBR GreenER qPCR SuperMix Universal (Thermo Fisher P/N 11762100) kit, using only forward and reverse amplification primers; alternatively, the Kapa Probe Force kit (Kapa Biosystems P/N KK4301) can be used with amplification primers and a TaqMan probe containing a FAM dye label at the 5′ end, an internal ZEN quencher, and a minor groove binder and fluorescent quencher at the 3′ end (Integrated DNA Technologies).

(710) Certain primer, probe, and non-native junction sequences—which can be used in the qPCR methods—are listed in the below Table 30. Specifically, the non-native junction sequences can be found in SEQ ID NOs: 372-405 and 425-457.

(711) TABLE-US-00031 TABLE 30 Microbial Detection up/down SEQ SEQ SEQ Junction SEQ F R base Junction stream ID 100 bp upstream ID 100 bp downstream ID “/” indicating Junction primer primer Probe CI Name junction NO of junction NO of junction NO

junction des. SEQ SEQ SEQ 1021 dsl131 up 304 TGGTGTCCGGGC 338 TTCTTGGTTCTCT 372 5′- disrupted N/A N/A N/A GAACGTCGCCAG GGAGCGCTTTAT TGGTGTCCGGGC nifL gene/ GTGGCACAAATT CGGCATCCTGAC GAACGTCGCCAG PinfC GTCAGAACTACG TGAAGAATTTGC GTGGCACAAATT ACACGACTAACC AGGCTTCTTCCCA GTCAGAACTACG GACCGCAGGAGT ACCTGGCTTGCA ACACGACTAACC GTGCGATGACCC CCCGTGCAGGTA GACCGCAGGAGT TGAATATGATGA GTTGTGATGAAC GTGCGATGACCC TGGA AT TGAATATGATGA TGGA/ TTCTTGGTTCTCT GGAGCGCTTTAT CGGCATCCTGAC TGAAGAATTTGC AGGCTTCTTCCCA ACCTGGCTTGCA CCCGTGCAGGTA GTTGTGATGAAC AT-3′ 1021 dsl131 down 305 CGGAAAACGAGT 339 GCGATAGAACTC 373 5′- PinfC/ N/A N/A N/A TCAAACGGCGCG ACTTCACGCCCC CGGAAAACGAGT disrupted TCCCAATCGTATT GAAGGGGGAAGC TCAAACGGCGCG nifL gene AATGGCGAGATT TGCCTGACCCTAC TCCCAATCGTATT CGCGCCACGGAA GATTCCCGCTATT AATGGCGAGATT GTTCGCTTAACAG TCATTCACTGACC CGCGCCACGGAA GTCTGGAAGGCG GGAGGTTCAAAA GTTCGCTTAACA AGCAGCTTGGTA TGACCCAGCGAA GGTCTGGAAGGC TT C GAGCAGCTTGGT ATT/ GCGATAGAACTC ACTTCACGCCCC GAAGGGGGAAGC TGCCTGACCCTA CGATTCCCGCTAT TTCATTCACTGAC CGGAGGTTCAAA ATGACCCAGCGA AC-3′ 1021 dsl133 N/A 306 CGCCAGAGAGTT 340 TCCCTGTGCGCCG 374 5′- 5′UTR N/A N/A N/A GAAATCGAACAT CGTCGCCGATGG CGCCAGAGAGTT and ATG/ TTCCGTAATACCG TGGCCAGCCAAC GAAATCGAACAT truncated CCATTACCCAGG TGGCGCGCTACC TTCCGTAATACC glnE gene AGCCGTTCTGGTT CGATCCTGCTCG GCCATTACCCAG GCACAGCGGAAA ATGAACTGCTCG GAGCCGTTCTGG ACGTTAACGAAA ACCCGAACACGC TTGCACAGCGGA GGATATTTCGCAT TCTATCAACCGA AAACGTTAACGA G CGG AAGGATATTTCG CATG/ TCCCTGTGCGCC GCGTCGCCGATG GTGGCCAGCCAA CTGGCGCGCTAC CCGATCCTGCTC GATGAACTGCTC GACCCGAACACG CTCTATCAACCG ACGG-3′ 1021 dsl145 up 307 CGGGCGAACGTC 341 CGTTCTGTAATAA 375 5′- disrupted N/A N/A N/A GCCAGGTGGCAC TAACCGGACAAT CGGGCGAACGTC nifL gene/ AAATTGTCAGAA TCGGACTGATTA GCCAGGTGGCAC Prm1 CTACGACACGAC AAAAAGCGCCCT AAATTGTCAGAA TAACCGACCGCA CGCGGCGCTTTTT CTACGACACGAC GGAGTGTGCGAT TTATATTCTCGAC TAACCGACCGCA GACCCTGAATAT TCCATTTAAAATA GGAGTGTGCGAT GATGATGGATGC AAAAATCCAATC GACCCTGAATAT CAGC GATGATGGATGC CAGC/ CGTTCTGTAATA ATAACCGGACAA TTCGGACTGATT AAAAAAGCGCCC TCGCGGCGCTTTT TTTATATTCTCGA CTCCATTTAAAAT AAAAAATCCAAT C-3′ 1021 dsl145 down 308 TCAACCTAAAAA 342 AACTCACTTCAC 376 5′- Prm1/ N/A N/A N/A AGTTTGTGTAATA GCCCCGAAGGGG TCAACCTAAAAA disrupted CTTGTAACGCTAC GAAGCTGCCTGA AGTTTGTGTAAT nifL gene ATGGAGATTAAC CCCTACGATTCCC ACTTGTAACGCT TCAATCTAGAGG GCTATTTCATTCA ACATGGAGATTA GTATTAATAATG CTGACCGGAGGT ACTCAATCTAGA AATCGTACTAAA TCAAAATGACCC GGGTATTAATAA CTGGTACTGGGC AGCGAACCGAGT TGAATCGTACTA GC CG AACTGGTACTGG GCGC/ AACTCACTTCAC GCCCCGAAGGGG GAAGCTGCCTGA CCCTACGATTCCC GCTATTTCATTCA CTGACCGGAGGT TCAAAATGACCC AGCGAACCGAGT CG-3′ 1021 dsl148 up 309 CGGGCGAACGTC 343 CGCGTCAGGTTG 377 5′- disrupted N/A N/A N/A GCCAGGTGGCAC AACGTAAAAAAG CGGGCGAACGTC nifL gene/ AAATTGTCAGAA TCGGTCTGCGCA GCCAGGTGGCAC Prm7 CTACGACACGAC AAGCACGTCGTC AAATTGTCAGAA TAACCGACCGCA GTCCGCAGTTCTC CTACGACACGAC GGAGTGTGCGAT CAAACGTTAATT TAACCGACCGCA GACCCTGAATAT GGTTTCTGCTTCG GGAGTGTGCGAT GATGATGGATGC GCAGAACGATTG GACCCTGAATAT CAGC GC GATGATGGATGC

CAGC/ CGCGTCAGGTTG AACGTAAAAAAG TCGGTCTGCGCA AAGCACGTCGTC
GTCCGCAGTTCTC CAAACGTTAATT GGTTTCTGCTTCG GCAGAACGATTG GC-3′ 1021
dsl148 down 310 AATTTTCTGCCCA 344 AACTCACTTCAC 378 5′- Prm4/ N/A N/A N/A
AATGGCTGGGAT GCCCCGAAGGGG AATTTTCTGCCCA disrupted TGTTCATTTTTTG
GAAGCTGCCTGA AATGGCTGGGAT nifL gene TTTGCCTTACAAC CCCTACGATTCCC
TGTTCATTTTTTG GAGAGTGACAGT GCTATTTCATTCA TTTGCCTTACAAC
ACGCGCGGGTAG CTGACCGGAGGT GAGAGTGACAGT TTAACTCAACATC
TCAAAATGACCC ACGCGCGGGTAG TGACCGGTCGAT AGCGAACCGAGT
TTAACTCAACAT CG CTGACCGGTCGA T/ AACTCACTTCAC GCCCCGAAGGGG
GAAGCTGCCTGA CCCTACGATTCCC GCTATTTCATTCA CTGACCGGAGGT
TCAAAATGACCC AGCGAACCGAGT CG-3′ CI006 ds126 N/A 311 GTAACCAATAAA 345
CCGATCCCCATC 379 5′- 5′ UTR up N/A N/A N/A GGCCACCACGCC
ACTGTGTGTCTTG GTAACCAATAAA to ATG- AGACCACACGAT TATTACAGTGCC
GGCCACCACGCC 4 bp of AGTGATGGCAAC GCTTCGTCGGCTT AGACCACACGAT
amtB gene/ ACTTTCCAGCTGC CGCCGGTACGAA AGTGATGGCAAC disrupted
ACCAGCACCTGA TACGAATGACGC ACTTTCCAGCTGC amtB gene TGGCCCATGGTC
GTTGCAGCTCAG ACCAGCACCTGA ACACCTTCAGCG CAACGAAAATTT
TGGCCCATGGTC AAA TG ACACCTTCAGCG AAA/ CCGATCCCCATC ACTGTGTGTCTTG
TATTACAGTGCC GCTTCGTCGGCTT CGCCGGTACGAA TACGAATGACGC
GTTGCAGCTCAG CAACGAAAATTT TG-3′ CI019 ds172 down 312 TGGTATTGTCAGT 346
CCGTCTCTGAAG 380 5′- Prm1.2/ SEQ SEQ N/A CTGAATGAAGCT CTCTCGGTGAAC
TGGTATTGTCAGT disrupted ID ID CTTGAAAAAGCT ATTGTTGCGAGG
CTGAATGAAGCT nifL gene NO: NO: GAGGAAGCGGGC CAGGATGCGAGC
CTTGAAAAAGCT 406 407 GTCGATTTAGTAG TGGTTGTGTTTTG GAGGAAGCGGGC
CAAG TGCC AAATCAGTCCGA ACATTACCGATA GTCGATTTAGTA AAGT TCGC
ATGCCGAGCCGC ATGTGCCGCGTG GAAATCAGTCCG TCGC AACA CAGTTTGTCGAAT
AACGGGTGCGTT AATGCCGAGCCG CTCA ATGT C ATG CCAGTTTGTCGA CAGG TCAC
ATC/ CCGTCTCTGAAG CTCTCGGTGAAC ATTGTTGCGAGG CAGGATGCGAGC
TGGTTGTGTTTTG ACATTACCGATA ATGTGCCGCGTG AACGGGTGCGTT ATG-3′ CI019
ds172 up 313 ACCGATCCGCAG 347 TGAACATCACTG 381 5′- disrupted N/A N/A N/A
GCGCGCATTTGTT ATGCACAAGCTA ACCGATCCGCAG nifL gene/ ATGCCAATCCGG
CCTATGTCGAAG GCGCGCATTTGTT Prm1.2 CATTCTGCCGCCA AATTAACTAAAA
ATGCCAATCCGG GACGGGTTTTGC AACTGCAAGATG CATTCTGCCGCC
ACTTGAGACACTT CAGGCATTCGCG AGACGGGTTTTG TTGGGCGAGAAC
TTAAAGCCGACT CACTTGAGACAC CACCGTCTGCTGG TGAGAAATGAGA
TTTTGGGCGAGA AGAT ACCACCGTCTGC TGG/ TGAACATCACTG ATGCACAAGCTA
CCTATGTCGAAG AATTAACTAAAA AACTGCAAGATG CAGGCATTCGCG
TTAAAGCCGACT TGAGAAATGAGA AGAT-3′ CI019 ds175 down 314 CGGGAACCGGTG
348 CCGTCTCTGAAG 382 5′- Prm3.1/ SEQ SEQ SEQ TTATAATGCCGCG CTCTCGGTGAAC
CGGGAACCGGTG disrupted ID ID ID CCCTCATATTGTG ATTGTTGCGAGG
TTATAATGCCGC nifL gene NO: NO: NO: GGGATTTCTTAAT CAGGATGCGAGC
GCCCTCATATTGT 408 409 410 GACCTATCCTGG TGGTTGTGTTTTG GGGGATTTCTTA
CGCC GGCA /56- GTCCTAAAGTTGT ACATTACCGATA ATGACCTATCCT CTCA TAAC
FAM/ AGTTGACATTAG ATGTGCCGCGTG GGGTCCTAAAGT TATT GCAC TA
CGGAGCACTAAC AACGGGTGCGTT TGTAGTTGACATT GTGG CCGT ACC ATG
AGCGGAGCACTA GGAT TCA CGT AC/ C/ CCGTCTCTGAAG ZEN/T CTCTCGGTGAAC
CTG ATTGTTGCGAGG AAG CAGGATGCGAGC CTC TGGTTGTGTTTTG TCG
ACATTACCGATA GT/ ATGTGCCGCGTG 3IABkFQ/ AACGGGTGCGTT ATG-3′ CI019 ds175
up 315 ACCGATCCGCAG 349 TACAGTAGCGCC 383 5′- disrupted N/A N/A N/A
GCGCGCATTTGTT TCTCAAAAATAG ACCGATCCGCAG nifL gene ATGCCAATCCGG

ATAAACGGCTCA GCGCGCATTTGTT /Prm3.1 CATTCTGCCGCCA TGTACGTGGGCC
ATGCCAATCCGG GACGGGTTTTGC GTTTATTTTTTCT CATTCTGCCGCC
ACTTGAGACACTT ACCCATAATCGG AGACGGGTTTTG TTGGGCGAGAAC
GAACCGGTGTTA CACTTGAGACAC CACCGTCTGCTGG TAATGCCGCGCC
TTTTGGGCGAGA CTC ACCACCGTCTGC TGG/ TACAGTAGCGCC TCTCAAAAATAG
ATAAACGGCTCA TGTACGTGGGCC GTTTATTTTTTCT ACCCATAATCGG
GAACCGGTGTTA TAATGCCGCGCC CTC-3′ CI006 ds20 down 316 TCAACCTAAAAA 350
AACTCACTTCAC 384 5′- Prm1/ SEQ SEQ SEQ AGTTTGTGTAATA ACCCCGAAGGGG
TCAACCTAAAAA disrupted ID ID ID CTTGTAACGCTAC GAAGTTGCCTGA
AGTTTGTGTAAT nifL gene NO: NO: NO: ATGGAGATTAAC CCCTACGATTCCC
ACTTGTAACGCT 411 412 413 TCAATCTAGAGG GCTATTTCATTCA ACATGGAGATTA
TAAA CAAA /56- GTATTAATAATG CTGACCGGAGGT ACTCAATCTAGA CTGG TCGA
FAM/ AATCGTACTAAA TCAAAATGACCC GGGTATTAATAA TACT AGCG AAG
CTGGTACTGGGC AGCGAACCGAGT TGAATCGTACTA GGGC CCAG TTGC GC CG
AACTGGTACTGG GCAA ACGG CT/ GCGC/ CT TAT ZEN/G AACTCACTTCAC ACC
ACCCCGAAGGGG CTAC GAAGTTGCCTGA GATT CCCTACGATTCCC CCC/
GCTATTTCATTCA 3IABkFQ/ CTGACCGGAGGT TCAAAATGACCC AGCGAACCGAGT
CG-3′ CI006 ds20 up 317 GGGCGACAAACG 351 CGTCCTGTAATA 385 5′4 disrupted N/A N/A
N/A GCCTGGTGGCAC ATAACCGGACAA GGGCGACAAACG nifL gene/
AAATTGTCAGAA TTCGGACTGATTA GCCTGGTGGCAC Prm1 CTACGACACGAC
AAAAAGCGCCCT AAATTGTCAGAA TAACTGACCGCA TGTGGCGCTTTTT
CTACGACACGAC GGAGTGTGCGAT TTATATTCCCGCC TAACTGACCGCA
GACCCTGAATAT TCCATTTAAAATA GGAGTGTGCGAT AAAAATCCAATC
GACCCTGAATAT GATGATGGATGC GATGATGGATGC CGGC CGGC/ CGTCCTGTAATA
ATAACCGGACAA TTCGGACTGATT AAAAAAGCGCCC TTGTGGCGCTTTT
TTTATATTCCCGC CTCCATTTAAAAT AAAAAATCCAAT C-3′ CI006 ds24 up 318
GGGCGACAAACG 352 GGACATCATCGC 386 5′- disrupted SEQ SEQ SEQ
GCCTGGTGGCAC GACAAACAATAT GGGCGACAAACG nifL gene/ ID ID ID
AAATTGTCAGAA TAATACCGGCAA GCCTGGTGGCAC Prm5 NO: NO: NO:
CTACGACACGAC CCACACCGGCAA AAATTGTCAGAA 414 415 416 TAACTGACCGCA
TTTACGAGACTG CTACGACACGAC GGTG GCGC /56- GGAGTGTGCGAT
CGCAGGCATCCT TAACTGACCGCA CACT AGTC FAM/ GACCCTGAATAT
TTCTCCCGTCAAT GGAGTGTGCGAT CTTT TCGT CA GATGATGGATGC
TTCTGTCAAATAA GACCCTGAATAT GCAT AAAT GGA CGGC AG GATGATGGATGC
GGTT TGCC GTG CGGC/ T/ GGACATCATCGC ZEN/G GACAAACAATAT CGA
TAATACCGGCAA TGA CCACACCGGCAA CCC TTTACGAGACTG TGA CGCAGGCATCCT
AT/ TTCTCCCGTCAAT 3IABkFQ TTCTGTCAAATA AAG-3′ CI006 ds24 down 319
TAAGAATTATCTG 353 AACTCACTTCAC 387 5′- Prm5/ N/A N/A N/A GATGAATGTGCC
ACCCCGAAGGGG TAAGAATTATCT disrupted ATTAAATGCGCA GAAGTTGCCTGA
GGATGAATGTGC nifL gene GCATAATGGTGC CCCTACGATTCCC CATTAAATGCGC
GTTGTGCGGGAA GCTATTTCATTCA AGCATAATGGTG AACTGCTTTTTTT
CTGACCGGAGGT CGTTGTGCGGGA TGAAAGGGTTGG TCAAAATGACCC
AAACTGCTTTTTT TCAGTAGCGGAA AGCGAACCGAGT TTGAAAGGGTTG AC CG
GTCAGTAGCGGA AAC/ AACTCACTTCAC ACCCCGAAGGGG GAAGTTGCCTGA
CCCTACGATTCCC GCTATTTCATTCA CTGACCGGAGGT TCAAAATGACCC
AGCGAACCGAGT CG-3′ CI006 ds30 N/A 320 CGCCAGAGAGTC 354 TTTAACGATCTGA
388 5′4 5′UTR N/A N/A N/A GAAATCGAACAT TTGGCGATGATG CGCCAGAGAGTC and
ATG/ TTCCGTAATACCG AAACGGATTCGC GAAATCGAACAT truncated CGATTACCCAGG
CGGAAGATGCGC TTCCGTAATACC glnE gene AGCCGTTCTGGTT TTTCTGAGAGCTG
GCGATTACCCAG GCACAGCGGAAA GCGCGAATTGTG GAGCCGTTCTGG

ACGTTAACGAAA GCAGGATGCGTT TTGCACAGCGGA GGATATTTCGCAT GCAGGAGGAGGA AAACGTTAACGA G TT AAGGATATTTCG CATG/ TTTAACGATCTG ATTGGCGATGAT GAAACGGATTCG CCGGAAGATGCG CTTTCTGAGAGCT GGCGCGAATTGT GGCAGGATGCGT TGCAGGAGGAGG ATT-3′ CI006 ds31 N/A 321 CGCCAGAGAGTC 355 GCACTGAAACAC 389 5′4 5′UTR N/A N/A N/A GAAATCGAACAT CTCATTTCCCTGT CGCCAGAGAGTC and ATG/ TTCCGTAATACCG GTGCCGCGTCGC GAAATCGAACAT truncated CGATTACCCAGG CGATGGTTGCCA TTCCGTAATACC glnE gene AGCCGTTCTGGTT GTCAGCTGGCGC GCGATTACCCAG GCACAGCGGAAA GCTACCCGATCCT GAGCCGTTCTGG ACGTTAACGAAA GCTTGATGAATT TTGCACAGCGGA GGATATTTCGCAT GCTCGACCCGAA AAACGTTAACGA G TA AAGGATATTTCG CATG/ GCACTGAAACAC CTCATTTCCCTGT GTGCCGCGTCGC CGATGGTTGCCA GTCAGCTGGCGC GCTACCCGATCC TGCTTGATGAATT GCTCGACCCGAA TA-3′ CI019 ds34 N/A 322 GATGATGGATGC 356 GCGCTCAAACAG 390 5′- 5′UTR N/A N/A N/A TTTCTGGTTAAAC TTAATCCGTCTGT GATGATGGATGC and ATG/ GGGCAACCTCGT GTGCCGCCTCGC TTTCTGGTTAAAC truncated TAACTGACTGACT CGATGGTCGCGA GGGCAACCTCGT glnE gene AGCCTGGGCAAA CACAACTTGCAC TAACTGACTGAC CTGCCCGGGCTTT GTCATCCTTTATT TAGCCTGGGCAA TTTTTGCAAGGAA GCTCGATGAACT ACTGCCCGGGCT TCTGATTTCATG GCTCGACCCGCG TTTTTTTGCAAGG CA AATCTGATTTCAT G/ GCGCTCAAACAG TTAATCCGTCTGT GTGCCGCCTCGC CGATGGTCGCGA CACAACTTGCAC GTCATCCTTTATT GCTCGATGAACT GCTCGACCCGCG CA-3′ CI019 ds70 up 323 ACCGATCCGCAG 357 AGTCTGAACTCA 391 5′4 disrupted N/A N/A N/A GCGCGCATTTGTT TCCTGCGGCAGT ACCGATCCGCAG nifL gene/ ATGCCAATCCGG CGGTGAGACGTA GCGCGCATTTGTT Prm4 CATTCTGCCGCCA TTTTTGACCAAAG ATGCCAATCCGG GACGGGTTTTGC AGTGATCTACAT CATTCTGCCGCC ACTTGAGACACTT CACGGAATTTTGT AGACGGGTTTTG TTGGGCGAGAAC GGTTGTTGCTGCT CACTTGAGACAC CACCGTCTGCTGG TAAAAGGGCAAA TTTTGGGCGAGA T ACCACCGTCTGC TGG/ AGTCTGAACTCA TCCTGCGGCAGT CGGTGAGACGTA TTTTTGACCAAA GAGTGATCTACA TCACGGAATTTT GTGGTTGTTGCTG CTTAAAAGGGCA AAT-3′ CI019 ds70 down 324 CATCGGACACCA 358 CCGTCTCTGAAG 392 5′- Prm4/ N/A N/A N/A CCAGCTTACAAA CTCTCGGTGAAC CATCGGACACCA disrupted TTGCCTGATTGCG ATTGTTGCGAGG CCAGCTTACAAA nifL gene GCCCCGATGGCC CAGGATGCGAGC TTGCCTGATTGCG GGTATCACTGAC TGGTTGTGTTTTG GCCCCGATGGCC CGACCATTTCGTG ACATTACCGATA GGTATCACTGAC CCTTATGTCATGC ATGTGCCGCGTG CGACCATTTCGT GATGGGGGCTGG AACGGGTGCGTT GCCTTATGTCATG G ATG CGATGGGGGCTG GG/ CCGTCTCTGAAG CTCTCGGTGAAC ATTGTTGCGAGG CAGGATGCGAGC TGGTTGTGTTTTG ACATTACCGATA ATGTGCCGCGTG AACGGGTGCGTT ATG-3′ 137 ds799 down 325 TCTTCAACAACTG 359 GCCATTGAGCTG 393 5′- PinfC/ SEQ SEQ SEQ GAGGAATAAGGT GCTTCCCGACCG TCTTCAACAACT disrupted ID ID ID ATTAAAGGCGGA CAGGGCGGCACC GGAGGAATAAGG nifL gene NO: NO: NO: AAACGAGTTCAA TGCCTGACCCTGC TATTAAAGGCGG 417 418 419 ACGGCACGTCCG GTTTCCCGCTGTT AAAACGAGTTCA CTCG AGGG /56- AATCGTATCAAT TAACACCCTGAC AACGGCACGTCC GCAG TGTT FAM/ GGCGAGATTCGC CGGAGGTGAAGC GAATCGTATCAA CATG AAAC AA GCCCTGGAAGTT ATGATCCCTGAA TGGCGAGATTCG GACG AGCG CGG CGC TC CGCCCTGGAAGT TAA GGAA CAC TCGC/ A G/ GCCATTGAGCTG ZEN/T GCTTCCCGACCG CCG CAGGGCGGCACC AAT TGCCTGACCCTG CGT CGTTTCCCGCTGT ATC TTAACACCCTGA AA/ CCGGAGGTGAAG 3IABkFQ/ CATGATCCCTGA ATC-3′ 137 ds799 up 326 TCCGGGTTCGGCT 360 AGCGTCAGGTAC 394 5′- disrupted N/A N/A N/A

TACCCCGCCGCGT CGGTCATGATTC TCCGGGTTCGGC nifL gene/ TTTGCGCACGGTG
ACCGTGCGATTCT TTACCCCGCCGC PinfC TCGGACAATTTGT CGGTTCCCTGGA
GTTTTGCGCACG CATAACTGCGAC GCGCTTCATTGGC GTGTCGGACAAT
ACAGGAGTTTGC ATCCTGACCGAA TTGTCATAACTGC GATGACCCTGAA
GAGTTCGCTGGC GACACAGGAGTT TATGATGCTCGA TTCTTCCCAACCT
TGCGATGACCCT G GAATATGATGCT CGA/ AGCGTCAGGTAC CGGTCATGATTC
ACCGTGCGATTC TCGGTTCCCTGG AGCGCTTCATTG GCATCCTGACCG
AAGAGTTCGCTG GCTTCTTCCCAAC CTG-3′ 137 ds809 N/A 327 ATCGCAGCGTCTT 361
GCGCTGAAGCAC 395 5′- 5′UTR SEQ SEQ SEQ TGAATATTTCCGT CTGATCACGCTCT
ATCGCAGCGTCT and ATG/ ID ID ID CGCCAGGCGCTG GCGCGGCGTCGC
TTGAATATTTCCG truncated NO: NO: NO: GCTGCCGAGCCG CGATGGTCGCCA
TCGCCAGGCGCT glnE gene 420 421 422 TTCTGGCTGCATA GCCAGCTGGCGC
GGCTGCCGAGCC GAGC GCCG /56- GTGGAAAACGAT GCCACCCGCTGC
GTTCTGGCTGCAT CGTT TCGG FAM/ AATTTCAGGCCA TGCTGGATGAGC
AGTGGAAAACGA CTGG CTGA TTAT GGGAGCCCTTAT TGCTGGATCCCA
TAATTTCAGGCC CTGC TAGA GGC G ACA AGGGAGCCCTTA ATAG GG GC/ TG/ ZEN/
GCGCTGAAGCAC TGAA CTGATCACGCTCT GCA GCGCGGCGTCGC CCTG
CGATGGTCGCCA ATC GCCAGCTGGCGC A/ GCCACCCGCTGC 3IABkFQ
TGCTGGATGAGC / TGCTGGATCCCA ACA-3′ 137 ds843 up 328 TCCGGGTTCGGCT 362
GCCCGCTGACCG 396 5′- disrupted N/A N/A N/A TACCCCGCCGCGT ACCAGAACTTCC
TCCGGGTTCGGC nifL gene/ TTTGCGCACGGTG ACCTTGGACTCG TTACCCCGCCGC
Prm1.2 TCGGACAATTTGT GCTATACCCTTGG GTTTTGCGCACG CATAACTGCGAC
CGTGACGGCGCG GTGTCGGACAAT ACAGGAGTTTGC CGATAACTGGGA
TTGTCATAACTGC GATGACCCTGAA CTACATCCCCATT GACACAGGAGTT
TATGATGCTCGA CCGGTGATCTTAC TGCGATGACCCT c GAATATGATGCT CGA/
GCCCGCTGACCG ACCAGAACTTCC ACCTTGGACTCG GCTATACCCTTG
GCGTGACGGCGC GCGATAACTGGG ACTACATCCCCA TTCCGGTGATCTT ACC-3′ 137
ds843 down 329 TCACTTTTTAGCA 363 GCCATTGAGCTG 397 5′- Prm1.2/ N/A N/A N/A
AAGTTGCACTGG GCTTCCCGACCG TCACTTTTTAGCA disrupted ACAAAAGGTACC
CAGGGCGGCACC AAGTTGCACTGG nifL gene ACAATTGGTGTA TGCCTGACCCTGC
ACAAAAGGTACC CTGATACTCGAC GTTTCCCGCTGTT ACAATTGGTGTA
ACAGCATTAGTG TAACACCCTGAC CTGATACTCGAC TCGATTTTTCATA
CGGAGGTGAAGC ACAGCATTAGTG TAAAGGTAATTTT ATGATCCCTGAA
TCGATTTTTCATA G TC TAAAGGTAATTT TG/ GCCATTGAGCTG GCTTCCCGACCG
CAGGGCGGCACC TGCCTGACCCTG CGTTTCCCGCTGT TTAACACCCTGA
CCGGAGGTGAAG CATGATCCCTGA ATC-3′ 137 ds853 up 330 TCCGGGTTCGGCT 364
GCTAAAGTTCTC 398 5′- disrupted N/A N/A N/A TACCCCGCCGCGT GGCTAATCGCTG
TCCGGGTTCGGC nifL gene/ TTTGCGCACGGTG ATAACATTTGAC TTACCCCGCCGC
Prm6.2 TCGGACAATTTGT GCAATGCGCAAT GTTTTGCGCACG CATAACTGCGAC
AAAAGGGCATCA GTGTCGGACAAT ACAGGAGTTTGC TTTGATGCCCTTT
TTGTCATAACTGC GATGACCCTGAA TTGCACGCTTTCA GACACAGGAGTT
TATGATGCTCGA TACCAGAACCTG TGCGATGACCCT GC GAATATGATGCT CGA/
GCTAAAGTTCTC GGCTAATCGCTG ATAACATTTGAC GCAATGCGCAAT
AAAAGGGCATCA TTTGATGCCCTTT TTGCACGCTTTCA TACCAGAACCTG GC-3′ 137
ds853 down 331 GTTCTCCTTTGCA 365 GCCATTGAGCTG 399 5′- Prm6.2/ N/A N/A N/A
ATAGCAGGGAAG GCTTCCCGACCG GTTCTCCTTTGCA disrupted AGGCGCCAGAAC
CAGGGCGGCACC ATAGCAGGGAAG nifL gene CGCCAGCGTTGA TGCCTGACCCTGC
AGGCGCCAGAAC AGCAGTTTGAAC GTTTCCCGCTGTT CGCCAGCGTTGA
GCGTTCAGTGTAT TAACACCCTGAC AGCAGTTTGAAC AATCCGAAACTT
CGGAGGTGAAGC GCGTTCAGTGTA AATTTCGGTTTGG ATGATCCCTGAA

TAATCCGAAACT A TC TAATTTCGGTTTG GAZ GCCATTGAGCTG GCTTCCCGACCG
CAGGGCGGCACC TGCCTGACCCTG CGTTTCCCGCTGT TTAACACCCTGA
CCGGAGGTGAAG CATGATCCCTGA ATC-3′ 137 ds857 up 332 TCCGGGTTCGGCT 366
CGCCGTCCTCGC 400 5′- disrupted N/A N/A N/A TACCCCGCCGCGT AGTACCATTGCA
TCCGGGTTCGGC nifL gene/ TTTGCGCACGGTG ACCGACTTTACA TTACCCCGCCGC
Prm8.2 TCGGACAATTTGT GCAAGAAGTGAT GTTTTGCGCACG CATAACTGCGAC
TCTGGCACGCAT GTGTCGGACAAT ACAGGAGTTTGC GGAACAAATTCT
TTGTCATAACTGC GATGACCCTGAA TGCCAGTCGGGC GACACAGGAGTT
TATGATGCTCGA TTTATCCGATGAC TGCGATGACCCT GAA GAATATGATGCT CGA/
CGCCGTCCTCGC AGTACCATTGCA ACCGACTTTACA GCAAGAAGTGAT
TCTGGCACGCAT GGAACAAATTCT TGCCAGTCGGGC TTTATCCGATGAC GAA-3′ 137
ds857 down 333 GATATGCCTGAA 367 GCCATTGAGCTG 401 5′- Prm8.2/ N/A N/A N/A
GTATTCAATTACT GCTTCCCGACCG GATATGCCTGAA disrupted TAGGCATTTACTT
CAGGGCGGCACC GTATTCAATTACT nifL gene AACGCAGGCAGG TGCCTGACCCTGC
TAGGCATTTACTT CAATTTTGATGCT GTTTCCCGCTGTT AACGCAGGCAGG
GCCTATGAAGCG TAACACCCTGAC CAATTTTGATGCT TTTGATTCTGTAC
CGGAGGTGAAGC GCCTATGAAGCG TTGAGCTTGATC ATGATCCCTGAA
TTTGATTCTGTAC TC TTGAGCTTGATC/ GCCATTGAGCTG GCTTCCCGACCG
CAGGGCGGCACC TGCCTGACCCTG CGTTTCCCGCTGT TTAACACCCTGA
CCGGAGGTGAAG CATGATCCCTGA ATC-3′ 63 ds908 down 334 TGGTATTGTCAGT 368
TCTTTAGATCTCT 402 5′- PinfC/ SEQ SEQ N/A CTGAATGAAGCT CGGTCCGCCCTG
TGGTATTGTCAGT disrupted ID ID CTTGAAAAAGCT ATGGCGGCACCT
CTGAATGAAGCT nifL gene NO: NO: GAGGAAGCGGGC TGCTGACGTTAC
CTTGAAAAAGCT 423 424 GTCGATTTAGTAG GCCTGCCGGTAC GAGGAAGCGGGC
GGAA GGGC AAATCAGTCCGA AGCAGGTTATCA GTCGATTTAGTA AACG GGAC
ATGCCGAGCCGC CCGGAGGCTTAA GAAATCAGTCCG AGTT CGAG CAGTTTGTCGAAT
AATGACCCAGTT AATGCCGAGCCG CAAC AGAT C ACC CCAGTTTGTCGA CGGC CTAA
ATC/ TCTTTAGATCTCT CGGTCCGCCCTG ATGGCGGCACCT TGCTGACGTTAC
GCCTGCCGGTAC AGCAGGTTATCA CCGGAGGCTTAA AATGACCCAGTT ACC-3′ 63
ds908 up 335 TGCAAATTGCAC 369 TGAATATCACTG 403 5′- disrupted N/A N/A N/A
GGTTATTCCGGGT ACTCACAAGCTA TGCAAATTGCAC nifL gene/ GAGTATATGTGT
CCTATGTCGAAG GGTTATTCCGGG PinfC GATTTGGGTTCCG AATTAACTAAAA
TGAGTATATGTG GCATTGCGCAAT AACTGCAAGATG TGATTTGGGTTCC
AAAGGGGAGAAA CAGGCATTCGCG GGCATTGCGCAA GACATGAGCATC
TTAAAGCCGACT TAAAGGGGAGAA ACGGCGTTATCA TGAGAAATGAGA
AGACATGAGCAT GC AGAT CACGGCGTTATC AGC/ TGAATATCACTG ACTCACAAGCTA
CCTATGTCGAAG AATTAACTAAAA AACTGCAAGATG CAGGCATTCGCG
TTAAAGCCGACT TGAGAAATGAGA AGAT-3′ 910 ds960 up 336 TCAGGGCTGCGG 370
CTGGGGTCACTG 404 5′- disrupted N/A N/A N/A ATGTCGGGCGTTT GAGCGCTTTATC
TCAGGGCTGCGG nifL gene/ CACAACACAAAA GGCATCCTGACC ATGTCGGGCGTT
PinfC TGTTGTAAATGCG GAAGAATTTGCC TCACAACACAAA ACACAGCCGGGC
GGTTTCTTCCCGA ATGTTGTAAATG CTGAAACCAGGA CCTGGCTGGCCC
CGACACAGCCGG GCGTGTGATGAC CTGTTCAGGTTGT GCCTGAAACCAG
CTTTAATATGATG GGTGATGAATAT GAGCGTGTGATG C CA ACCTTTAATATG ATGC/
CTGGGGTCACTG GAGCGCTTTATC GGCATCCTGACC GAAGAATTTGCC
GGTTTCTTCCCGA CCTGGCTGGCCC CTGTTCAGGTTGT GGTGATGAATAT CA-3′ 910
ds960 down 337 CGGAAAACGAGT 371 GCAATAGAACTA 405 5′- PinfC/ N/A N/A N/A
TCAAACGGCACG ACTACCCGCCCT CGGAAAACGAGT disrupted TCCGAATCGTATC
GAAGGCGGTACC TCAAACGGCACG nifL gene AATGGCGAGATT TGCCTGACCCTGC
TCCGAATCGTAT CGCGCCCAGGAA GATTCCCGTTATT CAATGGCGAGAT

GTTCGCTTAACTG TCATTCACTGACC TCGCGCCCAGGA GTCTGGAAGGTG
GGAGGCCCACGA AGTTCGCTTAACT AGCAGCTGGGTA TGACCCAGCGAC
GGTCTGGAAGGT TT C GAGCAGCTGGGT ATT/ GCAATAGAACTA ACTACCCGCCCT
GAAGGCGGTACC TGCCTGACCCTG CGATTCCCGTTAT TTCATTCACTGAC
CGGAGGCCCACG ATGACCCAGCGA CC-3′ 137 ds843 up 425 TCCGGGTTCGGCT 436
GCCCGCTGACCG 447 TCCGGGTTCGGC 5′ N/A N/A N/A TACCCCGCCGCGT
ACCAGAACTTCC TTACCCCGCCGC upstream TTTGCGCACGGTG ACCTTGGACTCG
GTTTTGCGCACG region of TCGGACAATTTGT GCTATACCCTTGG GTGTCGGACAAT
nifL/ CATAACTGCGAC CGTGACGGCGCG TTGTCATAACTGC Prm1.2 ACAGGAGTTTGC
CGATAACTGGGA GACACAGGAGTT GATGACCCTGAA CTACATCCCCATT
TGCGATGACCCT TATGATGCTCGA CCGGTGATCTTAC GAATATGATGCT C CGA/
GCCCGCTGACCG ACCAGAACTTCC ACCTTGGACTCG GCTATACCCTTG
GCGTGACGGCGC GCGATAACTGGG ACTACATCCCCA TTCCGGTGATCTT ACC 137
ds843 down 426 TCACTTTTTAGCA 437 GCCATTGAGCTG 448 TCACTTTTTAGCA Prm1.2/
N/A N/A N/A AAGTTGCACTGG GCTTCCCGACCG AAGTTGCACTGG nifA
ACAAAAGGTACC CAGGGCGGCACC ACAAAAGGTACC ACAATTGGTGTA
TGCCTGACCCTGC ACAATTGGTGTA CTGATACTCGAC GTTTCCCGCTGTT
CTGATACTCGAC ACAGCATTAGTG TAACACCCTGAC ACAGCATTAGTG
TCGATTTTTCATA CGGAGGTGAAGC TCGATTTTTCATA TAAAGGTAATTTT
ATGATCCCTGAA TAAAGGTAATTT G TC TG/ GCCATTGAGCTG GCTTCCCGACCG
CAGGGCGGCACC TGCCTGACCCTG CGTTTCCCGCTGT TTAACACCCTGA
CCGGAGGTGAAG CATGATCCCTGA ATC 137 ds809 up 427 ATCGCAGCGTCTT 438
GCGCTGAAGCAC 449 ATCGCAGCGTCT 1647 bp N/A N/A N/A TGAATATTTCCGT
CTGATCACGCTCT TTGAATATTTCCG deletion of CGCCAGGCGCTG GCGCGGCGTCGC
TCGCCAGGCGCT glnE N- GCTGCCGAGCCG CGATGGTCGCCA GGCTGCCGAGCC
terminus TTCTGGCTGCATA GCCAGCTGGCGC GTTCTGGCTGCAT after the
GTGGAAAACGAT GCCACCCGCTGC AGTGGAAAACGA start AATTTCAGGCCA
TGCTGGATGAGC TAATTTCAGGCC codon. GGGAGCCCTTAT TGCTGGATCCCA
AGGGAGCCCTTA G ACA TG/ GCGCTGAAGCAC CTGATCACGCTCT GCGCGGCGTCGC
CGATGGTCGCCA GCCAGCTGGCGC GCCACCCGCTGC TGCTGGATGAGC
TGCTGGATCCCA ACA 137 ds2974 up 428 ACGCGCGCTCAC 439 GCTATCCGTATGC 450
ACGCGCGCTCAC 5′ region N/A N/A N/A CGGAGCCGGCTT CGGGAATGGATG
CGGAGCCGGCTT ofNtrC GAGCTGCACAAC GTCTGGCGCTGCT GAGCTGCACAAC
upstream GTTCGAAAGCGG CAAACAGATTAA GTTCGAAAGCGG of D54A
CAATGAGGTGCT GCAGCGTCATCC CAATGAGGTGCT (GAT-> AGATGCCCTCAC
AATGCTTCCGGTC AGATGCCCTCAC GCT) CACCAAAACCCC ATCATAATGACC
CACCAAAACCCC GGATGTACTGCT GCACATTCCGAT GGATGTACTGCT GTCA C GTCA/
GCTATCCGTATG CCGGGAATGGAT GGTCTGGCGCTG CTCAAACAGATT
AAGCAGCGTCAT CCAATGCTTCCG GTCATCATAATG ACCGCACATTCC GATC 137
ds2974 down 429 CGCGCTCACCGG 440 ATCCGTATGCCG 451 CGCGCTCACCGG NtrC N/A
N/A N/A AGCCGGCTTGAG GGAATGGATGGT AGCCGGCTTGAG sequence
CTGCACAACGTTC CTGGCGCTGCTC CTGCACAACGTT downstream GAAAGCGGCAAT
AAACAGATTAAG CGAAAGCGGCAA of the GAGGTGCTAGAT CAGCGTCATCCA
TGAGGTGCTAGA D54A GCCCTCACCACC ATGCTTCCGGTCA TGCCCTCACCAC (GAT->
AAAACCCCGGAT TCATAATGACCG CAAAACCCCGGA GCT) GTACTGCTGTCAG
CACATTCCGATCT TGTACTGCTGTCA mutation CT GG GCT/ ATCCGTATGCCG
GGAATGGATGGT CTGGCGCTGCTC AAACAGATTAAG CAGCGTCATCCA
ATGCTTCCGGTC ATCATAATGACC GCACATTCCGAT CTGG 137 799 up 430
TCCGGGTTCGGCT 441 AGCGTCAGGTAC 452 TCCGGGTTCGGC 5′ N/A N/A N/A
TACCCCGCCGCGT CGGTCATGATTC TTACCCCGCCGC upstream TTTGCGCACGGTG

ACCGTGCGATTCT GTTTTGCGCACG region of TCGGACAATTTGT CGGTTCCCTGGA GTGTCGGACAAT nifL/ CATAACTGCGAC GCGCTTCATTGGC TTGTCATAACTGC PinfC ACAGGAGTTTGC ATCCTGACCGAA GACACAGGAGTT GATGACCCTGAA GAGTTCGCTGGC TGCGATGACCCT TATGATGCTCGA TTCTTCCCAACCT GAATATGATGCT G CGA/ AGCGTCAGGTAC CGGTCATGATTC ACCGTGCGATTC TCGGTTCCCTGG AGCGCTTCATTG GCATCCTGACCG AAGAGTTCGCTG GCTTCTTCCCAAC CTG 137 799 down 431 TCTTCAACAACTG 442 GCCATTGAGCTG 453 TCTTCAACAACT PinfC/ N/A N/A N/A GAGGAATAAGGT GCTTCCCGACCG GGAGGAATAAGG nifA ATTAAAGGCGGA CAGGGCGGCACC TATTAAAGGCGG AAACGAGTTCAA TGCCTGACCCTGC AAAACGAGTTCA ACGGCACGTCCG GTTTCCCGCTGTT AACGGCACGTCC AATCGTATCAAT TAACACCCTGAC GAATCGTATCAA GGCGAGATTCGC CGGAGGTGAAGC TGGCGAGATTCG GCCCTGGAAGTT ATGATCCCTGAA CGCCCTGGAAGT CGC TC TCGC/ GCCATTGAGCTG GCTTCCCGACCG CAGGGCGGCACC TGCCTGACCCTG CGTTTCCCGCTGT TTAACACCCTGA CCGGAGGTGAAG CATGATCCCTGA ATC 137 ds2538 up 432 AGCTCATTGCGG 443 CCTTGATTATGGC 454 AGCTCATTGCGG 5′ N/A N/A N/A CGCGCACCGAAT CGCGGCGAGCTG CGCGCACCGAAT upstream TTATCGACCAGCT CACCCGCTCTCTG TTATCGACCAGC region of GCTGCAGCGGTT ACGTCGCACTGC TGCTGCAGCGGT glnD- GTGGATCGCCTA TGATCCTCAGCC TGTGGATCGCCT Utase CGGTTTTGAATCC GCAAAAAACTGC ACGGTTTTGAAT de- GTCTGCGATCTGG CTGACGACCAGG CCGTCTGCGATCT activation CGCTGGTGGCCG CGCAAAAGGTCG GGCGCTGGTGGC mutation. T GC CGT/ CCTTGATTATGGC CGCGGCGAGCTG CACCCGCTCTCTG ACGTCGCACTGC TGATCCTCAGCC GCAAAAAACTGC CTGACGACCAGG CGCAAAAGGTCG GC 137 ds2538 down 433 GTTGTGGATCGCC 444 CTGCTGATCCTCA 455 GTTGTGGATCGC 3′ N/A N/A N/A TACGGTTTTGAAT GCCGCAAAAAAC CTACGGTTTTGA downstream CCGTCTGCGATCT TGCCTGACGACC ATCCGTCTGCGA region GGCGCTGGTGGC AGGCGCAAAAGG TCTGGCGCTGGT of glnD- CGTCCTTGATTAT TCGGCGAACTGC GGCCGTCCTTGA Utase GGCCGCGGCGAG TGACGCTACTGT TTATGGCCGCGG de- CTGCACCCGCTCT GGGACGTCAAGC CGAGCTGCACCC activation CTGACGTCGCA TGGAGGTGGGCC GCTCTCTGACGTC mutation. ACA GCA/ CTGCTGATCCTCA GCCGCAAAAAAC TGCCTGACGACC AGGCGCAAAAGG TCGGCGAACTGC TGACGCTACTGT GGGACGTCAAGC TGGAGGTGGGCC ACA 137 ds2969 up 434 ACGGCAGGGTTT 445 GCCCGCTGACCG 456 ACGGCAGGGTTT 5′ N/A N/A N/A TGTGTTTTTGAAA ACCAGAACTTCC TGTGTTTTTGAAA upstream ACAAATGCCTGA ACCTTGGACTCG ACAAATGCCTGA of an extra AATCGGCTATAA GCTATACCCTTGG AATCGGCTATAA copy of AGTGTGATCTGC CGTGACGGCGCG AGTGTGATCTGC Prm1.2_ ATCAAAATGCCA CGATAACTGGGA ATCAAAATGCCA nifA gene TGCGCCAAACTT CTACATCCCCATT TGCGCCAAACTT inserted in AAGGAATATTAA CCGGTGATCTTAC AAGGAATATTAA anon- GGA C GGA/ coding GCCCGCTGACCG site of ACCAGAACTTCC Klebsiella ACCTTGGACTCG genome GCTATACCCTTG between GCGTGACGGCGC two GCGATAACTGGG hypothetical ACTACATCCCCA coding TTCCGGTGATCTT sequences. ACC 137 ds2969 down 435 GGAACGCGACAA 446 GCGTTAAAAGAT 457 GGAACGCGACAA 3′ N/A N/A N/A TGTTGTGCCGCAG ATTTTTGTGCGTA TGTTGTGCCGCA downstream GGATGCGGGATA CCGAACCTCGCA GGGATGCGGGAT of an ATGCTTTATTTTT GACGGCATTATG AATGCTTTATTTT extra copy CAGCCAGATAAA GCGTTGCATTGTT TCAGCCAGATAA of AAATTCGTCACTG TATCGGGCTTATT AAAATTCGTCAC Prm1.2_ GTACGTCGTTTGC TCTGGGGTTGTTT TGGTACGTCGTTT nifA gene AGCAGGAAGGTA CAGCATTTGTTA GCAGCAGGAAGG inserted in TA/ anon- GCGTTAAAAGAT coding ATTTTTGTGCGTA site of CCGAACCTCGCA Klebsiella GACGGCATTATG genome

GCGTTGCATTGTT between TATCGGGCTTATT two TCTGGGGTTGTTT hypothetical CAGCATTTGTTA coding sequences.

(712) TABLE-US-00032 TABLE 31 WT and Remodeled Non-intergeneric Microbes Strain Name Genotype SEQ ID NO CI006 16S rDNA - contig 5 62 CI006 16S rDNA - contig 8 63 CI019 16S rDNA 64 CI006 nifH 65 CI006 nifD 66 CI006 nifK 67 CI006 nifL 68 CI006 nifA 69 CI019 nifH 70 CI019 nifD 71 CI019 nifK 72 CI019 nifL 73 CI019 nifA 74 CI006 Prm5 with 500 bp 75 flanking regions CI006 nifLA operon - upstream 76 intergenic region plus nifL and nifA CDSs CI006 nifL (Amino Acid) 77 CI006 nifA (Amino Acid) 78 CI006 glnE 79 CI006 glnE_KO1 80 CI006 glnE (Amino Acid) 81 CI006 glnE_KO1 (Amino Acid) 82 CI006 GlnE ATase domain 83 (Amino Acid) CM029 Prm5 inserted into nifL 84 region

(713) TABLE-US-00033 TABLE 32 Remodeled Non-intergeneric Microbes Associated Novel Strain SEQ ID Junction If Strain ID NO Genotype Description Applicable CI63; 63 SEQ ID 16S N/A N/A CI063 NO 85 CI63; 63 SEQ ID nifH N/A N/A CI063 NO 86 CI63; 63 SEQ ID nifD1 1 of 2 unique genes annotated as nifD N/A CI063 NO 87 in 63 genome CI63; 63 SEQ ID nifD2 2 of 2 unique genes annotated as nifD N/A CI063 NO 88 in 63 genome CI63; 63 SEQ ID nifK1 1 of 2 unique genes annotated as nifK N/A CI063 NO 89 in 63 genome CI63; 63 SEQ ID nifK2 2 of 2 unique genes annotated as nifK N/A CI063 NO 90 in 63 genome CI63; 63 SEQ ID nifL N/A N/A CI063 NO 91 CI63; 63 SEQ ID nifA N/A N/A CI063 NO 92 CI63; 63 SEQ ID glnE N/A N/A CI063 NO 93 CI63; 63 SEQ ID amtB N/A N/A CI063 NO 94 CI63; 63 SEQ ID PinfC 500 bp immediately upstream of the N/A CI063 NO 95 ATG start codon of the infC gene CI137 137 SEQ ID 16S N/A N/A NO 96 CI137 137 SEQ ID nifH1 1 of 2 unique genes annotated as nifH N/A NO 97 in 137 genome CI137 137 SEQ ID nifH2 2 of 2 unique genes annotated as nifH N/A NO 98 in 137 genome CI137 137 SEQ ID nifD1 1 of 2 unique genes annotated as nifD N/A NO 99 in 137 genome CI137 137 SEQ ID nifD2 2 of 2 unique genes annotated as nifD N/A NO 100 in 137 genome CI137 137 SEQ ID nifK1 1 of 2 unique genes annotated as nifK N/A NO 101 in 137 genome CI137 137 SEQ ID nifK2 2 of 2 unique genes annotated as nifK N/A NO 102 in 137 genome CI137 137 SEQ ID nifL N/A N/A NO 103 CI137 137 SEQ ID nifA N/A N/A NO 104 CI137 137 SEQ ID glnE N/A N/A NO 105 CI137 137 SEQ ID PinfC 500 bp immediately upstream of the N/A NO 106 TTG start codon of infC CI137 137 SEQ ID amtB N/A N/A NO 107 CI137 137 SEQ ID Prm8.2 internal promoter located in nlpI gene; N/A NO 108 299 bp starting at 81 bp after the A of the ATG of the nlpI gene CI137 137 SEQ ID Prm6.2 300 bp upstream of the secE gene N/A NO 109 starting at 57 bp upstream of the A of the ATG of secE CI137 137 SEQ ID Prm1.2 400 bp immediately upstream of the N/A NO 110 ATG of cspE gene none 728 SEQ ID 16S N/A N/A NO 111 none 728 SEQ ID nifH N/A N/A NO 112 none 728 SEQ ID nifD1 1 of 2 unique genes annotated as nifD N/A NO 113 in 728 genome none 728 SEQ ID nifD2 2 of 2 unique genes annotated as nifD N/A NO 114 in 728 genome none 728 SEQ ID nifK1 1 of 2 unique genes annotated as nifK N/A NO 115 in 728 genome none 728 SEQ ID nifK2 2 of 2 unique genes annotated as nifK N/A NO 116 in 728 genome none 728 SEQ ID nifL N/A N/A NO 117 none 728 SEQ ID nifA N/A N/A NO 118 none 728 SEQ ID glnE N/A N/A NO 119 none 728 SEQ ID amtB N/A N/A NO 120 none 850 SEQ ID 16S N/A N/A NO 121 none 852 SEQ ID 16S N/A N/A NO 122 none 853 SEQ ID 16S N/A N/A NO 123 none 910 SEQ ID 16S N/A N/A NO 124 none 910 SEQ ID nifH N/A N/A NO 125 none 910 SEQ ID Dinitrogenase iron- N/A N/A NO 126 molybdenum cofactor CDS none 910 SEQ ID nifD1 N/A N/A NO 127 none 910 SEQ ID nifD2 N/A N/A NO 128 none 910 SEQ ID nifK1 N/A N/A NO 129 none 910 SEQ ID nifK2 N/A N/A NO 130 none 910 SEQ ID nifL N/A N/A NO 131 none 910 SEQ ID nifA N/A N/A NO 132 none 910 SEQ ID glnE N/A N/A NO 133 none 910 SEQ ID amtB N/A N/A NO 134 none 910 SEQ ID PinfC 498 bp immediately upstream of the N/A NO 135 ATG of the infC gene none 1021 SEQ ID 16S N/A N/A NO 136 none 1021 SEQ ID nifH N/A N/A NO 137 none 1021 SEQ ID nifD1 1 of 2 unique genes annotated as nifD N/A NO 138 in 910 genome none 1021 SEQ ID nifD2 2 of 2 unique genes annotated as nifD N/A NO 139 in 910 genome none 1021 SEQ ID nifK1 1 of 2

unique genes annotated as nifK N/A NO 140 in 910 genome none 1021 SEQ ID nifK2 2 of 2 unique genes annotated as nifK N/A NO 141 in 910 genome none 1021 SEQ ID nifL N/A N/A NO 142 none 1021 SEQ ID nifA N/A N/A NO 143 none 1021 SEQ ID glnE N/A N/A NO 144 none 1021 SEQ ID amtB N/A N/A NO 145 none 1021 SEQ ID PinfC 500 bp immediately upstream of the N/A NO 146 ATG start codon of the infC gene none 1021 SEQ ID Prm1 348 bp includes the 319 bp immediately N/A NO 147 upstream of the ATG start codon of the lpp gene and the first 29 bp of the lpp gene none 1021 SEQ ID Prm7 339 bp upstream of the sspA gene, N/A NO 148 ending at 46 bp upstream of the ATG of the sspA gene none 1113 SEQ ID 16S N/A N/A NO 149 none 1113 SEQ ID nifH N/A N/A NO 150 none 1113 SEQ ID nifD1 1 of 2 unique genes annotated as nifD N/A NO 151 in 1113 genome none 1113 SEQ ID nifD2 2 of 2 unique genes annotated as nifD N/A NO 152 in 1113 genome none 1113 SEQ ID nifK N/A N/A NO 153 none 1113 SEQ ID nifL N/A N/A NO 154 none 1113 SEQ ID nifA partial gene due to a gap in the sequence assembly, N/A NO 155 we can only identify a partial gene from the 1113 genome none 1113 SEQ ID glnE N/A N/A NO 156 none 1116 SEQ ID 16S N/A NO 157 none 1116 SEQ ID nifH N/A NO 158 none 1116 SEQ ID nifD1 1 of 2 unique genes annotated as nifD N/A NO 159 in 1116 genome none 1116 SEQ ID nifD2 2 of 2 unique genes annotated as nifD N/A NO 160 in 1116 genome none 1116 SEQ ID nifK1 1 of 2 unique genes annotated as nifK N/A NO 161 in 1116 genome none 1116 SEQ ID nifK2 2 of 2 unique genes annotated as nifK N/A NO 162 in 1116 genome none 1116 SEQ ID nifL N/A N/A NO 163 none 1116 SEQ ID nifA N/A N/A NO 164 none 1116 SEQ ID glnE N/A N/A NO 165 none 1116 SEQ ID amtB N/A N/A NO 166 none 1293 SEQ ID 16S N/A N/A NO 167 none 1293 SEQ ID nifH N/A N/A NO 168 none 1293 SEQ ID nifD1 1 of 2 unique genes annotated as nifD N/A NO 169 in 1293 genome none 1293 SEQ ID nifD2 2 of 2 unique genes annotated as nifD N/A NO 170 in 1293 genome none 1293 SEQ ID nifK 1 of 2 unique genes annotated as nifK N/A NO 171 in 1293 genome none 1293 SEQ ID nifK1 2 of 2 unique genes annotated as nifK N/A NO 172 in 1293 genome none 1293 SEQ ID nifA N/A N/A NO 173 none 1293 SEQ ID glnE N/A N/A NO 174 none 1293 SEQ ID amtB1 1 of 2 unique genes annotated as amtB N/A NO 175 in 1293 genome none 1293 SEQ ID amtB2 2 of 2 unique genes annotated as amtB N/A NO 176 in 1293 genome none 1021-1612 SEQ ID ΔnifL::PinfC starting at 24 bp after the A of the ATG ds1131 NO 177 start codon, 1375 bp of nifL have been deleted and replaced with the 1021 PinfC promoter sequence none 1021-1612 SEQ ID ΔnifL::PinfC with starting at 24 bp after the A of the ATG ds1131 NO 178 500 bp flank start codon, 1375 bp of nifL have been deleted and replaced with the 1021 PinfC promoter sequence; 500 bp flanking the nifL gene upstream and downstream are included none 1021-1612 SEQ ID glnEΔAR-2 glnE gene with 1673 bp immediately ds1133 NO 179 downstream of the ATG start codon deleted, resulting in a truncated glnE protein lacking the adenylyl-removing (AR) domain none 1021-1612 SEQ ID glnEΔAR-2 with glnE gene with 1673 bp immediately ds1133 NO 180 500 bp flank downstream of the ATG start codon deleted, resulting in a truncated glnE protein lacking the adenylyl-removing (AR) domain; 500 bp flanking the glnE gene upstream and downstream are included none 1021-1615 SEQ ID ΔnifL::Prm1 starting at 24 bp after the A of the ATG ds1145 NO 181 start codon, 1375 bp of nifL have been deleted and replaced with the 1021 Prm1 promoter sequence none 1021-1615 SEQ ID ΔnifL::Prm1 with starting at 24 bp after the A of the ATG ds1145 NO 182 500 bp flank start codon, 1375 bp of nifL have been deleted and replaced with the 1021 rm 1 promoter sequence; 500 bp flanking the nifL gene upstream and downstream are included none 1021-1615 SEQ ID glnEΔAR-2 glnE gene with 1673 bp immediately ds1133 NO 183 downstream of the ATG start codon deleted, resulting in a truncated glnE protein lacking the adenylyl-removing (AR) domain none 1021-1615 SEQ ID glnEΔAR-2 with glnE gene with 1673 bp immediately ds1133 NO 184 500 bp flank downstream of the ATG start codon deleted, resulting in a truncated glnE protein lacking the adenylyl-removing (AR) domain; 500 bp flanking the glnE gene upstream and downstream are included none 1021-1619 SEQ ID ΔnifL::Prm1 starting at 24 bp after the A of the ATG ds1145 NO 185 start codon, 1375 bp of nifL have been deleted and replaced with the 1021 Prm1 promoter sequence none 1021-

1619 SEQ ID ΔnifL::Prm1 with starting at 24 bp after the A of the ATG ds1145 NO 186 500 bp flank start codon, 1375 bp of nifL have been deleted and replaced with the 1021 rm1 promoter sequence; 500 bp flanking the nifL gene upstream and downstream are included none 1021-1623 SEQ ID glnEΔAR-2 glnE gene with 1673 bp immediately ds1133 NO 187 downstream of the ATG start codon deleted, resulting in a truncated glnE protein lacking the adenylyl-removing (AR) domain none 1021-1623 SEQ ID glnEΔAR-2 with glnE gene with 1673 bp immediately ds1133 NO 188 500 bp flank downstream of the ATG start codon deleted, resulting in a truncated glnE protein lacking the adenylyl-removing (AR) domain; 500 bp flanking the glnE gene upstream and downstream are included none 1021-1623 SEQ ID ΔnifL::Prm7 starting at 24 bp after the A of the ATG ds1148 NO 189 start codon, 1375 bp of nifL have been deleted and replaced with the 1021 Prm7 promoter sequence none 1021-1623 SEQ ID ΔnifL::Prm7 with starting at 24 bp after the A of the ATG ds1148 NO 190 500 bp flank start codon, 1375 bp of nifL have been deleted and replaced with the 1021 rm7 promoter sequence; 500 bp flanking the nifL gene upstream and downstream are included none 137-1034 SEQ ID glnEΔAR-2 glnE gene with 1290 bp immediately ds809 NO 191 downstream of the ATG start codon deleted, resulting in a truncated glnE protein lacking the adenylyl-removing (AR) domain none 137-1034 SEQ ID glnEΔAR-2 with glnE gene with 1290 bp immediately ds809 NO 192 500 bp flank downstream of the ATG start codon deleted, resulting in a truncated glnE protein lacking the adenylyl-removing (AR) domain; 500 bp flanking the glnE gene upstream and downstream are included none 137-1036 SEQ ID ΔnifL::PinfC starting at 24 bp after the A of the ATG ds799 NO 193 start codon, 1372 bp of nifL have been deleted and replaced with the 137 PinfC promoter sequence none 137-1036 SEQ ID ΔnifL::PinfC with starting at 24 bp after the A of the ATG ds799 NO 194 500 bp flank start codon, 1372 bp of nifL have been deleted and replaced with the 137 PinfC promoter sequence; 500 bp flanking the nifL gene upstream and downstream are included none 137-1314 SEQ ID glnEΔAR-2 36 bp glnE gene with 1290 bp immediately none NO 195 deletion downstream of the ATG start codon deleted AND 36 bp deleted beginning at 1472 bp downstream of the start codon, resulting in a truncated glnE protein lacking the adenylyl-removing (AR) domain none 137-1314 SEQ ID glnEΔAR-2 36 bp glnE gene with 1290 bp immediately none NO 196 deletion downstream of the ATG start codon deleted AND 36 bp deleted beginning at 1472 bp downstream of the start codon, resulting in a truncated glnE protein lacking the adenylyl-removing (AR) domain; 500 bp flanking the nifL gene upstream and downstream are included none 137-1314 SEQ ID ΔnifL::Prm8.2 starting at 24 bp after the A of the ATG ds857 NO 197 start codon, 1372 bp of nifL have been deleted and replaced with the 137 Prm8.2 promoter sequence none 137-1314 SEQ ID ΔnifL::Prm8.2 with starting at 24 bp after the A of the ATG ds857 NO 198 500 bp flank start codon, 1372 bp of nifL have been deleted and replaced with the 137 Prm8.2 promoter sequence; 500 bp flanking the nifL gene upstream and downstream are included none 137-1329 SEQ ID glnEΔAR-2 36 bp glnE gene with 1290 bp immediately none NO 199 deletion downstream of the ATG start codon deleted AND 36 bp deleted beginning at 1472 bp downstream of the start codon, resulting in a truncated glnE protein lacking the adenylyl-removing (AR) domain none 137-1329 SEQ ID glnEΔAR-2 36 bp glnE gene with 1290 bp immediately none NO 200 deletion downstream of the ATG start codon deleted AND 36 bp deleted beginning at 1472 bp downstream of the start codon, resulting in a truncated glnE protein lacking the adenylyl-removing (AR) domain; 500 bp flanking the nifL gene upstream and downstream are included none 137-1329 SEQ ID ΔnifL::Prm6.2 starting at 24 bp after the A of the ATG ds853 NO 201 start codon, 1372 bp of nifL have been deleted and replaced with the 137 Prm6.2 promoter sequence none 137-1329 SEQ ID ΔnifL::Prm6.2 with starting at 24 bp after the A of the ATG ds853 NO 202 500 bp flank start codon, 1372 bp of nifL have been deleted and replaced with the 137 Prm6.2 promoter sequence; 500 bp flanking the nifL gene upstream and downstream are included none 137-1382 SEQ ID ΔnifL::Prm1.2 starting at 24 bp after the A of the ATG ds843 NO 203 start codon, 1372 bp of nifL have been deleted and replaced with the 137 Prm1.2 promoter sequence none 137-1382 SEQ ID ΔnifL::Prm1.2 with starting at 24

bp after the A of the ATG ds843 NO 204 500 bp flank start codon, 1372 bp of nifL have been deleted and replaced with the 137 Prm1.2 promoter sequence; 500 bp flanking the nifL gene upstream and downstream are included none 137-1382 SEQ ID glnEΔAR-2 36 bp glnE gene with 1290 bp immediately none NO 205 deletion downstream of the ATG start codon deleted AND 36 bp deleted beginning at 1472 bp downstream of the start codon, resulting in a truncated glnE protein lacking the adenylyl-removing (AR) domain none 137-1382 SEQ ID glnEΔAR-2 36 bp glnE gene with 1290 bp immediately none NO 206 deletion downstream of the ATG start codon deleted AND 36 bp deleted beginning at 1472 bp downstream of the start codon, resulting in a truncated glnE protein lacking the adenylyl-removing (AR) domain; 500 bp flanking the nifL gene upstream and downstream are included none 137-1586 SEQ ID ΔnifL::PinfC starting at 24 bp after the A of the ATG ds799 NO 207 start codon, 1372 bp of nifL have been deleted and replaced with the 137 PinfC promoter sequence none 137-1586 SEQ ID ΔnifL::PinfC with starting at 24 bp after the A of the ATG ds799 NO 208 500 bp flank start codon, 1372 bp of nifL have been deleted and replaced with the 137 PinfC promoter sequence; 500 bp flanking the nifL gene upstream and downstream are included none 137-1586 SEQ ID glnEΔAR-2 glnE gene with 1290 bp immediately ds809 NO 209 downstream of the ATG start codon deleted, resulting in a truncated glnE protein lacking the adenylyl-removing (AR) domain none 137-1586 SEQ ID glnEΔAR-2 with glnE gene with 1290 bp immediately ds809 NO 210 500 bp flank downstream of the ATG start codon deleted, resulting in a truncated glnE protein lacking the adenylyl-removing (AR) domain; 500 bp flanking the glnE gene upstream and downstream are included none 19-594 SEQ ID glnEΔAR-2 glnE gene with 1650 bp immediately ds34 NO 211 downstream of the ATG start codon deleted, resulting in a truncated glnE protein lacking the adenylyl-removing (AR) domain none 19-594 SEQ ID glnEΔAR-2 with glnE gene with 1650 bp immediately ds34 NO 212 500 bp flank downstream of the ATG start codon deleted, resulting in a truncated glnE protein lacking the adenylyl-removing (AR) domain; 500 bp flanking the glnE gene upstream and downstream are included none 19-594 SEQ ID ΔnifL::Prm6.1 starting at 221 bp after the A of the ds180 NO 213 ATG start codon, 845 bp of nifL have been deleted and replaced with the CI019 Prm6.1 promoter sequence none 19-594 SEQ ID ΔnifL::Prm6.1 with starting at 221 bp after the A of the ds180 NO 214 500 bp flank ATG start codon, 845 bp of nifL have been deleted and replaced with the CI019 Prm6.1promoter sequence; 500 bp flanking the nifL gene upstream and downstream are included none 19-714 SEQ ID ΔnifL::Prm6.1 starting at 221 bp after the A of the ds180 NO 215 ATG start codon, 845 bp of nifL have been deleted and replaced with the CI019 Prm6.1 promoter sequence none 19-714 SEQ ID ΔnifL::Prm6.1 with starting at 221 bp after the A of the ds180 NO 216 500 bp flank ATG start codon, 845 bp of nifL have been deleted and replaced with the CI019 Prm6.1promoter sequence; 500 bp flanking the nifL gene upstream and downstream are included none 19-715 SEQ ID ΔnifL::Prm7.1 starting at 221 bp after the A of the ds181 NO 217 ATG start codon, 845 bp of nifL have been deleted and replaced with the CI019 Prm7.1 promoter sequence none 19-715 SEQ ID ΔnifL::Prm7.1 with starting at 221 bp after the A of the ds181 NO 218 500 bp flank ATG start codon, 845 bp of nifL have been deleted and replaced with the CI019 Prm76. lpromoter sequence; 500 bp flanking the nifL gene upstream and downstream are included 19-713 19-750 SEQ ID ΔnifL::Prm1.2 starting at 221 bp after the A of the ds172 NO 219 ATG start codon, 845 bp of nifL have been deleted and replaced with the CI019 Prm1.2 promoter sequence 19-713 19-750 SEQ ID ΔnifL::Prm1.2 with starting at 221 bp after the A of the ds172 NO 220 500 bp flank ATG start codon, 845 bp of nifL have been deleted and replaced with the CI019 Prm1.2 promoter sequence; 500 bp flanking the nifL gene upstream and downstream are included 17-724 19-804 SEQ ID ΔnifL::Prm1.2 starting at 221 bp after the A of the ds172 NO 221 ATG start codon, 845 bp of nifL have been deleted and replaced with the CI019 Prm1.2 promoter sequence 17-724 19-804 SEQ ID ΔnifL::Prm1.2 with starting at 221 bp after the A of the ds172 NO 222 500 bp flank ATG start codon, 845 bp of nifL have been deleted and replaced with the CI019 Prm1.2 promoter sequence; 500 bp flanking the nifL gene upstream and downstream are included 17-724 19-804 SEQ ID

glnEΔAR-2 glnE gene with 1650 bp immediately ds34 NO 223 downstream of the ATG start codon deleted, resulting in a truncated glnE protein lacking the adenylyl-removing (AR) domain 17-724 19-804 SEQ ID glnEΔAR-2 with glnE gene with 1650 bp immediately ds34 NO 224 500 bp flank downstream of the ATG start codon deleted, resulting in a truncated glnE protein lacking the adenylyl-removing (AR) domain; 500 bp flanking the glnE gene upstream and downstream are included 19-590 19-806 SEQ ID ΔnifL::Prm3.1 starting at 221 bp after the A of the ds175 NO 225 ATG start codon, 845 bp of nifL have been deleted and replaced with the CI019 Prm3.1 promoter sequence 19-590 19-806 SEQ ID ΔnifL::Prm3.1 with starting at 221 bp after the A of the ds175 NO 226 500 bp flank ATG start codon, 845 bp of nifL have been deleted and replaced with the CI019 Prm3.1 promoter sequence; 500 bp flanking the nifL gene upstream and downstream are included 19-590 19-806 SEQ ID glnEΔAR-2 glnE gene with 1650 bp immediately ds34 NO 227 downstream of the ATG start codon deleted, resulting in a truncated glnE protein lacking the adenylyl-removing (AR) domain 19-590 19-806 SEQ ID glnEΔAR-2 with glnE gene with 1650 bp immediately ds34 NO 228 500 bp flank downstream of the ATG start codon deleted, resulting in a truncated glnE protein lacking the adenylyl-removing (AR) domain; 500 bp flanking the glnE gene upstream and downstream are included none 63-1146 SEQ ID ΔnifL::PinfC starting at 24 bp after the A of the ATG ds908 NO 229 start codon, 1375 bp of nifL have been deleted and replaced with the 63 PinfC promoter sequence none 63-1146 SEQ ID ΔnifL::PinfC with starting at 24 bp after the A of the ATG ds908 NO 230 500 bp flank start codon, 1375 bp of nifL have been deleted and replaced with the 63 PinfC promoter sequence; 500 bp flanking the nifL gene upstream and downstream are included CM015; 6-397 SEQ ID ΔnifL::Prm5 starting at 31 bp after the A of the ATG ds24 PBC6.15 NO 231 start codon, 1375 bp of nifL have been deleted and replaced with the CI006 Prm5 promoter sequence CM015; 6-397 SEQ ID ΔnifL::Prm5 with starting at 31 bp after the A of the ATG ds24 PBC6.15 NO 232 500 bp flank start codon, 1375 bp of nifL have been deleted and replaced with the CI006 Prm5 promoter sequence; 500 bp flanking the nifL gene upstream and downstream are included CM014 6-400 SEQ ID ΔnifL::Prm1 starting at 31 bp after the A of the ATG ds20 NO 233 start codon, 1375 bp of nifL have been deleted and replaced with the CI006 Prm1 promoter sequence CM014 6-400 SEQ ID ΔnifL::Prm1 with starting at 31 bp after the A of the ATG ds20 NO 234 500 bp flank start codon, 1375 bp of nifL have been deleted and replaced with the CI006 Prm1 promoter sequence; 500 bp flanking the nifL gene upstream and downstream are included CM037; 6-403 SEQ ID ΔnifL::Prm1 starting at 31 bp after the A of the ATG ds20 PBC6.37 NO 235 start codon, 1375 bp of nifL have been deleted and replaced with the CI006 Prm1 promoter sequence CM037; 6-403 SEQ ID ΔnifL::Prm1 with starting at 31 bp after the A of the ATG ds20 PBC6.38 NO 236 500 bp flank start codon, 1375 bp of nifL have been deleted and replaced with the CI006 Prm1 promoter sequence; 500 bp flanking the nifL gene upstream and downstream are included CM037; 6-403 SEQ ID glnEΔAR-2 glnE gene with 1644 bp immediately ds31 PBC6.39 NO 237 downstream of the ATG start codon deleted, resulting in a truncated glnE protein lacking the adenylyl-removing (AR) domain CM037; 6-403 SEQ ID glnEΔAR-2 with glnE gene with 1644 bp immediately ds31 PBC6.40 NO 238 500 bp flank downstream of the ATG start codon deleted, resulting in a truncated glnE protein lacking the adenylyl-removing (AR) domain; 500 bp flanking the glnE gene upstream and downstream are included CM038; 6-404 SEQ ID glnEΔAR-1 glnE gene with 1287 bp immediately ds30 PBC6.38 NO 239 downstream of the ATG start codon deleted, resulting in a truncated glnE protein lacking the adenylyl-removing (AR) domain CM038; 6-404 SEQ ID ΔnifL::Prm1 starting at 31 bp after the A of the ATG ds20 PBC6.38 NO 240 start codon, 1375 bp of nifL have been deleted and replaced with the CI006 Prm1 promoter sequence CM038; 6-404 SEQ ID ΔnifL::Prm1 with starting at 31 bp after the A of the ATG ds20 PBC6.38 NO 241 500 bp flank start codon, 1375 bp of nifL have been deleted and replaced with the CI006 Prm1 promoter sequence; 500 bp flanking the nifL gene upstream and downstream are included CM038; 6-404 SEQ ID glnEΔAR-1 with glnE gene with 1287 bp immediately ds30 PBC6.38 NO 242 500 bp flank downstream of the ATG start

codon deleted, resulting in a truncated glnE protein lacking the adenylyl-removing (AR) domain; 500 bp flanking the glnE gene upstream and downstream are included CM029; 6-412 SEQ ID glnEΔAR-1 glnE gene with 1287 bp immediately ds30 PBC6.29 NO 243 downstream of the ATG start codon deleted, resulting in a truncated glnE protein lacking the adenylyl-removing (AR) domain CM029; 6-412 SEQ ID glnEΔAR-1 with glnE gene with 1287 bp immediately ds30 PBC6.29 NO 244 500 bp flank downstream of the ATG start codon deleted, resulting in a truncated glnE protein lacking the adenylyl-removing (AR) domain; 500 bp flanking the glnE gene upstream and downstream are included CM029; 6-412 SEQ ID ΔnifL::Prm5 starting at 31 bp after the A of the ATG ds24 PBC6.29 NO 245 start codon, 1375 bp of nifL have been deleted and replaced with the CI006 Prm5 promoter sequence CM029; 6-412 SEQ ID ΔnifL::Prm5 with starting at 31 bp after the A of the ATG ds24 PBC6.29 NO 246 500 bp flank start codon, 1375 bp of nifL have been deleted and replaced with the CI006 Prm5 promoter sequence; 500 bp flanking the nifL gene upstream and downstream are included CM093; 6-848 SEQ ID ΔnifL::Prm1 starting at 31 bp after the A of the ATG ds20 PBC6.93 NO 247 start codon, 1375 bp of nifL have been deleted and replaced with the CI006 Prm1 promoter sequence CM093; 6-848 SEQ ID ΔnifL::Prm1 with starting at 31 bp after the A of the ATG ds20 PBC6.93 NO 248 500 bp flank start codon, 1375 bp of nifL have been deleted and replaced with the CI006 Prm1 promoter sequence; 500 bp flanking the nifL gene upstream and downstream are included CM093; 6-848 SEQ ID glnEΔAR-2 glnE gene with 1644 bp immediately ds31 PBC6.93 NO 249 downstream of the ATG start codon deleted, resulting in a truncated glnE protein lacking the adenylyl-removing (AR) domain CM093; 6-848 SEQ ID glnEΔAR-2 with glnE gene with 1644 bp immediately ds31 PBC6.93 NO 250 500 bp flank downstream of the ATG start codon deleted, resulting in a truncated glnE protein lacking the adenylyl-removing (AR) domain; 500 bp flanking the glnE gene upstream and downstream are included CM093; 6-848 SEQ ID ΔamtB First 1088 bp of amtB gene and 4 bp ds126 PBC6.93 NO 251 upstream of start codon deleted; 199 bp of gene remaining lacks a start codon; no amtB protein is translated CM093; 6-848 SEQ ID ΔamtB with 500 bp First 1088 bp of amtB gene and 4 bp ds126 PBC6.93 NO 252 flank upstream of start codon deleted; 199 bp of gene remaining lacks a start codon; no amtB protein is translated CM094; 6-881 SEQ ID glnEΔAR-1 glnE gene with 1287 bp immediately ds30 PBC6.94 NO 253 downstream of the ATG start codon deleted, resulting in a truncated glnE protein lacking the adenylyl-removing (AR) domain CM094; 6-881 SEQ ID glnEΔAR-l with glnE gene with 1287 bp immediately ds30 PBC6.94 NO 254 500 bp flank downstream of the ATG start codon deleted, resulting in a truncated glnE protein lacking the adenylyl-removing (AR) domain; 500 bp flanking the glnE gene upstream and downstream are included CM094; 6-881 SEQ ID ΔnifL::Prm1 starting at 31 bp after the A of the ATG ds20 PBC6.94 NO 255 start codon, 1375 bp of nifL have been deleted and replaced with the CI006 Prm1 promoter sequence CM094; 6-881 SEQ ID ΔnifL::Prm1 with starting at 31 bp after the A of the ATG ds20 PBC6.94 NO 256 500 bp flank start codon, 1375 bp of nifL have been deleted and replaced with the CI006 Prm1 promoter sequence; 500 bp flanking the nifL gene upstream and downstream are included CM094; 6-881 SEQ ID ΔamtB First 1088 bp of amtB gene and 4 bp ds126 PBC6.94 NO 257 upstream of start codon deleted; 199 bp of gene remaining lacks a start codon; no amtB protein is translated CM094; 6-881 SEQ ID ΔamtB with 500 bp First 1088 bp of amtB gene and 4 bp ds126 PBC6.94 NO 258 flank upstream of start codon deleted; 199 bp of gene remaining lacks a start codon; no amtB protein is translated none 910-1246 SEQ ID ΔnifL::PinfC starting at 20 bp after the A of the ATG ds960 NO 259 start codon, 1379 bp of nifL have been deleted and replaced with the 910 PinfC promoter sequence none 910-1246 SEQ ID ΔnifL::PinfC with starting at 20 bp after the A of the ATG ds960 NO 260 500 bp flank start codon, 1379 bp of nifL have been deleted and replaced with the 910 PinfC promoter sequence; 500 bp flanking the nifL gene upstream and downstream are included PBC6.1, CI006 SEQ ID 16S-1 1 of 3 unique 16S rDNA genes in the N/A 6, CI6 NO 261 CI006 genome PBC6.1, CI006 SEQ ID 16S-2 2 of 3 unique 16S rDNA genes in the N/A 6, CI6 NO 262 CI006 genome PBC6.1, CI006 SEQ ID nifH N/A N/A

6, CI6 NO 263 PBC6.1, CI006 SEQ ID nifD2 2 of 2 unique genes annotated as nifD N/A 6, CI6 NO 264 in CI006 genome PBC6.1, CI006 SEQ ID nifK2 2 of 2 unique genes annotated as nifK N/A 6, CI6 NO 265 in CI006 genome PBC6.1, CI006 SEQ ID nifL N/A N/A 6, CI6 NO 266 PBC6.1, CI006 SEQ ID nifA N/A N/A 6, CI6 NO 267 PBC6.1, CI006 SEQ ID glnE N/A N/A 6, CI6 NO 268 PBC6.1, CI006 SEQ ID 16S-3 3 of 3 unique 16S rDNA genes in the N/A 6, CI6 NO 269 CI006 genome PBC6.1, CI006 SEQ ID nifD1 1 of 2 unique genes annotated as nifD N/A 6, CI6 NO 270 in CI006 genome PBC6.1, CI006 SEQ ID nifK1 1 of 2 unique genes annotated as nifK N/A 6, CI6 NO 271 in CI006 genome PBC6.1, CI006 SEQ ID amtB N/A N/A 6, CI6 NO 272 PBC6.1, CI006 SEQ ID Prm1 348 bp includes the 319 bp immediately N/A 6, CI6 NO 273 upstream of the ATG start codon of the lpp gene and the first 29 bp of the lpp gene PBC6.1, CI006 SEQ ID Prm5 313 bp starting at 432 bp upstream of the N/A 6, CI6 NO 274 ATG start codon of the ompX gene and ending 119 bp upstream of the ATG start codon of the ompX gene 19, CI19 CI019 SEQ ID nifL N/A N/A NO 275 19, CI19 CI019 SEQ ID nifA N/A N/A NO 276 19, CI19 CI019 SEQ ID 16S-1 1 of 7 unique 16S rDNA genes in the N/A NO 277 CI019 genome 19, CI19 CI019 SEQ ID 16S-2 2 of 7 unique 16S rDNA genes in the N/A NO 278 CI019 genome 19, CI19 CI019 SEQ ID 16S-3 3 of 7 unique 16S rDNA genes in the N/A NO 279 CI019 genome 19, CI19 CI019 SEQ ID 16S-4 4 of 7 unique 16S rDNA genes in the N/A NO 280 CI019 genome 19, CI19 CI019 SEQ ID 16S-5 5 of 7 unique 16S rDNA genes in the N/A NO 281 CI019 genome 19, CI19 CI019 SEQ ID 16S-6 6 of 7 unique 16S rDNA genes in the N/A NO 282 CI019 genome 19, CI19 CI019 SEQ ID 16S-7 7 of 7 unique 16S rDNA genes in the N/A NO 283 CI019 genome 19, CI19 CI019 SEQ ID nifH1 1 of 2 unique genes annotated as nifH N/A NO 284 in CI019 genome 19, CI19 CI019 SEQ ID nifH2 2 of 2 unique genes annotated as nifH N/A NO 285 in CI019 genome 19, CI19 CI019 SEQ ID nifD1 1 of 2 unique genes annotated as nifD N/A NO 286 in CI019 genome 19, CI19 CI019 SEQ ID nifD2 2 of 2 unique genes annotated as nifD N/A NO 287 in CI019 genome 19, CI19 CI019 SEQ ID nifK1 1 of 2 unique genes annotated as nifK N/A NO 288 in CI019 genome 19, CI19 CI019 SEQ ID nifK2 2 of 2 unique genes annotated as nifK N/A NO 289 in CI019 genome 19, CI19 CI019 SEQ ID glnE N/A N/A NO 290 19, CI19 CI019 SEQ ID Prm4 449 bp immediately upstream of the N/A NO 291 ATG of the dscC 2 gene 19, CI19 CI019 SEQ ID Prm1.2 500 bp immediately upstream of the N/A NO 292 TTG start codon of the infC gene 19, CI19 CI019 SEQ ID Prm3.1 170 bp immediately upstream of the N/A NO 293 ATG start codon of the rplN gene 19, CI20 CI020 SEQ ID Prm6.1 142 bp immediately upstream of the N/A NO 294 ATG of a highly-expressed hypothetical protein (annotated as PROKKA_00662 in CI019 assembly 82) 19, CI21 CI021 SEQ ID Prm7.1 293 bp immediately upstream of the N/A NO 295 ATG of the lpp gene 19-375, CM67 SEQ ID glnEΔAR-2 glnE gene with 1650 bp immediately ds34 19-417, NO 296 downstream of the ATG start codon CM067 deleted, resulting in a truncated glnE protein lacking the adenylyl-removing (AR) domain 19-375, CM67 SEQ ID glnEΔAR-2 with glnE gene with 1650 bp immediately ds34 19-417, NO 297 500 bp flank downstream of the ATG start codon CM067 deleted, resulting in a truncated glnE protein lacking the adenylyl-removing (AR) domain; 500 bp flanking the glnE gene upstream and downstream are included 19-375, CM67 SEQ ID ΔnifL::null-v1 starting at 221 bp after the A of the none 19-417, NO 298 ATG start codon, 845 bp of nifL have CM067 been deleted and replaced with the 31 bp sequence “GGAGTCTGAACTCATCCTGCGATGGGGGCTG” 19-375, CM67 SEQ ID ΔnifL::null-v1 with starting at 221 bp after the A of the none 19-417, NO 299 500 bp flank ATG start codon, 845 bp of nifL have CM067 been deleted and replaced with the 31 bp sequence “GGAGTCTGAACTCATCCTGCGATGGGGGCTG”; 500 bp flanking the nifL gene upstream and downstream are included 19-377, CM69 SEQ ID ΔnifL::null-v2 starting at 221 bp after the A of the none CM069 NO 300 ATG start codon, 845 bp of nifL have been deleted and replaced with the 5 bp sequence “TTAAA” 19-377, CM69 SEQ ID ΔnifL::null-v2 with starting at 221 bp after the A of the none CM069 NO 301 500 bp flank ATG start codon, 845 bp of nifL have been deleted and replaced with the 5 bp sequence “TTAAA”; 500 bp flanking the nifL gene upstream and

downstream are included 19-389, CM81 SEQ ID ΔnifL::Prm4 starting at 221 bp after the A of the ds70 19-418, NO 302 ATG start codon, 845 bp of nifL have CM081 been deleted and replaced with the CI19 Prm4 sequence 19-389, CM81 SEQ ID ΔnifL::Prm4 with starting at 221 bp after the A of the ds70 19-418, NO 303 500 bp flank ATG start codon, 845 bp of nifL have CM081 been deleted and replaced with the CI19 Prm4 sequence; 500 bp flanking the nifL gene upstream and downstream are included none 137-3890 SEQ ID ΔnifL-Prm1.2 starting at 24 bp after the A of the ATG ds843 NO 458 start codon, 1372 bp of nifL have been deleted and replaced with the 137 Prm1.2 promoter sequence none 137-3890 SEQ ID ΔnifL-Prm1.2 with starting at 24 bp after the A of the ATG ds843 NO 459 500 bp flank start codon, 1372 bp of nifL have been deleted and replaced with the 137 Prm1.2 promoter sequence; 500 bp flanking the nifL gene upstream and downstream are included none 137-3890 SEQ ID glnE_KO2 glnE gene with 1290 bp immediately ds809 NO 460 downstream of the ATG start codon deleted, resulting in a truncated glnE protein lacking the adenylyl-removing (AR) domain none 137-3890 SEQ ID glnE_KO2 with glnE gene with 1290 bp immediately ds809 NO 461 500 bp flank downstream of the ATG start codon deleted, resulting in a truncated glnE protein lacking the adenylyl-removing (AR) domain; 500 bp flanking the glnE gene upstream and downstream are included none 137-3890 SEQ ID NtrC_D54A Deactivation of the phosphorylation ds2974 NO 462 site of the DNA-binding transcriptional regulator NrtC by swapping the 54th amino acid from aspartate to alanine (D to A) by changing the GAT codon to GCT. Disables the ability of NtrC to be phosphorylated. none 137-3890 SEQ ID NtrC_D54A with Deactivation of the phosphorylation ds2974 NO 463 flanking sequences site of the DNA-binding transcriptional regulator NrtC by swapping the 54th amino acid from aspartate to alanine (D to A) by changing the GAT codon to GCT. Disables the ability of NtrC to be phosphorylated. 693 bp upstream and 549 bp downstream NtrC sequences flanking NtrCD54A mutation are included. none 137-3896 SEQ ID ΔnifT::PinfC Deletion of the nifL gene from 20 bp ds799 NO 464 after the ATG (start) to 87 bp before the TGA (stop) of the gene. A 500 bp fragment from the region upstream of the infC gene was inserted (PinfC) upstream of nifA replacing the deleted portion. none 137-3896 SEQ ID ΔnifL::PinfC with Deletion of the nifL gene from 20 bp ds799 NO 465 flanking sequences after the ATG (start) to 87 bp before the TGA (stop) of the gene. A 500 bp fragment from the region upstream of the infC gene was inserted (PinfC) upstream of nifA replacing the deleted portion; 332 bp upstream and 324 bp downstream flanking the nifL gene are included. none 137-3896 SEQ ID glnD_UTase_Deactivation Deactivation of the uridylyltransferase ds2538 NO 466 (UT) domain of the bifunctional uridylyltransferase/uridylyl-removing enzyme, glnD, by mutating amino acid residues 90 and 91 from GG to DV as well as residue 104 from D to A. none 137-3896 SEQ ID glnD_UTase_Deactivation Deactivation of the uridylyltransferase ds2538 NO 467 with flanking (UT) domain of the bifunctional sequences uridylyltransferase/uridylyl-removing enzyme, glnD, by mutating amino acid residues 90 and 91 from GG to DV as well as residue 104 from D to A; 450 bp flanking the mutated sites upstream and downstream are included. none 137-3896 SEQ ID NC- Insertion of a copy of the nifA gene ds2969 NO 468 nifA_copy::Prm1.2 into a noncoding region of 137. This copy is being driven by a 400 bp promoter (Prm1.2) derived from a region upstream of the cspE gene. none 137-3896 SEQ ID NC- Insertion of a copy of the nifA gene ds2969 NO 469 nifA_copy::Prm1.2 into a noncoding region of 137. This with flanking copy is being driven by a 400 bp sequences promoter (Prm1.2) derived from a region upstream of the cspE gene; 2000 bp flanking the insertion site upstream and downstream are included.

Example 7: Ecological Sidedressing and Weatherproof Nitrogen

(714) Sustainable production of grains such as corn, wheat, and rice require the application of some source of nitrogen. Growers apply nitrogen that plants can use in a number of forms. In geographies where livestock production is intense, livestock manure can meet a significant portion of the nitrogen needs of a corn crop. Where no organic form of nitrogen is available, commercial nitrogen fertilizers either in the form of a gas held under pressure as a liquid (NH3) a dry

formulation such as ammonium nitrate or urea, or in liquid formulations such as combinations of urea and ammonia nitrate (UAN).
(715) The point in time when nitrogen is applied to corn depends upon a number of factors. The first of these may be local or state regulations. Other factors that may affect when a grower chooses to apply nitrogen would be field-working conditions in the fall (still a popular application timing for many geographies) due to uncertainty around cropping plans, Spring weather, and planting conditions and the size of the operation.
(716) Growers may apply nitrogen in the fall, after the previous crop is removed. This application timing, while popular, is under attack by regulatory agencies who are seeking to limit either the number of pounds that can be applied in the Fall or the Fall application entirely. If no Fall application occurs, then growers will usually apply nitrogen prior to planting the corn crop, after crop emergence, or a combination of the two, which is referred to as a split application.
(717) In any of the aforementioned nitrogen delivery regimes, the second application of nitrogen, which normally occurs at the V4-V6 stage, is referred to as a sidedress application. The sidedress application of nitrogen is often applied between the rows.
(718) Due to the instability of nitrogen molecules once they are in the soil, research has demonstrated that if a grower can apply the nitrogen as close to when the corn crop needs the nitrogen, there are significant benefits for the crop as well as for the environment. The nitrogen use efficiency increases, meaning it takes less pounds of nitrogen to produce a bushel.
(719) Sidedressing is not without risks. The ability to get across all of a grower's acres in a timely manner is not ensured. These risks increase as the size of the operation increases and as potential changes to the climate make the number of days suitable for fieldwork less predictable.
(720) An alternative to the use of commercial fertilizer for legumes (primarily soybeans) has been biological nitrogen fixing (BNF) systems, which exist in nature. These systems fall into one of three types and differ in their use of substrate and efficiency. See FIG. **26**.
(721) An example of where the majority of the nitrogen needs of the crop are met through a symbiotic relationship with the plant would be that of soybeans or alfalfa. They are capable of converting almost enough molecular nitrogen (N.sub.2) to meet the nitrogen needs of the crop. In the case of soybeans, many farmers apply *Rhizobium* at the time of planting, but some *Rhizobium* are ubiquitous in most soils and populations are able to survive in the soil from year to year.
(722) The ability to produce a microbe that would be able to convert N.sub.2 to NH.sub.3 through root association in cereals such as corn, rice, or wheat would be revolutionary and the equivalent of BNF in soybeans. It could also replace sidedressing since both practices would allow for the timely delivery of nitrogen to the growing plant in season. BNF for cereals would also allow growers to reduce the risks associated with sidedressing. These risks include reduced yields due to untimely applications, variable in-season cost of nitrogen, the cost of application, and consistency of nitrogen availability in years when environmental conditions are conducive to loss through de-nitrification or leaching. BNF for cereals would also create value through ease of use and reducing passes over the field for specific nitrogen applications.
(723) As can be seen from the below Table B, Fall and Spring nitrogen application strategies always use sidedress. The split application also features sidedressing. The state of the art is such that sidedressing is an energy intensive mechanical process that is applied by a tractor that compacts the soil. Often at stage V4-V6, additional nitrogen is applied as sidedressing.
(724) The disclosed remodeled nitrogen fixing bacteria are able to eliminate the practice of sidedressing, as these bacteria live in intimate association with the plant's root system and “spoonfeed” the plant nitrogen.
(725) TABLE-US-00034 TABLE B Comparison of Current Nitrogen Application Timing Practices and Proposed Microbial Introduction Practices Benefits of the Proposed Microbial Introduction Nitrogen Application Proposed Microbial Over Previous Nitrogen Timing Practices Introduction Practices Application Timing Fall application - 100% of At planting either as seed Potential to

reduce rates crop needs treatment or in furrow applied in the fall application No need to apply supplemental applications in crop if spring weather conditions are conducive to nitrogen loss More consistent yields across the geography due to supplemental nitrogen being available in soil types where conditions for nitrogen loss are higher than in other parts of the field Early spring applications - At planting either as a seed No need to apply 100% of crop needs treatment or in furrow supplemental applications in application crop if weather conditions are conducive to nitrogen loss after application More consistent yields across the geography due to supplemental nitrogen being available in soil types where conditions for nitrogen loss are higher than in other parts of the field Planned Split applications At planting either as a seed Reduces the needs for the 150 lb followed by 30 lbs treatment or in furrow second application application Ensures that split application is applied to all acres Ensures that the application is applied in a timely manner to prevent yield loss Ensures that the application is done in a timely manner as to prevent damage to the crop through the pruning of roots More consistent yields across the geography due to supplemental nitrogen being available in soil types where conditions for nitrogen loss are higher than in other parts of the field

(726) Thus, as can be seen in Table B, the present disclosure provides an alternative to traditional synthetic fertilizer sidedressing, by allowing a farmer to utilize an “ecological sidedressing” comprised of non-intergeneric remodeled bacteria that are capable of fixing atmospheric nitrogen and delivering such to the corn plant throughout the corn's growth cycle.

Example 8: Remodeling Microbial Systems for Temporally and Spatially Targeted Dynamic Nitrogen Delivery

(727) The microbes of the disclosure are engineered with one or more of the following features, in order to develop non-intergeneric remodeled microbes that are capable of colonizing corn and supplying fixed nitrogen to the corn, at physiologically relevant periods of the corn's life cycle.

(728) These genetic modifications, in some aspects, have been discussed previously, inter alia, in Examples 2-6. They are discussed again here, in order to provide the building blocks of a Guided Microbial Remodeling (GMR) campaign, which will be elaborated upon below.

(729) Feature: Nitrogenase Expression—nifL Deletion and Promoter Insertion Upstream of nifA.

(730) NifA activates the nif gene complex and drives nitrogen fixation when there is insufficient fixed nitrogen available to the microbe. NifL inhibits NifA when there is sufficient fixed N available to the microbe. The nifL and nifA genes are present in an operon and are driven by the same promoter upstream of nifL, which is activated in conditions of nitrogen insufficiency and repressed in conditions of nitrogen sufficiency (FIG. **1**, Dixon and Kahn 2004). In this feature, we have deleted most of the nifL coding sequence and replaced it with a constitutive promoter naturally present elsewhere in the genome of the wild-type strain which we have observed is highly expressed in nitrogen-replete conditions. This allows NifA to be both expressed and active in nitrogen-replete conditions, such as a fertilized field.

(731) Feature: Nitrogenase Expression—Promoter Swap of the rpoN Gene to Increase Availability of Sigma Factor 54

(732) Sigma factors are required for initiation of transcription of prokaryotic genes, and sometimes specific sigma factors initiate the transcription of a set of genes in a common regulatory network. Sigma 54 (σ.sup.54), encoded by the gene rpoN, is responsible for transcription of many genes involved in nitrogen metabolism, including the nif cluster and nitrogen assimilation genes (Klipp et al. 2005, *Genetics and Regulation of Nitrogen Fixation in Free-Living Bacteria*, Kluwer Academic Publishers (Vol. 2). doi.org/10.1007/1-4020-2179-8). In strains where nifA is controlled by a strong promoter active in nitrogen replete conditions, the availability of σ.sup.54 to initiate transcription of the nif genes may become limiting. In this feature, the promoter of the rpoN gene has been disrupted by deleting the intergenic sequence immediately upstream of the gene. The deleted sequence was replaced by a different promoter naturally present elsewhere in the genome of the wild-type strain, which we have observed is highly expressed in nitrogen-replete conditions. This

results in increased expression of σ.sup.54 which relieves any limitation on transcription initiation in strains highly expressing nifA.
(733) Feature: Nitrogen Assimilation—Deletion of the Adenylyl-Removing Domain of GlnE
(734) Fixed nitrogen is primarily assimilated by the microbe by the glutamine synthetase/glutamine oxoglutarate aminotransferase (GS-GOGAT) pathway. The resulting glutamine and glutamate pools in the cell control nitrogen metabolism, with glutamate serving as the main nitrogen pool for biosynthesis and glutamine serving as the signaling molecule for nitrogen status. The glnE gene encodes an enzyme, known as glutamine synthetase adenylyl transferase or glutamine-ammonia-ligase adenylyl transferase, that regulates the activity of glutamine synthetase (GS), in response to intracellular levels of glutamine. The GlnE protein consists of two domains with independent and distinct enzymatic activities: an adenylyltransferase (ATase) domain, which covalently modifies the GS protein with an adenylyl group, thus reducing GS activity; and an adenylyl-removing (AR) domain, which removes the adenylyl group from GS, thus increasing its activity. Clancy et al. (2007) showed that truncation of the *Escherichia coli* K12 GlnE protein to remove the AR domain lead to expression of a protein that retains ATase activity. In this feature, we have deleted the N-terminal AR domain of GlnE, resulting in a strain lacking the AR activity, but functionally expressing the ATase domain. This leads to constitutively adenylated GS with attenuated activity, causing a reduction in assimilation of ammonium and excretion of ammonium out of the cell.
(735) Feature: Nitrogen Assimilation—Decrease Transcription and/or Translation Rates of Gene Encoding GS
(736) The glnA gene, which encodes the GS enzyme, is controlled by a promoter which is activated under nitrogen depletion, and repressed under nitrogen replete conditions (Van Heeswijk et al. 2013). In this feature, the amount of GS enzyme in the cell has been decreased in at least one of two ways (or a combination of the following two ways into one cell). First, the “A” of the ATG start codon of the glnA gene, which encodes glutamine synthetase (GS), has been changed to “G”. The rest of the glnA gene and GS protein sequence remains unaltered. The resulting GTG start codon is hypothesized to result in a decreased translation initiation rate of the glnA transcript, leading to a decrease in the intracellular level of GS. Second, the promoter upstream of the glnA gene has been disrupted by deleting the intergenic sequence immediately upstream of the gene. The deleted sequence was replaced by the promoter of the glnD, glnE or glnB genes, which are expressed constitutively at a very low level regardless of nitrogen status (Van Heeswijk et al 2013). This leads to a decrease in glnA transcription levels s and therefore a decrease in GS levels in the cell. As aforementioned, the previous two scenarios (alteration of start codon and promoter disruption) can be combined into a host. The decreased GS activity in the cell leads to a decrease in the bacterial assimilation of the ammonium produced by nitrogen fixation, resulting in excretion of ammonium outside of the bacterial cell, making nitrogen more available for plant uptake (Ortiz-Marquez, J. C. F., Do Nascimento, M., & Curatti, L. (2014) “Metabolic engineering of ammonium release for nitrogen-fixing multispecies microbial cell-factories,” *Metabolic Engineering*, 23, 1-11. doi.org/10.1016/j.ymben.2014.03.002).
(737) Feature: Nitrogen Assimilation—Promoter Swap of the glsA2 Gene to Increase Glutaminase Activity
(738) Glutaminase enzymes catalyze the release of ammonium from glutamine and may play an important role in controlling the intracellular glutamine pool (Van Heeswijk et al. 2012). In this feature, the glsA2 gene encoding glutaminase has been upregulated by deleting a sequence immediately upstream of the gene and replacing it with different promoter naturally present elsewhere in the genome which is highly expressed in nitrogen-replete conditions. This results in increased expression of glutaminase enzyme in the cell, leading to release of ammonium from the glutamine pool and therefore increased excretion of ammonium out of the cell.
(739) Feature: Ammonium Excretion—amtB Deletion
(740) The amtB gene encodes a transport protein that functions to import ammonium from the

extracellular space into the cell interior. It is believed that in nitrogen-fixing bacteria, the AmtB protein functions to ensure that any ammonium that passively diffuses out of the cell during nitrogen fixation is imported back into the cell, thus preventing loss of fixed nitrogen (Zhang et al. 2012). In this feature, the amtB coding sequence has been deleted, leading to net diffusion of ammonium out of the cell and thus an increase in ammonium excretion (Barney et al. 2015). The amtB promoter has been left intact.

(741) Feature: Robustness and Colonization—Promoter Swap of bcsII and bcsIII Operons to Increase Bacterial Cellulose Production

(742) Bacterial cellulose biosynthesis is an important factor for both attachment to the root and biofilm formation on root surfaces (Rodriguez-Navarro et al. 2007). The bcsII and bcsIII operons each encode a set of genes involved in bacterial cellulose biosynthesis (Ji et al. 2016). In this feature, the native promoter of the bcsII operon has been disrupted by deleting the intergenic region upstream of the first gene in the operon and replacing it with a different promoter naturally present elsewhere in the genome of the wild-type strain which we have observed is highly expressed in nitrogen-replete conditions. This results in increased expression of the bcsII operon in a fertilized-field environment, which leads to an increase in bacterial cellulose production and thus attachment to corn roots.

(743) Feature: Promoter Swap of pehA Operon to Increase Polygalacturonase Production

(744) Polygalacturonases are implicated as important factors for colonization of plant roots by non-nodule-forming bacteria (Compant, S., Clement, C., & Sessitsch, A. (2010), “Plant growth-promoting bacteria in the rhizo- and endosphere of plants: Their role, colonization, mechanisms involved and prospects for utilization,” *Soil Biology and Biochemistry,* 42(5), 669-678. doi.org/10.1016/j.soilbio.2009.11.024.)

(745) The pehA gene encodes a polygalacturonase in an operon with two uncharacterized protein coding regions, with the pehA at the downstream end of the operon. In this feature, the promoter of the pehA operon has been disrupted by deleting a sequence immediately upstream of the first gene in the operon. The deleted sequence was replaced by a different promoter naturally present elsewhere in the genome of the wild-type strain, which we have observed is highly expressed in nitrogen-replete conditions. This results in increased expression of the PehA polygalacturonase protein in a fertilized-field environment, which leads to enhanced colonization of corn roots by the microbe.

(746) Feature: Robustness and Colonization—Promoter Swap of the fhaB Gene to Increase Expression of Adhesins

(747) Bacterial surface adhesins, such as agglutinins, have been implicated in attachment, colony and biofilm formation on plant roots (Danhorn, T., & Fuqua, C. (2007), “Biofilm formation by plant-associated bacteria. *Annual Review of Microbiology,* 61, 401-422. doi.org/10.1146/annurev.micro.61.080706.093316).

(748) The fhaB gene encodes a filamentous hemagglutinin protein. In this feature, the promoter of the fhaB gene has been disrupted by deleting the intergenic sequence immediately upstream of the gene. The deleted sequence was replaced by a different promoter naturally present elsewhere in the genome of the wild-type strain, which we have observed is highly expressed in nitrogen-replete conditions. This results in increased expression of the hemagglutinin protein, leading to increased root attachment and colonization.

(749) Feature: Robustness and Colonization—Promoter Swap of the dctA Gene to Increase Expression of Organic Acid Transporters

(750) For successful colonization of the rhizosphere, a bacterium must have the ability to utilize carbon sources found in root exudates, such as organic acids. The gene dctA encodes an organic acid transporter that has been shown to be necessary for effective colonization in rhizosphere bacteria and repressed in response to exogenous nitrogen (Nam, H. S., Anderson, A. J., Yang, K. Y., Cho, B. H., & Kim, Y. C. (2006), “The dctA gene of *Pseudomonas chlororaphis* O6 is under RpoN

control and is required for effective root colonization and induction of systemic resistance," *FEMS Microbiology Letters*, 256(1), 98-104. doi.org/10.1111/j.1574-6968.2006.00092.x). In this feature, the promoter of the dctA gene has been disrupted by deleting the intergenic sequence immediately upstream of the gene. The deleted sequence was replaced by a different promoter naturally present elsewhere in the genome of the wild-type strain, which we have observed is highly expressed in the rhizosphere in nitrogen-replete conditions. This results in increased expression of the DctA transporter, enhanced utilization of root exudate carbon and thus improved robustness in fertilized-field conditions.

(751) Feature: Robustness and Colonization—Promoter Swap of the PhoB Gene to Promote Biofilm Formation

(752) In rhizosphere bacteria, the PhoR-PhoB two-component system mediates a response to phosphorous limitation and has been linked to colony and biofilm formation on plant roots (Danhorn and Fuqua 2007). In this feature, the promoter of the phoB1 gene has been disrupted by deleting the intergenic sequence immediately upstream of the gene. The deleted sequence was replaced by a different promoter naturally present elsewhere in the genome of the wild-type strain, which we have observed is highly expressed in the rhizosphere in nitrogen-replete conditions. This results in increased expression of the PhoB component of the PhoR-PhoB system, leading to enhanced colony and biofilm formation on roots.

(753) Feature: Correlated Metabolic and Regulatory Networks—Altering Nitrogen Signaling to Influence Stress Response

(754) GlnD is the central nitrogen-sensing enzyme in the cell. The GlnD protein consists of three domains: a uridylyl-transferase (UTase) domain, and (UR) uridylyl-removing domain, and a glutamine-binding ACT domain. In nitrogen-excess conditions, intracellular glutamine binds to the ACT domain of GlnD, causing the UTase domain to uridylylate the PII proteins GlnB and GlnK, causing a regulatory cascade upregulating genes involved in nitrogen fixation and assimilation. In nitrogen starvation conditions, glutamine is not available to bind to the ACT domain of GlnD, which causes the UR domain to de-uridylylate GlnK and GlnB, which causes repression of genes involved in nitrogen assimilation and repression. These PII regulatory cascades regulate several pathways, including nitrogen starvation stress responses, nitrogen assimilation and nitrogen fixation in diazotrophs (Dixon and Kahn 2004; van Heeswijk et al. 2013). In this feature, either the UTase domain, the UR domain, the ACT domain, or the entire gene encoding the GlnD protein has been modified in order to alter the transduction of nitrogen starvation signals which cause stress responses.

(755) Feature: Correlated Metabolic and Regulatory Networks—Deletion of the glgA Glycogen Synthase Gene

(756) Because nitrogen fixation is such an energy-intensive process, it is believed to be limited by the availability of ATP in the cell. It has therefore been hypothesized that diverting carbon away from energy storage pathways and towards oxidative phosphorylation could enhance nitrogen fixation in diazotrophs (Glick 2012). One study suggested that deletion of glgA gene, which encodes glycogen synthase, led to enhanced nitrogen fixation in legume-*Rhizobia* symbiosis (Marroqui et al. 2001). In this feature, the entire glgA gene has been deleted in order to abolish glycogen synthesis. The deletion of the glgA gene leads to increased levels of nitrogen fixation in both nitrogen-starvation and nitrogen-replete conditions.

(757) GMR Campaign Utilizing Genetic Features

(758) The microbes of the disclosure have been engineered to contain one or more of the aforementioned features. The overall goal of the GMR campaigns is to develop microbes that are capable of supplying all of the nitrogen needs of a corn plant throughout the entirety of a growing season. In FIG. **27**, the inventors have calculated that in order for a nitrogen fixing microbe to supply a corn plant with all of its nitrogen needs over a growing season, and thus completely replace synthetic fertilizer, then the microbes (in the aggregate) need to produce about 200 pounds

of nitrogen per acre. FIG. **27** also illustrates that strain PBC 137-1036 (i.e. the remodeled *Klebsiella variicola*) supplies about 20 pounds of nitrogen per acre.

(759) FIG. **28** of the provisional application is updated in the present application. Specifically, FIG. **28**A of the present application is identical to FIG. **28** of the provisional application and FIG. **28**B of the present application is new showing the nitrogen produced by PBC 137-3890, a further remodeled strain of *Klebsiella variicola*. FIG. **28**A provides a scenario whereby fertilizer could be replaced by the remodeled microbes of the disclosure. As aforementioned in FIG. **27**, the large dashed line is the nitrogen required by the corn (about 200 pounds per acre). The solid line, as already discussed, is the current nitrogen amount that can be supplied by the remodeled 137-1036 strain (about 20 pounds per acre). In the gray-shaded oval "A" scenario of FIG. **28**A, the inventors expect to increase the activity of the 137-1036 strain by 5 fold (see FIG. **29** for GMR campaign strategy to achieve such). In the gray-shaded oval "B" scenario of FIG. **28**A, the inventors expect to utilize a remodeled microbe with a particular colonization profile that is complementary to that of the 137-1036 strain, and which will supply nitrogen to the plant at later stages of the growth cycle. Since the filing of the provisional application, the inventors have been successful in improving the nitrogen production activity of the 137-1036 strain through the GMR campaign. Specifically, FIG. **28**B shows the nitrogen production by the strain 137-3890, which is a further remodeled strain of 137-1036 obtained by employing the GMR campaign described in the application. As shown in FIG. **28**B, the nitrogen production activity of 137-3890 is substantially improved compared to 137-1036.

(760) FIG. **29** of the provisional application is updated in the present application. Specifically, FIG. **29**A of the present application is identical to FIG. **29** of the provisional application and FIG. **29**B of the present application is new showing the predicted N produced (lbs of N per acre) after the features F2 and F3 were incorporated in the PBC137 (*Klebsiella variicola*) since the filing of the provisional application.

(761) In FIG. **29**A, left panel, the discussed features (i.e. non-intergeneric genetic modifications) are illustrated with respect to a historical GMR campaign for PBC6.1 (*Kosakonia sacchari*), which was also discussed in Example 2. As can be seen in FIG. **29**A, left panel, the predicted N produced (lbs of N per acre) increased with each additional feature engineered into the microbial strain.

(762) In addition to the historical GMR campaign for PBC6.1 depicted in FIG. **29**A, left panel, one can also see the GMR campaign being executed for the PBC137 (*Klebsiella variicola*), FIG. **29**A, right panel. At the time the provisional application was filed, the nitrogenase expression feature (F1) was engineered into the host strain and features 2-6 were being executed. The expected contribution of each of these features to N produced (lbs of N per acre) was depicted in the provisional application by the dashed bar graphs in FIG. **29** (right panel), of the provisional application, which is now FIG. **29**A. These expectations were informed by the data from the PBC6.1 historical GMR campaign shown in the left panel of FIG. **29** of the provisional application. As can be seen in FIG. **28**A, the gray-shaded oval scenario "A", once the GMR campaign is completed in PBC137, it is anticipated that the non-intergeneric remodeled strain (in the aggregate, considering all microbes/colonized plants in an acre) will be capable of supplying nearly all of the nitrogen needs of a corn plant throughout the plant's early growth cycle. Further, FIG. **30** of the provisional, which is now FIG. **30**A, depicted the same expectation, and mapped the expected gains in nitrogen production to the applicable feature set at the time the provisional application was filed. Since the filing of the provisional application, the inventors have been working on engineering features F2-F6 into the host strain. At the time of filing the present application, the features F2 (nitrogen assimilation) and F3 (ammonium excretion) have been engineered into the PBC137 host strain. FIG. **29**B, right panel, depicts the N produced by the remodeled strains upon incorporation of the features F1-F3. As can be seen from the right panel of FIG. **29**B, the N produced (lbs of N per acre) increased with each additional feature engineered into the microbial strain. FIG. **30**B depicts N produced as mmol of N/CFU per hour by the remodeled strains of

PBC137 once the features F1 (nitrogenase expression), F2 (nitrogen assimilation), and F3 (ammonium excretion) were incorporated.

(763) The mutations made to the PBC137 WT strain to incorporate the features F1-F3 are summarized in Table 33 below.

(764) TABLE-US-00035 TABLE 33 List of isolated and derivative PBC137 strains Strain ID Genotype Mutation Mutation Description 137 WT WT Wild type *Klebsiella variicola* strain. 137-ΔnifL::PinfC ΔnifL::PinfC Deletion of the nifL gene from 20 bp 1036 after the ATG (start) to 87 bp before the TGA (stop) of the gene. A 500 bp fragment of the region upstream of the infC gene containing the promoter of the infC gene was inserted (PinfC) upstream of nifA replacing the deleted portion. 137- ΔnifL::PinfC, ΔnifL::PinfC Deletion of the nifL gene from 20 bp 3896 ΔglnD_UTase_deactivation, after the ATG (start) to 87 bp before the NC_nifA_copy::Prm1.2 TGA (stop) of the gene. A 500 bp fragment of the region upstream of the infC gene containing the promoter of the infC gene was inserted (PinfC) upstream of nifA replacing the deleted portion. ΔglnD_UTase_Deactivation Deactivation of the uridylyltransferase (UT) domain of the bifunctional uridylyltransferase/uridylyl-removing enzyme, glnD, by mutating amino acid residues 90 and 91 from GG to DV as well as residue 104 from D to A. NC- Insertion of a copy of the nifA gene into nifA_copy::Prm1.2 a noncoding region of 137. This copy is being driven by a 400 bp promoter (Prm1.2) derived from a region upstream of the cspE gene. 137- ΔnifL: Prm1.2, ΔnifL ::Prm 1.2 Deletion of the nifL gene from 20 bp 3890 ΔglnE.sub.AR-KO2, after the ATG (start) to 87 bp before the NtrC_D54A TGA (stop) of the gene. A 400 bp fragment from the region upstream of the cspE gene containing the promoter of the cspE gene was inserted (Prm1. 2) upstream of nifA replacing the deleted portion. ΔglnE.sub.AR-KO2 Deletion of 1647 bp after the start codon of the glnE gene. NtrC_D54A Deactivation of the phosphorylation site of the DNA-binding transcriptional regulator NrtC by swapping the 54.sup.th amino acid from aspartate to alanine (D to A). Disables the ability of NtrC to be phosphorylated.

Case I: Current Gen1 Microbe Providing 17 lbs of N from Strain 137-1036

(765) FIG. **31** depicts the colonization days 1-130 and the total CFU per acre of the non-intergeneric remodeled microbe of 137-1036, which was discussed previously. As mentioned, this microbe produces about 20 pounds of nitrogen per acre (in the aggregate) (17 pounds). The remodeled 137-1036 microbe has the following activity: 5.49E-13 mmol of N/CFU per hour or 4.07E-16 pounds of N/CFU per day.

(766) Case II: Current Gen1 Microbe Strain 137-1036 after Activity Improved 5-Fold to Provide First Half of N Requirement

(767) FIG. **32** depicts the colonization days 1-130 and the total CFU per acre of the proposed non-intergeneric remodeled microbe (progeny of 137-1036, see FIG. **29** and FIG. **30** for proposed genetic alteration features), which was discussed previously. As mentioned, this microbe is expected to produce about 100 pounds of nitrogen per acre (in the aggregate) (scenario “A”). The remodeled 137-1036 progeny microbe is targeted to have the following activity: 2.75E-12 mmol of N/CFU per hour or 2.03E-15 pounds of N/CFU per day. As noted above, since the filing of the provisional application, the features F2 and F3 have been incorporated and the activity of the remodeled strain 137-3890 with features F1-F3 is 4.03E-13 mmol of N/CFU per hour.

(768) Case III: Microbe with Later Stage Colonization with 5× Improved Activity

(769) FIG. **33** depicts the colonization days 1-130 and the total CFU per acre of a proposed non-intergeneric remodeled microbe that has a complimentary colonization profile to the 137-1036 microbe. As mentioned, this microbe is expected to produce about 100 pounds of nitrogen per acre (in the aggregate) (scenario “B” in FIG. **28**), and should start colonizing at about the same time that the 137-1036 microbe begins to decline. The microbe is targeted to have the following activity: 2.75E-12 mmol of N/CFU per hour or 2.03E-15 pounds of N/CFU per day.

(770) FIG. **34** provides the colonization profile of the 137-1036 in the top panel and the colonization profile of the microbe with a later stage/complimentary colonization dynamic in the

bottom panel.
(771) Case IV: Combine Microbe from Case II and III into a Consortia, or Find and Remodel a Single Microbe that has the Depicted Colonization Profile and Stated Activity
(772) FIG. **35** depicts two scenarios: (1) the colonization days 1-130 and the total CFU per acre of a proposed consortia of non-intergeneric remodeled microbes that have a colonization profile as depicted in Case II and Case III explained above, or (2) the colonization days 1-130 and the total CFU per acre of a proposed single non-intergeneric remodeled microbe that has the depicted colonization profile. The microbe (whether two microbes in a consortia, or single microbe) is targeted to have the following activity: 2.75E-12 mmol of N/CFU per hour or 2.03E-15 pounds of N/CFU per day.
Example 9: GMR Campaigns Utilizing Microbes with Distinct Spatial Colonization Patterns in the Corn Root Zone
(773) As aforementioned in Example 8, the present disclosure provides a GMR campaign, which seeks to provide a farmer with a complete replacement for traditional synthetic fertilizer delivery. The “ecological sidedressing” discussed above in Example 7, which eliminates the need for a farmer to supply an in-season nitrogen application, is one step toward the ultimate goal of supplying a BNF product for cereal crops.
(774) In order to remodel a microbe to be a successful BNF product for a cereal crop, it is paramount that the microbe colonizes a corn plant at a physiologically relevant time period of the corn's growth cycle, as well as colonizing said corn plant to a sufficient degree.
(775) The inventors have surprisingly discovered a functional genus of microbes, which have a desirable spatial colonization pattern, which make this group of microbes particularly useful for GMR campaigns.
(776) FIG. **36** sets forth the general experimental design utilized in this study, which entailed collecting colonization and transcript samples from corn over the course of 10 weeks. These samples allowed for the calculation of colonization ability of the microbes, as well as activity of the microbes. FIG. **37** and FIG. **38** provide a visual representation of aspects of the sampling scheme utilized in the experiment, which allows for differentiation of colonization patterns between a “standard” seminal node root sample and a more “peripheral” root sample.
(777) As can be seen in FIG. **39**, the WT 137 (*Klebsiella variicola*), 019 (*Rahnella aquatilis*), and 006 (*Kosakonia sacchari*), all have a similar colonization pattern, which demonstrates a dropoff in colonization toward the later weeks. This pattern is mirrored in the remodeled forms of each strain, which are depicted in the right hand side of the graphic
(778) FIG. **40** depicts the experimental scheme utilized to sample the corn roots. The plots: each square is a time point, the Y axis is the distance, and the X axis is the node. The standard sample was always collected along with the leading edge of growth. The periphery and intermediate samples changed week to week, but an attempt at consistency was made.
(779) FIG. **41** depicts the overall results from the experiment, which utilized and averaged all the data taken in the sampling scheme of FIG. **40**. As can be seen from FIG. **41**, strain 137 maintains higher colonization in peripheral roots than strain 6 or strain 19. The ‘standard sample’ was most representative for this strain when compared to samples from other root locations.
Example 10: Higher Corn Planting Density Enabled by Remodeled Microbes
(780) Corn yields have increased significantly since the 1930s largely due to genetic improvement and better crop management. Grain yield is the product of the number of plants per acre, kernels per plant, and weight per kernel. Of the three components that make up grain yield, the number of plants per acre is the factor that the farmer has the most direct control over. Kernel number and kernel weight can be managed indirectly through proper fertility, weed, pest and disease management to optimize plant health, and weather also plays a major role. Currently the average U.S. corn planting density is just under 32,000 plants per acre and has increased 400 plants per acre per year since the 1960s.

(781) However, ever-increasing planting populations are resulting in smaller and less expansive root systems available to acquire nutrients. Placing nutrients directly in the root zone at the right time using the correct source and rate increases the probability that roots will take up and utilize those nutrients.
(782) Integrating this understanding of seeding rates, row spacing, and product placement with advanced fertility management practices such as applying the right source, right rate, right timing, and right place for nutrient management is critical to maximize grain yield and input efficiency at higher planting densities.
(783) The microbes of the disclosure enable more densely planted corn crops, as the microbes live in intimate association with the plant (i.e. root surface) and provide the plant with a constant source of readily useable fixed atmospheric nitrogen.
(784) The disclosure's teachings of a BNF source for cereal crops will provide farmers with a tool that enables more densely planted acreage, as all the plants in the field will have a ready source of nitrogen delivered to their root systems throughout the growing season. This type of nitrogen delivery will not only remove the need for an in-season “sidedressing” application of nitrogen, but will also enable the farmer to realize a higher yield per acre due to the increased planting density per acre.
Example 11: Reduced Infield Variability of Corn Crop Enabled by Remodeled Microbes
(785) The present inventors have further determined that the microbes of the disclosure are able to improve yield stability through a more consistent and uniform delivery of nitrogen. The microbes of the disclosure enable reduced infield variability of a corn crop exposed to said microbes, which translates into improved yield stability for the farmer.
(786) Experimental Protocol for NDVI Field Trial
(787) NDVI measurements were taken through satellite imaging about 1.5 months after corn planting to monitor the Normalized Difference Vegetation Index measurement. NDVI is calculated from the visible and near-infrared light reflected by vegetation. The remodeled microbe 137-1036 was applied to treat the corn, i.e. the remodeled *Klebsiella variicola*, which was deposited as NCMA 201712002 and can be found, inter alia, in Table 1.
(788) With respect to FIG. **42** that illustrates the results of the field experiment, healthy vegetation absorbs most of the visible light that hits it, and reflects a large portion of the near-infrared light. Unhealthy or sparse vegetation reflects more visible light and less near-infrared light.
(789) In the two plots that are shown in FIG. **42**, the microbes of the disclosure (137-1036) were applied to the field area plots demarcated with the “pins” (left panel) and the “cross markers” right panel. The treated area has also been illustrated with a square border. In both cases (left and right panels of FIG. **42**), a more consistent NDVI measurement across the whole treated area was observed, compared to areas not treated with the 137-1036 microbe.
(790) Data on mean yield of corn from a field trial showing reduced in field variability for the field treated with the remodeled strain of the present disclosure (137-1036 strain) compared to untreated field is shown in Table 34 below.
(791) TABLE-US-00036 TABLE 34 Average side- Average Average Average Average dress reduction PBM mean check mean PBM sd check sd lbs N/ac yield yield bpa yield yield bpa 35 227.8 228.4 16.5 19.9
(792) The data in Table 34 is an average from 5 different locations comparing untreated field (check) and ProveN (137-1036 strain) treated field (PBM). The untreated/check fields were not treated with the microbes of the present disclosure and had exogenous N applied. The PBM fields were treated with the microbes of the present disclosure, but did not have sidedress applied. As shown in Table 34, the PBM field needed 35 lbs less side-dressing (first column); at the same time, the mean yield from the PBM field and untreated field was similar. The standard deviation for the mean yield obtained from the PBM field is considerably less than that of the check (16.5 vs 19.9 bushels per acre (bpa)). The lesser standard deviation for the PBM-treated field indicates more

uniform vegetation and reduced heterogeneity compared to the control field which is consistent with the NDVI data shown in FIG. **42**.

Example 12: Nitrogen Delivery by Sustainable Nitrogen Producing Microbes Across Challenging Soil Types in Corn Fields

(793) The present inventors determined that over the course of evaluating the performance of the presently disclosed nitrogen producing microbes across a variety of soil types and conditions, the microbes consistently colonized corn roots and supplied N to corn plants, even in challenging soil types where traditional N fertilizer was not very effective. The present study evaluated 47 different soil types in variable weather conditions across 13 states in the U.S., which revealed the microbes thrived in all of the evaluated soil types and weather conditions. In this study, the soil with a high sand content was considered a “challenging” or “problematic” soil type as growers can lose nitrogen in these type of coils quickly whereas the soil with a low sand content was considered a “typical” or “non-problematic” soil type. The % sand content of 47 evaluated soil types was measured; it was observed that 5 of them had a very high sand content. Specifically, 5 of the 47 evaluated soil types had an average sand content of about 50.90% and were considered a “challenging” or “problematic” soil type and the remaining soil types with an average of about 26.64% sand content were considered a “typical” or “non-problematic” soil type. The individual sand content of the 5 challenging soil types is listed in Table 35.

(794) Growers typically lose nitrogen in heavy rains and/or challenging soil types. The microbes exhibited strong performance in a variety of challenging soil types, as well as soil exposed to heavy rains.

(795) The data from field trials showing improvement in corn yield for challenging soil types treated with the remodeled microbes of the present disclosure compared to the same soil type not treated with the remodel microbes is summarized in Table 35 below. The column “Pivot Yield” in Table 35 shows the yield from the challenging soil type fields treated with the remodeled strains of the present disclosure. For challenging soil types, the remodeled microbes conferred a ˜17 bushel per acre average against fields in comparable conditions using only chemical nitrogen fertilizer. This superior improvement in yield in challenging soil types and soil exposed to heavy rains is surprising because under typical soil and weather conditions, the application of the microbes exhibited a ˜7.7 bushel per acre advantage compared to fields without the microbes.

(796) Utilizing the present microbes reduced the need for chemical fertilizer and delivers a return on investment to the growers who use the microbes, while decreasing the complexity and risk typically associated with chemical fertilizer use

(797) As illustrated in Example 11 relating to reduced infield variability, as measured by NDVI, the current data of Example 12, demonstrating improved performance across a wide range of soil types, further illustrates that the microbe taught herein are able to lend yield predictability and reduce yield heterogeneity across a farmer's field.

(798) The ability for a farmer to realize relatively homogeneous yield gains across their growing acreage, even in acres normally susceptible to low yields, is a dramatic step forward in the art. Farmers will now be able to more reliably predict yields and realize value on acreage that traditionally would be low performing.

(799) TABLE-US-00037 TABLE 35 Cation Soil Type Texture Organic Exchange field.id Names Class Matter Coefficient pH % Sand % Silt % Clay 18PB12J1 Kandota Sandy 0.486576 8.91133 6.582414 57.75961 17.08227 15.15813 sandy loam loam, 2 to 6 percent slopes 18PB12K1 Nicollet Loam 1.332813 14.82493 7.404525 43.412 37.51325 19.07475 loam, 1 to 3 percent slopes 18PB1A1 Hamerly- Loam 1.832989 21.28688 7.626457 31.62567 39.39813 28.9762 Tonka- Parnell complex, 0 to 3 percent slopes 18PB12H1 Fieldon- Loam 2.730757 13.67336 7.11 53.23678 21.08033 15.68289 Canisteo loams 18PB1E1 Tracy Sandy 0.701942 6.156816 5.358657 68.46489 19.47012 12.06499 sandy loam loam, 0 to 2 percent slopes Saturated Pivot Untreated Hydrolic Erodability Drainage Water Yield Yield Difference field.id Coefficient Factor Class

Storage (bu/acre) (bu/acre) in Yield 18PB12J1 10.00583 0.214759 Well 24.21 184.6102 171.8642 12.74599 drained 18PB12K1 9.077925 0.342763 Somewhat 28.04 219.4818 207.4651 12.01668 poorly drained 18PB1A1 6.175194 0.301595 Somewhat 24.64 257.7206 229.7333 27.98731 poorly drained 18PB12H1 28.68618 0.202908 Poorly 21.26 214.953 195.3212 19.63179 drained 18PB1E1 42.98403 0.1522 Well 21.03 195.4602 180.0739 15.38628 drained

Example 13: Improving Activity of Microbial Strains

(800) In this example, Steps A-F described in Example 1 were used to generate several non-transgenic derivative strains of *Klebsiella variicola* Wild type (WT) strain, CI137. First, the WT strain, CI137, was isolated from a rhizosphere, characterized, and domesticated using the approaches described in steps A-C of Example 1.

(801) Then using the approaches described in steps D-F of Example 1, the nitrogen fixation trait of CI137 was rationally improved without the use of transgenes. To test whether the nitrogen fixation trait of the WT strain can be improved, various genes involved in nitrogen fixation as described throughout this application were targeted to engineer non-intergeneric mutations, the engineered/remodeled microbes were analyzed for nitrogen fixation, and the engineering and the analytics steps were iterated to test whether further improvements can be made in the nitrogen fixation ability. Using this iterative approach, beneficial mutations were stacked to increase the nitrogen fixation ability.

(802) Non-intergeneric mutations made through this iterative remodeling process to generate remodeled CI137 strains that showed improvement in nitrogen fixation are summarized in Table 36 below. The stepwise improvement in the nitrogen fixation trait of the remodeled strains is shown in FIG. **43**.

(803) TABLE-US-00038 TABLE 36 137 Strain and Mutation Description Associated SEQ Novel Strain ID Junction If Strain ID NO Genotype Mutation Mutation Description Applicable 137-ΔnifL::PinfC ΔnifL::PinfC Deletion of the nifL gene from 1036 20 bp after the ATG (start) to 87 bp before the TGA (stop) of the gene. A 500 bp fragment of the region upstream of the infC gene was inserted (PinfC) upstream of nifA replacing the deleted portion. 137- ΔglnE.sub.AR-KO2 ΔglnE.sub.AR-KO2 Deletion of 1647 bp after the start 1034 codon of the glnE gene. 137-ΔnifL::PinfC ΔnifL::PinfC Deletion of the nifL gene from 2249 20 bp after the ATG (start) to 87 bp before the TGA (stop) of the gene. A 500 bp fragment of the region upstream of the infC gene was inserted (PinfC) upstream of nifA replacing the deleted portion. glnE.sub.AR-DxD glnE.sub.AR-DxD Modification of the “GAT” found 513 bp after the start codon of glnE to a “GCG” codon. 137- ΔnifL::Prm8.2 ΔnifL::Prm8.2 Deletion of the nifL gene from 1968 20 bp after the ATG (start) to 87 bp before the TGA (stop) of the gene. A 299 bp fragment (Prm8.2), found 77 bp after the start codon of nlpI to 376 bp after the start codon of nlpI was inserted upstream of nifA replacing the deleted portion. ΔglnE.sub.AR-KO2 ΔglnE.sub.AR-KO2 Deletion of 1647 bp after the start codon of the glnE gene. 137- ΔnifL::PinfC ΔnifL::PinfC Deletion of the nifL gene from 1586 20 bp after the ATG (start) to 87 bp before the TGA (stop) of the gene. A 500 bp fragment of the region upstream of the infC gene was inserted (PinfC) upstream of nifA replacing the deleted portion. ΔglnE.sub.AR-KO2 ΔglnE.sub.AR-KO2 Deletion of 1647 bp after the start codon of the glnE gene. 137- ΔnifL::Prm1.2 ΔnifL::Prm1.2 Deletion of the nifL gene from 2084 20 bp after the ATG (start) to 87 bp before the TGA (stop) of the gene. A 400 bp fragment from the region upstream of the cspE gene was inserted (Prm1.2) upstream of nifA replacing the deleted portion. ΔglnE.sub.AR-KO2 ΔglnE.sub.AR-KO2 Deletion of 1647 bp after the start codon of the glnE gene. 137- ΔnifL::Prm1.2 ΔnifL::Prm1.2 Deletion of the nifL gene from 2251 20 bp after the ATG (start) to 87 bp before the TGA (stop) of the gene. A 400 bp fragment from the region upstream of the cspE gene was inserted (Prm1.2) upstream of nifA replacing the deleted portion. ΔglnE.sub.AR-KO2 ΔglnE.sub.AR-KO2 Deletion of 1647 bp after the start codon of the glnE gene. rpoN-Prm8.2 rpoN-Prm8.2 Deletion of the 47 bp between ibtB2 and rpoN and insertion of a fragment (Prm8.2), found 77 bp after the start codon of nlpI to 376 bp after the start codon of nlpI,

directly upstream of rpoN. 137- ΔnifL::Prm1.2 ΔnifL::Prm1.2 Deletion of the nifL gene from 2219 20 bp after the ATG (start) to 87 bp before the TGA (stop) of the gene. A 400 bp fragment from the region upstream of the cspE gene was inserted (Prm1.2) upstream of nifA replacing the deleted portion. ΔglnE.sub.AR-KO2 ΔglnE.sub.AR-KO2 Deletion of 1647 bp after the start codon of the glnE gene. ΔglnD.sub.ACT1/2 ΔglnD.sub.ACT1/2 Deletion of the 546 bp before the stop codon of the glnD gene.

(804) The feature sets indicated in Table 37 correspond to the Features List in FIG. **29**, which recites F0, F1, F2, F3, F4, F5, and F6. The features amount to targeted improvements in strains to facilitate reduced exogenous nitrogen use in fields or complete replacement of exogenous nitrogen use in fields. The improvement in nitrogen fixation exhibited by the strains listed in Table 37 is shown in FIG. **43**.

(805) TABLE-US-00039 TABLE 37 Ammonium Excretion in Modified Cells Strain ID Genotype Feature Sets 137-1036 ΔnifL::PinfC F1 137-2249 ΔnifL::PinfC, glnE.sub.AR-DxD F1, F2 137-1034 ΔglnE.sub.AR-KO2 F2 137-1586 ΔnifL::PinfC, ΔglnE.sub.AR-KO2 F1, F2 137-2084 ΔnifL::Prm1.2, ΔglnE.sub.AR-KO2 F1, F2 137-1968 ΔnifL::Prm8.2, ΔglnE.sub.AR-KO2 F1, F2 137-2251 ΔnifL::Prm1.2, rpoN-Prm8.2 F1, F4 137-2219 ΔnifL::Prm1.2, ΔglnE.sub.AR-KO2, ΔglnD.sub.ACT1/2 F1, F2, F3

Numbered Embodiments of the Disclosure

(806) Notwithstanding the appended claims, the disclosure sets forth the following numbered embodiments: 1. A method of providing fixed atmospheric nitrogen to a cereal plant, comprising: a. providing to a locus a plurality of non-intergeneric remodeled bacteria that each produce fixed N of at least about 5.49×10.sup.−13 mmol of N per CFU per hour; and b. providing to the locus a plurality of cereal plants, wherein said plurality of non-intergeneric remodeled bacteria colonize the root surface of said plurality of cereal plants and supply the cereal plants with fixed N, and wherein said plurality of non-intergeneric remodeled bacteria produce in the aggregate at least about 15 pounds of fixed N per acre over the course of at least about 10 days to about 60 days. 2. The method according to embodiment 1, wherein the plurality of non-intergeneric remodeled bacteria each produce fixed N of at least about 2.75×10.sup.−12 mmol of N per CFU per hour. 3. The method according to any of embodiments 1 or 2, wherein the plurality of non-intergeneric remodeled bacteria each produce fixed N of at least about 4.03×10.sup.−13 mmol of N per CFU per hour. 4. The method according to any one of embodiments 1-3, wherein the plurality of non-intergeneric remodeled bacteria each produce fixed N of at least about 2.75×10.sup.−12 mmol of N per CFU per hour and wherein said plurality of bacteria produce in the aggregate at least about 25 pounds of fixed N per acre over the course of at least about 10 days to about 60 days. 5. The method according to any one of embodiments 1-4, wherein the plurality of non-intergeneric remodeled bacteria each produce fixed N of at least about 2.75×10.sup.−12 mmol of N per CFU per hour and wherein said plurality of bacteria produce in the aggregate at least about 50 pounds of fixed N per acre over the course of at least about 10 days to about 60 days. 6. The method according to any one of embodiments 1-5, wherein the plurality of non-intergeneric remodeled bacteria each produce fixed N of at least about 2.75×10.sup.−12 mmol of N per CFU per hour and wherein said plurality of bacteria produce in the aggregate at least about 75 pounds of fixed N per acre over the course of at least about 10 days to about 60 days. 7. The method according to any one of embodiments 1-6, wherein the plurality of non-intergeneric remodeled bacteria each produce fixed N of at least about 2.75×10.sup.−12 mmol of N per CFU per hour and wherein said plurality of bacteria produce in the aggregate at least about 100 pounds of fixed N per acre over the course of at least about 10 days to about 60 days. 8. The method according to any one of embodiments 1-7, wherein the plurality of non-intergeneric remodeled bacteria colonize the root surface of the plurality of cereal plants at a total aggregate CFU per acre concentration according to FIG. **31**, **32**, **33**, **34**, or **35**. 9. The method according to any one of embodiments 1-8, wherein the plurality of non-intergeneric remodeled bacteria colonize the root surface of the plurality of cereal plants at a

total aggregate CFU per acre concentration of about 5×10.sup.13 for at least about 20 days. 10. The method according to any one of embodiments 1-9, wherein the plurality of non-intergeneric remodeled bacteria colonize the root surface of the plurality of cereal plants at a total aggregate CFU per acre concentration of about 5×10.sup.13 for at least about 30 days. 11. The method according to any one of embodiments 1-10, wherein the plurality of non-intergeneric remodeled bacteria colonize the root surface of the plurality of cereal plants at a total aggregate CFU per acre concentration of about 5×10.sup.13 for at least about 60 days. 12. The method according to any one of embodiments 1-11, wherein the plurality of non-intergeneric remodeled bacteria colonize the root surface of peripheral roots of the plurality of cereal plants equally as well as they colonize other roots. 13. The method according to any one of embodiments 1-12, wherein the plurality of non-intergeneric remodeled bacteria colonize the root surface of peripheral roots of the plurality of cereal plants to a higher degree than they colonize other roots. 14. The method according to any one of embodiments 1-13, wherein exogenous nitrogen is not applied to said locus after the plurality of non-intergeneric remodeled bacteria and cereal plants are applied. 15. The method according to any one of embodiments 1-14, wherein a sidedressing containing exogenous nitrogen is not applied to said locus after the plurality of non-intergeneric remodeled bacteria and cereal plants are applied. 16. The method according to any one of embodiments 1-15, further comprising before step a) and b), applying exogenous nitrogen to said locus. 17. The method according to any one of embodiments 1-16, further comprising before step a) and b), applying exogenous nitrogen to said locus, and not applying exogenous nitrogen to said locus after step a) and b). 18. The method according to any one of embodiments 1-17, wherein said cereal plant is corn, rice, wheat, barley, *sorghum*, millet, oat, rye, or triticale. 19. The method according to any one of embodiments 1-18, wherein the cereal plant is a corn plant and the plurality of non-intergeneric remodeled bacteria produce in the aggregate at least about 15 pounds of fixed N per acre before the corn plant's VT stage. 20. The method according to any one of embodiments 1-19, wherein the cereal plant is a corn plant and the plurality of non-intergeneric remodeled bacteria produce in the aggregate at least about 15 pounds of fixed N per acre before the corn plant's R1 stage. 21. The method according to any one of embodiments 1-20, wherein the cereal plant is a corn plant and the plurality of non-intergeneric remodeled bacteria produce in the aggregate at least about 15 pounds of fixed N per acre before the corn plant's R6 stage. 22. The method according to any one of embodiments 1-21, wherein the plurality of non-intergeneric remodeled bacteria produce 1% or more of the fixed nitrogen in an individual cereal plant of said plurality exposed thereto. 23. The method according to any one of embodiments 1-22, wherein the plurality of non-intergeneric remodeled bacteria are capable of fixing atmospheric nitrogen in the presence of exogenous nitrogen. 24. The method according to any one of embodiments 1-23, wherein each member of the plurality of non-intergeneric remodeled bacteria comprises at least one genetic variation introduced into at least one gene, or non-coding polynucleotide, of the nitrogen fixation or assimilation genetic regulatory network. 25. The method according to any one of embodiments 1-24, wherein each member of the plurality of non-intergeneric remodeled bacteria comprises an introduced control sequence operably linked to at least one gene of the nitrogen fixation or assimilation genetic regulatory network. 26. The method according to any one of embodiments 1-25, wherein each member of the plurality of non-intergeneric remodeled bacteria comprises a heterologous promoter operably linked to at least one gene of the nitrogen fixation or assimilation genetic regulatory network. 27. The method according to any one of embodiments 1-26, wherein each member of the plurality of non-intergeneric remodeled bacteria comprises at least one genetic variation introduced into a member selected from the group consisting of: nifA, nifL, ntrB, ntrC, polynucleotide encoding glutamine synthetase, glnA, glnB, glnK, drat, amtB, polynucleotide encoding glutaminase, glnD, glnE, nifJ, nifH, nifD, nifK, nifY, nifE, nifN, nifU, nifS, nifV, nifW, nifZ, nifM, nifF, nifB, nifQ, a gene associated with biosynthesis of a nitrogenase enzyme, and combinations thereof. 28. The method according to any one of embodiments 1-27, wherein each member of the plurality of non-

intergeneric remodeled bacteria comprises at least one genetic variation introduced into at least one gene, or non-coding polynucleotide, of the nitrogen fixation or assimilation genetic regulatory network that results in one or more of: increased expression or activity of NifA or glutaminase; decreased expression or activity of NifL, NtrB, glutamine synthetase, GlnB, GlnK, DraT, AmtB; decreased adenylyl-removing activity of GlnE; or decreased uridylyl-removing activity of GlnD. 29. The method according to any one of embodiments 1-28, wherein each member of the plurality of non-intergeneric remodeled bacteria comprises a mutated nifL gene that has been altered to comprise a heterologous promoter inserted into said nifL gene. 30. The method according to any one of embodiments 1-29, wherein each member of the plurality of non-intergeneric remodeled bacteria comprises a mutated glnE gene that results in a truncated GlnE protein lacking an adenylyl-removing (AR) domain. 31. The method according to any one of embodiments 1-30, wherein each member of the plurality of non-intergeneric remodeled bacteria comprises a mutated amtB gene that results in the lack of expression of said amtB gene. 32. The method according to any one of embodiments 1-31, wherein each member of the plurality of non-intergeneric remodeled bacteria comprises at least one of: a mutated nifL gene that has been altered to comprise a heterologous promoter inserted into said nifL gene; a mutated glnE gene that results in a truncated GlnE protein lacking an adenylyl-removing (AR) domain; a mutated amtB gene that results in the lack of expression of said amtB gene; and combinations thereof. 33. The method according to any one of embodiments 1-32, wherein each member of the plurality of non-intergeneric remodeled bacteria comprises a mutated nifL gene that has been altered to comprise a heterologous promoter inserted into said nifL gene and a mutated glnE gene that results in a truncated GlnE protein lacking an adenylyl-removing (AR) domain. 34. The method according to any one of embodiments 1-33, wherein each member of the plurality of non-intergeneric remodeled bacteria comprises a mutated nifL gene that has been altered to comprise a heterologous promoter inserted into said nifL gene and a mutated glnE gene that results in a truncated GlnE protein lacking an adenylyl-removing (AR) domain and a mutated amtB gene that results in the lack of expression of said amtB gene. 35. The method according to any one of embodiments 1-34, wherein each member of the plurality of non-intergeneric remodeled bacteria comprises at least one genetic variation introduced into genes involved in a pathway selected from the group consisting of: exopolysaccharide production, endo-polygalaturonase production, trehalose production, and glutamine conversion. 36. The method according to any one of embodiments 1-35, wherein each member of the plurality of non-intergeneric remodeled bacteria comprises at least one genetic variation introduced into genes selected from the group consisting of: bcsii, bcsiii, yjbE, fhaB, pehA, otsB, treZ, glsA2, and combinations thereof. 37. The method according to any one of embodiments 1-36, wherein the plurality of non-intergeneric remodeled bacteria comprise at least two different species of bacteria. 38. The method according to any one of embodiments 1-37, wherein the plurality of non-intergeneric remodeled bacteria comprise at least two different strains of the same species of bacteria. 39. The method according to any one of embodiments 1-38, wherein the plurality of non-intergeneric remodeled bacteria comprise bacteria selected from: *Rahnella aquatilis, Klebsiella variicola, Achromobacter spiritinus, Achromobacter marplatensis, Microbacterium murale, Kluyvera intermedia, Kosakonia pseudosacchari, Enterobacter* sp *Azospirillum lipoferum, Kosakonia sacchari*, and combinations thereof. 40. The method according to any one of embodiments 1-39, wherein the plurality of non-intergeneric remodeled bacteria are epiphytic or rhizospheric. 41. The method according to any one of embodiments 1-40, wherein the plurality of non-intergeneric remodeled bacteria comprise bacteria selected from: a bacteria deposited as NCMA 201701002, a bacteria deposited as NCMA 201708004, a bacteria deposited as NCMA 201708003, a bacteria deposited as NCMA 201708002, a bacteria deposited as NCMA 201712001, a bacteria deposited as NCMA 201712002, and combinations thereof 42. The method according to any one of embodiments 1-41, wherein the plurality of non-intergeneric remodeled bacteria comprise bacteria with a nucleic acid sequence that shares at least about 90% sequence identity to a

nucleic acid sequence selected from SEQ ID NOs: 177-260, 296-303, and 458-469. 43. The method according to any one of embodiments 1-42, wherein the plurality of non-intergeneric remodeled bacteria comprise bacteria with a nucleic acid sequence that shares at least about 95% sequence identity to a nucleic acid sequence selected from SEQ ID NOs: 177-260, 296-303, and 458-469. 44. The method according to any one of embodiments 1-43, wherein the plurality of non-intergeneric remodeled bacteria comprise bacteria with a nucleic acid sequence that shares at least about 99% sequence identity to a nucleic acid sequence selected from SEQ ID NOs: 177-260, 296-303, and 458-469. 45. The method according to any one of embodiments 1-44, wherein the plurality of non-intergeneric remodeled bacteria comprise bacteria with a nucleic acid sequence selected from SEQ ID NOs: 177-260, 296-303, and 458-469. 46. A method of providing fixed atmospheric nitrogen to a corn plant that eliminates the need for the addition of in-season exogenous nitrogen application, comprising: a. providing to a locus a plurality of non-intergeneric remodeled bacteria that each produce fixed N of at least about 5.49×10.sup.−13 mmol of N per CFU per hour; and b. providing to the locus a plurality of corn plants, wherein said plurality of non-intergeneric remodeled bacteria colonize the root surface of said plurality of corn plants and supply the corn plants with fixed N, and wherein said plurality of non-intergeneric remodeled bacteria produce in the aggregate at least about 15 pounds of fixed N per acre over the course of at least about 10 days to about 60 days, and wherein exogenous nitrogen is not applied to said locus after the plurality of non-intergeneric remodeled bacteria and corn plants are applied. 47. The method according to embodiment 46, wherein exogenous nitrogen is not applied as a sidedressing. 48. The method according to any one of embodiments 46 or 47, wherein the plurality of non-intergeneric remodeled bacteria each produce fixed N of at least about 2.75×10.sup.−12 mmol of N per CFU per hour. 49. The method according to any one of embodiments 46-48, wherein the plurality of non-intergeneric remodeled bacteria each produce fixed N of at least about 4.03×10.sup.−13 mmol of N per CFU per hour. 50. The method according to any one of embodiments 46-49, wherein the plurality of non-intergeneric remodeled bacteria each produce fixed N of at least about 2.75×10.sup.−12 mmol of N per CFU per hour and wherein said plurality of bacteria produce in the aggregate at least about 25 pounds of fixed N per acre over the course of at least about 10 days to about 60 days. 51. The method according to any one of embodiments 46-50, wherein the plurality of non-intergeneric remodeled bacteria each produce fixed N of at least about 2.75×10.sup.−12 mmol of N per CFU per hour and wherein said plurality of bacteria produce in the aggregate at least about 50 pounds of fixed N per acre over the course of at least about 10 days to about 60 days. 52. The method according to any one of embodiments 46-51, wherein the plurality of non-intergeneric remodeled bacteria each produce fixed N of at least about 2.75×10.sup.−12 mmol of N per CFU per hour and wherein said plurality of bacteria produce in the aggregate at least about 75 pounds of fixed N per acre over the course of at least about 10 days to about 60 days. 53. The method according to any one of embodiments 46-52, wherein the plurality of non-intergeneric remodeled bacteria each produce fixed N of at least about 2.75×10.sup.−12 mmol of N per CFU per hour and wherein said plurality of bacteria produce in the aggregate at least about 100 pounds of fixed N per acre over the course of at least about 10 days to about 60 days. 54. The method according to any one of embodiments 46-53, wherein the plurality of non-intergeneric remodeled bacteria colonize the root surface of the plurality of corn plants at a total aggregate CFU per acre concentration according to FIG. **31**, **32**, **33**, **34**, or **35**. 55. The method according to any one of embodiments 46-54, wherein the plurality of non-intergeneric remodeled bacteria colonize the root surface of the plurality of corn plants at a total aggregate CFU per acre concentration of about 5×10.sup.13 for at least about 20 days. 56. The method according to any one of embodiments 46-55, wherein the plurality of non-intergeneric remodeled bacteria colonize the root surface of the plurality of corn plants at a total aggregate CFU per acre concentration of about 5×10.sup.13 for at least about 30 days. 57. The method according to any one of embodiments 46-56, wherein the plurality of non-intergeneric remodeled bacteria colonize the root surface of the plurality of corn plants at a total aggregate CFU per acre

concentration of about 5×10.sup.13 for at least about 60 days. 58. The method according to any one of embodiments 46-57, wherein the plurality of non-intergeneric remodeled bacteria colonize the root surface of peripheral roots of the plurality of corn plants equally as well as they colonize other roots. 59. The method according to any one of embodiments 46-58, wherein the plurality of non-intergeneric remodeled bacteria colonize the root surface of peripheral roots of the plurality of corn plants to a higher degree than they colonize other roots. 60. The method according to any one of embodiments 46-59, further comprising before step a) and b), applying exogenous nitrogen to said locus. 61. The method according to any one of embodiments 46-60, wherein the plurality of non-intergeneric remodeled bacteria produce in the aggregate at least about 15 pounds of fixed N per acre before the corn plant's VT stage. 62. The method according to any one of embodiments 46-61, wherein the plurality of non-intergeneric remodeled bacteria produce in the aggregate at least about 15 pounds of fixed N per acre before the corn plant's R1 stage. 63. The method according to any one of embodiments 46-62, wherein the plurality of non-intergeneric remodeled bacteria produce in the aggregate at least about 15 pounds of fixed N per acre before the corn plant's R6 stage. 64. The method according to any one of embodiments 46-63, wherein the plurality of non-intergeneric remodeled bacteria produce 1% or more of the fixed nitrogen in an individual corn plant of said plurality exposed thereto. 65. The method according to any one of embodiments 46-64, wherein the plurality of non-intergeneric remodeled bacteria are capable of fixing atmospheric nitrogen in the presence of exogenous nitrogen. 66. The method according to any one of embodiments 46-65, wherein each member of the plurality of non-intergeneric remodeled bacteria comprises at least one genetic variation introduced into at least one gene, or non-coding polynucleotide, of the nitrogen fixation or assimilation genetic regulatory network. 67. The method according to any one of embodiments 46-66, wherein each member of the plurality of non-intergeneric remodeled bacteria comprises an introduced control sequence operably linked to at least one gene of the nitrogen fixation or assimilation genetic regulatory network. 68. The method according to any one of embodiments 46-67, wherein each member of the plurality of non-intergeneric remodeled bacteria comprises a heterologous promoter operably linked to at least one gene of the nitrogen fixation or assimilation genetic regulatory network. 69. The method according to any one of embodiments 46-68, wherein each member of the plurality of non-intergeneric remodeled bacteria comprises at least one genetic variation introduced into a member selected from the group consisting of: nifA, nifL, ntrB, ntrC, polynucleotide encoding glutamine synthetase, glnA, glnB, glnK, drat, amtB, polynucleotide encoding glutaminase, glnD, glnE, nifJ, nifH, nifD, nifK, nifY, nifE, nifN, nifU, nifS, nifV, nifW, nifZ, nifM, nifF, nifB, nifQ, a gene associated with biosynthesis of a nitrogenase enzyme, and combinations thereof. 70. The method according to any one of embodiments 46-69, wherein each member of the plurality of non-intergeneric remodeled bacteria comprises at least one genetic variation introduced into at least one gene, or non-coding polynucleotide, of the nitrogen fixation or assimilation genetic regulatory network that results in one or more of: increased expression or activity of NifA or glutaminase; decreased expression or activity of NifL, NtrB, glutamine synthetase, GlnB, GlnK, DraT, AmtB; decreased adenylyl-removing activity of GlnE; or decreased uridylyl-removing activity of GlnD. 71. The method according to any one of embodiments 46-70, wherein each member of the plurality of non-intergeneric remodeled bacteria comprises a mutated nifL gene that has been altered to comprise a heterologous promoter inserted into said nifL gene. 72. The method according to any one of embodiments 46-71, wherein each member of the plurality of non-intergeneric remodeled bacteria comprises a mutated glnE gene that results in a truncated GlnE protein lacking an adenylyl-removing (AR) domain. 73. The method according to any one of embodiments 46-72, wherein each member of the plurality of non-intergeneric remodeled bacteria comprises a mutated amtB gene that results in the lack of expression of said amtB gene. 74. The method according to any one of embodiments 46-73, wherein each member of the plurality of non-intergeneric remodeled bacteria comprises at least one of: a mutated nifL gene that has been altered to comprise a heterologous promoter inserted into said

nifL gene; a mutated glnE gene that results in a truncated GlnE protein lacking an adenylyl-removing (AR) domain; a mutated amtB gene that results in the lack of expression of said amtB gene; and combinations thereof 75. The method according to any one of embodiments 46-74, wherein each member of the plurality of non-intergeneric remodeled bacteria comprises a mutated nifL gene that has been altered to comprise a heterologous promoter inserted into said nifL gene and a mutated glnE gene that results in a truncated GlnE protein lacking an adenylyl-removing (AR) domain. 76. The method according to any one of embodiments 46-75, wherein each member of the plurality of non-intergeneric remodeled bacteria comprises a mutated nifL gene that has been altered to comprise a heterologous promoter inserted into said nifL gene and a mutated glnE gene that results in a truncated GlnE protein lacking an adenylyl-removing (AR) domain and a mutated amtB gene that results in the lack of expression of said amtB gene. 77. The method according to any one of embodiments 46-76, wherein each member of the plurality of non-intergeneric remodeled bacteria comprises at least one genetic variation introduced into genes involved in a pathway selected from the group consisting of: exopolysaccharide production, endo-polygalaturonase production, trehalose production, and glutamine conversion. 78. The method according to any one of embodiments 46-77, wherein each member of the plurality of non-intergeneric remodeled bacteria comprises at least one genetic variation introduced into genes selected from the group consisting of: bcsii, bcsiii, yjbE, fhaB, pehA, otsB, treZ, glsA2, and combinations thereof 79. The method according to any one of embodiments 46-78, wherein the plurality of non-intergeneric remodeled bacteria comprise at least two different species of bacteria. 80. The method according to any one of embodiments 46-79, wherein the plurality of non-intergeneric remodeled bacteria comprise at least two different strains of the same species of bacteria. 81. The method according to any one of embodiments 46-80, wherein the plurality of non-intergeneric remodeled bacteria comprise bacteria selected from: *Rahnella aquatilis, Klebsiella variicola, Achromobacter spiritinus, Achromobacter marplatensis, Microbacterium murale, Kluyvera intermedia, Kosakonia pseudosacchari, Enterobacter* sp *Azospirillum lipoferum, Kosakonia sacchari*, and combinations thereof. 82. The method according to any one of embodiments 46-81, wherein the plurality of non-intergeneric remodeled bacteria are epiphytic or rhizospheric. 83. The method according to any one of embodiments 46-82, wherein the plurality of non-intergeneric remodeled bacteria comprise bacteria selected from: a bacteria deposited as NCMA 201701002, a bacteria deposited as NCMA 201708004, a bacteria deposited as NCMA 201708003, a bacteria deposited as NCMA 201708002, a bacteria deposited as NCMA 201712001, a bacteria deposited as NCMA 201712002, and combinations thereof 84. The method according to any one of embodiments 46-83, wherein the plurality of non-intergeneric remodeled bacteria comprise bacteria with a nucleic acid sequence that shares at least about 90% sequence identity to a nucleic acid sequence selected from SEQ ID NOs: 177-260, 296-303, and 458-469. 85. The method according to any one of embodiments 46-84, wherein the plurality of non-intergeneric remodeled bacteria comprise bacteria with a nucleic acid sequence that shares at least about 95% sequence identity to a nucleic acid sequence selected from SEQ ID NOs: 177-260, 296-303, and 458-469. 86. The method according to any one of embodiments 46-85, wherein the plurality of non-intergeneric remodeled bacteria comprise bacteria with a nucleic acid sequence that shares at least about 99% sequence identity to a nucleic acid sequence selected from SEQ ID NOs: 177-260, 296-303, and 458-469. 87. The method according to any one of embodiments 46-86, wherein the plurality of non-intergeneric remodeled bacteria comprise bacteria with a nucleic acid sequence selected from SEQ ID NOs: 177-260, 296-303, and 458-469. 88. A method for increasing corn yield per acre, comprising: a. providing to a locus a plurality of non-intergeneric remodeled bacteria that each produce fixed N of at least about 5.49×10.sup.−13 mmol of N per CFU per hour; and b. providing to the locus a plurality of corn plants at a density of at least 35,000 seeds per acre, wherein said plurality of non-intergeneric remodeled bacteria colonize the root surface of said plurality of corn plants and supply the corn plants with fixed N, and wherein said plurality of non-intergeneric

remodeled bacteria produce in the aggregate at least about 15 pounds of fixed N per acre over the course of at least about 10 days to about 60 days, and wherein exogenous nitrogen is not applied to said locus after the plurality of non-intergeneric remodeled bacteria and corn plants are applied. 89. The method according to embodiment 88, wherein exogenous nitrogen is not applied as a sidedressing. 90. The method according to any one of embodiments 88 or 89, wherein the plurality of non-intergeneric remodeled bacteria each produce fixed N of at least about 2.75×10.sup.−12 mmol of N per CFU per hour. 91. The method according to any one of embodiments 88-90, wherein the plurality of non-intergeneric remodeled bacteria each produce fixed N of at least about 4.03×10.sup.−12 mmol of N per CFU per hour. 92. The method according to any one of embodiments 88-91, wherein the plurality of non-intergeneric remodeled bacteria each produce fixed N of at least about 2.75×10.sup.−12 mmol of N per CFU per hour and wherein said plurality of bacteria produce in the aggregate at least about 25 pounds of fixed N per acre over the course of at least about 10 days to about 60 days. 93. The method according any one of embodiments 88-92, wherein the plurality of non-intergeneric remodeled bacteria each produce fixed N of at least about 2.75×10.sup.−12 mmol of N per CFU per hour and wherein said plurality of bacteria produce in the aggregate at least about 50 pounds of fixed N per acre over the course of at least about 10 days to about 60 days. 94. The method according to any one of embodiments 88-93, wherein the plurality of non-intergeneric remodeled bacteria each produce fixed N of at least about 2.75×10.sup.−12 mmol of N per CFU per hour and wherein said plurality of bacteria produce in the aggregate at least about 75 pounds of fixed N per acre over the course of at least about 10 days to about 60 days. 95. The method according to any one of embodiments 88-94, wherein the plurality of non-intergeneric remodeled bacteria each produce fixed N of at least about 2.75×10.sup.12 mmol of N per CFU per hour and wherein said plurality of bacteria produce in the aggregate at least about 100 pounds of fixed N per acre over the course of at least about 10 days to about 60 days. 96. The method according to any one of embodiments 88-95, wherein the plurality of non-intergeneric remodeled bacteria colonize the root surface of the plurality of corn plants at a total aggregate CFU per acre concentration according to FIG. **31**, **32**, **33**, **34**, or **35**. 97. The method according to any one of embodiments 88-96, wherein the plurality of non-intergeneric remodeled bacteria colonize the root surface of the plurality of corn plants at a total aggregate CFU per acre concentration of about 5×10.sup.13 for at least about 20 days. 98. The method according to any one of embodiments 88-97, wherein the plurality of non-intergeneric remodeled bacteria colonize the root surface of the plurality of corn plants at a total aggregate CFU per acre concentration of about 5×10.sup.13 for at least about 30 days. 99. The method according to any one of embodiments 88-98, wherein the plurality of non-intergeneric remodeled bacteria colonize the root surface of the plurality of corn plants at a total aggregate CFU per acre concentration of about 5×10.sup.13 for at least about 60 days. 100. The method according to any one of embodiments 88-99, wherein the plurality of non-intergeneric remodeled bacteria colonize the root surface of peripheral roots of the plurality of corn plants equally as well as they colonize other roots. 101. The method according to any one of embodiments 88-100, wherein the plurality of non-intergeneric remodeled bacteria colonize the root surface of peripheral roots of the plurality of corn plants to a higher degree than they colonize other roots. 102. The method according to any one of embodiments 88-101, further comprising before step a) and b), applying exogenous nitrogen to said locus. 103. The method according to any one of embodiments 88-102, wherein the plurality of non-intergeneric remodeled bacteria produce in the aggregate at least about 15 pounds of fixed N per acre before the corn plant's VT stage. 104. The method according to any one of embodiments 88-103, wherein the plurality of non-intergeneric remodeled bacteria produce in the aggregate at least about 15 pounds of fixed N per acre before the corn plant's R1 stage. 105. The method according to any one of embodiments 88-104, wherein the plurality of non-intergeneric remodeled bacteria produce in the aggregate at least about 15 pounds of fixed N per acre before the corn plant's R6 stage. 106. The method according to any one of embodiments 88-105, wherein the plurality of non-intergeneric remodeled bacteria

produce 1% or more of the fixed nitrogen in an individual corn plant of said plurality exposed thereto. 107. The method according to any one of embodiments 88-106, wherein the plurality of non-intergeneric remodeled bacteria are capable of fixing atmospheric nitrogen in the presence of exogenous nitrogen. 108. The method according to any one of embodiments 88-107, wherein each member of the plurality of non-intergeneric remodeled bacteria comprises at least one genetic variation introduced into at least one gene, or non-coding polynucleotide, of the nitrogen fixation or assimilation genetic regulatory network. 109. The method according to any one of embodiments 88-108, wherein each member of the plurality of non-intergeneric remodeled bacteria comprises an introduced control sequence operably linked to at least one gene of the nitrogen fixation or assimilation genetic regulatory network. 110. The method according to any one of embodiments 88-109, wherein each member of the plurality of non-intergeneric remodeled bacteria comprises a heterologous promoter operably linked to at least one gene of the nitrogen fixation or assimilation genetic regulatory network. 111. The method according to any one of embodiments 88-110, wherein each member of the plurality of non-intergeneric remodeled bacteria comprises at least one genetic variation introduced into a member selected from the group consisting of: nifA, nifL, ntrB, ntrC, polynucleotide encoding glutamine synthetase, glnA, glnB, glnK, drat, amtB, polynucleotide encoding glutaminase, glnD, glnE, nifJ, nifH, nifD, nifK, nifY, nifE, nifN, nifU, nifS, nifV, nifW, nifZ, nifM, nifF, nifB, nifQ, a gene associated with biosynthesis of a nitrogenase enzyme, and combinations thereof. 112. The method according to any one of embodiments 88-111, wherein each member of the plurality of non-intergeneric remodeled bacteria comprises at least one genetic variation introduced into at least one gene, or non-coding polynucleotide, of the nitrogen fixation or assimilation genetic regulatory network that results in one or more of: increased expression or activity of NifA or glutaminase; decreased expression or activity of NifL, NtrB, glutamine synthetase, GlnB, GlnK, DraT, AmtB; decreased adenylyl-removing activity of GlnE; or decreased uridylyl-removing activity of GlnD. 113. The method according to any one of embodiments 88-112, wherein each member of the plurality of non-intergeneric remodeled bacteria comprises a mutated nifL gene that has been altered to comprise a heterologous promoter inserted into said nifL gene. 114. The method according to any one of embodiments 88-113, wherein each member of the plurality of non-intergeneric remodeled bacteria comprises a mutated glnE gene that results in a truncated GlnE protein lacking an adenylyl-removing (AR) domain. 115. The method according to any one of embodiments 88-114, wherein each member of the plurality of non-intergeneric remodeled bacteria comprises a mutated amtB gene that results in the lack of expression of said amtB gene. 116. The method according to any one of embodiments 88-115, wherein each member of the plurality of non-intergeneric remodeled bacteria comprises at least one of: a mutated nifL gene that has been altered to comprise a heterologous promoter inserted into said nifL gene; a mutated glnE gene that results in a truncated GlnE protein lacking an adenylyl-removing (AR) domain; a mutated amtB gene that results in the lack of expression of said amtB gene; and combinations thereof. 117. The method according to any one of embodiments 88-116, wherein each member of the plurality of non-intergeneric remodeled bacteria comprises a mutated nifL gene that has been altered to comprise a heterologous promoter inserted into said nifL gene and a mutated glnE gene that results in a truncated GlnE protein lacking an adenylyl-removing (AR) domain. 118. The method according to any one of embodiments 88-117, wherein each member of the plurality of non-intergeneric remodeled bacteria comprises a mutated nifL gene that has been altered to comprise a heterologous promoter inserted into said nifL gene and a mutated glnE gene that results in a truncated GlnE protein lacking an adenylyl-removing (AR) domain and a mutated amtB gene that results in the lack of expression of said amtB gene. 119. The method according to any one of embodiments 88-118, wherein each member of the plurality of non-intergeneric remodeled bacteria comprises at least one genetic variation introduced into genes involved in a pathway selected from the group consisting of: exopolysaccharide production, endo-polygalaturonase production, trehalose production, and glutamine conversion. 120. The method according to any one of

embodiments 88-119, wherein each member of the plurality of non-intergeneric remodeled bacteria comprises at least one genetic variation introduced into genes selected from the group consisting of: bcsii, bcsiii, yjbE, fhaB, pehA, otsB, treZ, glsA2, and combinations thereof 121. The method according to any one of embodiments 88-120, wherein the plurality of non-intergeneric remodeled bacteria comprise at least two different species of bacteria. 122. The method according to any one of embodiments 88-121, wherein the plurality of non-intergeneric remodeled bacteria comprise at least two different strains of the same species of bacteria. 123. The method according to any one of embodiments 88-122, wherein the plurality of non-intergeneric remodeled bacteria comprise bacteria selected from: *Rahnella aquatilis, Klebsiella variicola, Achromobacter spiritinus, Achromobacter marplatensis, Microbacterium murale, Kluyvera intermedia, Kosakonia pseudosacchari, Enterobacter* sp *Azospirillum lipoferum, Kosakonia sacchari,* and combinations thereof. 124. The method according to any one of embodiments 88-123, wherein the plurality of non-intergeneric remodeled bacteria are epiphytic or rhizospheric. 125. The method according to any one of embodiments 88-124, wherein the plurality of non-intergeneric remodeled bacteria comprise bacteria selected from: a bacteria deposited as NCMA 201701002, a bacteria deposited as NCMA 201708004, a bacteria deposited as NCMA 201708003, a bacteria deposited as NCMA 201708002, a bacteria deposited as NCMA 201712001, a bacteria deposited as NCMA 201712002, and combinations thereof 126. The method according to any one of embodiments 88-125, wherein the plurality of non-intergeneric remodeled bacteria comprise bacteria with a nucleic acid sequence that shares at least about 90% sequence identity to a nucleic acid sequence selected from SEQ ID NOs: 177-260, 296-303, and 458-469. 127. The method according to any one of embodiments 88-126, wherein the plurality of non-intergeneric remodeled bacteria comprise bacteria with a nucleic acid sequence that shares at least about 95% sequence identity to a nucleic acid sequence selected from SEQ ID NOs: 177-260, 296-303, and 458-469. 128. The method according to any one of embodiments 88-127, wherein the plurality of non-intergeneric remodeled bacteria comprise bacteria with a nucleic acid sequence that shares at least about 99% sequence identity to a nucleic acid sequence selected from SEQ ID NOs: 177-260, 296-303, and 458-469. 129. The method according to any one of embodiments 88-128, wherein the plurality of non-intergeneric remodeled bacteria comprise bacteria with a nucleic acid sequence selected from SEQ ID NOs: 177-260, 296-303, and 458-469. 130. A method for increasing corn yield per acre in agriculturally challenging soil, comprising: a. providing to a locus located in an agriculturally challenging soil a plurality of non-intergeneric remodeled bacteria that each produce fixed N of at least about 5.49×10.sup.−13 mmol of N per CFU per hour; and b. providing to the locus located in an agriculturally challenging soil a plurality of corn plants, wherein said plurality of non-intergeneric remodeled bacteria colonize the root surface of said plurality of corn plants and supply the corn plants with fixed N, and wherein said agriculturally challenging soil comprises a soil that comprises at least about 30% sand, and wherein said plurality of corn plants achieve at least a 1 bushel per acre yield increase, as compared to a control plurality of corn plants when the control plurality of corn plants is provided to the locus. 131. The method according to embodiment 130, wherein said agriculturally challenging soil comprises at least about 40% sand. 132. The method according to any one of embodiments 130-131, wherein said agriculturally challenging soil comprises at least about 50% sand. 133. The method according to any one of embodiments 130-132, wherein said agriculturally challenging soil comprises less than about 30% silt. 134. The method according to any one of embodiments 130-133, wherein said agriculturally challenging soil comprises less than about 20% clay. 135. The method according to any one of embodiments 130-134, wherein said agriculturally challenging soil comprises a pH of about 5 to about 8. 136. The method according to any one of embodiments 130-135, wherein said agriculturally challenging soil comprises a pH of about 6.8. 137. The method according to any one of embodiments 130-136, wherein said agriculturally challenging soil comprises an organic matter content of about 0.40 to about 2.8. 138. The method according to any one of embodiments 130-137, wherein said agriculturally challenging soil

comprises an organic matter content of about 1.42. 139. The method according to any one of embodiments 130-138, wherein said agriculturally challenging soil is a sandy loam or loam soil. 140. The method according to any one of embodiments 130-139, wherein said agriculturally challenging soil comprises at least one of the soil variables in the approximate amounts as listed in Table 35. 141. The method according to any one of embodiments 130-140, wherein said plurality of corn plants achieve at least a 5 bushel per acre yield increase, as compared to a control plurality of corn plants. 142. The method according to any one of embodiments 130-141, wherein said plurality of corn plants achieve at least a 10 bushel per acre yield increase, as compared to a control plurality of corn plants. 143. The method according to any one of embodiments 130-142, wherein said control plurality of corn plants have exogenous nitrogen applied to said plants after said control plurality of corn plants have been planted. 144. The method according to any one of embodiments 130-143, wherein exogenous nitrogen is not applied to said locus after the plurality of non-intergeneric remodeled bacteria and corn plants are supplied. 145. The method according to any one of embodiments 130-144, wherein the control plurality of corn plants is provided to the locus without the plurality of non-intergeneric remodeled bacteria. 146. The method according to any one of embodiments 130-145, wherein exogenous nitrogen is not applied to said locus after the plurality of non-intergeneric remodeled bacteria and corn plants are supplied, but wherein exogenous nitrogen is applied to the control plurality of corn plants, said control plurality of corn plants not being provided with a plurality of non-intergeneric remodeled bacteria. 147. The method according to any one of embodiments 130-146, wherein exogenous nitrogen is not applied to said locus after the plurality of non-intergeneric remodeled bacteria and corn plants are supplied, but wherein exogenous nitrogen is applied to the control plurality of corn plants, said control plurality of corn plants not being provided with a plurality of non-intergeneric remodeled bacteria, wherein said plurality of corn plants with the remodeled bacteria achieve at least about a 17 bushel per acre yield increase, as compared to the control plurality of corn plants without said remodeled bacteria. 148. The method according to any one of embodiments 130-147, wherein said plurality of non-intergeneric remodeled bacteria produce in the aggregate at least about 15 pounds of fixed N per acre over the course of at least about 10 days to about 60 days. 149. The method according to any one of embodiments 130-148, wherein exogenous nitrogen is not applied as a sidedressing. 150. The method according to any one of embodiments 130-149, wherein the plurality of non-intergeneric remodeled bacteria each produce fixed N of at least about 2.75×10.sup.−12 mmol of N per CFU per hour. 151. The method according to any one of embodiments 130-150, wherein the plurality of non-intergeneric remodeled bacteria each produce fixed N of at least about 4.03×10.sup.−13 mmol of N per CFU per hour. 152. The method according to any one of embodiments 130-151, wherein said plurality of non-intergeneric remodeled bacteria produce in the aggregate at least about 25 pounds of fixed N per acre over the course of at least about 10 days to about 60 days. 153. The method according to any one of embodiments 130-152, wherein said plurality of non-intergeneric remodeled bacteria produce in the aggregate at least about 50 pounds of fixed N per acre over the course of at least about 10 days to about 60 days. 154. The method according to any one of embodiments 130-153, wherein said plurality of non-intergeneric remodeled bacteria produce in the aggregate at least about 75 pounds of fixed N per acre over the course of at least about 10 days to about 60 days. 155. The method according to any one of embodiments 130-154, wherein said plurality of non-intergeneric remodeled bacteria produce in the aggregate at least about 100 pounds of fixed N per acre over the course of at least about 10 days to about 60 days. 156. The method according to any one of embodiments 130-155, wherein the plurality of non-intergeneric remodeled bacteria colonize the root surface of the plurality of corn plants at a total aggregate CFU per acre concentration according to FIG. **31**, **32**, **33**, **34**, or **35**. 157. The method according to any one of embodiments 130-156, wherein the plurality of non-intergeneric remodeled bacteria colonize the root surface of the plurality of corn plants at a total aggregate CFU per acre concentration of about 5×10.sup.13 for at least about 20 days. 158. The

method according to any one of embodiments 130-157, wherein the plurality of non-intergeneric remodeled bacteria colonize the root surface of the plurality of corn plants at a total aggregate CFU per acre concentration of about 5×10.sup.13 for at least about 30 days. 159. The method according to any one of embodiments 130-158, wherein the plurality of non-intergeneric remodeled bacteria colonize the root surface of the plurality of corn plants at a total aggregate CFU per acre concentration of about 5×10.sup.13 for at least about 60 days. 160. The method according to any one of embodiments 130-159, wherein the plurality of non-intergeneric remodeled bacteria colonize the root surface of peripheral roots of the plurality of corn plants equally as well as they colonize other roots. 161. The method according to any one of embodiments 130-160, wherein the plurality of non-intergeneric remodeled bacteria colonize the root surface of peripheral roots of the plurality of corn plants to a higher degree than they colonize other roots. 162. The method according to any one of embodiments 130-161, wherein the plurality of non-intergeneric remodeled bacteria produce in the aggregate at least about 15 pounds of fixed N per acre before the corn plant's VT stage. 163. The method according to any one of embodiments 130-162, wherein the plurality of non-intergeneric remodeled bacteria produce in the aggregate at least about 15 pounds of fixed N per acre before the corn plant's R1 stage. 164. The method according to any one of embodiments 130-163, wherein the plurality of non-intergeneric remodeled bacteria produce in the aggregate at least about 15 pounds of fixed N per acre before the corn plant's R6 stage. 165. The method according to any one of embodiments 130-164, wherein the plurality of non-intergeneric remodeled bacteria produce 1% or more of the fixed nitrogen in an individual corn plant of said plurality exposed thereto. 166. The method according to any one of embodiments 130-165, wherein the plurality of non-intergeneric remodeled bacteria are capable of fixing atmospheric nitrogen in the presence of exogenous nitrogen. 167. The method according to any one of embodiments 130-166, wherein each member of the plurality of non-intergeneric remodeled bacteria comprises at least one genetic variation introduced into at least one gene, or non-coding polynucleotide, of the nitrogen fixation or assimilation genetic regulatory network. 168. The method according to any one of embodiments 130-167, wherein each member of the plurality of non-intergeneric remodeled bacteria comprises an introduced control sequence operably linked to at least one gene of the nitrogen fixation or assimilation genetic regulatory network. 169. The method according to any one of embodiments 130-168, wherein each member of the plurality of non-intergeneric remodeled bacteria comprises a heterologous promoter operably linked to at least one gene of the nitrogen fixation or assimilation genetic regulatory network. 170. The method according to any one of embodiments 130-169, wherein each member of the plurality of non-intergeneric remodeled bacteria comprises at least one genetic variation introduced into a member selected from the group consisting of: nifA, nifL, ntrB, ntrC, polynucleotide encoding glutamine synthetase, glnA, glnB, glnK, drat, amtB, polynucleotide encoding glutaminase, glnD, glnE, nifJ, nifH, nifD, nifK, nifY, nifE, nifN, nifU, nifS, nifV, nifW, nifZ, nifM, nifF, nifB, nifQ, a gene associated with biosynthesis of a nitrogenase enzyme, and combinations thereof. 171. The method according to any one of embodiments 130-170, wherein each member of the plurality of non-intergeneric remodeled bacteria comprises at least one genetic variation introduced into at least one gene, or non-coding polynucleotide, of the nitrogen fixation or assimilation genetic regulatory network that results in one or more of: increased expression or activity of NifA or glutaminase; decreased expression or activity of NifL, NtrB, glutamine synthetase, GlnB, GlnK, DraT, AmtB; decreased adenylyl-removing activity of GlnE; or decreased uridylyl-removing activity of GlnD. 172. The method according to any one of embodiments 130-171, wherein each member of the plurality of non-intergeneric remodeled bacteria comprises a mutated nifL gene that has been altered to comprise a heterologous promoter inserted into said nifL gene. 173. The method according to any one of embodiments 130-172, wherein each member of the plurality of non-intergeneric remodeled bacteria comprises a mutated glnE gene that results in a truncated GlnE protein lacking an adenylyl-removing (AR) domain. 174. The method according to any one of embodiments 130-173,

wherein each member of the plurality of non-intergeneric remodeled bacteria comprises a mutated amtB gene that results in the lack of expression of said amtB gene. 175. The method according to any one of embodiments 130-174, wherein each member of the plurality of non-intergeneric remodeled bacteria comprises at least one of: a mutated nifL gene that has been altered to comprise a heterologous promoter inserted into said nifL gene; a mutated glnE gene that results in a truncated GlnE protein lacking an adenylyl-removing (AR) domain; a mutated amtB gene that results in the lack of expression of said amtB gene; and combinations thereof. 176. The method according to any one of embodiments 130-175, wherein each member of the plurality of non-intergeneric remodeled bacteria comprises a mutated nifL gene that has been altered to comprise a heterologous promoter inserted into said nifL gene and a mutated glnE gene that results in a truncated GlnE protein lacking an adenylyl-removing (AR) domain. 177. The method according to any one of embodiments 130-176, wherein each member of the plurality of non-intergeneric remodeled bacteria comprises a mutated nifL gene that has been altered to comprise a heterologous promoter inserted into said nifL gene and a mutated glnE gene that results in a truncated GlnE protein lacking an adenylyl-removing (AR) domain and a mutated amtB gene that results in the lack of expression of said amtB gene. 178. The method according to any one of embodiments 130-177, wherein each member of the plurality of non-intergeneric remodeled bacteria comprises at least one genetic variation introduced into genes involved in a pathway selected from the group consisting of: exopolysaccharide production, endo-polygalaturonase production, trehalose production, and glutamine conversion. 179. The method according to any one of embodiments 130-178, wherein each member of the plurality of non-intergeneric remodeled bacteria comprises at least one genetic variation introduced into genes selected from the group consisting of: bcsii, bcsiii, yjbE, fhaB, pehA, otsB, treZ, glsA2, and combinations thereof 180. The method according to any one of embodiments 130-179, wherein the plurality of non-intergeneric remodeled bacteria comprise at least two different species of bacteria. 181. The method according to any one of embodiments 130-180, wherein the plurality of non-intergeneric remodeled bacteria comprise at least two different strains of the same species of bacteria. 182. The method according to any one of embodiments 130-181, wherein the plurality of non-intergeneric remodeled bacteria comprise bacteria selected from: *Rahnella aquatilis, Klebsiella variicola, Achromobacter spiritinus, Achromobacter marplatensis, Microbacterium murale, Kluyvera intermedia, Kosakonia pseudosacchari, Enterobacter* sp *Azospirillum lipoferum, Kosakonia sacchari,* and combinations thereof. 183. The method according to any one of embodiments 130-182, wherein the plurality of non-intergeneric remodeled bacteria are epiphytic or rhizospheric. 184. The method according to any one of embodiments 130-183, wherein the plurality of non-intergeneric remodeled bacteria comprise bacteria selected from: a bacteria deposited as NCMA 201701002, a bacteria deposited as NCMA 201708004, a bacteria deposited as NCMA 201708003, a bacteria deposited as NCMA 201708002, a bacteria deposited as NCMA 201712001, a bacteria deposited as NCMA 201712002, and combinations thereof. 185. The method according to any one of embodiments 130-184, wherein the plurality of non-intergeneric remodeled bacteria comprise bacteria with a nucleic acid sequence that shares at least about 90% sequence identity to a nucleic acid sequence selected from SEQ ID NOs: 177-260, 296-303, and 458-469. 186. The method according to any one of embodiments 130-185, wherein the plurality of non-intergeneric remodeled bacteria comprise bacteria with a nucleic acid sequence that shares at least about 95% sequence identity to a nucleic acid sequence selected from SEQ ID NOs: 177-260, 296-303, and 458-469. 187. The method according to any one of embodiments 130-186, wherein the plurality of non-intergeneric remodeled bacteria comprise bacteria with a nucleic acid sequence that shares at least about 99% sequence identity to a nucleic acid sequence selected from SEQ ID NOs: 177-260, 296-303, and 458-469. 188. The method according to any one of embodiments 130-187, wherein the plurality of non-intergeneric remodeled bacteria comprise bacteria with a nucleic acid sequence selected from SEQ ID NOs: 177-260, 296-303, and 458-469. 189. A method for reducing infield variability for corn

yield per acre, comprising: a. providing to a locus a plurality of non-intergeneric remodeled bacteria that each produce fixed N of at least about 5.49×10.sup.−13 mmol of N per CFU per hour; and b. providing to the locus a plurality of corn plants, wherein said plurality of non-intergeneric remodeled bacteria colonize the root surface of said plurality of corn plants and supply the corn plants with fixed N, and wherein the standard deviation of corn mean yield measured across the locus as measured in bushels per acre is lower for the plurality of corn plants colonized by said non-intergeneric remodeled bacteria, as compared to a control plurality of corn plants when the control plurality of corn plants is provided to the locus. 190. The method according to embodiment 189, wherein the standard deviation for the plurality of corn plants colonized by said non-intergeneric remodeled bacteria is less than 19 bushels per acre, as compared to the control plurality of corn plants, said control plurality of corn plants not being colonized by non-intergeneric remodeled bacteria. 191. The method according to any one of embodiments 189-190, wherein the yield between the plurality of corn plants colonized by the non-intergeneric remodeled bacteria is within 1-10% of the yield of the control plurality of corn plants, said control plurality of corn plants not being colonized by non-intergeneric remodeled bacteria. 192. The method according to any one of embodiments 189-191, wherein said control plurality of corn plants have exogenous nitrogen applied to said plants after said control plurality of corn plants have been planted. 193. The method according to any one of embodiments 189-192, wherein exogenous nitrogen is not applied to said locus after the plurality of non-intergeneric remodeled bacteria and corn plants are supplied. 194. The method according to any one of embodiments 189-193, wherein the control plurality of corn plants is provided to the locus without the plurality of non-intergeneric remodeled bacteria. 195. The method according to any one of embodiments 189-194, wherein exogenous nitrogen is not applied to said locus after the plurality of non-intergeneric remodeled bacteria and corn plants are supplied, but wherein exogenous nitrogen is applied to the control plurality of corn plants, said control plurality of corn plants not being provided with a plurality of non-intergeneric remodeled bacteria. 196. The method according to any one of embodiments 189-195, wherein exogenous nitrogen is not applied to said locus after the plurality of non-intergeneric remodeled bacteria and corn plants are supplied, but wherein exogenous nitrogen is applied to the control plurality of corn plants, said control plurality of corn plants not being provided with a plurality of non-intergeneric remodeled bacteria, wherein the yield between the plurality of corn plants colonized by the non-intergeneric remodeled bacteria is within 1-10% of the yield of the control plurality of corn plants. 197. The method according to any one of embodiments 189-196, wherein said plurality of non-intergeneric remodeled bacteria produce in the aggregate at least about 15 pounds of fixed N per acre over the course of at least about 10 days to about 60 days. 198. The method according to any one of embodiments 189-197, wherein exogenous nitrogen is not applied as a sidedressing. 199. The method according to any one of embodiments 189-198, wherein the plurality of non-intergeneric remodeled bacteria each produce fixed N of at least about 2.75×10.sup.−12 mmol of N per CFU per hour. 200. The method according to any one of embodiments 189-199, wherein the plurality of non-intergeneric remodeled bacteria each produce fixed N of at least about 4.03×10.sup.−13 mmol of N per CFU per hour. 201. The method according to any one of embodiments 189-200, wherein said plurality of non-intergeneric remodeled bacteria produce in the aggregate at least about 25 pounds of fixed N per acre over the course of at least about 10 days to about 60 days. 202. The method according to any one of embodiments 189-201, wherein said plurality of non-intergeneric remodeled bacteria produce in the aggregate at least about 50 pounds of fixed N per acre over the course of at least about 10 days to about 60 days. 203. The method according to any one of embodiments 189-202, wherein said plurality of non-intergeneric remodeled bacteria produce in the aggregate at least about 75 pounds of fixed N per acre over the course of at least about 10 days to about 60 days. 204. The method according to any one of embodiments 189-203, wherein said plurality of non-intergeneric remodeled bacteria produce in the aggregate at least about 100 pounds of fixed N per acre over the

course of at least about 10 days to about 60 days. 205. The method according to any one of embodiments 189-204, wherein the plurality of non-intergeneric remodeled bacteria colonize the root surface of the plurality of corn plants at a total aggregate CFU per acre concentration according to FIG. **31**, **32**, **33**, **34**, or **35**. 206. The method according to any one of embodiments 189-205, wherein the plurality of non-intergeneric remodeled bacteria colonize the root surface of the plurality of corn plants at a total aggregate CFU per acre concentration of about 5×10.sup.13 for at least about 20 days. 207. The method according to any one of embodiments 189-206, wherein the plurality of non-intergeneric remodeled bacteria colonize the root surface of the plurality of corn plants at a total aggregate CFU per acre concentration of about 5×10.sup.13 for at least about 30 days. 208. The method according to any one of embodiments 189-207, wherein the plurality of non-intergeneric remodeled bacteria colonize the root surface of the plurality of corn plants at a total aggregate CFU per acre concentration of about 5×10.sup.13 for at least about 60 days. 209. The method according to any one of embodiments 189-208, wherein the plurality of non-intergeneric remodeled bacteria colonize the root surface of peripheral roots of the plurality of corn plants equally as well as they colonize other roots. 210. The method according to any one of embodiments 189-209, wherein the plurality of non-intergeneric remodeled bacteria colonize the root surface of peripheral roots of the plurality of corn plants to a higher degree than they colonize other roots. 211. The method according to any one of embodiments 189-210, wherein the plurality of non-intergeneric remodeled bacteria produce in the aggregate at least about 15 pounds of fixed N per acre before the corn plant's VT stage. 212. The method according to any one of embodiments 189-211, wherein the plurality of non-intergeneric remodeled bacteria produce in the aggregate at least about 15 pounds of fixed N per acre before the corn plant's R1 stage. 213. The method according to any one of embodiments 189-212, wherein the plurality of non-intergeneric remodeled bacteria produce in the aggregate at least about 15 pounds of fixed N per acre before the corn plant's R6 stage. 214. The method according to any one of embodiments 189-213, wherein the plurality of non-intergeneric remodeled bacteria produce 1% or more of the fixed nitrogen in an individual corn plant of said plurality exposed thereto. 215. The method according to any one of embodiments 189-214, wherein the plurality of non-intergeneric remodeled bacteria are capable of fixing atmospheric nitrogen in the presence of exogenous nitrogen. 216. The method according to any one of embodiments 189-215, wherein each member of the plurality of non-intergeneric remodeled bacteria comprises at least one genetic variation introduced into at least one gene, or non-coding polynucleotide, of the nitrogen fixation or assimilation genetic regulatory network. 217. The method according to any one of embodiments 189-216, wherein each member of the plurality of non-intergeneric remodeled bacteria comprises an introduced control sequence operably linked to at least one gene of the nitrogen fixation or assimilation genetic regulatory network. 218. The method according to any one of embodiments 189-217, wherein each member of the plurality of non-intergeneric remodeled bacteria comprises a heterologous promoter operably linked to at least one gene of the nitrogen fixation or assimilation genetic regulatory network. 219. The method according to any one of embodiments 189-218, wherein each member of the plurality of non-intergeneric remodeled bacteria comprises at least one genetic variation introduced into a member selected from the group consisting of: nifA, nifL, ntrB, ntrC, polynucleotide encoding glutamine synthetase, glnA, glnB, glnK, drat, amtB, polynucleotide encoding glutaminase, glnD, glnE, nifJ, nifH, nifD, nifK, nifY, nifE, nifN, nifU, nifS, nifV, nifW, nifZ, nifM, nifF, nifB, nifQ, a gene associated with biosynthesis of a nitrogenase enzyme, and combinations thereof. 220. The method according to any one of embodiments 189-219, wherein each member of the plurality of non-intergeneric remodeled bacteria comprises at least one genetic variation introduced into at least one gene, or non-coding polynucleotide, of the nitrogen fixation or assimilation genetic regulatory network that results in one or more of: increased expression or activity of NifA or glutaminase; decreased expression or activity of NifL, NtrB, glutamine synthetase, GlnB, GlnK, DraT, AmtB; decreased adenylyl-removing activity of GlnE; or decreased uridylyl-removing activity of GlnD.

221. The method according to any one of embodiments 189-220, wherein each member of the plurality of non-intergeneric remodeled bacteria comprises a mutated nifL gene that has been altered to comprise a heterologous promoter inserted into said nifL gene. 222. The method according to any one of embodiments 189-221, wherein each member of the plurality of non-intergeneric remodeled bacteria comprises a mutated glnE gene that results in a truncated GlnE protein lacking an adenylyl-removing (AR) domain. 223. The method according to any one of embodiments 189-222, wherein each member of the plurality of non-intergeneric remodeled bacteria comprises a mutated amtB gene that results in the lack of expression of said amtB gene. 224. The method according to any one of embodiments 189-223, wherein each member of the plurality of non-intergeneric remodeled bacteria comprises at least one of: a mutated nifL gene that has been altered to comprise a heterologous promoter inserted into said nifL gene; a mutated glnE gene that results in a truncated GlnE protein lacking an adenylyl-removing (AR) domain; a mutated amtB gene that results in the lack of expression of said amtB gene; and combinations thereof. 225. The method according to any one of embodiments 189-224, wherein each member of the plurality of non-intergeneric remodeled bacteria comprises a mutated nifL gene that has been altered to comprise a heterologous promoter inserted into said nifL gene and a mutated glnE gene that results in a truncated GlnE protein lacking an adenylyl-removing (AR) domain. 226. The method according to any one of embodiments 189-225, wherein each member of the plurality of non-intergeneric remodeled bacteria comprises a mutated nifL gene that has been altered to comprise a heterologous promoter inserted into said nifL gene and a mutated glnE gene that results in a truncated GlnE protein lacking an adenylyl-removing (AR) domain and a mutated amtB gene that results in the lack of expression of said amtB gene. 227. The method according to any one of embodiments 189-226, wherein each member of the plurality of non-intergeneric remodeled bacteria comprises at least one genetic variation introduced into genes involved in a pathway selected from the group consisting of: exopolysaccharide production, endo-polygalaturonase production, trehalose production, and glutamine conversion. 228. The method according to any one of embodiments 189-227, wherein each member of the plurality of non-intergeneric remodeled bacteria comprises at least one genetic variation introduced into genes selected from the group consisting of: bcsii, bcsiii, yjbE, fhaB, pehA, otsB, treZ, glsA2, and combinations thereof 229. The method according to any one of embodiments 189-228, wherein the plurality of non-intergeneric remodeled bacteria comprise at least two different species of bacteria. 230. The method according to any one of embodiments 189-229, wherein the plurality of non-intergeneric remodeled bacteria comprise at least two different strains of the same species of bacteria. 231. The method according to any one of embodiments 189-230, wherein the plurality of non-intergeneric remodeled bacteria comprise bacteria selected from: *Rahnella aquatilis, Klebsiella variicola, Achromobacter spiritinus, Achromobacter marplatensis, Microbacterium murale, Kluyvera intermedia, Kosakonia pseudosacchari, Enterobacter* sp *Azospirillum lipoferum, Kosakonia sacchari*, and combinations thereof. 232. The method according to any one of embodiments 189-231, wherein the plurality of non-intergeneric remodeled bacteria are epiphytic or rhizospheric. 233. The method according to any one of embodiments 189-232, wherein the plurality of non-intergeneric remodeled bacteria comprise bacteria selected from: a bacteria deposited as NCMA 201701002, a bacteria deposited as NCMA 201708004, a bacteria deposited as NCMA 201708003, a bacteria deposited as NCMA 201708002, a bacteria deposited as NCMA 201712001, a bacteria deposited as NCMA 201712002, and combinations thereof. 234. The method according to any one of embodiments 189-233, wherein the plurality of non-intergeneric remodeled bacteria comprise bacteria with a nucleic acid sequence that shares at least about 90% sequence identity to a nucleic acid sequence selected from SEQ ID NOs: 177-260, 296-303, and 458-469. 235. The method according to any one of embodiments 189-234, wherein the plurality of non-intergeneric remodeled bacteria comprise bacteria with a nucleic acid sequence that shares at least about 95% sequence identity to a nucleic acid sequence selected from SEQ ID NOs: 177-260, 296-303, and 458-469. 236. The method

according to any one of embodiments 189-235, wherein the plurality of non-intergeneric remodeled bacteria comprise bacteria with a nucleic acid sequence that shares at least about 99% sequence identity to a nucleic acid sequence selected from SEQ ID NOs: 177-260, 296-303, and 458-469.
237. The method according to any one of embodiments 189-236, wherein the plurality of non-intergeneric remodeled bacteria comprise bacteria with a nucleic acid sequence selected from SEQ ID NOs: 177-260, 296-303, and 458-469.
(807) While preferred embodiments of the present disclosure have been shown and described herein, it will be obvious to those skilled in the art that such embodiments are provided by way of example only. Numerous variations, changes, and substitutions will now occur to those skilled in the art without departing from the disclosure. It should be understood that various alternatives to the embodiments of the disclosure described herein may be employed in practicing the disclosure. It is intended that the following Claims define the scope of the disclosure and that methods and structures within the scope of these claims and their equivalents be covered thereby.
INCORPORATION BY REFERENCE
(808) All references, articles, publications, patents, patent publications, and patent applications cited herein are incorporated by reference in their entireties for all purposes. However, mention of any reference, article, publication, patent, patent publication, and patent application cited herein is not, and should not be taken as, an acknowledgment or any form of suggestion that they constitute valid prior art or form part of the common general knowledge in any country in the world. Further, U.S. Pat. No. 9,975,817, issued on May 22, 2018, and entitled: Methods and Compositions for Improving Plant Traits, is hereby incorporated by reference. Further, PCT/US2018/013671, filed Jan. 12, 2018, and entitled: Methods and Compositions for Improving Plant Traits, is hereby incorporated by reference.

Claims

1. A method of providing fixed atmospheric nitrogen to a cereal plant, comprising: a. providing to a locus a plurality of non-intergeneric remodeled bacteria that each produce fixed N of at least about 5.49×10.sup.−13 mmol of N per CFU per hour, wherein the plurality of non-intergeneric remodeled bacteria comprise at least one genetic variation introduced into at least one gene, or non-coding polynucleotide, of the nitrogen fixation or assimilation genetic regulatory network that results in one or more of: increased expression or activity of NifA or glutaminase; decreased expression or activity of NifL, NtrB, glutamine synthetase, GlnB, GlnK, DraT, AmtB; decreased adenylyl-removing activity of GlnE; or decreased uridylyl-removing activity of GlnD; and b. providing to the locus a plurality of cereal plants, wherein said plurality of non-intergeneric remodeled bacteria colonize the root surface of said plurality of cereal plants and supply the cereal plants with fixed N, and wherein said plurality of non-intergeneric remodeled bacteria produce in the aggregate at least about 15 pounds of fixed N per acre over the course of at least about 10 days to about 60 days.
2. The method according to claim 1, wherein the plurality of non-intergeneric remodeled bacteria each produce fixed N of at least about 2.75×10.sup.−12 mmol of N per CFU per hour.
3. The method according to claim 1, wherein the plurality of non-intergeneric remodeled bacteria colonize the root surface of the plurality of cereal plants at a total aggregate CFU per acre concentration of about 5×10.sup.13 for at least about 20 days.
4. The method according to claim 1, wherein exogenous nitrogen is not applied to said locus after the plurality of non-intergeneric remodeled bacteria and cereal plants are applied.
5. The method according to claim 1, wherein said cereal plant is corn, rice, wheat, barley, *sorghum*, millet, oat, rye, or triticale.
6. The method according to claim 1, wherein each member of the plurality of non-intergeneric remodeled bacteria comprises at least one genetic variation introduced into at least one gene, or non-coding polynucleotide, involved in a pathway selected from the group consisting of: the

nitrogen fixation or assimilation genetic regulatory network, exopolysaccharide production, endo-polygalaturonase production, trehalose production, and glutamine conversion.
7. The method according to claim 1, wherein each member of the plurality of non-intergeneric remodeled bacteria comprises an introduced control sequence operably linked to at least one gene of the nitrogen fixation or assimilation genetic regulatory network.
8. The method according to claim 1, wherein each member of the plurality of non-intergeneric remodeled bacteria comprises a heterologous promoter operably linked to at least one gene of the nitrogen fixation or assimilation genetic regulatory network.
9. The method according to claim 1, wherein each member of the plurality of non-intergeneric remodeled bacteria comprises at least one genetic variation introduced into a member selected from the group consisting of: nifA, nifL, ntrB, ntrC, polynucleotide encoding glutamine synthetase, glnA, glnB, glnK, drat, amtB, polynucleotide encoding glutaminase, glnD, glnE, nifJ, nifH, nifD, nifK, nifY, nifE, nifN, nifU, nifS, nifV, nifW, nifZ, nifM, nifF, nifB, nifQ, a gene associated with biosynthesis of a nitrogenase enzyme, bcsii, bcsiii, yjbE, fhaB, pehA, otsB, treZ, glsA2, and combinations thereof.
10. The method according to claim 1, wherein each member of the plurality of non-intergeneric remodeled bacteria comprises at least one of: a mutated nifL gene that has been altered to comprise a heterologous promoter inserted into said nifL gene; a mutated glnE gene that results in a truncated GlnE protein lacking an adenylyl-removing (AR) domain; a mutated amtB gene that results in the lack of expression of said amtB gene; and combinations thereof.
11. The method according to claim 1, wherein the plurality of non-intergeneric remodeled bacteria comprise bacteria selected from: *Rahnella aquatilis, Klebsiella variicola, Achromobacter spiritinus, Achromobacter marplatensis, Microbacterium murale, Kluyvera intermedia, Kosakonia pseudosacchari, Enterobacter* sp., *Azospirillum lipoferum, Kosakonia sacchari*, and combinations thereof.
12. The method according to claim 1, wherein the plurality of non-intergeneric remodeled bacteria comprise bacteria selected from: a bacteria deposited as NCMA 201701002, a bacteria deposited as NCMA 201708004, a bacteria deposited as NCMA 201708003, a bacteria deposited as NCMA 201708002, a bacteria deposited as NCMA 201712001, a bacteria deposited as NCMA 201712002, and combinations thereof.
13. The method according to claim 1, wherein the plurality of non-intergeneric remodeled bacteria comprise bacteria comprising a nucleic acid sequence that shares at least about 90% sequence identity to a nucleic acid sequence selected from SEQ ID NOs: 177-260, 296-303, and 458-469.
14. A method for increasing corn yield per acre in agriculturally challenging soil, comprising: a. providing to a locus located in an agriculturally challenging soil a plurality of non-intergeneric remodeled bacteria that each produce fixed N of at least about 5.49×10.sup.−13 mmol of N per CFU per hour; and b. providing to the locus located in an agriculturally challenging soil a plurality of corn plants, wherein said plurality of non-intergeneric remodeled bacteria colonize the root surface of said plurality of corn plants and supply the corn plants with fixed N, and wherein said agriculturally challenging soil comprises a soil that comprises at least about 30% sand, and wherein said plurality of corn plants achieve at least a 1 bushel per acre yield increase, as compared to a control plurality of corn plants when the control plurality of corn plants is provided to the locus.
15. The method according to claim 14, wherein said agriculturally challenging soil comprises at least one of: a) at least about 40% sand; b) less than about 30% silt; c) less than about 20% clay; d) a pH of about 5 to 8; and e) an organic matter content of about 0.40 to about 2.8.
16. The method according to claim 14, wherein said agriculturally challenging soil is a sandy loam or loam soil.
17. The method according to claim 14, wherein said plurality of corn plants achieve at least a 5 bushel per acre yield increase, as compared to a control plurality of corn plants.
18. The method according to claim 14, wherein exogenous nitrogen is not applied to said locus

after the plurality of non-intergeneric remodeled bacteria and corn plants are supplied.
19. The method according to claim 14, wherein exogenous nitrogen is not applied to said locus after the plurality of non-intergeneric remodeled bacteria and corn plants are supplied, but wherein exogenous nitrogen is applied to the control plurality of corn plants, said control plurality of corn plants not being provided with a plurality of non-intergeneric remodeled bacteria.
20. The method according to claim 14, wherein said plurality of non-intergeneric remodeled bacteria produce in the aggregate at least about 15 pounds of fixed N per acre over the course of at least about 10 days to about 60 days.
21. The method according to claim 14, wherein the plurality of non-intergeneric remodeled bacteria each produce fixed N of at least about 2.75×10.sup.−12 mmol of N per CFU per hour.
22. The method according to claim 14, wherein the plurality of non-intergeneric remodeled bacteria colonize the root surface of the plurality of corn plants at a total aggregate CFU per acre concentration of about 5×10.sup.13 for at least about 20 days.
23. The method according to claim 14, wherein each member of the plurality of non-intergeneric remodeled bacteria comprises at least one genetic variation introduced into at least one gene, or non-coding polynucleotide, involved in a pathway selected from the group consisting of: the nitrogen fixation or assimilation genetic regulatory network, exopolysaccharide production, endo-polygalaturonase production, trehalose production, and glutamine conversion.
24. The method according to claim 14, wherein each member of the plurality of non-intergeneric remodeled bacteria comprises an introduced control sequence operably linked to at least one gene of the nitrogen fixation or assimilation genetic regulatory network.
25. The method according to claim 14, wherein each member of the plurality of non-intergeneric remodeled bacteria comprises a heterologous promoter operably linked to at least one gene of the nitrogen fixation or assimilation genetic regulatory network.
26. The method according to claim 14, wherein each member of the plurality of non-intergeneric remodeled bacteria comprises at least one genetic variation introduced into a member selected from the group consisting of: nifA, nifL, ntrB, ntrC, polynucleotide encoding glutamine synthetase, glnA, glnB, glnK, drat, amtB, polynucleotide encoding glutaminase, glnD, glnE, nifJ, nifH, nifD, nifK, nifY, nifE, nifN, nifU, nifS, nifV, nifW, nifZ, nifM, nifF, nifB, nifQ, a gene associated with biosynthesis of a nitrogenase enzyme, bcsii, bcsiii, yjbE, fhaB, pehA, otsB, treZ, glsA2, and combinations thereof.
27. The method according to claim 14, wherein each member of the plurality of non-intergeneric remodeled bacteria comprises at least one genetic variation introduced into at least one gene, or non-coding polynucleotide, of the nitrogen fixation or assimilation genetic regulatory network that results in one or more of: increased expression or activity of NifA or glutaminase; decreased expression or activity of NifL, NtrB, glutamine synthetase, GlnB, GlnK, DraT, AmtB; decreased adenylyl-removing activity of GlnE; or decreased uridylyl-removing activity of GlnD.
28. The method according to claim 14, wherein each member of the plurality of non-intergeneric remodeled bacteria comprises at least one of: a mutated nifL gene that has been altered to comprise a heterologous promoter inserted into said nifL gene; a mutated glnE gene that results in a truncated GlnE protein lacking an adenylyl-removing (AR) domain; a mutated amtB gene that results in the lack of expression of said amtB gene; and combinations thereof.
29. The method according to claim 14, wherein the plurality of non-intergeneric remodeled bacteria comprise bacteria selected from: *Rahnella aquatilis, Klebsiella variicola, Achromobacter spiritinus, Achromobacter marplatensis, Microbacterium murale, Kluyvera intermedia, Kosakonia pseudosacchari, Enterobacter* sp., *Azospirillum lipoferum, Kosakonia sacchari*, and combinations thereof.
30. The method according to claim 14, wherein the plurality of non-intergeneric remodeled bacteria comprise bacteria selected from: a bacteria deposited as NCMA 201701002, a bacteria deposited as NCMA 201708004, a bacteria deposited as NCMA 201708003, a bacteria deposited as NCMA

201708002, a bacteria deposited as NCMA 201712001, a bacteria deposited as NCMA 201712002, and combinations thereof.
31. The method according to claim 14, wherein the plurality of non-intergeneric remodeled bacteria comprise bacteria with a nucleic acid sequence that shares at least about 90% sequence identity to a nucleic acid sequence selected from SEQ ID NOs: 177-260, 296-303, and 458-469.
32. A method for reducing infield variability for corn yield per acre, comprising: a. providing to a locus a plurality of non-intergeneric remodeled bacteria that each produce fixed N of at least about 5.49×10.sup.−13 mmol of N per CFU per hour; and b. providing to the locus a plurality of corn plants, wherein said plurality of non-intergeneric remodeled bacteria colonize the root surface of said plurality of corn plants and supply the corn plants with fixed N, and wherein the standard deviation of corn mean yield measured across the locus as measured in bushels per acre is lower for the plurality of corn plants colonized by said non-intergeneric remodeled bacteria, as compared to a control plurality of corn plants when the control plurality of corn plants is provided to the locus.
33. The method according to claim 32, wherein the standard deviation for the plurality of corn plants colonized by said non-intergeneric remodeled bacteria is less than 19 bushels per acre, as compared to the control plurality of corn plants, said control plurality of corn plants not being colonized by non-intergeneric remodeled bacteria.
34. The method according to claim 32, wherein the yield between the plurality of corn plants colonized by the non-intergeneric remodeled bacteria is within 1-10% of the yield of the control plurality of corn plants, said control plurality of corn plants not being colonized by non-intergeneric remodeled bacteria.
35. The method according to claim 32, wherein exogenous nitrogen is not applied to said locus after the plurality of non-intergeneric remodeled bacteria and corn plants are supplied, but wherein exogenous nitrogen is applied to the control plurality of corn plants, said control plurality of corn plants not being provided with a plurality of non-intergeneric remodeled bacteria.
36. The method according to claim 32, wherein said plurality of non-intergeneric remodeled bacteria produce in the aggregate at least about 15 pounds of fixed N per acre over the course of at least about 10 days to about 60 days.
37. The method according to claim 32, wherein exogenous nitrogen is not applied as a sidedressing.
38. The method according to claim 32, wherein the plurality of non-intergeneric remodeled bacteria each produce fixed N of at least about 2.75×10.sup.−12 mmol of N per CFU per hour.
39. The method according to claim 32, wherein the plurality of non-intergeneric remodeled bacteria colonize the root surface of the plurality of corn plants at a total aggregate CFU per acre concentration of about 5×10.sup.13 for at least about 20 days.
40. The method according to claim 32, wherein each member of the plurality of non-intergeneric remodeled bacteria comprises at least one genetic variation introduced into at least one gene, or non-coding polynucleotide, involved in a pathway selected from the group consisting of: the nitrogen fixation or assimilation genetic regulatory network, exopolysaccharide production, endo-polygalaturonase production, trehalose production, and glutamine conversion.
41. The method according to claim 32, wherein each member of the plurality of non-intergeneric remodeled bacteria comprises an introduced control sequence operably linked to at least one gene of the nitrogen fixation or assimilation genetic regulatory network.
42. The method according to claim 32, wherein each member of the plurality of non-intergeneric remodeled bacteria comprises a heterologous promoter operably linked to at least one gene of the nitrogen fixation or assimilation genetic regulatory network.
43. The method according to claim 32, wherein each member of the plurality of non-intergeneric remodeled bacteria comprises at least one genetic variation introduced into a member selected from the group consisting of: nifA, nifL, ntrB, ntrC, polynucleotide encoding glutamine synthetase, glnA, glnB, glnK, drat, amtB, polynucleotide encoding glutaminase, glnD, glnE, nifJ, nifH, nifD, nifK, nifY, nifE, nifN, nifU, nifS, nifV, nifW, nifZ, nifM, nifF, nifB, nifQ, a gene associated with

biosynthesis of a nitrogenase enzyme, bcsii, bcsiii, yjbE, fhaB, pehA, otsB, treZ, glsA2, and combinations thereof.

44. The method according to claim 32, wherein each member of the plurality of non-intergeneric remodeled bacteria comprises at least one genetic variation introduced into at least one gene, or non-coding polynucleotide, of the nitrogen fixation or assimilation genetic regulatory network that results in one or more of: increased expression or activity of NifA or glutaminase; decreased expression or activity of NifL, NtrB, glutamine synthetase, GlnB, GlnK, DraT, AmtB; decreased adenylyl-removing activity of GlnE; or decreased uridylyl-removing activity of GlnD.

45. The method according to claim 32, wherein each member of the plurality of non-intergeneric remodeled bacteria comprises at least one of: a mutated nifL gene that has been altered to comprise a heterologous promoter inserted into said nifL gene; a mutated glnE gene that results in a truncated GlnE protein lacking an adenylyl-removing (AR) domain; a mutated amtB gene that results in the lack of expression of said amtB gene; and combinations thereof.

46. The method according to claim 32, wherein the plurality of non-intergeneric remodeled bacteria comprise bacteria selected from: *Rahnella aquatilis, Klebsiella variicola, Achromobacter spiritinus, Achromobacter marplatensis, Microbacterium murale, Kluyvera intermedia, Kosakonia pseudosacchari, Enterobacter* sp., *Azospirillum lipoferum, Kosakonia sacchari*, and combinations thereof.

47. The method according to claim 32, wherein the plurality of non-intergeneric remodeled bacteria comprise bacteria selected from: a bacteria deposited as NCMA 201701002, a bacteria deposited as NCMA 201708004, a bacteria deposited as NCMA 201708003, a bacteria deposited as NCMA 201708002, a bacteria deposited as NCMA 201712001, a bacteria deposited as NCMA 201712002, and combinations thereof.

48. The method according to claim 32, wherein the plurality of non-intergeneric remodeled bacteria comprise bacteria with a nucleic acid sequence that shares at least about 90% sequence identity to a nucleic acid sequence selected from SEQ ID NOs: 177-260, 296-303, and 458-469.