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(54) **METHODS AND COMPOSITIONS FOR IMPROVING CORN YIELD**

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See application file for complete search history.

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(57) **ABSTRACT**

The present invention provides yield and early vigor enhancing compositions comprising *Methylobacterium* sp., methods for improving corn yield and early vigor, and methods of making the compositions. Also provided are isolated yield enhancing *Methylobacterium* sp.

**19 Claims, No Drawings**

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## METHODS AND COMPOSITIONS FOR IMPROVING CORN YIELD

This application is a continuation of U.S. application Ser. No. 15/100,946, filed Jun. 1, 2016, issued as U.S. Pat. No. 10,980,240, which is the 371 national stage application of International Patent Application No. PCT/US2014/068657, filed Dec. 4, 2014, which claims the benefit of U.S. Patent Application No. 61/911,780, filed Dec. 4, 2013, the contents of each which are incorporated by reference herein.

### BACKGROUND

One-carbon organic compounds such as methane and methanol are found extensively in nature, and are utilized as carbon sources by bacteria classified as methanotrophs and methylotrophs. Methanotrophic bacteria include species in the genera *Methylobacter*, *Methylomonas*, *Methylomicrobium*, *Methylococcus*, *Methylosinus*, *Methylocystis*, *Methylosphaera*, *Methylorhabdus*, and *Methylocella* (Lidstrom, 2006). Methanotrophs possess the enzyme methane monooxygenase, that incorporates an atom of oxygen from O<sub>2</sub> into methane, forming methanol. All methanotrophs are obligate one-carbon utilizers that are unable to use compounds containing carbon-carbon bonds. Methylotrophs, on the other hand, can also utilize more complex organic compounds, such as organic acids, higher alcohols, sugars, and the like. Thus, methylotrophic bacteria are facultative methylotrophs. Methylotrophic bacteria include species in the genera *Methylobacterium*, *Hyphomicrobium*, *Methylophilus*, *Methylobacillus*, *Methylophaga*, *Aminobacter*, *Methylobacter*, *Methylophaga*, *Methylosulfonomonas*, *Marinosulfonomonas*, *Paracoccus*, *Xanthobacter*, *Ancylobacter* (also known as *Microcycylus*), *Thiobacillus*, *Rhodopseudomonas*, *Rhodobacter*, *Acetobacter*, *Bacillus*, *Mycobacterium*, *Arthobacter*, and *Nocardia* (Lidstrom, 2006).

Most methylotrophic bacteria of the genus *Methylobacterium* are pink-pigmented. They are conventionally referred to as PPFM bacteria, being pink-pigmented facultative methylotrophs. Green (2005, 2006) identified twelve validated species in the genus *Methylobacterium*, specifically *M. aminovorans*, *M. chloromethanicum*, *M. dichloromethanicum*, *M. extorquens*, *M. fujisawaense*, *M. mesophilicum*, *M. organophilum*, *M. radiotolerans*, *M. rhodesianum*, *M. rhodinum*, *M. thiocyanatum*, and *M. zatmanii*. However, *M. nidulans* is a nitrogen-fixing *Methylobacterium* that is not a PPFM (Sy et al., 2001). *Methylobacterium* are ubiquitous in nature, being found in soil, dust, fresh water, sediments, and leaf surfaces, as well as in industrial and clinical environments (Green, 2006).

### SUMMARY

Provided herein are isolated yield enhancing *Methylobacterium* sp., compositions comprising yield enhancing *Methylobacterium* sp., methods of using the compositions to increase yield of corn plants, plant parts, and corn plants derived therefrom, and methods of making the compositions. Such yield enhancing *Methylobacterium* sp. are in certain instances referred to herein as simply "*Methylobacterium*". In certain embodiments, yield enhancing *Methylobacterium* sp. can be distinguished from other yield neutral or yield negative *Methylobacterium* by assaying the *Methylobacterium* sp. for improved yield in a controlled environment (i.e. a growth chamber or greenhouse) or in a field test in comparison to untreated control plants or in comparison to control plants treated yield neutral or yield negative

*Methylobacterium*, and combinations thereof. In certain embodiments, the yield enhancing *Methylobacterium* sp. is a *Methylobacterium* isolate selected from the group consisting of ISO02 (NRRL B-50930), ISO03 (NRRL B-50931), ISO04 (NRRL B-50932), ISO11 (NRRL B-50939), and derivatives thereof.

Methods for improving corn plant yield that comprise applying a composition comprising a *Methylobacterium* sp. to a corn plant at about the V6 to about the R3 stage of development are provided herein. In certain embodiments, the methods comprise (a) applying a composition comprising a *Methylobacterium* sp. to a corn plant at about the V6 to about the R3 stage of development, wherein the composition comprises: (i) a solid substance with the *Methylobacterium* grown thereon and adhered thereto; (ii) an emulsion having the *Methylobacterium* grown therein; or (iii) a *Methylobacterium* isolate ISO02 (NRRL B-50930), ISO03 (NRRL B-50931), ISO04 (NRRL B-50932), ISO11 (NRRL B-50939), or a derivative thereof and an agriculturally acceptable adjuvant, excipient, or combination thereof; and, (b) growing the corn plant to maturity, thereby improving yield of the corn plant. In certain embodiments, the solid substance with the *Methylobacterium* grown thereon and adhered thereto is provided in a liquid or in an emulsion. In certain embodiments of the methods, the composition comprises a solid substance with the *Methylobacterium* grown thereon and adhered thereto or an emulsion having the *Methylobacterium* grown therein. In certain embodiments of the methods, the composition comprises the solid substance or the emulsion and wherein the *Methylobacterium* sp. is selected from the group consisting of ISO02 (NRRL B-50930), ISO03 (NRRL B-50931), ISO04 (NRRL B-50932), and derivatives thereof. In certain embodiments of the methods, the methods further comprise growing the corn plant to maturity. In certain embodiments of the methods, the composition is applied at about the V6 to about the R2 stage of development, at about the R1 to R2 stage of development, at about the R1 to R3 stage of development, or at about the R1 stage of development. In certain embodiments of the methods, the composition comprises: (i) a solid substance with the *Methylobacterium* grown thereon and adhered thereto. In certain embodiments of the methods, the composition is a solid that comprises the *Methylobacterium* sp. at a titer of about 1×10<sup>6</sup> CFU/gm to about 1×10<sup>14</sup> CFU/gm. In certain embodiments of the methods, the composition is a liquid containing the solid substance or an emulsion and has a *Methylobacterium* sp. titer of about 1×10<sup>6</sup> CFU/mL to about 1×10<sup>11</sup> CFU/mL. In certain embodiments of the methods, the *Methylobacterium* sp. is selected from the group consisting of ISO02 (NRRL B-50930), ISO03 (NRRL B-50931), ISO04 (NRRL B-50932), and derivatives thereof. In certain embodiments, the *Methylobacterium* sp. is a glyphosate resistant or glufosinate resistant derivative of ISO02 (NRRL B-50930), ISO03 (NRRL B-50931), or ISO04 (NRRL B-50932). In certain embodiments of any of the aforementioned methods, the applied composition coats or partially coats the corn plant or a part thereof. In certain embodiments of any of the aforementioned methods, the composition is applied to foliage of the corn plant. In certain embodiments of any of the aforementioned methods, the composition further comprises a fungicidal agent. In certain embodiments of any of the aforementioned methods, the methods further comprise the step of harvesting seed from the mature corn plant. In certain embodiments of any of the aforementioned methods, the yield of harvested seed is increased in comparison to

yield of harvested seed obtained from a control corn plant that did not receive an application of the *Methylobacterium* sp.

Also provided herein is a corn plant or corn plant part that is coated or partially coated with a composition comprising a *Methylobacterium* sp. In certain embodiments, the *Methylobacterium* sp. is selected from the group consisting of ISO02 (NRRL B-50930), ISO03 (NRRL B-50931), ISO04 (NRRL B-50932), ISO11 (NRRL B-50939), and derivatives thereof. In certain embodiments, the *Methylobacterium* sp. is ISO11 (NRRL B-50939) or a derivative thereof. In certain embodiments, the composition comprises: (i) a solid substance with the *Methylobacterium* grown thereon and adhered thereto; or (ii) an emulsion having the *Methylobacterium* grown therein. In certain embodiments, the composition comprises the *Methylobacterium* sp. at a titer of about  $1 \times 10^6$  CFU/gm to about  $1 \times 10^{14}$  CFU/gm for a solid composition or at a titer of about  $1 \times 10^6$  CFU/mL to about  $1 \times 10^{11}$  CFU/mL for a liquid composition containing the solid substance or for the emulsion. In certain embodiments, the *Methylobacterium* sp. is *Methylobacterium* isolate ISO11 or a derivative thereof. In certain of any of the aforementioned embodiments, the corn plant part is selected from the group consisting of a seed, a leaf, an ear, or a tassel.

Also provided herein are methods for improving corn plant yield that comprise: (i) applying a composition comprising a *Methylobacterium* sp. to a corn seed or a corn plant at about the VE to about the V5 stage of corn plant development. In certain embodiments, the methods for improving corn plant yield that comprise: (a) applying a composition comprising a *Methylobacterium* sp. to a corn seed or to a corn plant at about the VE to about the V5 stage of corn plant development, wherein the composition comprises: (i) a solid substance with the *Methylobacterium* grown thereon and adhered thereto; (ii) an emulsion having the *Methylobacterium* grown therein; or (iii) a *Methylobacterium* isolate ISO02 (NRRL B-50930), ISO03 (NRRL B-50931), ISO04 (NRRL B-50932), ISO11 (NRRL B-50939), or a derivative thereof and an agriculturally acceptable adjuvant, excipient, or combination thereof; and, (b) growing a corn plant from the seed or the corn plant to maturity, thereby improving yield of the corn plant. In certain embodiments of the methods, the composition comprises a solid substance with the *Methylobacterium* grown thereon and adhered thereto or an emulsion having the *Methylobacterium* grown therein. In certain embodiments, the solid substance with the *Methylobacterium* grown thereon and adhered thereto is provided in a liquid or in an emulsion. In certain embodiments of the methods, the methods further comprise growing a corn plant from the seed or the corn plant to maturity. In certain embodiments of the methods, the composition is applied at about the VE to about the V3 stage of development, about the V3 to about the V5 stage of development, about the V2 to V4, or V3 stage of development. In certain embodiments of the methods, the composition comprises the *Methylobacterium* sp. at a titer of about  $1 \times 10^6$  CFU/gm to about  $1 \times 10^{14}$  CFU/gm for a solid composition or at a titer of about  $1 \times 10^6$  CFU/mL to about  $1 \times 10^{11}$  CFU/mL for a liquid composition containing the solid substance or for the emulsion. In certain embodiments of the methods, the *Methylobacterium* sp. is *Methylobacterium* isolate ISO02 (NRRL B-50930), ISO03 (NRRL B-50931), ISO04 (NRRL B-50932), ISO11 (NRRL B-50939), or a derivative thereof. In certain embodiments, the derivative thereof is a strain selected for resistance to a bacteriocidal agent. In certain embodiments of any of the aforementioned methods, the derivative of the *Methylobac-*

*terium* isolate is selected for glyphosate resistance or for glufosinate resistance. In certain embodiments of any of the aforementioned methods, the corn plant is a glyphosate tolerant corn plant and a formulation containing glyphosate is also applied at about the V2 to about the V4 stage of corn plant development. In certain embodiments of the methods, the *Methylobacterium* sp. is *Methylobacterium* isolate ISO11 or a derivative thereof. In certain embodiments of any of the aforementioned methods, the method further comprises harvesting seed from the mature corn plant. In certain embodiments of any of the aforementioned methods, the yield of harvested seed is increased in comparison to yield of harvested seed obtained from a control corn plant that did not receive an application of the *Methylobacterium* sp. In certain embodiments of any of the aforementioned methods, the applied composition coats or partially coats the corn seed or the corn plant or a part thereof.

Also provided herein are methods for improving corn plant early vigor that comprise: (a) applying a composition comprising a *Methylobacterium* sp. to a corn seed or to a corn plant at about the VE to about the V3 stage of corn plant development, wherein the composition comprises: (i) a solid substance with the *Methylobacterium* grown thereon and adhered thereto; (ii) an emulsion having the *Methylobacterium* grown therein; or (iii) a *Methylobacterium* isolate ISO02 (NRRL B-50930), ISO03 (NRRL B-50931), ISO04 (NRRL B-50932), ISO11 (NRRL B-50939), or a derivative thereof and an agriculturally acceptable adjuvant, excipient, or combination thereof; and, (b) growing a corn plant from the seed or the corn plant to the V3 to V6 stage of development, thereby improving early vigor of the corn plant. In certain embodiments of the methods, the composition is applied at about the VE to about the V2 stage of development, about the VE to about the V1 stage of development, or VE stage of development. In certain embodiments, the solid substance with the *Methylobacterium* grown thereon and adhered thereto is provided in a liquid or in an emulsion. In certain embodiments of the methods, the composition comprises the *Methylobacterium* sp. at a titer of about  $1 \times 10^6$  CFU/gm to about  $1 \times 10^{14}$  CFU/gm for a solid composition or at a titer of about  $1 \times 10^6$  CFU/mL to about  $1 \times 10^{11}$  CFU/mL for a liquid composition containing the solid substance or for the emulsion. In certain embodiments of the methods, the composition comprises the solid or the emulsion and the *Methylobacterium* sp. is *Methylobacterium* isolate ISO02 (NRRL B-50930), ISO03 (NRRL B-50931), ISO04 (NRRL B-50932), ISO11 (NRRL B-50939), or a derivative thereof. In certain embodiments of the methods, the derivative thereof is selected for resistance to a bacteriocidal agent. In certain embodiments of the methods, the *Methylobacterium* isolate is selected for glyphosate resistance or for glufosinate resistance. In certain embodiments of the methods, the corn plant is a glyphosate tolerant corn plant and a formulation containing glyphosate is also applied at about the V2 to about the V4 stage of corn plant development. In certain embodiments of any of the aforementioned methods, the vigor of the corn plant in step (b) is increased in comparison to vigor of a control corn plant that did not receive an application of the *Methylobacterium* sp. In certain embodiments of any of the aforementioned methods, increased vigor comprises increased height, increased leaf area, increased chlorophyll content, increased stalk diameter, an advanced vegetative stage on a V1-V6 scale, root volume, root length, number of root tips, and combinations thereof. In certain embodiments of the aforementioned methods, the applied composition coats or partially coats the corn seed or the corn plant or a part thereof.

## Definitions

As used herein, the phrases “adhered thereto” and “adherent” refer to *Methylobacterium* that are associated with a solid substance by growing, or having been grown, on a solid substance.

As used herein, the phrase “agriculturally acceptable adjuvant” refers to a substance that enhances the performance of an active agent in a composition for treatment of plants and/or plant parts. In certain compositions, an active agent can comprise a mono-culture or co-culture of *Methylobacterium*.

As used herein, the phrase “agriculturally acceptable excipient” refers to an essentially inert substance that can be used as a diluent and/or carrier for an active agent in a composition for treatment of plants and/or plant parts. In certain compositions, an active agent can comprise a mono-culture or co-culture of *Methylobacterium*.

As used herein, the term “*Methylobacterium*” refers to bacteria that are facultative methylotrophs of the genus *Methylobacterium*. The term *Methylobacterium*, as used herein, thus does not encompass includes species in the genera *Methylobacter*, *Methylomonas*, *Methylomicrobium*, *Methylococcus*, *Methylosinus*, *Methylocystis*, *Methylosphaera*, *Methylocaldum*, and *Methylocella*, which are obligate methanotrophs.

As used herein, the phrase “control plant” refers to a plant that had not received treatment with a yield or early vigor enhancing *Methylobacterium* or composition comprising the same at either the seed or any subsequent stage of the control plant’s development. In certain embodiments, a control plant can be a plant that was treated with a yield neutral *Methylobacterium* sp.

As used herein, the phrase “co-culture of *Methylobacterium*” refers to a *Methylobacterium* culture comprising at least two strains of *Methylobacterium* or at least two species of *Methylobacterium*.

As used herein, the phrase “contaminating microorganism” refers to microorganisms in a culture, fermentation broth, fermentation broth product, or composition that were not identified prior to introduction into the culture, fermentation broth, fermentation broth product, or composition.

As used herein, the phrase “derivatives thereof”, when used in the context of a *Methylobacterium* isolate, refers to any strain that is obtained from the *Methylobacterium* isolate. Derivatives of a *Methylobacterium* isolate include, but are not limited to, variants of the strain obtained by selection, variants of the strain selected by mutagenesis and selection, and a genetically transformed strain obtained from the *Methylobacterium* isolate.

As used herein, the phrase “early corn vigor” or “early vigor”, when used in the context of apply compositions containing *Methylobacterium* to corn seed, plants or parts of plants, refers to any growth characteristic of a corn plant in the V3 to V6 stage of development that is indicative of improved growth in comparison to an untreated corn plant. Such growth characteristics can include, but are not limited to, increased height, increased leaf area, increased chlorophyll content, increased stalk diameter, an advanced vegetative stage on a V1-V6 scale, increased root volume, increased root length, increased number of root tips, and combinations thereof.

As used herein, the term “emulsion” refers to a colloidal mixture of two immiscible liquids wherein one liquid is the continuous phase and the other liquid is the dispersed phase. In certain embodiments, the continuous phase is an aqueous

liquid and the dispersed phase is liquid that is not miscible, or partially miscible, in the aqueous liquid.

As used herein, the phrase “essentially free of contaminating microorganisms” refers to a culture, fermentation broth, fermentation product, or composition where at least about 95% of the microorganisms present by amount or type in the culture, fermentation broth, fermentation product, or composition are the desired *Methylobacterium* or other desired microorganisms of pre-determined identity.

As used herein, the phrase “inanimate solid substance” refers to a substance which is insoluble or partially soluble in water or aqueous solutions and which is either non-living or which is not a part of a still-living organism from which it was derived.

As used herein, the phrase “mono-culture of *Methylobacterium*” refers to a *Methylobacterium* culture consisting of a single strain of *Methylobacterium*.

As used herein, the term “peptide” refers to any polypeptide of 50 amino acid residues or less.

As used herein, the term “protein” refers to any polypeptide having 51 or more amino acid residues.

As used herein, a “pesticide” refers to an agent that is insecticidal, fungicidal, nematocidal, bacteriocidal, or any combination thereof.

As used herein, the phrase “bacteriostatic agent” refers to agents that inhibit growth of bacteria but do not kill the bacteria.

As used herein, the phrase “pesticide does not substantially inhibit growth of said *Methylobacterium*” refers to any pesticide that when provided in a composition comprising a fermentation product comprising a solid substance wherein a mono-culture or co-culture of *Methylobacterium* is adhered thereto, results in no more than a 50% inhibition of *Methylobacterium* growth when the composition is applied to a plant or plant part in comparison to a composition lacking the pesticide. In certain embodiments, the pesticide results in no more than a 40%, 20%, 10%, 5%, or 1% inhibition of *Methylobacterium* growth when the composition is applied to a plant or plant part in comparison to a composition lacking the pesticide.

As used herein, the term “PPFM bacteria” refers without limitation to bacterial species in the genus *Methylobacterium* other than *M. nodulans*.

As used herein, the phrase “solid substance” refers to a substance which is insoluble or partially soluble in water or aqueous solutions.

As used herein, the phrase “solid phase that can be suspended therein” refers to a solid substance that can be distributed throughout a liquid by agitation.

As used herein, the term “non-regenerable” refers to either a plant part or processed plant product that cannot be regenerated into a whole plant.

As used herein, the phrase “substantially all of the solid phase is suspended in the liquid phase” refers to media wherein at least 95%, 98%, or 99% of solid substance(s) comprising the solid phase are distributed throughout the liquid by agitation.

As used herein, the phrase “substantially all of the solid phase is not suspended in the liquid phase” refers to media where less than 5%, 2%, or 1% of the solid is in a particulate form that is distributed throughout the media by agitation.

To the extent to which any of the preceding definitions is inconsistent with definitions provided in any patent or non-patent reference incorporated herein by reference, any patent or non-patent reference cited herein, or in any patent or non-patent reference found elsewhere, it is understood that the preceding definition will be used herein.

Yield and Early Vigor Enhancing *Methylobacterium*, Compositions Comprising Yield and Early Vigor Enhancing *Methylobacterium*, Methods of their Use, and Methods of Making

Various yield enhancing *Methylobacterium* isolates, compositions comprising these *Methylobacterium*, methods of using the compositions to improve corn plant yield, and methods of making the compositions are provided herein. Amounts of the compositions that comprise yield enhancing *Methylobacterium* sp. sufficient to provide for improved corn plant yield can be determined by measuring any or all of changes in yield relative to untreated plants or plant parts. In certain embodiments, yield can be assessed by measuring output of seed on a per unit area basis (i.e. bushels per acre, kilograms per hectare, and the like), where the yield enhancing *Methylobacterium* sp treated plants or plants grown from *Methylobacterium* sp treated seed are grown at about the same density as the control plants. In certain embodiments, yield can be assessed by measuring output on a per plant or per plant part basis (grams of seed per plant, grams of seed per cob, kernels per plant, kernels per cob and the like) of the yield enhancing *Methylobacterium* sp treated plants in comparison to untreated control plants.

Isolated yield enhancing *Methylobacterium* sp. are provided herein. In certain embodiments, the *Methylobacterium* is selected from the group consisting of *M. aminovorans*, *M. extorquens*, *M. fujisawaense*, *M. mesophilicum*, *M. radiotolerans*, *M. rhodesianum*, *M. nodulans*, *M. phyllosphaerae*, *M. thiocyanatum*, and *M. oryzae*. In certain embodiments, *Methylobacterium* is not *M. radiotolerans* or *M. oryzae*. In certain embodiments, the yield or early vigor enhancing *Methylobacterium* isolate is selected from the group consisting of ISO02, ISO03, ISO04, ISO11, and derivatives thereof. In certain embodiments, the yield enhancing *Methylobacterium* isolate can enhance yield when applied prior to or during reproductive stages of corn development and is a *Methylobacterium* sp. selected from the group consisting of ISO02, ISO03, and ISO04. In certain embodiments, the yield enhancing *Methylobacterium* isolate can enhance yield when applied to a corn seed or in vegetative stages of corn development. In certain embodiments where the yield enhancing *Methylobacterium* isolate is applied to a corn seed or in vegetative stages of corn development, the *Methylobacterium* sp. is ISO11. In certain embodiments, the yield enhancing *Methylobacterium* provides for at least about 2%, at least about 5%, at least about 10%, or at least about 15% increases in yield of a treated plant or a plant arising from a treated seed in comparison to untreated control plants or plants grown from untreated seeds. In certain embodiments, the yield enhancing *Methylobacterium* provides for at least about 2% or at least about 5% to at least about a 10% or at least about a 20% increases in yield of a treated plant or a plant grown from a treated seed in comparison to untreated control plants or plants arising from untreated seeds.

In certain embodiments, the *Methylobacterium* is not *M. radiotolerans* or *M. oryzae*. In certain embodiments, the yield or early vigor enhancing *Methylobacterium* provides for increases in yield and/or early vigor when applied to a seed. In certain embodiments, the yield enhancing *Methylobacterium* provides for increases in yield when applied just prior to or during corn reproductive stages of development. In certain embodiments of any of the aforementioned compositions, the composition comprises a solid substance wherein a mono-culture or co-culture of *Methylobacterium* is adhered thereto. In certain embodiments where the *Methylobacterium* is adhered to a solid substance, the composition comprises a colloid formed by the solid substance

wherein a mono-culture or co-culture of *Methylobacterium* is adhered thereto and a liquid. In certain embodiments, the colloid is a gel. In certain embodiments of certain aforementioned compositions, composition is an emulsion that does not contain a solid substance. In certain embodiments of any of the aforementioned compositions, the yield or early vigor enhancing *Methylobacterium* is selected from the group consisting of ISO02, ISO03, ISO04, ISO11, and derivatives thereof. In certain embodiments of any of the aforementioned compositions, the yield enhancing *Methylobacterium* is selected from the group consisting of ISO02, ISO03, ISO04, and derivatives thereof.

In certain embodiments, isolated yield or early vigor enhancing *Methylobacterium* sp. can be identified by treating a plant, a seed, soil in which the plant or a plant arising from the seed are grown, or other plant growth media in which the plant or a plant arising from the seed are grown and assaying for increased yield or improved early vigor.

In certain embodiments, corn seed or plants in the vegetative stages of development are treated with the yield or early vigor enhancing *Methylobacterium* sp. The vegetative stages of corn are as follows: VE (coleoptile emergence to just prior to first leaf collaring), V1 (first leaf collared), V2 (first and second leaf collared), V3 (first through third leaf collared), V4 (first through fourth leaf collared), V5 (first through fifth leaf collared), V6 (first through sixth leaf collared), and so on up to V18 (plants with the 18th leaf collared). A description of the corn vegetative stages can be found on the world wide web (internet) at [agronext.iastate.edu/corn/production/management/growth/Corn-GrowthandDevelopment.html](http://agronext.iastate.edu/corn/production/management/growth/Corn-GrowthandDevelopment.html) and in "Corn Growth and Development", Abendroth et al. Iowa State University Extension and Outreach publication PMR 19 Mar. 2011). In certain embodiments, the yield enhancing *Methylobacterium* sp. are applied at about the VE to about the V4, V5, or V6 stage of development. In certain embodiments, the yield enhancing *Methylobacterium* sp. are applied at about the VE, V1, V2, or V3 to about the V4, V5, or V6 stage of development. In certain embodiments, the yield or early vigor enhancing *Methylobacterium* that is applied to the seed or during the vegetative stage is ISO11.

In certain embodiments, the yield or early vigor enhancing *Methylobacterium* are applied before, during, or after the application of glyphosate to a transgenic corn plant that is glyphosate tolerant. Commercially available glyphosate formulations that can be used include, but are not limited to, Roundup Original MAX®, Roundup PowerMAX®, Roundup UltraMax®, or RoundUp WeatherMAX® (Monsanto Co., St. Louis, MO., USA); Touchdown IQ® or Touchdown Total® (Syngenta, Wilmington, Delaware, USA); Glyphomax®, Glyphomax Plus®, or Glyphomax XRT® (Dow Agrosiences LLC, Indianapolis, IN, USA). Corn plants are typically sprayed with glyphosate at about the V3 and/or at about the V6 vegetative development stage. In certain embodiments, the yield enhancing *Methylobacterium* that is applied before, during, or after the application of glyphosate a *Methylobacterium* that is selected for glyphosate resistance. Selections for glyphosate resistant bacteria that have been described (Comai et al., Science 221 (4608): 370-371) can be adapted for selection of yield enhancing *Methylobacterium*. The selection and use of glyphosate resistant yield or early vigor enhancing *Methylobacterium* from mutagenized or other populations of *Methylobacterium* such as ISO02, ISO03, ISO04, ISO11, and derivatives thereof is provided herein.

In certain embodiments, corn seed or plants in the late vegetative stages to reproductive stages of development are

treated with the yield enhancing *Methylobacterium* sp. As used herein, the late vegetative stages of corn are the V6, to the V(n) (nth leaf, where the final number of leaves depend on the corn variety and environmental conditions) or VT (tasselling) stages of development. The reproductive stages of corn development are: R1 (beginning flowering—at least one flower on any node); R2 (full flowering—an open flower at one of the two uppermost nodes); R3 (beginning pod-pods are 5 mm at one of the four uppermost nodes); R4 (full pod-pods at 2 cm at one of the four uppermost nodes); R5 (Beginning seed-seed is 3 mm long in the pod at one of the four uppermost nodes on the main stem); R6 (full seed-pod containing a green seed that fills the pod capacity at one of the four uppermost nodes on the main stem); R7 (beginning maturity—one normal pod on the main stem has reached its mature pod color); and R8 (full maturity—95% of the pods have reached their full mature color). A description of the corn reproductive and vegetative stages can be found in “Corn Growth and Development”, Abendroth et al. Iowa State University Extension and Outreach publication PMR 19 Mar. 2011). In certain embodiments, the yield enhancing *Methylobacterium* sp. are applied at about the V5, V6 to about the Vn stage or VT stage of development to about the R2, R<sup>3</sup>, R4, R5, or R6 stage of development. In certain embodiments, the yield enhancing *Methylobacterium* sp. are applied at about the V12, V16, V18, Vn, or VT stage of development to about the R2, R<sup>3</sup>, or R4 stage of development. In certain embodiments, the yield enhancing *Methylobacterium* sp. are applied at about the R1 stage of development. In certain embodiments, the yield enhancing *Methylobacterium* that is applied to the late vegetative or reproductive stage corn plant is selected from the group consisting of ISO02, ISO03, ISO04, and derivatives thereof.

Various *Methylobacterium* sp. isolates provided herein are disclosed in Table 1.

TABLE 1

<i>Methylobacterium</i> sp. isolates		
ISOLATE No.	NLS No.	USDA ARS NRRL No. <sup>1</sup>
ISO01	NLS0046	NRRL B-50929
ISO02	NLS0020	NRRL B-50930
ISO03	NLS0017	NRRL B-50931
ISO04	NLS0042	NRRL B-50932
ISO05	NLS0089	NRRL B-50933
ISO06	NLS0068	NRRL B-50934
ISO07	NLS0065	NRRL B-50935
ISO08	NLS0069	NRRL B-50936
ISO09	NLS0062	NRRL B-50937
ISO10	NLS0064	NRRL B-50938
ISO11	NLS0021	NRRL B-50939
ISO12	NLS0066	NRRL B-50940
ISO13	NLS0037	NRRL B-50941
ISO14	NLS0038	NRRL B-50942

<sup>1</sup>Deposit number for strain deposited with the AGRICULTURAL RESEARCH SERVICE CULTURE COLLECTION (NRRL) of the National Center for Agricultural Utilization Research, Agricultural Research Service, U.S. Department of Agriculture, 1815 North University Street, Peoria, Illinois 61604 U.S.A. under the terms of the Budapest Treaty on the International Recognition of the Deposit of Microorganisms for the Purposes of Patent Procedure. Subject to 37 CFR §1.808(b), all restrictions imposed by the depositor on the availability to the public of the deposited material will be irrevocably removed upon the granting of any patent from this patent application.

Co-assigned patent applications that disclose additional specific uses of the *Methylobacterium* strains of Table 1 such as: (1) increasing soybean yield (U.S. 61/911,698, filed Dec. 4, 2013; and International Application claiming benefit of the same filed on Dec. 4, 2014); (2) improving lettuce cultivation (International Patent Application PCT/US14/68558, filed on Dec. 4, 2014); (3) improving tomato growth

(International Patent Application PCT/US14/68611 filed on Dec. 4, 2014) and are each incorporated herein by reference. Specifically incorporated herein by reference in their entireties are the amino acid and genomic nucleic acid sequences of NLS017, NLS020, NLS037, NLS042, NLS065, and NLS066 that are disclosed in International Application PCT/US14/68558 filed on Dec. 4, 2014. Also specifically incorporated herein by reference in their entireties are the genomic nucleic acid sequences of NLS017 and NLS066 disclosed in the International Patent Application PCT/US14/68611, filed Feb. 4, 2014. Such amino acid and genomic nucleic acid sequences can be used to identify compositions, plant parts, plant seeds, or processed plant products comprising *Methylobacterium* sp. NLS017, NLS020, NLS037, NLS042, NLS065, and NLS066.

Also provided herein are methods for improving corn yield that comprise applying any of the aforementioned compositions provided herein to a plant or a plant part in an amount that provides for increased corn yield in the plant, plant part, or a plant obtained therefrom relative to yield of a control plant, plant part, or plant obtained therefrom that had not received an application of the composition. In certain embodiments, application of the composition provides for at least about 50%, at least about 75%, at least about 85%, or at least about 95% increased corn yield in the plant, plant part, or a plant derived therefrom relative to infection of the control plant, plant part, or plant obtained therefrom. In certain embodiments, the plant part is selected from the group consisting of a leaf, a stem, a flower, a root, and a seed. In certain embodiments, the method further comprises the step of harvesting at least one plant part selected from the group consisting of a leaf, a stem, a flower, a root, or a seed from the plant or plant part. In certain embodiments of any of the aforementioned methods, the methods further comprise obtaining a processed food or feed composition from the plant or plant part. In certain embodiments, the processed food or feed composition is a meal or a paste. In certain embodiments of any of the aforementioned methods, the yield enhancing *Methylobacterium* is selected from the group consisting of ISO02, ISO03, ISO04, ISO11, and derivatives thereof. In certain embodiments where the composition is applied prior to or during the reproductive stages of corn development, the yield enhancing *Methylobacterium* is selected from the group consisting of ISO02, ISO03, ISO04, and derivatives thereof. In certain embodiments where the composition is applied to the seed or during the vegetative stages of corn development, the yield enhancing *Methylobacterium* is ISO11.

Also provided are methods of making the compositions useful for improving corn yield or early vigor that comprise combining a yield or early vigor enhancing *Methylobacterium* with an agriculturally acceptable excipient and/or with an agriculturally acceptable adjuvant. In certain embodiments of the methods, the *Methylobacterium* sp. is selected from the group consisting of *M. aminovorans*, *M. extorquens*, *M. fujisawaense*, *M. mesophilicum*, *M. radiotolerans*, *M. rhodesianum*, *M. nodulans*, *M. phyllosphaerae*, *M. thiocyanatum*, and *M. oryzae*. In certain embodiments of the methods, the *Methylobacterium* is not *M. radiotolerans* or *M. oryzae*. In certain embodiments of the methods, the *Methylobacterium* is adhered to a solid substance. In certain embodiments of the methods, the *Methylobacterium* is adhered to the solid substance is combined with a liquid to form a composition that is a colloid. In certain embodiments of the methods, the colloid is a gel. In certain embodiments of the methods, the *Methylobacterium* adhered to the solid substance is provided by culturing the *Methylobacterium* in



the presence of the solid substance. In certain embodiments of the methods, the composition comprises an emulsion. In certain embodiments of the methods, the *Methylobacterium* is provided by culturing the *Methylobacterium* in an emulsion. In certain embodiments of any of the aforementioned methods, the yield or early vigor enhancing *Methylobacterium* is selected from the group consisting of ISO02, ISO03, ISO04, ISO11, and derivatives thereof. In certain embodiments where the composition is applied prior to or during the reproductive stages of corn development, the yield enhancing *Methylobacterium* is selected from the group consisting of ISO02, ISO03, ISO04, and derivatives thereof. In certain embodiments where the composition is applied to the seed or during the vegetative stages of corn development, the yield or early vigor enhancing *Methylobacterium* is ISO11.

Methods where *Methylobacterium* are cultured in biphasic media comprising a liquid phase and a solid substance have been found to significantly increase the resultant yield of *Methylobacterium* relative to methods where the *Methylobacterium* are cultured in liquid media alone. In certain embodiments, the methods can comprise growing the *Methylobacterium* in liquid media with a particulate solid substance that can be suspended in the liquid by agitation under conditions that provide for *Methylobacterium* growth. In certain embodiments where particulate solid substances are used, at least substantially all of the solid phase can thus be suspended in the liquid phase upon agitation. Such particulate solid substances can comprise materials that are about 1 millimeter or less in length or diameter. In certain embodiments, the degree of agitation is sufficient to provide for uniform distribution of the particulate solid substance in the liquid phase and/or optimal levels of culture aeration. However, in other embodiments provided herein, at least substantially all of the solid phase is not suspended in the liquid phase, or portions of the solid phase are suspended in the liquid phase and portions of the solid phase are not suspended in the liquid phase. Non-particulate solid substances can be used in certain biphasic media where the solid phase is not suspended in the liquid phase. Such non-particulate solid substances include, but are not limited to, materials that are greater than about 1 millimeter in length or diameter. Such particulate and non-particulate solid substances also include, but are not limited to, materials that are porous, fibrous, or otherwise configured to provide for increased surface areas for adherent growth of the *Methylobacterium*. Biphasic media where portions of the solid phase are suspended in the liquid phase and portions of the solid phase are not suspended in the liquid phase can comprise a mixture of particulate and non-particulate solid substances. Such particulate and non-particulate solid substances used in any of the aforementioned biphasic media also include, but are not limited to, materials that are porous, fibrous, or otherwise configured to provide for increased surface areas for adherent growth of the *Methylobacterium*. In certain embodiments, the media comprises a colloid formed by a solid and a liquid phase. A colloid comprising a solid and a liquid can be pre-formed and added to liquid media or can be formed in media containing a solid and a liquid. Colloids comprising a solid and a liquid can be formed by subjecting certain solid substances to a chemical and/or thermal change. In certain embodiments, the colloid is a gel. In certain embodiments, the liquid phase of the media is an emulsion. In certain embodiments, the emulsion comprises an aqueous liquid and a liquid that is not miscible, or only partially miscible, in the aqueous liquid. Liquids that are not miscible, or only partially miscible, in water include, but are not limited to, any of the following: (1) liquids having a miscibility in water

that is equal to or less than that of pentanol, hexanol, or heptanol at 25 degrees C.; (2) liquids comprising an alcohol, an aldehyde, a ketone, a fatty acid, a phospholipid, or any combination thereof; (3) alcohols selected from the group consisting of aliphatic alcohols containing at least 5 carbons and sterols; (4) an animal oil, microbial oil, synthetic oil, plant oil, or combination thereof; and/or, (5) a plant oil is selected from the group consisting of corn, soybean, cotton, peanut, sunflower, olive, flax, coconut, palm, rapeseed, sesame seed, safflower, and combinations thereof. In certain embodiments, the immiscible or partially immiscible liquid can comprises at least about 0.02% to about 20% of the liquid phase by mass. In certain embodiments, the methods can comprise obtaining a biphasic culture media comprising the liquid, the solid, and *Methylobacterium* and incubating the culture under conditions that provide for growth of the *Methylobacterium*. Biphasic culture medias comprising the liquid, the solid, and *Methylobacterium* can be obtained by a variety of methods that include, but are not limited to, any of: (a) inoculating a biphasic media comprising the liquid and the solid substance with *Methylobacterium*; (b) inoculating the solid substance with *Methylobacterium* and then introducing the solid substance comprising the *Methylobacterium* into the liquid media; (c) inoculating the solid substance with *Methylobacterium*, incubating the *Methylobacterium* on the solid substance, and then introducing the solid substance comprising the *Methylobacterium* into the liquid media; or (d) any combination of (a), (b), or (c). Methods and compositions for growing *Methylobacterium* in biphasic media comprising a liquid and a solid are disclosed in co-assigned U.S. patent application Ser. No. 13/907,161, filed May 31, 2013, which is incorporated herein by reference in its entirety, and in co-assigned International Patent Application PCT/US13/43722, filed May 31, 2013, which is incorporated herein by reference in its entirety.

Methods where *Methylobacterium* are cultured in media comprising an emulsion have also been found to significantly increase the resultant yield of *Methylobacterium* relative to methods where the *Methylobacterium* are cultured in liquid media alone. In certain embodiments, the methods for making the compositions provided herein can comprise growing the yield enhancing *Methylobacterium* agent in an emulsion under conditions that provide for *Methylobacterium* growth. Medias comprising the emulsion and yield enhancing *Methylobacterium* can be obtained by a variety of methods that include, but are not limited to, any of: (a) inoculating a media comprising the emulsion with *Methylobacterium*; (b) inoculating the aqueous liquid with the *Methylobacterium*, introducing the non-aqueous liquid, and mixing to form an emulsion; (c) inoculating the aqueous liquid with the *Methylobacterium*, introducing the non-aqueous liquid, and mixing to form an emulsion; or (d) any combination of (a), (b), or (c). In certain embodiments, the emulsion comprises an aqueous liquid and a liquid that is not miscible, or only partially miscible, in the aqueous liquid. Non-aqueous liquids that are not miscible, or only partially miscible, in water include, but are not limited to, any of the following: (1) liquids having a miscibility in water that is equal to or less than that of n-pentanol, n-hexanol, or n-heptanol at 25 degrees C.; (2) liquids comprising an alcohol, an aldehyde, a ketone, a fatty acid, a phospholipid, or any combination thereof; (3) alcohols is selected from the group consisting of aliphatic alcohols containing at least 5, 6, or 7 carbons and sterols; (4) an animal oil, microbial oil, synthetic oil, plant oil, or combination thereof; and/or, (5) a plant oil is selected from the group consisting of corn,

soybean, cotton, peanut, sunflower, olive, flax, coconut, palm, rapeseed, sesame seed, safflower, and combinations thereof. In certain embodiments, the immiscible or partially immiscible non-aqueous liquid can comprise at least about 0.02% to about 20% of the emulsion by mass. In certain

embodiments, the immiscible or partially immiscible non-aqueous liquid can comprise at least about any of about 0.05%, 0.1%, 0.5%, or 1% to about 3%, 5%, 10%, or 20% of the emulsion by mass. Methods and compositions for growing *Methylobacterium* in media comprising an emulsion are disclosed in co-assigned International Patent Application PCT/US2014/040218, filed May 30, 2014, which is incorporated herein by reference in its entirety.

In certain embodiments, the fermentation broth, fermentation broth product, or compositions that comprise yield or early vigor enhancing *Methylobacterium* sp. can further comprise one or more introduced microorganisms of predetermined identity other than *Methylobacterium*. Other microorganisms that can be added include, but are not limited to, microorganisms that are biopesticidal or provide some other benefit when applied to a plant or plant part. Biopesticidal or otherwise beneficial microorganisms thus include, but are not limited to, various *Bacillus* sp., *Pseudomonas* sp., *Coniothyrium* sp., *Pantoea* sp., *Streptomyces* sp., and *Trichoderma* sp. Microbial biopesticides can be a bacterium, fungus, virus, or protozoan. Particularly useful biopesticidal microorganisms include various *Bacillus subtilis*, *Bacillus thuringiensis*, *Bacillus pumilis*, *Pseudomonas syringae*, *Trichoderma harzianum*, *Trichoderma virens*, and *Streptomyces lydicus* strains. Other microorganisms that are added can be genetically engineered or wild-type isolates that are available as pure cultures. In certain embodiments, it is anticipated that the bacterial or fungal microorganism can be provided in the fermentation broth, fermentation broth product, or composition in the form of a spore.

In certain embodiments, the liquid culture medium is prepared from inexpensive and readily available components, including, but not limited to, inorganic salts such as potassium phosphate, magnesium sulfate and the like, carbon sources such as glycerol, methanol, glutamic acid, aspartic acid, succinic acid and the like, and amino acid blends such as peptone, tryptone, and the like. Exemplary liquid media that can be used include, but are not limited to, ammonium mineral salts (AMS) medium (Whittenbury et al., 1970), Vogel-Bonner (VB) minimal culture medium (Vogel and Bonner, 1956), and LB broth ("Luria-Bertani Broth").

In general, the solid substance used in the methods and compositions that provide for the efficient growth of *Methylobacterium* can be any suitable solid substance which is insoluble or only partially soluble in water or aqueous solutions. Such suitable solid substances are also non-bacteriocidal or non-bacteriostatic with respect to yield enhancing *Methylobacterium* sp. when the solid substances are provided in the liquid culture media. In certain embodiments, such suitable solid substances are also solid substances that are readily obtained in sterile form or rendered sterile. Solid substances used herein can be sterilized by any method that provides for removal of contaminating microorganisms and thus include, but are not limited to, methods such as autoclaving, irradiation, chemical treatment, and any combination thereof. These solid substances include natural substances of animal, plant, microbial, fungal, or mineral origin, manmade substances, or combinations of natural and manmade substances. In certain embodiments, the solid substances are inanimate solid substances. Inanimate solid

substances of animal, plant, microbial, or fungal origin can be obtained from animals, plants, microbes, or fungi that are inviable (i.e. no longer living) or that have been rendered inviable. Diatom shells are thus inanimate solid substances when previously associated diatom algae have been removed or otherwise rendered inviable. Since diatom shells are inanimate solid substances, they are not considered to be photosynthetic organisms or photosynthetic microorganisms. In certain embodiments, solid substances include, but are not limited to, sand, silt, soil, clay, ash, charcoal, diatomaceous earth and other similar minerals, ground glass or glass beads, ground ceramic materials, ceramic beads, bentonite, kaolin, talc, perlite, mica, vermiculite, silicas, quartz powder, montmorillonite, and combinations thereof. In certain embodiments, the solid substance can be a polymer or polymeric beads. Polymers that can be used as a solid substance include, but are not limited to, various polysaccharides such as cellulosic polymers and chitinous polymers which are insoluble or only partially soluble in water or aqueous solutions, agar (i.e. galactans), and combinations thereof. In certain embodiments, the solid substance can be an insoluble or only partially soluble salt crystal. Salt crystals that can be used include, but are not limited to, insoluble or only partially soluble carbonates, chromates, sulfites, phosphates, hydroxides, oxides, and sulfides. In certain embodiments, the solid substance can be a microbial cell, fungal cell, microbial spore, or fungal spore. In certain embodiments, the solid substance can be a microbial cell or microbial spore wherein the microbial cell or microbial spore is not a photosynthetic microorganism. In certain embodiments, the microbial cell or microbial spore is not a photosynthetic microorganism, where the photosynthetic microorganism is selected from the group consisting of algae, cyanobacteria, diatoms, *Botryococcus braunii*, *Chlorella*, *Dunaliella tertiolecta*, *Gracilaria*, *Pleurochrysis carterae*, *Sargassum*, and *Ulva*. In still other embodiments, the solid substance can be an inactivated (i.e. inviable) microbial cell, fungal cell, microbial spore, or fungal spore. In still other embodiments, the solid substance can be a quiescent (i.e. viable but not actively dividing) microbial cell, fungal cell, microbial spore, or fungal spore. In still other embodiments, the solid substance can be cellular debris of microbial origin. In still other embodiments, the solid substance can be particulate matter from any part of a plant. Plant parts that can be used to obtain the solid substance include, but are not limited to, cobs, husks, hulls, leaves, roots, flowers, stems, barks, seeds, and combinations thereof. Products obtained from processed plant parts including, but not limited to, bagasse, wheat bran, soy grits, crushed seed cake, stover, and the like can also be used. Such plant parts, processed plants, and/or processed plant parts can be milled to obtain the solid material in a particulate form that can be used. In certain embodiments, wood or a wood product including, but not limited to, wood pulp, sawdust, shavings, and the like can be used. In certain embodiments, the solid substance can be a particulate matter from an animal(s), including, but not limited to, bone meal, gelatin, ground or powdered shells, hair, macerated hide, and the like.

In certain embodiments, the solid substance is provided in a particulate form that provides for distribution of the solid substance in the culture media. In certain embodiments, the solid substance is comprised of particle of about 2 microns to about 1000 microns in average length or average diameter. In certain embodiments, the solid substance is comprised of particle of about 1 microns to about 1000 microns in average length or average diameter. In certain embodiments, the solid substance is a particle of about 1, 2, 4, 10,

20, or 40 microns to any of about 100, 200, 500, 750, or 1000 microns in average length or average diameter. Desirable characteristics of particles used in the methods and compositions provided herein include suitable wettability such that the particles can be suspended throughout the media upon agitation.

In certain embodiments, the solid substance is provided in the media as a colloid wherein the continuous phase is a liquid and the dispersed phase is the solid. Suitable solids that can be used to form colloids in liquid media used to grow yield enhancing *Methylobacterium* sp. include, but are not limited to, various solids that are referred to as hydrocolloids. Such hydrocolloids used in the media, methods and compositions provided herein can be hydrophilic polymers, of plant, animal, microbial, or synthetic origin. Hydrocolloid polymers used in the methods can contain many hydroxyl groups and/or can be polyelectrolytes. Hydrocolloid polymers used in the compositions and methods provided herein include, but are not limited to, agar, alginate, arabinoxylan, carrageenan, carboxymethylcellulose, cellulose, curdlan, gelatin, gellan,  $\beta$ -glucan, guar gum, gum arabic, locust bean gum, pectin, starch, xanthan gum, and mixtures thereof. In certain embodiments, the colloid used in the media, methods, and compositions provided herein can comprise a hydrocolloid polymer and one or more proteins.

In certain embodiments, the solid substance can be a solid substance that provides for adherent growth of the yield enhancing *Methylobacterium* sp. on the solid substance. Yield enhancing *Methylobacterium* sp. that are adhered to a solid substance are *Methylobacterium* that cannot be substantially removed by simply washing the solid substance with the adherent yield enhancing *Methylobacterium* sp. with growth media whereas non-adherent *Methylobacterium* can be substantially removed by washing the solid substance with liquid growth media. In this context, "substantially removed" means that at least about 30%, 40%, 50%, 60%, 70%, or 80% the *Methylobacterium* present are removed when the solid substance is washed with three volumes of liquid growth media. Such washing can be effected by a variety of methods including, but not limited to, decanting liquid from a washed solid phase or passing liquid through a solid phase on a filter that permits flow through of bacteria in the liquid. In certain embodiments, the adherent yield enhancing *Methylobacterium* sp. that are associated with the solid can include both *Methylobacterium* that are directly attached to the solid and/or *Methylobacterium* that are indirectly attached to the solid substance. *Methylobacterium* that are indirectly attached to the solid substance include, but are not limited to, *Methylobacterium* that are attached to another *Methylobacterium* or to another microorganism that is attached to the solid substance, *Methylobacterium* that are attached to the solid substance by being attached to another substance that is attached to the solid substance, and the like. In certain embodiments, at least 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, 95%, 98%, 99%, 99.5% or 99.9% of the *Methylobacterium* in the fermentation broth, fermentation broth product, or compositions are *Methylobacterium* that are adhered to the solid substance. In certain embodiments, adherent yield enhancing *Methylobacterium* sp. can be present on the surface of the solid substance in the fermentation broth, fermentation broth product, or composition at a density of at least about 1 *Methylobacterium*/20 square micrometers, of at least about 1 *Methylobacterium*/10 square micrometers, of at least about 1 *Methylobacterium*/5 square micrometers, of at least about 1 *Methylobacterium*/2 square micrometers, or of at least about 1 *Methylobacte-*

*rium*/square micrometer. In certain embodiments, adherent yield enhancing *Methylobacterium* sp. can be present on the surface of the solid substance in the fermentation broth, fermentation broth product, or composition at a density of at least about 1 *Methylobacterium*/20 square micrometers to about 1 *Methylobacterium*/square micrometer, of at least about 1 *Methylobacterium*/10 square micrometers to about 1 *Methylobacterium*/square micrometer, of at least about 1 *Methylobacterium*/10 square micrometers to about 1 *Methylobacterium*/square micrometer, of at least about 1 *Methylobacterium*/5 square micrometers to about 1 *Methylobacterium*/square micrometer, or of at least about 1 *Methylobacterium*/2 square micrometers to about 1 *Methylobacterium*/square micrometer. In certain embodiments, adherent yield enhancing *Methylobacterium* sp. can be present on the surface of the solid substance in the fermentation broth, fermentation broth product, or composition at a density of at least about 1 *Methylobacterium*/20 square micrometers to about 1 *Methylobacterium*/2 square micrometers, of at least about 1 *Methylobacterium*/10 square micrometers to about 1 *Methylobacterium*/2 square micrometers, of at least about 1 *Methylobacterium*/10 square micrometers to about 1 *Methylobacterium*/10 square micrometers, or of at least about 1 *Methylobacterium*/5 square micrometers to about 1 *Methylobacterium*/2 square micrometers. Biphasic fermentation broths provided herein can comprise a liquid phase that contains non-adherent *Methylobacterium*. In certain embodiments, titers of non-adherent *Methylobacterium* in the liquid phase can be less than about 100,000, 10,000, or 1,000 CFU/ml. In certain embodiments of any of the aforementioned compositions, the yield or early vigor enhancing *Methylobacterium* is selected from the group consisting of ISO02, ISO03, ISO04, ISO11, and derivatives thereof. In certain embodiments where the composition is applied prior to or during the reproductive stages of corn development, the yield enhancing *Methylobacterium* is selected from the group consisting of ISO02, ISO03, ISO04, and derivatives thereof. In certain embodiments where the composition is applied to the seed or during the vegetative stages of corn development, the yield enhancing *Methylobacterium* is ISO11 or a derivative thereof.

Biphasic culture methods provided can yield fermentation broths with yield or early vigor enhancing *Methylobacterium* sp. at a titer of greater than about  $5 \times 10^8$  colony-forming units per milliliter, at a titer of greater than about  $1 \times 10^9$  colony-forming units per milliliter, at a titer of greater than about  $1 \times 10^{10}$  colony-forming units per milliliter, at a titer of at least about  $3 \times 10^{10}$  colony-forming units per milliliter. In certain embodiments, fermentation broths provided herein can comprise yield enhancing *Methylobacterium* sp. at a titer of at least about  $5 \times 10^8$  colony-forming units per milliliter to at least about  $3 \times 10^{10}$  colony-forming units per milliliter, at least about  $5 \times 10^8$  colony-forming units per milliliter to at least about  $4 \times 10^{10}$  colony-forming units per milliliter, or at least about  $5 \times 10^8$  colony-forming units per milliliter to at least about  $6 \times 10^{10}$  colony-forming units per milliliter. In certain embodiments, fermentation broths provided herein can comprise yield enhancing *Methylobacterium* sp. at a titer of at least about  $1 \times 10^9$  colony-forming units per milliliter to at least about  $3 \times 10^{10}$  colony-forming units per milliliter, at least about  $1 \times 10^9$  colony-forming units per milliliter to at least about  $4 \times 10^{10}$  colony-forming units per milliliter, or at least about  $1 \times 10^9$  colony-forming units per milliliter to at least about  $6 \times 10^{10}$  colony-forming units per milliliter. In certain embodiments, fermentation broths provided herein will comprise yield enhancing *Methylobacterium* sp. at a titer of at least about  $1 \times 10^{10}$  colony-forming

units per milliliter to at least about  $3 \times 10^{10}$  colony-forming units per milliliter, at least about  $1 \times 10^{10}$  colony-forming units per milliliter to at least about  $4 \times 10^{10}$  colony-forming units per milliliter, or at least about  $1 \times 10^{10}$  colony-forming units per milliliter to at least about  $6 \times 10^{10}$  colony-forming units per milliliter. In certain embodiments, fermentation broths provided herein will comprise yield enhancing *Methylobacterium* sp. at a titer of, at least about  $3 \times 10^{10}$  colony-forming units per milliliter to at least about  $4 \times 10^{10}$  colony-forming units per milliliter, or at least about  $3 \times 10^{10}$  colony-forming units per milliliter to at least about  $6 \times 10^{10}$  colony-forming units per milliliter. In certain embodiments of any of the aforementioned compositions, the yield enhancing *Methylobacterium* is selected from the group consisting of ISO02, ISO03, ISO04, ISO11, and derivatives thereof. In certain embodiments where the composition is applied prior to or during the reproductive stages of corn development, the yield enhancing *Methylobacterium* is selected from the group consisting of ISO02, ISO03, and ISO04. In certain embodiments where the composition is applied to the seed or during the vegetative stages of corn development, the yield or early vigor enhancing *Methylobacterium* is ISO11.

Solid substances with adherent yield or early vigor enhancing *Methylobacterium* sp. can be obtained as fermentation products can be used to make various compositions useful for treating plants or plant parts to improve corn yield or early vigor. Alternatively, compositions provided herein comprising yield or early vigor enhancing *Methylobacterium* sp., solid substances with yield or early vigor enhancing *Methylobacterium* sp. grown thereon, or comprising emulsions with yield or early vigor enhancing *Methylobacterium* sp. grown therein can be used to treat plants or plant parts. Plants, plant parts, and, in particular, plant seeds that have been at least partially coated or coated with the fermentation broth products or compositions comprising yield enhancing *Methylobacterium* sp. are thus provided. Partial coating of a plant, a plant part, or a seed includes, but is not limited to coating at least about 5%, 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, 95%, 98%, 99%, or about 99.5% of the surface area of the plant, plant part, or plant seed. Also provided are processed plant products that contain the fermentation broth products or compositions with yield enhancing *Methylobacterium* sp. or adherent yield enhancing *Methylobacterium* sp. Solid substances with adherent yield enhancing *Methylobacterium* sp. can be used to make various compositions that are particularly useful for treating plant seeds. Seeds that have been at least partially coated with the fermentation broth products or compositions are thus provided. Partial coating of a seed includes, but is not limited to coating at least about 5%, 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, 95%, 98%, 99%, or about 99.5% of the surface area of the seed. Also provided are processed seed products, including, but not limited to, meal, flour, feed, and flakes that contain the fermentation broth products or compositions provided herein. In certain embodiments, the processed plant product will be non-regenerable (i.e. will be incapable of developing into a plant). In certain embodiments, the solid substance used in the fermentation product or composition that at least partially coats the plant, plant part, or plant seed or that is contained in the processed plant, plant part, or seed product comprises a solid substance and associated or adherent yield enhancing *Methylobacterium* sp. that can be readily identified by comparing a treated and an untreated plant, plant part, plant seed, or processed product thereof. In certain

ISO03, ISO04, ISO11, and derivatives thereof. In certain embodiments, the yield or early vigor enhancing *Methylobacterium* is selected from the group consisting of ISO02, ISO03, and ISO04.

Compositions useful for treating plants or plant parts that comprise yield or early vigor enhancing *Methylobacterium* sp., a solid substance with adherent yield or early vigor enhancing *Methylobacterium* sp., or comprising emulsions with yield or early vigor enhancing *Methylobacterium* sp. grown therein can also further comprise an agriculturally acceptable adjuvant or an agriculturally acceptable excipient. An agriculturally acceptable adjuvant or an agriculturally acceptable excipient is typically an ingredient that does not cause undue phytotoxicity or other adverse effects when exposed to a plant or plant part. In certain embodiments, the solid substance can itself be an agriculturally acceptable adjuvant or an agriculturally acceptable excipient so long as it is not bacteriocidal or bacteriostatic to the *Methylobacterium*. In other embodiments, the composition further comprises at least one of an agriculturally acceptable adjuvant or an agriculturally acceptable excipient. Any of the aforementioned compositions can also further comprise a pesticide. Pesticides used in the composition include, but are not limited to, an insecticide, a fungicide, a nematocide, and a bactericide. In certain embodiments, the pesticide used in the composition is a pesticide that does not substantially inhibit growth of the *Methylobacterium*. As *Methylobacterium* are gram negative bacteria, suitable bacteriocides used in the compositions can include, but are not limited to, bacteriocides that exhibit activity against gram positive bacteria but not gram negative bacteria. Compositions provided herein can also comprise a bacteriostatic agent that does not substantially inhibit growth of the *Methylobacterium*. Bacteriostatic agents suitable for use in compositions provided herein include, but are not limited to, those that exhibit activity against gram positive bacteria but not gram negative bacteria. Any of the aforementioned compositions can also be an essentially dry product (i.e. having about 5% or less water content), a mixture of the composition with an emulsion, or a suspension.

Agriculturally acceptable adjuvants used in the compositions that comprise yield or early vigor enhancing *Methylobacterium* sp. include, but are not limited to, components that enhance product efficacy and/or products that enhance ease of product application. Adjuvants that enhance product efficacy can include various wetters/spreaders that promote adhesion to and spreading of the composition on plant parts, stickers that promote adhesion to the plant part, penetrants that can promote contact of the active agent with interior tissues, extenders that increase the half-life of the active agent by inhibiting environmental degradation, and humectants that increase the density or drying time of sprayed compositions. Wetters/spreaders used in the compositions can include, but are not limited to, non-ionic surfactants, anionic surfactants, cationic surfactants, amphoteric surfactants, organo-silicate surfactants, and/or acidified surfactants. Stickers used in the compositions can include, but are not limited to, latex-based substances, terpene/pinylene, and pyrrolidone-based substances. Penetrants can include mineral oil, vegetable oil, esterified vegetable oil, organo-silicate surfactants, and acidified surfactants. Extenders used in the compositions can include, but are not limited to, ammonium sulphate, or menthene-based substances. Humectants used in the compositions can include, but are not limited to, glycerol, propylene glycol, and diethyl glycol. Adjuvants that improve ease of product application include, but are not limited to, acidifying/buffering agents, anti-

foaming/de-foaming agents, compatibility agents, drift-reducing agents, dyes, and water conditioners. Anti-foaming/de-foaming agents used in the compositions can include, but are not limited to, dimethopolysiloxane. Compatibility agents used in the compositions can include, but are not limited to, ammonium sulphate. Drift-reducing agents used in the compositions can include, but are not limited to, polyacrylamides, and polysaccharides. Water conditioners used in the compositions can include, but are not limited to, ammonium sulphate.

Methods of treating plants and/or plant parts with the fermentation broths, fermentation broth products, and compositions comprising yield or early vigor enhancing *Methylobacterium* sp. are also provided herein. Treated plants, and treated plant parts obtained therefrom, include, but are not limited to, corn. Corn plant parts that are treated include, but are not limited to, leaves, stalks, primary roots, nodal roots, seeds, fruit, tassels, silks, husks, sheaths, shanks, coleoptiles, and the like. Seeds or other propagules of any of the aforementioned corn plants can be treated with the fermentation broths, fermentation broth products, fermentation products, and/or compositions provided herein.

In certain embodiments, plants and/or plant parts are treated by applying the fermentation broths, fermentation broth products, fermentation products, and compositions that comprise yield or early vigor enhancing *Methylobacterium* sp. as a spray. Such spray applications include, but are not limited to, treatments of a single plant part or any combination of plant parts. Spraying can be achieved with any device that will distribute the fermentation broths, fermentation broth products, fermentation products, and compositions to the plant and/or plant part(s). Useful spray devices include a boom sprayer, a hand or backpack sprayer, crop dusters (i.e. aerial spraying), and the like. Spraying devices and or methods providing for application of the fermentation broths, fermentation broth products, fermentation products, and compositions to either one or both of the adaxial surface and/or abaxial surface can also be used. Plants and/or plant parts that are at least partially coated with any of a biphasic fermentation broth, a fermentation broth product, fermentation product, or compositions that comprise a solid substance with yield enhancing *Methylobacterium* sp. adhered thereto are also provided herein. Partial coating of a plant, a plant part, or a seed includes, but is not limited to coating at least about 5%, 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, 95%, 98%, 99%, or about 99.5% of the surface area of the plant, plant part, or plant seed. Also provided herein are processed plant products that comprise a solid substance with yield or early vigor enhancing *Methylobacterium* sp. adhered thereto.

In certain embodiments, seeds are treated by exposing the seeds to the fermentation broths, fermentation broth products, fermentation products, and compositions that comprise yield or early vigor enhancing *Methylobacterium* sp. Seeds can be treated with the fermentation broths, fermentation broth products, and compositions provided herein by methods including, but not limited to, imbibition, coating, spraying, and the like. Seed treatments can be effected with both continuous and/or a batch seed treaters. In certain embodiments, the coated seeds can be prepared by slurring seeds with a coating composition containing a fermentation broth, fermentation broth product, or compositions that comprise the solid substance with yield enhancing *Methylobacterium* sp. and air drying the resulting product. Air drying can be accomplished at any temperature that is not deleterious to the seed or the *Methylobacterium*, but will typically not be greater than 30 degrees Centigrade. The proportion of coat-

ing that comprises a solid substance and yield enhancing *Methylobacterium* sp. includes, but is not limited to, a range of 0.1 to 25% by weight of the seed, 0.5 to 5% by weight of the seed, and 0.5 to 2.5% by weight of seed. In certain embodiments, a solid substance used in the seed coating or treatment will have yield enhancing *Methylobacterium* sp. adhered thereon. In certain embodiments, a solid substance used in the seed coating or treatment will be associated with yield enhancing *Methylobacterium* sp. and will be a fermentation broth, fermentation broth product, or composition obtained by the methods provided herein. Various seed treatment compositions and methods for seed treatment disclosed in U.S. Pat. Nos. 5,106,648, 5,512,069, and 8,181,388 are incorporated herein by reference in their entireties and can be adapted for use with an active agent comprising the fermentation broths, fermentation broth products, or compositions provided herein. In certain embodiments, the composition used to treat the seed can contain agriculturally acceptable excipients that include, but are not limited to, woodflours, clays, activated carbon, diatomaceous earth, fine-grain inorganic solids, calcium carbonate and the like. Clays and inorganic solids that can be used with the fermentation broths, fermentation broth products, or compositions provided herein include, but are not limited to, calcium bentonite, kaolin, china clay, talc, perlite, mica, vermiculite, silicas, quartz powder, montmorillonite and mixtures thereof. Agriculturally acceptable adjuvants that promote sticking to the seed that can be used include, but are not limited to, polyvinyl acetates, polyvinyl acetate copolymers, hydrolyzed polyvinyl acetates, polyvinylpyrrolidone-vinyl acetate copolymer, polyvinyl alcohols, polyvinyl alcohol copolymers, polyvinyl methyl ether, polyvinyl methyl ether-maleic anhydride copolymer, waxes, latex polymers, celluloses including ethylcelluloses and methylcelluloses, hydroxy methylcelluloses, hydroxypropylcellulose, hydroxymethylpropylcelluloses, polyvinyl pyrrolidones, alginates, dextrans, malto-dextrans, polysaccharides, fats, oils, proteins, karaya gum, jaguar gum, tragacanth gum, polysaccharide gums, mucilage, gum arabics, shellacs, vinylidene chloride polymers and copolymers, soybean-based protein polymers and copolymers, lignosulfonates, acrylic copolymers, starches, polyvinylacrylates, zeins, gelatin, carboxymethylcellulose, chitosan, polyethylene oxide, acrylamide polymers and copolymers, polyhydroxyethyl acrylate, methylacrylamide monomers, alginate, ethylcellulose, polychloroprene and syrups or mixtures thereof. Other useful agriculturally acceptable adjuvants that can promote coating include, but are not limited to, polymers and copolymers of vinyl acetate, polyvinylpyrrolidone-vinyl acetate copolymer and water-soluble waxes. Various surfactants, dispersants, anticaking-agents, foam-control agents, and dyes disclosed herein and in U.S. Pat. No. 8,181,388 can be adapted for use with an active agent comprising the fermentation broths, fermentation broth products, or compositions provided herein.

Provided herein are compositions that comprise yield or early vigor enhancing *Methylobacterium* sp. that provide for increase yield or early vigor of corn plants relative to untreated plants, plant parts, and plants obtained therefrom that have not been exposed to the compositions. In certain embodiments, plant parts, including, but not limited to, a seed, a leaf, a fruit, a stem, a root, or a coleoptile can be treated with the compositions provided herein to increase corn plant yield. Treatments or applications can include, but are not limited to, spraying, coating, partially coating, immersing, and/or imbibing the plant or plant parts with the compositions provided herein. In certain embodiments, a

seed, a leaf, a fruit, a stem, a root, a tuber, or a coleoptile can be immersed and/or imbibed with a liquid, semi-liquid, emulsion, or slurry of a composition provided herein. Such seed immersion or imbibition can be sufficient to provide for increased yield in a treated corn plant or corn plant grown from a treated seed in comparison to an untreated corn plant or corn plant grown from an untreated seed. In certain embodiments, plant seeds can be immersed and/or imbibed for at least 1, 2, 3, 4, 5, or 6 hours. Such immersion and/or imbibition can, in certain embodiments, be conducted at temperatures that are not deleterious to the plant seed or the *Methylobacterium*. In certain embodiments, the seeds can be treated at about 15 to about 30 degrees Centigrade or at about 20 to about 25 degrees Centigrade. In certain embodiments, seed imbibition and/or immersion can be performed with gentle agitation. In certain embodiments, the seed and/or coleoptile is exposed to the composition by providing the composition in furrow. Providing the composition in furrow represents one of several methods provided herein for applying a composition to a corn seed or to a corn plant at about the VE stage of corn plant development.

Compositions provided herein comprising yield or early vigor enhancing *Methylobacterium* sp. and related methods are therefore expected to be useful in improving yield and/or early vigor in a wide variety of corn plants, including, but not limited to, various *Zea mays* hybrids, inbreds, haploids, subspecies, and varieties. In certain embodiments, yield and/or early vigor can be improved in dent corn (*Zea mays* var. *indentata*), flint corn (*Zea mays* var. *indurata*), flour corn (*Zea mays* var. *amylacea*), popcorn (*Zea mays* var. *everta*), pod corn (*Zea mays* var. *tunicata* Larrañaga ex A. St. Hil.) striped maize (*Zea mays* var. *japonica*), sweet corn (*Zea mays* var. *saccharata* and *Zea mays* var. *rugosa*), and/or waxy corn (*Zea mays* var. *ceratina*).

In certain embodiments, an amount of a composition provided herein that is sufficient to provide for increased corn yield and/or early vigor can be a composition with yield or early vigor enhancing *Methylobacterium* sp. at a titer of at least about  $1 \times 10^6$  colony-forming units per milliliter, at least about  $5 \times 10^6$  colony-forming units per milliliter, at least about  $1 \times 10^7$  colony-forming units per milliliter, at least about  $5 \times 10^8$  colony-forming units per milliliter, at least about  $1 \times 10^9$  colony-forming units per milliliter, at least about  $1 \times 10^{10}$  colony-forming units per milliliter, or at least about  $3 \times 10^{10}$  colony-forming units per milliliter. In certain embodiments, an amount of a composition provided herein that is sufficient to provide for increased corn yield and/or early vigor to a plant or plant part can be a composition with yield or early vigor enhancing *Methylobacterium* sp. at a titer of about least about  $1 \times 10^6$  colony-forming units per milliliter, at least about  $5 \times 10^6$  colony-forming units per milliliter, at least about  $1 \times 10^7$  colony-forming units per milliliter, or at least about  $5 \times 10^8$  colony-forming units per milliliter to at least about  $6 \times 10^{10}$  colony-forming units per milliliter of a liquid or an emulsion. In certain embodiments, an amount of a composition provided herein that is sufficient to provide for increased corn yield and/or early vigor can be a fermentation broth product with a yield or early vigor enhancing *Methylobacterium* sp. titer of a solid phase of that product is at least about  $1 \times 10^6$  colony-forming units per milliliter, at least about  $5 \times 10^6$  colony-forming units per milliliter, at least about  $1 \times 10^7$  colony-forming units per milliliter, or at least about  $5 \times 10^8$  colony-forming units per gram to at least about  $6 \times 10^{10}$  colony-forming units of *Methylobacterium* per gram of the solid phase. In certain embodiments, an amount of a composition provided herein that is sufficient to provide for increased corn yield and/or

early vigor can be a composition with a *Methylobacterium* titer of at least about  $1 \times 10^6$  colony-forming units per gram, at least about  $5 \times 10^6$  colony-forming units per gram, at least about  $1 \times 10^7$  colony-forming units per gram, or at least about  $5 \times 10^8$  colony-forming units per gram to at least about  $6 \times 10^{10}$  colony-forming units of *Methylobacterium* per gram of particles in the composition containing the particles that comprise a solid substance wherein a mono-culture or co-culture of yield enhancing *Methylobacterium* sp. is adhered thereto. In certain embodiments, an amount of a composition provided herein that is sufficient to provide for increased corn yield and/or early vigor to a plant or plant part can be a composition with a *Methylobacterium* titer of at least about  $1 \times 10^6$  colony-forming units per mL, at least about  $5 \times 10^6$  colony-forming units per mL, at least about  $1 \times 10^7$  colony-forming units per mL, or at least about  $5 \times 10^8$  colony-forming units per mL to at least about  $6 \times 10^{10}$  colony-forming units of *Methylobacterium* per mL in a composition comprising an emulsion wherein a mono-culture or co-culture of a yield or early vigor enhancing *Methylobacterium* sp. adhered to a solid substance is provided therein or grown therein. In certain embodiments, an amount of a composition provided herein that is sufficient to provide for increased corn yield and/or early vigor to a plant or plant part can be a composition with a *Methylobacterium* titer of at least about  $1 \times 10^6$  colony-forming units per mL, at least about  $5 \times 10^6$  colony-forming units per mL, at least about  $1 \times 10^7$  colony-forming units per mL, or at least about  $5 \times 10^8$  colony-forming units per mL to at least about  $6 \times 10^{10}$  colony-forming units of *Methylobacterium* per mL of in a composition comprising an emulsion wherein a mono-culture or co-culture of a yield or early vigor enhancing *Methylobacterium* sp. is provided therein or grown therein.

In certain embodiments, an amount of a composition provided herein that is sufficient to provide for increased corn yield and/or early vigor can be a composition with a *Methylobacterium* sp. at a titer of at least about  $1 \times 10^4$  colony-forming units per milliliter, at least about  $1 \times 10^5$  colony-forming units per milliliter, at least about  $1 \times 10^6$  colony-forming units per milliliter, at least about  $5 \times 10^6$  colony-forming units per milliliter, at least about  $1 \times 10^7$  colony-forming units per milliliter, at least about  $5 \times 10^8$  colony-forming units per milliliter, at least about  $1 \times 10^9$  colony-forming units per milliliter, at least about  $1 \times 10^{10}$  colony-forming units per milliliter, or at least about  $3 \times 10^{10}$  colony-forming units per milliliter. In certain embodiments, an amount of a composition provided herein that is sufficient to provide for increased corn yield and/or early vigor can be a composition with *Methylobacterium* sp. at a titer of at least about  $1 \times 10^4$  colony-forming units per milliliter, at least about  $1 \times 10^5$  colony-forming units per milliliter, at least about  $1 \times 10^6$  colony-forming units per milliliter, at least about  $5 \times 10^6$  colony-forming units per milliliter, at least about  $1 \times 10^7$  colony-forming units per milliliter, or at least about  $5 \times 10^8$  colony-forming units per milliliter to at least about  $6 \times 10^{10}$  colony-forming units per milliliter of a liquid or an emulsion. In certain embodiments, an amount of a composition provided herein that is sufficient to provide for increased corn yield and/or early vigor can be a fermentation broth product with a *Methylobacterium* sp. titer of a solid phase of that product is at least about  $1 \times 10^4$  colony-forming units per gram, at least about  $1 \times 10^5$  colony-forming units per gram, at least about  $1 \times 10^6$  colony-forming units per gram, at least about  $5 \times 10^6$  colony-forming units per gram, at least about  $1 \times 10^7$  colony-forming units per gram, or at least about  $5 \times 10^8$  colony-forming units per gram to at least about  $6 \times 10^{10}$  colony-forming units of *Methylobacterium* per gram,

at least about  $1 \times 10^{11}$  colony-forming units of *Methylobacterium* per gram, at least about  $1 \times 10^{12}$  colony-forming units of *Methylobacterium* per gram, at least about  $1 \times 10^{13}$  colony-forming units of *Methylobacterium* per gram, or at least about  $5 \times 10^{13}$  colony-forming units of *Methylobacterium* per gram of the solid phase. In certain embodiments, an amount of a composition provided herein that is sufficient to provide for increased corn yield and/or early vigor can be a composition with a *Methylobacterium* titer of at least about  $1 \times 10^6$  colony-forming units per gram, at least about  $5 \times 10^6$  colony-forming units per gram, at least about  $1 \times 10^7$  colony-forming units per gram, or at least about  $5 \times 10^8$  colony-forming units per gram to at least about  $6 \times 10^{10}$  colony-forming units of *Methylobacterium* per gram, at least about  $1 \times 10^{11}$  colony-forming units of *Methylobacterium* per gram, at least about  $1 \times 10^{12}$  colony-forming units of *Methylobacterium* per gram, at least about  $1 \times 10^{13}$  colony-forming units of *Methylobacterium* per gram, or at least about  $5 \times 10^{13}$  colony-forming units of *Methylobacterium* per gram of particles in the composition containing the particles that comprise a solid substance wherein a mono-culture or co-culture of *Methylobacterium* sp. is adhered thereto. In certain embodiments, an amount of a composition provided herein that is sufficient to provide for increased corn yield and/or early vigor can be a composition with a *Methylobacterium* titer of at least about  $1 \times 10^6$  colony-forming units per mL, at least about  $5 \times 10^6$  colony-forming units per mL, at least about  $1 \times 10^7$  colony-forming units per mL, or at least about  $5 \times 10^8$  colony-forming units per mL to at least about  $6 \times 10^{10}$  colony-forming units of *Methylobacterium* per mL in a composition comprising an emulsion wherein a mono-culture or co-culture of a *Methylobacterium* sp. adhered to a solid substance is provided therein or grown therein. In certain embodiments, an amount of a composition provided herein that is sufficient to provide for increased corn yield and/or early vigor can be a composition with a *Methylobacterium* titer of at least about  $1 \times 10^6$  colony-forming units per mL, at least about  $5 \times 10^6$  colony-forming units per mL, at least about  $1 \times 10^7$  colony-forming units per mL, or at least about  $5 \times 10^8$  colony-forming units per mL to at least about  $6 \times 10^{10}$  colony-forming units of *Methylobacterium* per mL of in a composition comprising an emulsion wherein a mono-culture or co-culture of a *Methylobacterium* sp. is provided therein or grown therein. In certain embodiments of any of the aforementioned compositions, the *Methylobacterium* sp. is *Methylobacterium* isolate ISO02 (NRRL B-50930), ISO03 (NRRL B-50931), ISO04 (NRRL B-50932), ISO11 (NRRL B-50939), or a derivative thereof. Also provided are corn plants and corn plant parts (e.g. seeds) that are coated or partially coated with any of the aforementioned compositions. Also provided are methods for improving corn yield or early corn vigor by using any of the aforementioned compositions.

### EXAMPLES

The following examples are included to demonstrate illustrative, non-limiting embodiments of the disclosure. It will be appreciated by those of skill in the art that the techniques disclosed in the following examples represent techniques discovered by the Applicants to function well in the practice of the invention. However, those of skill in the art should, in light of the instant disclosure, appreciate that many changes can be made in the specific embodiments that

are disclosed, while still obtaining like or similar results, without departing from the scope of the disclosure.

#### Example 1. Increases in Corn Yield by Application of *Methylobacterium* Compositions

Corn field trials were established at six Illinois, USA locations for the purpose of evaluating 14 PPFM (pink-pigmented-facultative-methylotrophs of the species *Methylobacterium*) isolates applied as a foliar spray to corn plants at an early vegetative stage (V3) and also at a reproductive stage (R1). The locations were established at Cropsey, Dana, Homer, Farmer City, Farmington and Homer, all in the state of Illinois. The trial at Cropsey experienced severe corn rootworm feeding damage during vegetative growth and the trial at Homer experienced greensnap breakage due to an early July straight line wind event; these two sites were thus not included in the corn foliar trial analysis.

#### Experimental Design

The trial was conducted as a split-plot design consisting of four 30-inch rows and were 20 feet long. The two middle rows were the treatment rows, the two outside rows were used as untreated border rows. There were eight replications of each of the 14 PPFM treatments for application at growth stages V3 and R1. The 14 PPFM treatments plus the control (no PPFM, also referred to as "check") comprised the whole plot, and the growth stage V3 and R1 comprised the split plot. There was a V3 and R1 control included in each of the 8 replications.

#### Methods

In preparation for the field trials, the PPFM cultures were grown in AMS+glycerol+peptone+diatomaceous earth, at 30° C. for 6 days. The ammonium mineral salts (AMS) medium contains, per liter, 700 milligrams of dibasic potassium phosphate anhydrous, 540 milligrams of monobasic potassium phosphate anhydrous, one gram of magnesium sulfate heptahydrate, 500 milligrams of ammonium chloride anhydrous, and 200 milligrams of calcium chloride dihydrate.

AMS base medium was prepared from three stock solutions, listed below:

Stock solution I: for one liter at 50× concentration  
dibasic potassium phosphate, anhydrous 35 grams  
monobasic potassium phosphate, anhydrous 27 grams

Stock solution II: for one liter at 50× concentration  
magnesium sulfate heptahydrate 50 grams  
ammonium chloride, anhydrous 25 grams

Stock solution III: for one liter at 50× concentration  
calcium chloride dihydrate 10 grams

Stock solutions I, II, and III were autoclaved separately.

To prepare one liter of liquid AMS medium with glycerol, peptone, and diatomaceous earth, the following were added to 920 ml of distilled water:

20 ml of stock solution I  
20 ml of stock solution II  
20 ml of stock solution III  
20 ml of a 50% glycerol stock solution  
10 grams of peptone  
2 grams of diatomaceous earth

The resulting solution with suspended diatomaceous earth was sterilized by autoclaving. The cultures were harvested by centrifugation at 5000 rpm for 15 minutes and then re-suspended in AMS+glycerol+peptone with 20% glycerol as a cryoprotectant at 10× concentration. The cultures were aliquoted and frozen at -80 until thawed for use. The liquid PPFM preparations were applied to the corn plants at the V3 or R1 stages at a rate of 15 gal per acre using a backpack

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chemical sprayer. Titers of the PPFMs applied at the various locations for both the R1 and V3 PPFM applications are provided in Tables 5 and 6, respectively. The trials were established within existing farmer field sites and were managed with local agronomic methods that the farmer practices throughout the growing season. All hybrids used were Roundup Ready™ hybrids, and the trials were sprayed with glyphosate at the V4 stage of growth. The trials were harvested for yield at physiological maturity with a commercial harvest combine. Table 2 indicates the hybrid planted, planting date and harvest date at the four corn foliar sites.

TABLE 2

Hybrid planted, planting date and harvest date at four corn foliar sites				
Location	Dana IL	Farmer City IL	Farmington IL	Pesotum IL
Hybrid	DuPont Pioneer™ P32V16 (Johnston, IA, USA)	AgriGold™ A6517VT3PRIB	Becks™ 5552	DuPont Pioneer™ P1319 (Johnston, IA, USA)
Planting date	May 14, 2013	May 14, 2013	May 15, 2013	May 13, 2013
Harvest date	Oct. 28, 2013	Oct. 19, 2013	Oct. 24, 2013	Oct. 21, 2013
Nitrogen applied	190 lbs/acre	220 lbs/acre	225 lbs/acre	156 lbs/acre
Fungicide applied at tassel stage	No fungicide applied	4 oz Stratego™ (Bayer CropScience, NC, USA)	Headline™ AMP 10 oz (BASF Crop Protection., NC, USA)	No fungicide applied

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random effect of the interaction of isolate i and replicate k nested within location h,  $LS_{hj}$  is the random effect of the interaction of location h and stage j,  $LIS_{hij}$  is the random effect of the three-way interaction of location h with isolate i and stage j, and  $e_{hijk}$  is the random error.

Thirteen of the 14 PPFM isolates showed a significant ( $p=0.25$ ) yield response vs the V3 or R1 check they were compared to in at least one location (Table 3). Only one location, Pesotum, showed no significant yield increase. Six isolates showed a significant increase vs the check across four combined locations at stage R1 (Table 4).

## Results

Analysis of variance (ANOVA) was conducted with the Analyze-Fit Model routine in JMP version 11.0 (SAS Institute). After the parameter estimates were obtained from the models, plots of residuals and tables of studentized residuals were examined for conformity with the assumptions of normality and constant variance. Comparisons of isolates with the check within the same growth stage at application were performed with two-tailed t-tests applied to the pairwise differences between least-squares means estimated from the ANOVA model, under the null hypothesis that the difference in means was zero.

The following model was applied to the split plot design at the four individual locations:

$$Y_{ijk} = M + I_i + S_j + IS_{ij} + R_k + IR_{ik} + e_{ijk}, \quad [1]$$

where  $Y_{ijk}$  is the yield of isolate i at stage j in replicate k, M represents the overall mean,  $I_i$  is the fixed effect of isolate i,  $S_j$  is the fixed effect of stage j,  $IS_{ij}$  is the fixed effect of the interaction of isolate i and stage j,  $R_k$  is the random effect of replicate k,  $IR_{ik}$  is the random effect of the interaction of isolate i and replicate k, and  $e_{ijk}$  is the random error.

Across-locations analyses for the four locations were conducted according to the following model:

$$Y_{hijk} = M + I_i + S_j + IS_{ij} + L_h + R(L)_{k(h)} + LI_{hi} + IR(L)_{ik(h)} + LIS_{hij} + e_{hijk}, \quad [2]$$

where  $Y_{hijk}$  is the yield at location h of isolate i at stage j in replicate k, M represents the overall mean,  $I_i$  is the fixed effect of isolate i,  $S_j$  is the fixed effect of stage j,  $IS_{ij}$  is the fixed effect of the interaction of isolate i and stage j,  $L_h$  is the random effect of location h,  $R(L)_{k(h)}$  is the random effect of replicate k nested within location h,  $LI_{hi}$  is the random effect of the interaction of location h and isolate i,  $IR(L)_{ik(h)}$  is the

TABLE 3

Mean yield, yield ranking, and p values of PPFM isolates at each of four locations by growth stage					
Location	PPFM Treatment	Stage of PPFM Applic.	Yield bu/acre	Rank	P value
Dana	ISO04	V3	215.6	1	0.129
Dana	ISO11	V3	215.4	2	0.134
Dana	ISO06	V3	212.3	3	0.282
Dana	ISO09	V3	204.8	4	0.936
Dana	ISO03	V3	204.3	5	0.993
Dana	Check	V3	204.2	6	—
Dana	ISO02	V3	204.1	7	0.984
Dana	ISO10	V3	203.3	8	0.904
Dana	ISO01	V3	202.8	9	0.848
Dana	ISO05	V3	201.5	10	0.711
Dana	ISO12	V3	201.2	11	0.681
Dana	ISO14	V3	200.7	12	0.157
Dana	ISO07	V3	200.3	13	0.596
Dana	ISO13	V3	198.9	14	0.474
Dana	ISO08	V3	196.9	15	0.326
Dana	ISO04	R1	213.2	1	0.114
Dana	ISO14	R1	212.0	2	0.156
Dana	ISO05	R1	205.6	3	0.767
Dana	ISO13	R1	205.4	4	0.589
Dana	ISO06	R1	205.3	5	0.600
Dana	ISO09	R1	205.0	6	0.630
Dana	ISO11	R1	205.0	7	0.631
Dana	ISO12	R1	204.1	8	0.719
Dana	ISO10	R1	203.1	9	0.819
Dana	ISO03	R1	201.6	10	0.985
Dana	Check	R1	201.4	11	—
Dana	ISO02	R1	199.2	12	0.769
Dana	ISO01	R1	198.0	13	0.648
Dana	ISO08	R1	196.3	14	0.494
Dana	ISO07	R1	194.5	15	0.351
F. City	ISO02	V3	265.0	1	0.201
F. City	ISO13	V3	264.6	2	0.221
F. City	ISO05	V3	258.3	3	0.790
F. City	ISO14	V3	257.6	4	0.868
F. City	ISO10	V3	257.1	5	0.937
F. City	ISO08	V3	256.7	6	0.986



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TABLE 3-continued

Mean yield, yield ranking, and p values of PPFM isolates at each of four locations by growth stage					
Location	PPFM Treatment	Stage of PPFM Applic.	Yield bu/acre	Rank	P value
F. City	Check	V3	256.6	7	—
F. City	ISO06	V3	256.2	8	0.957
F. City	ISO11	V3	255.2	9	0.835
F. City	ISO01	V3	252.7	10	0.561
F. City	ISO09	V3	249.8	11	0.309
F. City	ISO07	V3	249.8	12	0.304
F. City	ISO12	V3	249.2	13	0.268
F. City	ISO04	V3	248.4	14	0.218
F. City	ISO03	V3	246.5	15	0.129
F. City	ISO06	R1	260.0	1	0.012
F. City	ISO03	R1	259.9	2	0.013
F. City	ISO05	R1	259.5	3	0.015
F. City	ISO09	R1	258.6	4	0.022
F. City	ISO07	R1	257.3	5	0.036
F. City	ISO02	R1	256.8	6	0.043
F. City	ISO01	R1	256.0	7	0.057
F. City	ISO04	R1	255.6	8	0.064
F. City	ISO12	R1	254.8	9	0.084
F. City	ISO13	R1	254.0	10	0.106
F. City	ISO11	R1	253.6	11	0.122
F. City	ISO10	R1	251.6	12	0.215
F. City	ISO08	R1	248.5	13	0.441
F. City	Check	R1	243.4	14	—
F. City	ISO14	R1	240.7	15	0.688
Farmington	ISO11	V3	269.8	1	0.021
Farmington	ISO02	V3	266.9	2	0.048
Farmington	ISO06	V3	264.4	3	0.087
Farmington	ISO03	V3	260.5	4	0.201
Farmington	ISO10	V3	258.2	5	0.306
Farmington	ISO14	V3	258.0	6	0.319
Farmington	ISO13	V3	257.0	7	0.374
Farmington	ISO01	V3	256.1	8	0.428
Farmington	ISO12	V3	255.6	9	0.461
Farmington	ISO05	V3	255.2	10	0.489
Farmington	ISO04	V3	254.9	11	0.510
Farmington	ISO09	V3	253.5	12	0.620
Farmington	ISO07	V3	252.2	13	0.724
Farmington	ISO08	V3	250.3	14	0.883
Farmington	Check	V3	249.0	15	—
Farmington	ISO02	R1	267.5	1	0.067
Farmington	ISO01	R1	265.6	2	0.105
Farmington	ISO14	R1	260.9	3	0.271
Farmington	ISO07	R1	260.6	4	0.285
Farmington	ISO10	R1	257.5	5	0.463
Farmington	ISO13	R1	256.5	6	0.533
Farmington	ISO12	R1	254.2	7	0.717
Farmington	ISO05	R1	253.7	8	0.755
Farmington	ISO06	R1	252.5	9	0.864
Farmington	Check	R1	250.9	10	—
Farmington	ISO09	R1	250.3	11	0.946
Farmington	ISO04	R1	250.0	12	0.919
Farmington	ISO03	R1	247.1	13	0.675
Farmington	ISO11	R1	243.0	14	0.378
Farmington	ISO08	R1	239.9	15	0.220
Pesotum	Check	V3	177.8	1	—
Pesotum	ISO13	V3	176.0	2	0.853
Pesotum	ISO03	V3	168.0	3	0.316
Pesotum	ISO08	V3	167.2	4	0.277
Pesotum	ISO07	V3	167.0	5	0.269
Pesotum	ISO11	V3	166.8	6	0.262
Pesotum	ISO12	V3	166.6	7	0.254
Pesotum	ISO01	V3	166.0	8	0.229
Pesotum	ISO04	V3	166.0	9	0.228
Pesotum	ISO02	V3	165.7	10	0.218
Pesotum	ISO10	V3	164.6	11	0.177
Pesotum	ISO14	V3	163.5	12	0.144
Pesotum	ISO06	V3	162.6	13	0.122
Pesotum	ISO05	V3	161.7	14	0.100
Pesotum	ISO09	V3	159.6	15	0.064
Pesotum	ISO03	R1	175.2	1	0.479
Pesotum	ISO02	R1	172.4	2	0.676
Pesotum	ISO13	R1	170.3	3	0.842
Pesotum	ISO10	R1	169.7	4	0.891

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TABLE 3-continued

Mean yield, yield ranking, and p values of PPFM isolates at each of four locations by growth stage					
Location	PPFM Treatment	Stage of PPFM Applic.	Yield bu/acre	Rank	P value
Pesotum	ISO12	R1	169.7	5	0.892
Pesotum	ISO01	R1	169.7	6	0.892
Pesotum	ISO09	R1	169.0	7	0.943
Pesotum	ISO04	R1	168.9	8	0.953
Pesotum	Check	R1	168.3	9	—
Pesotum	ISO11	R1	166.3	10	0.833
Pesotum	ISO14	R1	166.1	11	0.815
Pesotum	ISO05	R1	165.8	12	0.797
Pesotum	ISO06	R1	158.2	13	0.298
Pesotum	ISO07	R1	154.0	14	0.144
Pesotum	ISO08	R1	146.3	15	0.025

TABLE 4

Mean yield, yield ranking, and p values of PPFM isolates across combined four locations by growth stage					
Location	PPFM Treatment	Stage	Yield bu/acre	Rank	P value
Across 4 locs.	ISO11	V3	226.8	1	0.244
Across 4 locs.	ISO02	V3	225.4	2	0.403
Across 4 locs.	ISO13	V3	224.1	3	0.596
Across 4 locs.	ISO06	V3	223.9	4	0.637
Across 4 locs.	Check	V3	221.9	5	—
Across 4 locs.	ISO04	V3	221.2	6	0.874
Across 4 locs.	ISO10	V3	220.8	7	0.795
Across 4 locs.	ISO14	V3	220.0	8	0.645
Across 4 locs.	ISO03	V3	219.8	9	0.624
Across 4 locs.	ISO01	V3	219.4	10	0.557
Across 4 locs.	ISO05	V3	219.2	11	0.519
Across 4 locs.	ISO12	V3	218.2	12	0.377
Across 4 locs.	ISO08	V3	217.8	13	0.328
Across 4 locs.	ISO07	V3	217.3	14	0.276
Across 4 locs.	ISO09	V3	216.9	15	0.240
Across 4 locs.	ISO02	R1	224.0	1	0.061
Across 4 locs.	ISO01	R1	222.3	2	0.136
Across 4 locs.	ISO04	R1	221.9	3	0.161
Across 4 locs.	ISO13	R1	221.6	4	0.188
Across 4 locs.	ISO05	R1	221.2	5	0.223
Across 4 locs.	ISO03	R1	221.0	6	0.242
Across 4 locs.	ISO09	R1	220.7	7	0.262
Across 4 locs.	ISO12	R1	220.7	8	0.268
Across 4 locs.	ISO10	R1	220.5	9	0.290
Across 4 locs.	ISO14	R1	219.9	10	0.355
Across 4 locs.	ISO06	R1	219.0	11	0.478
Across 4 locs.	ISO11	R1	217.0	12	0.821
Across 4 locs.	ISO07	R1	216.6	13	0.890
Across 4 locs.	Check	R1	216.0	14	—
Across 4 locs.	ISO08	R1	207.7	15	0.051

TABLE 5

Titers of PPFMs Applied at the R1 Stage at Indicated Locations (in CFU/mL)						
NLS #	Isolate	Cropsey Titer	Pesotum Titer	Farmer City Titer	Dana Titer	Farmington Titer
0046	ISO01	8.6E+08	5.6E+08	5.6E+08	8.6E+08	8.6E+08
0020	ISO02	1.2E+09	1.2E+09	1.2E+09	7.9E+08	1.2E+09
0017	ISO03	2.8E+08	5.7E+08	5.7E+08	2.8E+08	2.8E+08

TABLE 5-continued

Titers of PPFMs Applied at the R1 Stage at Indicated Locations (in CFU/mL)						
NLS #	Isolate	Cropsey Titer	Pesotum Titer	Farmer City Titer	Dana Titer	Farmington Titer
0042	ISO04	2.4E+08	1.4E+08	1.4E+08	2.4E+08	2.4E+08
0089	ISO05	6.7E+08	4.8E+08	6.7E+08	4.8E+08	6.7E+08
0068	ISO06	3.1E+08	2.6E+08	ND <sup>1</sup>	1.9E+08	2.6E+08
0065	ISO07	3.8E+08	3.7E+08	3.7E+08	3.8E+08	3.8E+08
0069	ISO08	2.0E+08	2.7E+08	2.7E+08	2.0E+08	2.0E+08
0062	ISO09	1.0E+08	2.9E+08	2.9E+08	5.5E+07	1.0E+08
0064	ISO10	8.9E+08	5.9E+08	5.9E+08	8.4E+08	9.5E+08
0021	ISO11	9.7E+07	1.2E+08	1.2E+08	9.7E+07	9.7E+07
0066	ISO12	5.6E+08	4.8E+08	4.8E+08	5.6E+08	5.6E+08
0037	ISO13	ND <sup>1</sup>	ND <sup>1</sup>	ND <sup>1</sup>	ND <sup>1</sup>	ND <sup>1</sup>
0038	ISO14	1.3E+08	1.3E+08	1.30E+08	1.3E+08	1.3E+08

<sup>1</sup>ND: Not determined.

TABLE 6

Titers of PPFMs Applied at the V3 Stage at Indicated Locations (in CFU/mL)							
NLS #	Isolate	Cropsey Titer	Pesotum Titer	Farmer City Titer	Homer Titer	Dana Titer	Farmington Titer
0046	ISO01	5.3E+08	4.2E+08	3.2E+08	4.2E+08	3.2E+08	5.3E+08
0020	ISO02	1.0E+09	9.8E+08	7.5E+08	1.0E+09	7.5E+08	9.8E+08
0017	ISO03	4.4E+08	4.8E+08	4.6E+08	3.1E+08	2.8E+08	4.3E+08
0042	ISO04	5.6E+08	3.9E+08	2.3E+08	2.2E+08	4.2E+08	2.8E+08
0089	ISO05	7.0E+07	4.8E+08	5.6E+08	4.2E+08	4.2E+08	3.4E+08
0068	ISO06	2.9E+08	2.9E+08	6.2E+08	6.2E+08	2.9E+08	6.2E+08
0065	ISO07	3.7E+08	2.4E+08	2.0E+08	2.0E+08	2.4E+08	2.0E+08
0069	ISO08	4.3E+08	1.9E+08	3.7E+07	3.7E+08	1.9E+08	3.7E+07
0062	ISO09	ND <sup>1</sup>	1.3E+08	ND <sup>1</sup>	ND <sup>1</sup>	1.1E+08	ND <sup>1</sup>
0064	ISO10	1.1E+09	9.3E+08	1.0E+09	5.6E+07	8.3E+08	8.9E+08
0021	ISO11	ND <sup>1</sup>	ND <sup>1</sup>	ND <sup>1</sup>	ND <sup>1</sup>	ND <sup>1</sup>	7.8E+07
0066	ISO12	2.9E+08	2.7E+08	3.0E+08	3.0E+08	2.7E+08	3.0E+08
0037	ISO13	1.5E+08	ND <sup>1</sup>	ND <sup>1</sup>	ND <sup>1</sup>	ND <sup>1</sup>	ND <sup>1</sup>
0038	ISO14	2.4E+08	1.4E+08	1.4E+08	1.4E+08	1.4E+08	1.4E+08

<sup>1</sup>ND: Not determined.

### Example 2. Increases in Corn Yield by Application of *Methylobacterium* Compositions in 2014 Field Tests

#### Experimental Design

Corn field trials were established at seven locations for the purpose of evaluating three PPFM (pink-pigmented-facultative-methylobacteria of the species *Methylobacterium*). Isolates were applied as a foliar spray to corn plants at an early vegetative stage (V3) and in furrow at planting. Foliar applications were made at five and 2.5-liters per acre. In furrow application were applied at seeding using 1.25-L and 0.625-L/acre. The field plots were established in Iowa, Illinois, Nebraska, Missouri, Ohio, South Dakota and Wisconsin. The trials were conducted using conventional row spacing (30 inches) with a minimal plot size of four rows by 20 feet. Each treatment was conducted using six replications in a Randomized Complete Block Design (unless otherwise noted). All observations were taken from center two rows of the plot. All destructive sampling was taken from outside two rows. Treatments were applied in-furrow at planting with a nozzle over the open seed furrow before covering. Straight stream nozzles or flat fan nozzles were adjusted so the fan pattern was parallel to the seed furrow. Foliar sprays

were applied with a conventional boom using flat fan or cone jet nozzles. A minimum of five gallons/acre total volume was used for the in-furrow applications and 15 gallons/acre for foliar applications. Early plant vigor was rated 20 and 60 days after emergence. Visual assessment of plant vigor was based on a 1 to 5 scale, with 1 being poor and 5 being excellent. The visual assessments of vigor considered factors such as height, leaf area, leaf color, and/or percent canopy closure. Plants were harvested per standard grower practices using a conventional metered combine.

#### Methods

In preparation for the field trials, the PPFM cultures were grown in AMS+glycerol+peptone+diatomaceous earth, at 30° C. for 6 days. The ammonium mineral salts (AMS) medium contains, per liter, 700 milligrams of dibasic potassium phosphate anhydrous, 540 milligrams of monobasic potassium phosphate anhydrous, one gram of magnesium sulfate heptahydrate, 500 milligrams of ammonium chloride anhydrous, and 200 milligrams of calcium chloride dihydrate.

AMS base medium was prepared from three stock solutions, listed below:

Stock solution I: for one liter at 50× concentration

dibasic potassium phosphate, anhydrous 35 grams

monobasic potassium phosphate, anhydrous 27 grams

Stock solution II: for one liter at 50× concentration

magnesium sulfate heptahydrate 50 grams

ammonium chloride, anhydrous 25 grams

Stock solution III: for one liter at 50× concentration

calcium chloride dihydrate 10 grams

Stock solutions I, II, and III were autoclaved separately.

To prepare one liter of liquid AMS medium with glycerol, peptone, and diatomaceous earth, the following were added to 920 ml of distilled water:

20 ml of stock solution I

20 ml of stock solution II

20 ml of stock solution III

20 ml of a 50% glycerol stock solution

10 grams of peptone

2 grams of diatomaceous earth

The resulting solution with suspended diatomaceous earth was sterilized by autoclaving.

The cultures were harvested by centrifugation at 5000 rpm for 15 minutes and then re-suspended in AMS+glycerol+peptone with 20% glycerol as a cryoprotectant at 10× concentration. The cultures were aliquoted and frozen at -80 until thawed for use. Trials were established within existing farmer field sites and were managed with local agronomic methods that the farmer practices throughout the growing season. Titer ranges for the different NLS strains used in the field sites were as follows: NLS0017=4.7×10<sup>8</sup>-2.2×10<sup>9</sup> CFU/mL; NLS0020=3.0×10<sup>8</sup>-3.1×10<sup>9</sup> CFU/mL; and NLS021=2.3×10<sup>8</sup>-3.7×10<sup>8</sup> CFU/mL.

#### Results

Analysis of variance (ANOVA), Tukey HSD, and mean separations using LSD were conducted with Statistix software, version 9.0. Initial Tukey HSD analysis were run at 90% confidence intervals evaluating all-pairwise comparisons tests of vigor and yield for treatment effects. At alpha equals 0.1 there were no significant pairwise differences among the means. However, when LSD all-pairwise com-

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parisons tests of vigor and yield were analyzed at alpha equals 0.20, (80% CI) several treatments demonstrated significantly better vigor and higher yield than the check across all locations by treatment.

TABLE 7

LSD All-Pairwise Comparisons Test of Vigor at 60 days after planting for TRT			
TRT	Mean	Homogeneous Groups	
Check	3.6042	D	
NLS017_L_Fur	3.7083	BCD	
NLS017_H_Fur	3.7292	ABC	8
NLS017_L_Fol	3.7606	AB	5
NLS017_H_Fol	3.7368	ABC	6
NLS020_L_Fur	3.8333	A	1
NLS020_H_Fur	3.7083	BCD	
NLS020_L_Fol	3.713	ABCD	
NLS020_H_Fol	3.7606	AB	3
NLS021_L_Fur	3.7292	ABC	7
NLS021_H_Fur	3.75	ABC	4
NLS021_L_Fol	3.713	ABCD	

Means followed by the same letter are not significantly different at alpha = 0.20.

"H" = 1.25-L acres in furrow treatment (Fur)

"L" = 0.625-L/acres in furrow treatment (Fur)

"H" = 5.0-L acres foliar treatment (Fol)

"L" = 2.5-L/acres foliar treatment (Fol)

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TABLE 8

LSD Pairwise Comparisons Test of Yield in Bushels per acre for TRT			
TRT	Mean	Homogeneous Groups	
Check	201.83	CD	
NLS017_L_Fur	201.74	CD	
NLS017_H_Fur	205.36	AB	3
NLS017_L_Fol	200.08	D	
NLS017_H_Fol	205.84	AB	2
NLS020_L_Fur	204.28	BC	
NLS020_H_Fur	202.79	BCD	
NLS020_L_Fol	203.63	BCD	
NLS020_H_Fol	203.59	BCD	
NLS021_L_Fur	207.95	A	1
NLS021_H_Fur	204.17	BC	
NLS021_L_Fol	203.14	BCD	
NLS021_H_Fol	203.74	BC	
14	201.99	BCD	

Means followed by the same letter are not significantly different at alpha = 0.20.

"H" = 1.25-L acres in furrow treatment (Fur)

"L" = 0.625-L/acres in furrow treatment (Fur)

"H" = 5.0-L acres foliar treatment (Fol)

"L" = 2.5-L/acres foliar treatment (Fol)

When evaluated by treatment using forced ranking, trends were visible that supported the vigor and yield data assessments.

TABLE 9

Corn yield analysis: Average bushels per acre across all locations.								
Corn	AgPro, IA	Rains, MO	Maloney, WI	CropSmith Farmers City, IL	CropSmith Homer, IL	Buckeye, OH	Auch, SD	AVG
Check (Avg)	174	211	155	260	227	199	152	197
AVG of Trts	175	225	158	249	225	200	156	202
NLS017_L_Fur	170	<b>222</b>	<b>158</b>	241	224	<b>202</b>	<b>152</b>	195
NLS017_H_Fur	<b>177</b>	<b>223</b>	<b>159</b>	252	<b>228</b>	<b>202</b>	<b>153</b>	<b>199</b>
NLS017_L_Fol	<u>167</u>	<b>227</b>	<b>155</b>	246	<u>227</u>	<u>186</u>		<b>201</b>
NLS017_H_Fol	<b>177</b>	<b>225</b>	<b>160</b>	258	227	199		<b>207</b>
NLS020_L_Fur	<b>177</b>	<b>221</b>	<b>156</b>	247	<b>229</b>	<b>204</b>	<b>156</b>	<b>199</b>
NLS020_H_Fur	<u>171</u>	<b>219</b>	<b>161</b>	248	<u>217</u>	<b>203</b>	<b>155</b>	196
NLS020_L_Fol	<b>179</b>	<b>225</b>	<b>156</b>	247	222	<b>201</b>		<b>205</b>
NLS020_H_Fol	<b>178</b>	<b>226</b>	<b>156</b>	254	222	<u>197</u>		<b>205</b>
NLS021_L_Fur	<b>180</b>	<b>227</b>	<b>160</b>	251	<b>228</b>	<b>210</b>	<b>159</b>	202
NLS021_H_Fur	<b>168</b>	<b>233</b>	<b>162</b>	253	<u>223</u>	<u>195</u>	<u>158</u>	199
NLS021_L_Fol	<u>173</u>	<b>230</b>	<b>157</b>	244	225	<b>202</b>		<b>205</b>
NLS021_H_Fol	<b>181</b>	<b>220</b>	<b>161</b>	245	227	<b>204</b>		<b>207</b>

Column Values in **Bold and Underlined** are Average Treatment Where Yields  $\geq$  Averaged Check

TABLE 7-continued

LSD All-Pairwise Comparisons Test of Vigor at 60 days after planting for TRT			
TRT	Mean	Homogeneous Groups	
NLS021_H_Fol	3.8082	AB	2
14	3.6132	CD	

Of the three PPFM isolates tested (NLS017, NLS020, and NLS021), two showed a significant (alpha=0.20) yield response when they were compared across all seven locations by these analyses. Isolate NLS021 gave the largest yield increase when applied at 625 ml/acre as an in furrow treatment. Isolate NLS017 delivered significant improvements in yield when applied at the high in-furrow (1,250 ml/acre) and foliar application rate (5-L/acre).

Yield data were also analyzed using the JMP statistical analysis software package (Version 9.0). The full model with all random effects was fit first and then reduced to the best fitting model based on Akaike information criterion (AIC) values. Across locations means comparisons were conducted using Fisher's LSD test with  $\alpha=0.05$ , 0.10, and 0.20 (Table 10). Raw means reported in Table 9 differ from adjusted means calculated by the mixed effects model used to analyze the data presented in Table 10. This model adjusted for random effects of location and replicate. As a result, statis-

tical differences do not necessarily reflect numerical differences in the raw yield values. Additionally, the approach to statistical analysis described in paragraph differs slightly from this approach, resulting in marginally different results between the two analyses. The analysis and results presented in Table 10 do not include a second check, 'Treatment 14' that was included at only four of the seven sites.

TABLE 10

Increase in yield over check across locations						
Number	Treatment	Mean <sup>1</sup> yield (bu/acre)	Yield > Check at $\alpha = 0.05$	Yield > Check at $\alpha = 0.10$	Yield > Check at $\alpha = 0.20$	Rank
1	Check	196.64				10
2	NLS017_L_Inf	195.42				12
3	NLS017_H_Inf	199.06				5
4	NLS017_L_Fol	194.01				13
5	NLS017_H_Fol	200.27			X	2
6	NLS020_L_Inf	198.54				6
7	NLS020_H_Inf	196.19				11
8	NLS020_L_Fol	197.81				9
9	NLS020_H_Fol	198.14				7
10	NLS021_L_Inf	202.18	X	X	X	1
11	NLS021_H_Inf	199.20				4
12	NLS021_L_Fol	197.86				8
13	NLS021_H_Fol	199.39				3

<sup>1</sup>least squares means

Treatment yield relative to the check for individual locations was analyzed in the same manner as the across locations data (Table 11). In the table below, yields significantly greater than the check at  $\alpha=0.05$ , 0.10, and 0.20 are represented by 'XXX,' 'XX,' and 'X,' respectively.

TABLE 11

Numerical increases in yield over check across locations						
Num- ber	Treatment	AgPro	Buck- eye	FC	Ma- loney	Rains Auch
1	Check					
2	NLS017_L_Inf					X
3	NLS017_H_Inf				X	XX
4	NLS017_L_Fol					XXX
5	NLS017_H_Fol				XXX	XXX
6	NLS020_L_Inf					X
7	NLS020_H_Inf				XXX	
8	NLS020_L_Fol					XXX
9	NLS020_H_Fol					XXX
10	NLS021_L_Inf		XXX	XXX	XXX	XXX X
11	NLS021_H_Inf				XXX	XXX
12	NLS021_L_Fol					XXX
13	NLS021_H_Fol			XXX	XXX	X

## Results

Of the three PPFM isolates tested (NLS017, NLS020, and NLS021), two showed a significant ( $\alpha=0.20$ ) yield response when they were compared across all seven locations. Isolate NLS021 gave the largest yield increase when applied at 625 ml/acre as an in furrow treatment. Isolate NLS017 delivered significant improvements in yield when applied at the high in-furrow (1,250 ml/acre) and foliar application rate (5-L/acre).

Individual location data indicate that PPFMs generally have a beneficial effect on corn yield. Farmers City, a site where PPFMs did not positively influence corn yield, had particularly high overall yields. This could indicate that Farmers City had an 'ideal' yield environment and suggests that the PPFM treatments used in these experiments offered yield protection and increased yield in the presence of

various biotic and/or abiotic stressors at other locations but did not significantly affect yield under the ideal growth conditions at Farmers City.

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- Having illustrated and described the principles of the present disclosure, it should be apparent to persons skilled in the art that the invention can be modified in arrangement and detail without departing from such principles.

Although the materials and methods of this invention have been described in terms of various embodiments and illustrative examples, it will be apparent to those of skill in the art that variations can be applied to the materials and methods described herein without departing from the concept, spirit and scope of the invention. All such similar substitutes and modifications apparent to those skilled in the art are deemed to be within the spirit, scope and concept of the invention as defined by the appended claims.

What is claimed is:

1. A method for improving corn plant yield wherein said method comprises:
  - i) spraying, immersing, imbibing, coating, or partially coating a corn plant, corn plant part, or corn seed with:
    - a) a composition comprising an essentially dry fermentation product having about 5% or less water content, wherein said fermentation product comprises a deposited *Methylobacterium* selected from the group consisting of NLS0017, deposited as NRRL B-50931; NLS0020, deposited as NRRL B-50930; NLS0021, deposited as NRRL B-50939; and NLS0042, deposited as NRRL B-50932; and
    - b) an agriculturally acceptable excipient selected from the group consisting of woodflours, clays, activated carbon, diatomaceous earth, fine-grain inorganic solids and calcium carbonate; or
  - ii) an agriculturally acceptable adjuvant selected from the group consisting of polyvinyl acetates, polyvinyl acetate copolymers, hydrolyzed polyvinyl acetates, polyvinylpyrrolidone-vinyl acetate copolymer, polyvinyl alcohols, polyvinyl alcohol copolymers, polyvinyl methyl ether, polyvinyl methyl ether-maleic anhydride copolymer, waxes, latex polymers, cellulose, methylcelluloses, hydroxy methylcelluloses, hydroxypropylcellulose, hydroxymethylpropylcelluloses, polyvinyl pyrrolidones, alginates, dextrans, malto-dextrans, polysaccharides, fats, oils, proteins, karaya gum, jaguar gum, tragacanth gum, polysaccharide gums, mucilage, gum arabics, shellacs,

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- vinylidene chloride polymers and copolymers, soybean-based protein polymers and copolymers, lignosulfonates, acrylic copolymers, starches, polyvinylacrylates, zeins, gelatin, carboxymethylcellulose, chitosan, polyethylene oxide, acrylamide polymers and copolymers, polyhydroxyethyl acrylate, methylacrylamide monomers, alginate, ethylcellulose, polychloroprene and syrups or mixtures thereof;
- 2) growing a corn plant from said corn plant, corn plant part, or corn seed; and
  - 3) harvesting at least one corn plant or corn plant part from said corn plant, wherein the *Methylobacterium* provides for increased yield of said corn plant or corn plant part in comparison to yield of said corn plant or corn plant part from a corn plant grown from an untreated corn plant, corn plant part, or corn seed.
2. The method of claim 1, wherein said clays and inorganic solids are selected from the group consisting of calcium bentonite, kaolin, china clay, talc, perlite, mica, vermiculite, silicas, quartz powder, montmorillonite and mixtures thereof.
  3. The method of claim 1, wherein said harvested plant part is corn seed.
  4. The method of claim 3, wherein said corn seed is harvested from a mature corn plant.
  5. The method of claim 1, wherein said method further comprises obtaining a processed food or feed composition from said harvested plant part.
  6. The method of claim 5, wherein said processed food or feed composition is a meal or a paste.
  7. The method of claim 1, wherein the composition comprises the *Methylobacterium* at a titer of about  $5 \times 10^8$  CFU/gm to about  $6 \times 10^{10}$  CFU/gm.
  8. The method of claim 1, wherein the composition further comprises one or more additional *Methylobacterium* strains.
  9. The method of claim 8, wherein the one or more additional *Methylobacterium* strains are selected from the group consisting of NLS0037, deposited as NRRL B-50941; NLS0038, deposited as NRRL B-50942; NLS0046, deposited as NRRL-B-50929; NLS0062, deposited as NRRL B-50937; NLS0064, deposited as NRRL B-50938; NLS0065, deposited as NRRL B-50935; NLS0066, deposited as NRRL B-50940; NLS0068, deposited as NRRL B-50934; NLS0069; deposited as NRRL B-50936; and NLS0089, deposited as NRRL B-50933.
  10. A composition comprising:
    - i) a population of corn seeds for planting;
    - ii) an agriculturally acceptable excipient selected from the group consisting of woodflours, clays, activated carbon, diatomaceous earth, fine-grain inorganic solids and calcium carbonate; or
 an agriculturally acceptable adjuvant selected from the group consisting of polyvinyl acetates, polyvinyl acetate copolymers, hydrolyzed polyvinyl acetates, polyvinylpyrrolidone-vinyl acetate copolymer, polyvinyl alcohols, polyvinyl alcohol copolymers, polyvinyl methyl ether, polyvinyl methyl ether-maleic anhydride copolymer, waxes, latex polymers, celluloses, methylcelluloses, hydroxy methylcelluloses, hydroxypropylcellulose, hydroxymethylpropylcelluloses, polyvinyl pyrrolidones, alginates, dextrans, malto-dextrans, polysaccharides, fats, oils, proteins, karaya gum, jaguar gum, tragacanth gum, polysaccharide gums, mucilage, gum arabics, shellacs, vinylidene chloride polymers and copolymers, soybean-based protein polymers and copolymers, lignosulfonates, acrylic copolymers, starches, polyvinylacrylates, zeins, gelatin, carboxym-

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ethylcellulose, chitosan, polyethylene oxide, acrylamide polymers and copolymers, polyhydroxyethyl acrylate, methylacrylamide monomers, alginate, ethylcellulose, polychloroprene and syrups or mixtures thereof; and

iii) an essentially dry fermentation product having about 5% or less water content, wherein said fermentation product comprises a deposited *Methylobacterium* selected from the group consisting of NLS0017, deposited as NRRL B-50931; NLS0020, deposited as NRRL B-50930; NLS0021, deposited as NRRL B-50939; and NLS0042, deposited as NRRL B-50932; wherein said fermentation product is essentially free of contaminating microorganisms.

11. The composition of claim 10, wherein said essentially dry fermentation product is obtained by lyophilization or spray drying.

12. The composition of claim 10, wherein said clays and inorganic solids are selected from the group consisting of calcium bentonite, kaolin, china clay, talc, perlite, mica, vermiculite, silicas, quartz powder, montmorillonite and mixtures thereof.

13. The composition of claim 10, wherein said composition further comprises an agriculturally acceptable adjuvant selected from the group consisting of a wetter/spreader, sticker, penetrant, extender, and humectant.

14. The composition of claim 10, wherein said *Methylobacterium* is at a titer of about  $1 \times 10^6$  CFU/gm to about  $1 \times 10^{14}$  CFU/gm.

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15. The composition of claim 10, wherein said composition comprises at least two strains of *Methylobacterium* selected from the group consisting of: NLS0017, deposited as NRRL B-50931; NLS0020, deposited as NRRL B-50930; NLS0021, deposited as NRRL B-50939; and NLS0042, deposited as NRRL B-50932.

16. The composition of claim 10, wherein the composition further comprises an active selected from the group consisting of an insecticide, a nematocide, a fungicide, and a biopesticidal or otherwise beneficial microbe.

17. The composition of claim 16, wherein said biopesticidal or otherwise beneficial microbe is selected from the group consisting of *Bacillus subtilis*, *Bacillus thuringiensis*, *Bacillus pumilis*, *Pseudomonas syringae*, *Trichoderma harzianum*, *Trichoderma vixens*, and *Streptomyces lydicus*.

18. The composition of claim 10, which further comprises one or more additional *Methylobacterium* strains.

19. The composition of claim 18, wherein the one or more additional *Methylobacterium* strains are selected from the group consisting of NLS0037, deposited as NRRL B-50941; NLS0038, deposited as NRRL B-50942; NLS0046, deposited as NRRL B-50929; NLS0062, deposited as NRRL B-50937; NLS0064, deposited as NRRL B-50938; NLS0065, deposited as NRRL B-50935; NLS0066, deposited as NRRL B-50940; NLS0068, deposited as NRRL B-50934; NLS0069, deposited as NRRL B-50936; and NLS0089, deposited as NRRL B-50933.

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