(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property Organization
International Bureau

(43) International Publication Date
17 February 2022 (17.02.2022)

WIPO | PCT

(10) International Publication Number
WO 2022/032382 A1

(51) International Patent Classification:
C12N 1/20 (2006.01) *A01P 1/00* (2006.01)
A01N 63/22 (2020.01) *A01P 21/00* (2006.01)

(21) International Application Number:
PCT/CA2021/051105

(22) International Filing Date:
10 August 2021 (10.08.2021)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:
63/063,649 10 August 2020 (10.08.2020) US

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(81) Designated States *(unless otherwise indicated, for every kind of national protection available)*: AE, AG, AL, AM, AO, AT, AU, AZ, BA, BB, BG, BH, BN, BR, BW, BY, BZ, CA, CH, CL, CN, CO, CR, CU, CZ, DE, DJ, DK, DM, DO, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, GT, HN, HR, HU, ID, IL, IN, IR, IS, IT, JO, JP, KE, KG, KH, KN, KP, KR, KW, KZ, LA, LC, LK, LR, LS, LU, LY, MA, MD, ME, MG, MK, MN, MW, MX, MY, MZ, NA, NG, NI, NO, NZ, OM, PA, PE, PG, PH, PL, PT, QA, RO, RS, RU, RW, SA, SC, SD, SE, SG, SK, SL, ST, SV, SY, TH, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, WS, ZA, ZM, ZW.

(84) Designated States *(unless otherwise indicated, for every kind of regional protection available)*: ARIPO (BW, GH, GM, KE, LR, LS, MW, MZ, NA, RW, SD, SL, ST, SZ, TZ, UG, ZM, ZW), Eurasian (AM, AZ, BY, KG, KZ, RU, TJ, TM), European (AL, AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HR, HU, IE, IS, IT, LT, LU, LV, MC, MK, MT, NL, NO, PL, PT, RO, RS, SE, SI, SK, SM, TR), OAPI (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, KM, ML, MR, NE, SN, TD, TG).

Declarations under Rule 4.17:
— *as to applicant's entitlement to apply for and be granted a patent (Rule 4.17(ii))*
— *as to the applicant's entitlement to claim the priority of the earlier application (Rule 4.17(iii))*

Published:
— *with international search report (Art. 21(3))*
— *with (an) indication(s) in relation to deposited biological material furnished under Rule 13bis separately from the description (Rules 13bis.4(d)(i) and 48.2(a)(viii))*
— *with sequence listing part of description (Rule 5.2(a))*
— *in black and white; the international application as filed contained color or greyscale and is available for download from PATENTSCOPE*

(54) Title: PLANT GROWTH PROMOTING BACTERIA

(57) Abstract: A novel bacteria strain Bacillus velezensis *ABN1001* was deposited at the International Depositary Authority of Canada. The bacteria, or mutants thereof, or metabolites produced by the bacteria, can be used in compositions for controlling plant disease, treating plant disease, and/or promoting plant growth.

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PLANT GROWTH PROMOTING BACTERIA

[0001] This application claims priority to US Provisional Application No. 63/063,649, filed on August 10, 2020, which is incorporated herein by reference in its entirety.

TECHNICAL FIELD

[0002] The present invention relates to a novel bacteria for promoting plant growth and for use as biopesticide.

BACKGROUND OF THE ART

[0003] There is a need for improvements in agriculture productivity because of a growing world population. The agriculture field is under pressure to produce more from less land. Pests in the present application refer to microorganisms that are a major cause for the loss of productivity in agriculture around the world. In the last forty years, synthetic chemical pesticides for pest control have been responsible for the increase in food production and productivity. However, the use of such chemicals is not a sustainable option for the future of agriculture.

[0004] The United States Environmental Protection Agency (EPA) defines biopesticides as a substance, or microorganisms, or a pesticidal substance produced by plants containing added genetic material, that control pests. Biopesticides are generally safer, more biodegradable, and can be less expensive to develop than synthetic chemical pesticides. In addition, pathogens have shown their capacity to develop resistance to synthetic chemical pesticides. There remains concerns over the adverse effects of synthetic pesticides on the environment and on human health. Due to the issues regarding the safety and sustainability of synthetic chemical pesticides, the field of agriculture is looking for alternatives such as biopesticides.

[0005] Many of the currently available biopesticides only target a simple major pest. Contans®, based on the fungus *Coniothyrium minitans*, targets only a single pathogen genus: *Sclerotinia*. Bioshield™, based on the bacterium *Serratia entomophila*, controls only a single insect pest. Other biopesticides on the market protect against multiple pests. Serenade®, provides protection for multiple fungal diseases. Chontrol® and Sarritor®, based on *Chondrosterum purpureum* and *Sclerotinia minor* respectively, target multiple weed species.

[0006] *Bacillus* bacteria have been investigated for their pathogenic relationships in plant disease protection (Castagnola, A.; Stock, S.P. Common virulence factors and tissue targets of entomopathogenic bacterial for biological control of Lepidopteran pests. Insects

2014, 5, 139–166). In fact, United States patents 7,094,592 and 6,077,506 relate to novel bacteria of the *Bacillus* genus, respectively *Bacillus* sp. D747 and *Bacillus thuringiensis* AQ52. *Bacillus* sp. D747 was identified to be a strain that exhibits effects of controlling several varieties of plant disease and pests without harming plant growth. *Bacillus thuringiensis* AQ52 was identified to be a novel antibiotic producing strain that demonstrates broad fungicidal and bactericidal activity.

[0007] Plant-growth-promoting rhizobacteria (PGPR) are known to be an efficient and environment-friendly alternative to chemical pesticides and fertilizers. Endospore forming bacilli are PGPRs that demonstrate similar long term stability to that of agrochemicals. The endospore forming bacilli are therefore performant biofertilizers. For example, *Bacillus amyloliquefaciens* FZB42 and has been commercialized by ABiTEP Gmbh as biofertilizer.

SUMMARY OF THE INVENTION

[0008] In one aspect of the present invention, there is provided a novel bacterial strain *Bacillus velezensis ABN1001*.

[0009] In accordance with another aspect of the present invention, there is provided an agent for controlling plant disease comprising *Bacillus velezensis ABN1001* bacteria, spores or metabolites obtained from a conditioned culture media of *Bacillus velezensis ABN1001*.

[0010] In accordance with another aspect of the present invention, there is provided an agent for promoting plant growth comprising *Bacillus velezensis ABN1001* bacteria, spores or metabolites obtained from a conditioned culture media of *Bacillus velezensis ABN1001*.

[0011] In accordance with another aspect of the present invention, there is provided a method to control plant disease using an agent comprising *Bacillus velezensis ABN1001* bacteria, spores or metabolites obtained from a conditioned culture media of *Bacillus velezensis ABN1001*.

[0012] In accordance with another aspect of the present invention, there is provided a method to promote plant growth using an agent comprising *Bacillus velezensis ABN1001* bacteria, spores or metabolites obtained from a conditioned culture media of *Bacillus velezensis ABN1001*.

[0013] Many further features and combinations thereof concerning the present improvements will appear to those skilled in the art following a reading of the instant disclosure.

DEFINITIONS

[0014] The term “whole broth culture” refers to a solution of liquid culture comprising both cells and media. The term “supernatant” or “conditioned culture media” refers herein to a culture media used to culture a bacteria but from which the bacteria and spores have been removed.

[0015] The term “biopesticide” refers herein to a pesticide containing a microorganism. The term “pathogen” refers to microorganisms that are harmful for plants.

DETAILED DESCRIPTION OF THE INVENTION

[0016] The strain *Bacillus velezensis* ABN1001 was isolated from young corn plant (10 to 17 after planting) The root sample was subjected to vigorous shaking to remove most of the freely attached soil (although soil was still noticeably present). The root sample was then shaken in sterile water to solubilize the bacteria on the root surface (and the closely-associated rhizosphere). The solution was then subjected to serial dilutions with sterile water and then pasteurized (80°C for 20 minutes) in order to make spore-forming bacteria the predominant type in the collection, but not to necessarily eliminate all others. The dilutions were then spread-plated for colony selection at 2 and again at 5 days. ABN1001 was singled out of 10-100 colonies . The cultures from the initial colony selection was maintained at -80ºC in tryptic soy broth amended with 30% glycerol.

[0017] An initial growth promotion assay was performed to quickly screen the bacterial strains. The initial growth promotion assay consisted of a quick screen on soy bean in a greenhouse. Five replicate plants were treated with a 10^7 cell suspension of the overnight bacterial growth in water and compared to five replicate controls treated with water only. Parameters were measured after about three to five weeks depending on the season the assay is performed in. The initial growth promotion assay demonstrated that *Bacillus velezensis ABN1001* promotes plant growth.

[0018] Molecular identification of *Bacillus velezensisBacillus velezensis ABN1001* was performed by sequencing the target genes of *Bacillus* that are known to be plant-growth promoting: *ssu*16s, *gyrA, gyrB, phoR, groEL, purH, rpoB* and *polC*. The sequences were analyzed using the software Basic Local Alignment Search Tool (BLAST).

[0019] The target gene *ssu*16s was found to have sequence identity to *ssu*16s of *Bacillus amyloliquefaciens* strain YP6, *Bacillus amyloliquefaciens* strain BA17 and *Bacillus*

velezensis strain MH25. The target gene sequence *ssu*16s obtained from *Bacillus velezensis ABN1001* is the following:

```
gatgcgtagc cgacctgaga gggtgatcgg ccacactggg actgagacac ggcccagact 60
cctacgggag gcagcagtag ggaatcttcc gcaatggacg aaagtctgac ggagcaacgc 120
cgcgtgagtg atgaaggttt tcggatcgta aagctctgtt gttagggaag aacaagtgcc 180
gttcaaatag ggcggcacct tgacggtacc taaccagaaa gccacggcta actacgtgcc 240
agcagccgcg gtaatacgta ggtggcaagc gttgtccgga attattgggc gtaaagggct 300
cgcaggcggt ttcttaagtc tgatgtgaaa gcccccggct caaccgggga gggtcattgg 360
aaactgggga acttgagtgc agaagaggag agtggaattc cacgtgtagc ggtgaaatgc 420
gtagagatgt ggaggaacac cagtggcgaa ggcgactctc tggtctgtaa ctgacgctga 480
ggagcgaaag cgtggggagc gaacaggatt agataccctg gtagtccacg ccgtaaacga 540
tgagtgctaa gtgttagggg gtttccgccc cttagtgctg cagctaacgc attaagcact 600
ccgcctgggg agtacggtcg                                           620
```

[0020] The target gene *gyrA* was found to have sequence identity to *Bacillus velezensis* strain JT3-1, *Bacillus velezensis* strain ZF2 and *Bacillus velezensis* strain LDO2. The target gene sequence *gyrA* obtained from *Bacillus velezensis ABN1001* is the following:

```
atgagcgtta tcgtatcccg ggcgcttccg gatgtgcgtg acggtctgaa gccggttcac 60
aggcggattt tgtacgcaat gaatgattta ggcatgacca gtgacaaacc atataaaaaa 120
tctgcccgta tcgtcggtga agttatcggt aagtaccacc cgcacggtga ctcagcggtt 180
tacgaatcaa tggtcagaat ggcgcaggat tttaactacc gctacatgct tgttgacgga 240
cacggcaact tcggttcggt tgacggcgac tcagcggccg cgatgcgtta cacagaagcg 300
agaatgtcaa aaatcgcaat ggaaatcctc cgggacatta cgaaagatac gattgattat 360
caagataact atgacggcgc agaaagagaa cctgtcgtca tgccttcgag atttccgaat 420
ctgctcgtaa acggagctgc cggtattg                                   448
```

[0021] The target gene *gyrB* was found to have sequence identity to *Bacillus velezensis* strain JT3-1, *Bacillus velezensis* strain ZF2 and *Bacillus velezensis* strain A2. The target gene sequence *gyrB* obtained from *Bacillus velezensis ABN1001* is the following:

```
gacggaaaaa aaaactatca ggcgtacgag cgcggtgtac ctgtggccga tcttgaagtg 60
atcggtgata ctgataagac cggaacgatt acgcacttcg ttccggatcc ggaaatttttc 120
aaagaaacaa ccgaatacga ctatgacctg ctttcaaacc gtgtccggga attggccttc 180
ctgacaaaag gtgtaaacat cacgattgaa gacaaacgtg aaggacaaga acggaaaaac 240
gagtaccact acgaaggcgg aatcaaaagc tatgttgagt acttaaaccg ttccaaagaa 300
gtcgttcatg aagagccgat ttatatcgaa ggcgagaaag acggcataac ggttgaagtt 360
gcattgcaat acaacgacag ctatacaagc aatatttatt ctttcacaaa taatatcaac 420
acatacgaag gcggcacgca cgaagccgga tttaaaaccg gtctgacccg tgttataaac 480
gactatgcaa gaagaaaagg gattttcaaa ga                               512
```

[0022] The target gene *phoR* was found to have sequence identity to *Bacillus velezensis* strain JT3-1, *Bacillus velezensis* strain ZF2 and *Bacillus velezensis* strain LDO2. The target gene sequence *phoR* obtained from *Bacillus velezensis ABN1001* is the following:

[0023]

```
ttttttaatt tccgtcatat cgtgaaagac gaggacgatg cccttccatt catcgtcggg 60
gcccatgatc ggaacgccgt caacttcaaa gtagcgccgc tcaatattga tgggaagtct 120
gagcaactgg catttttttcg tctccgtcat aaaaatgtct tcgacaagcc gaatgatctc 180
ctcatgttca aacgcatcat ggtaaaggcg ccgaagcaga cgttcaggct ttgtatgaaa 240
ctgcttcgta tatgaccggt tcacgagatt gataaaacct ctcccgtcta ttaaaatcaa 300
accggatccg atattttcaa taacggttag cagacgatcg cgctgcatat cctgcgttct 360
cgtcatttcc attaaatcga ccgccaggct gttcatcgca cgcccgagac ggtccgaccg 420
ccttgcgtag ccgctgtagg agcgggcgtc gtaattccct ttggacagct ccgtcgccac 480
ctttgtcgcg gcgtcgattg attttttgta acgggacgtc atgtttgtat aaaagaaaac 540
gataatgata aaagcggtac aaagacttgc tgccagcatg ccccacattt ctccggtaac 600
acttgaaccg ccgttgatct cagaggaaac gagtacatac cccgcaattt tcccggcgtc 660
atttttacg gcagtgccgc ggatgacttt gtttttcg                          698
```

[0024] The target gene *groEL* was found to have sequence identity to *Bacillus velezensis* strain JT3-1, *Bacillus velezensis* strain ZF2 and *Bacillus velezensis* strain LDO2. The target gene sequence *groEL* obtained from *Bacillus velezensis ABN1001* is the following:

```
tccttacatg gtgactgact ctgataagat ggaagcggtt cttgacaatc cttacatctt 60
aatcacagac aaaaaaatca caaacattca agaaatcctt cctgtgcttg agcaagttgt 120
acagcaaggc aaaccattgc ttctgatcgc tgaagatgtt gaaggggaag ctcttgctac 180
actcgttgtc aacaaacttc gcggcacatt caacgctgtt gccgttaaag ctcctggctt 240
cggtgaccgc cgtaaagcaa tgcttgaaga catctctgtt cttacaggcg gagaagtgat 300
cacagaagac ttaggccttg acctgaaatc tactgaaatc ggacaattgg gacgcgcttc 360
taaagttgtg gtaacgaaag aaaacacaac aatcgtagaa ggcgccggcg acactgaaaa 420
aattgctgca cgcgtcaacc aaatccgcgc tcaagtggaa gaaacaactt ctgaattcga 480
cagagaaaaa ttacaagagc gtcttgcgaa acttgccggc ggcgtagctg tcatcaaagt 540
cggcgctgcg actgaaactg agctgaaaga gcgtaaactt cgcatcgaag acgccctcaa 600
ctcaactcgc gcagctgttg aagaaggcat cgtatccggc ggtggtacag cgcttgtcaa 660
cgtatacaac aaagtcgctg cagtggaagc tgaaggcgat gcgcaaacag gtatcaacat 720
tgtgcttcgc gcgcttgaag agccgat                                     747
```

[0025] The target gene *purH* was found to have sequence identity to *Bacillus velezensis* strain JT3-1, *Bacillus velezensis* strain ZF2 and *Bacillus velezensis* strain LDO2. The target gene sequence *purH* obtained from *Bacillus velezensis ABN1001* is the following:

```
ttgcccccat tgaccttgtg gtcgtcaacc tttacccgtt taaagaaacg atttcaaaag 60
aagacgtaac atacgatgaa gcgatagaaa acattgatat cggcggtccc ggcatgctgc 120
gcgccgcctc gaaaaaccat caggatgtga cggtcatcac agatccggcc gattacagtt 180
ccgtgctcaa tgagattaaa gaacacggcg gcgtttctct taaaagaaaa cgcgagcttg 240
cggccaaagt attccgccat accgcggcat acgacgcatt aatcgctgat tacttaacac 300
gcgaggccga tgagaaagac cctgagcaat tcaccgttac atttgagaaa aaacaatcgc 360
tccgctacgg tgaaaaccct caccaagagg cggttttcta ccaaagcgca cttcccgtct 420
ccggttccat cgcggcggca aaacagcttc acggcaaaga gctttcttac aacaatatta 480
aggacgcaga tgcggccgtt caaatcgtcc gggaatttac agaacccgca gctgttgccg 540
ttaaacatat gaacccgtgc ggagtcggta cgggagcttc aattgagaag cattcaataa 600
agcgtatgaa gctgataaac ctccattttc gcggcatcat cgcgctgaac cgtgagttga 660
tcagcacggc tgagcccttc acggcatctt ttagaat                          697
```

[0026] The target gene *rpoB* was found to have sequence identity to *Bacillus velezensis* strain JT3-1, *Bacillus velezensis* strain ZF2 and *Bacillus velezensis* strain LDO2. The target gene sequence *rpoB* obtained from *Bacillus velezensis ABN1001* is the following:

```
cacgtgatac aaagcttggg cctgaagaga tcacccgcga tattccaaac gtaggggaag 60
acgcgcttcg caatcttgat gaccgcggaa ttatccgtat cggtgcggaa gtcaacgacg 120
gagaccttct cgtaggtaaa gtaacgccta aaggtgtaac tgagcttacg gctgaagaac 180
gccttctgca tgcgatcttt ggagaaaaag cgcgtgaagt ccgtgatact tctctccgtg 240
tgcctcacgg cggcggcgga attatccacg acgtaaaagt cttcaaccgt gaagacggcg 300
acgaacttcc tccgggagtg aaccagcttg tacgcgtata tatcgttcag aaacgtaaga 360
tttctgaagg tgataaaatg gccggacgtc acggaaacaa aggggttatc tcgaagattc 420
ttcctgaaga agatatgcct taccttcctg acggcacgcc gatcgatatc atgcttaacc 480
cgctgggtgt accatcacgt atgaatatcg gtcaggtatt agaacttcac atgggtatgg 540
ctgcccgcta cctcggcatt cacatcgcgt cacctgtatt tgacggcgcg cgtgaagaag 600
atgtgtggga aacacttgaa gaagcaggca tgtcaagaga cgctaaaaca gttctttatg 660
acggccgtac gggagaaccg tttgacaacc gtgtatctgt cggaatcatg tacatgatca 720
aactggcgca catggttgat gataaacttc atgcccg                          757
```

[0027] Finally, the target gene *polC* was found to have sequence identity to *Bacillus velezensis* strain JT3-1, *Bacillus velezensis* strain ZF2 and *Bacillus velezensis* strain LDO2. The target gene sequence *polC* obtained from *Bacillus velezensis ABN1001* is the following:

```
tcttttgaac ggaaaaagcg aaaaatccgg tcattgatac gctggaactc gcgcgtttcc 60
tgtatcctga gtttaaaaat caccgcttaa atacgttatg taagaagttt gatatcgaat 120
taacccagca tcaccgagcg gtctttgacg ctgaagcaac gggctacctg ctgttgaaaa 180
tgctcaaaga tgccgctgaa aaagacattt tttatcatga tcagctgaat gagaatatgg 240
gacaatccaa tgcttatcag agatcaagac cttatcacgc tacattgctt gccgtgaatg 300
agaccggcct taaaaatctg tttaagctcg tgtccatttc tcatattcaa tatttctaca 360
gagtgccgcg cattccgagg tcgcagctta ataaatacag agaaggtctg ttaatcggct 420
ctgcctgtga caggggtgag gtctttgaag gcatgatgca aaaatctcct gaagaggttg 480
aagatatcgc atccttctat gattatcttg aagtgcagcc gccggaagta tacagacacc 540
ttctgcagct tgagctcgtc cgagatgaaa aagcgctgaa agaaatcatc gccaacatta 600
cgaagctcgg agaaaaattg aataagccgg tcgtggctac gggaaatgtc cactatttaa 660
acgatgagga taaaatttac cggaagatct taatatcttc ccaaggcggc gccaacccgt 720
taaacagaca cgaactgcct aaagtgcact tcagaacgac agacgaaaaa tgctttgaaa 780
a                                                                 781
```

[0028] The molecular identification revealed that ABN1001 is a novel bacteria of the genus *Bacillus* and was named *Bacillus velezensis ABN1001*.

[0029] The morphology of *Bacillus velezensis ABN1001* was compared to *Bacillus velezensis* and *Bacillus amyloliquefaciens*. This approach revealed rapid and abundant growth of *Bacillus velezensis ABN1001* in aerobic conditions (about 18h) and slow growth in anaerobic conditions (about 72 to 96h) on agar Brain Heart Infusion (BHI). *Bacillus velezensis ABN1001* was found to be mobile in wet mount. *Bacillus velezensis ABN1001* is gram positive, has colonies that are of medium size (about 3mm on BHI agar after 24 hr), have an irregular shape, have a white cream color, have a convex shape, and are mucoid.

It was further identified that *Bacillus velezensis ABN1001* is a sporulating strain (endospores).

[0030] *Bacillus velezensis ABN1001* grows on cereus selective agar (CSA) media (meat peptone 10.0, meat extract 1.0, D(-)-mannitol 10.0,s chloride 10.0, phenol red 0.025, agar 12.0, final pH 7.1 +/- 0.2 at 25°C) and it fermented mannitol.

[0031] The freezing protocol consist of starting with a pure culture on a rich agar medium such as BHI agar. Two colonies are used to inoculate a tube containing steril BHI broth and growth for 16-20 hours at 28°C. One ml of the culture is mixed with 1ml of steril glycerol 30% and let at room temperature for 15 min. 1.8 ml is transferred in a 2 ml cryogenic screwable tube and stored at minus 80°C.

[0032] In order to grow back the bacteria from freezing, the tube (or a portion of the tube content taken aseptically) is thawed at room temperature and transferred in a rich medium such as BHI broth or stricken onto a rich agar medium such as BHI agar. The culture is grown overnight at 28°C. Plants can be administered a culture of *Bacillus velezensis ABN1001*, a bacterial culture thereof supplemented with other ingredients, a pure bacteria isolated from the bacteria culture, a conditioned culture media or an antifungal or antibacterial metabolite produced by *Bacillus velezensis ABN1001* in culture (isolated from a conditioned culture media). The plant can be treated directly for example on the roots, stems, leaves, seeds, or into the soil in the vicinity of the plant to be treated.

[0033] The agents of the present invention for controlling plant disease and for promoting plant growth comprise the strain of the present invention *Bacillus velezensis ABN1001*. The strain can be utilised alone or in combination with one or more variants of ABN1001. The variants include but are not limited to spontaneous mutant strains, mutant strains obtained by ultra-violet or chemical mutagen treatment, cell fusion strains, and genetic recombination strains. The culture can be used to create formulations in which the strain is diluted with at least one of an inert liquid or solid carrier, a surfactant, protective agents, and other auxiliary agents if necessary.

[0034] The agents of the present invention for controlling plant disease and for promoting plant growth can be utilised alone or in combination with one or more plant growth-promoting bacteria such as *Bacillus amyloliquefaciens* strains D747, QST713, GB03, MBI600, FZB24, or FZB42, or *Bacillus pumilus* strains INR7 (also known as GB34) or QST2808.

[0035] The agents of the present invention for controlling plant disease and for promoting plant growth can comprise for example wettable powders, dry flowables, microencapsulation agents, liquid or solid formulations, antibiotic extracted from microbial cultures, whole broth culture, conditioned culture media, granules, suspensions, or emulsifiable concentrate. Biopesticides may be applied in combination with one or more chemical pesticide, herbicide, or fungicide.

[0036] As known in the art, carriers for the agent of the present invention can comprise, for example, one of porous solid carriers such as talc, bentonite, clay, kaolin, diatomaceous earth, white carbon, vermiculite, slaked lime, siliceous sand, ammonium sulfate, and urea. Liquid carriers can, for example, be one of water, isopropyl alcohol, xylene, cyclohexanone, methylnaphthalene, and alkyl glycol.

[0037] As known in the art surfactants and dispersants for the agent of the present invention can comprise, for example, one of dinaphthylmethanesulfonates, alcohol sulfates, alkyl aryl sulfonates, lignin sulfonates, polyoxyethylene glycol ethers, polyoxyethylene alkyl aryl ethers, and polyoxyethylene sorbitan monoalkylates.

[0038] As known in the art auxiliary agents for the agent of the present invention can comprise, for example, one of carboxymethylcellulose, polyethylene glycol, propylene glycol, gum Arabic, and xanthan gum.

[0039] As known in the art auxiliary agents for the agent of the present invention can further comprise, for example, skim milk or pH buffers.

[0040] The method to apply biopesticides or biofertilizers on plants is well known in the art. For example, *Bacillus velezensis* ABN1001, or a composition containing same, can be applied in the form of wettable powders, dry flowables, microencapsulation of agents, liquid or solid formulations, antibiotic extracted from microbial cultures, whole broth culture, granules, suspensions, or emulsifiable concentrate. It may also be applied in combination with one or more chemical pesticide, herbicide, or fungicide.

EXAMPLE 1

Protection against plant disease

[0041] Acetoin is a compound that confers plant immunity against a wide range of diseases by activating plant defences against pathogens (Rudrappa, Thimmaraju, et al. "The rhizobacterial elicitor acetoin induces systemic resistance in Arabidopsis thaliana." *Communicative & Integrative Biology* 3.2 (2010): 130-138). A colorimetric assay was used

to measure the secretion of acetoin of *Bacillus velezensis ABN1001* with *Enterobacter aerogenes* as the positive control. The measurement was performed using the Voges-Proskauer to infer the concentration level from the colorimetric measurement (Westerfeld, W. W. "A colorimetric determination of blood acetoin." *J. biol. Chem* 161.2 (1945): 495-502). The concentration level comparisons are summarized in Table 1.

Table 1: Results for the production of acetoin by *Bacillus velezensis ABN1001*

Strains	**First colorimetric assay**
Enterobacter aerogenes	+++
Bacillus velezensis ABN1001	+++

[0042] As it is known that acetoin is responsible for conferring protection against a wide range of diseases and as it is also known to promote plant growth, it is understood that *Bacillus velezensis ABN1001* also promotes plant growth and confer plant resistance to a wide range of diseases.

EXAMPLE 2

Antifungal activity against Trichoderma harzianum T-22

[0043] *Bacillus velezensis ABN1001* was cultured for 72 hours in yeast extracts-sugar media (20g/L yeast peptone, 15g/L molasses, 15g/L saccharose) and stirred in an Erlenmeyer. A volume of the supernatant of the culture mixture (100µL) was extracted and tested in tubes placed on a surface of fungus *Trichoderma harzianum* T-22. The control strain used was *Bacillus amiloliquefaciens* FZB24. The inhibition zone was measured and the results are summarized in Table 2.

Table 2: Results of the antifungal activity of *Bacillus velezensis ABN1001* against *Trichoderma harzianum* T-22

	First assay	Second assay
Strains	**Inhibition zone (average in mm)**	**Inhibition zone (average in mm)**
FZB24	20.33	17.14
ABN1001	14.73	19.57 Δ = + 12%

[0044] The strain of the present invention *Bacillus velezensis ABN1001* showed a larger inhibition zone than the control. Therefore, *Bacillus velezensis ABN1001* exhibits antifungal activity against *Trichoderma harzianum* T-22.

EXAMPLE 3

Broad antifungal activity

[0045] *Bacillus velezensis ABN1001* was cultured for 72 hours in yeast extracts-sugar media (20g/L yeast peptone, 15g/L molasses, 15g/L saccharose) and stirred in an Erlenmeyer. A volume of the supernatant of the culture mixture (100µL) was extracted and tested in tubes (also known as penicylinder) placed on Petri dishes inoculated with *Fusarium solani, Botrytis cinerea, Pythium splendens, Colletotrichum acutatum, Rhizoctonia solani, Verticillium dahliae* or *Sclerotinia sclerotiorum*. The positive control strain used was *Bacillus amyloliquefaciens* FZB24, a commercially available strain isolated from the product FZB24 of the company ABiTEP GmbH (Berlin, Germany). The inhibition zone was measured and the results are summarized in Table 3.

Table 3: Results for the broad antifungal activity of *Bacillus velezensis ABN1001*

	First assay	Second assay
Strains	**Inhibition zone (average in mm)**	**Inhibition zone (average in mm)**
FZB24	19.86	18.72
ABN1001	9.22 Δ = -56%	16.74 Δ = -11%

[0046] Therefore, the strain of the present invention *Bacillus velezensis ABN1001* has shown antifungal activity comparable to that of the positive control, hence having broad antifungal effects.

EXAMPLE 4

Broad antibacterial activity

[0047] To demonstrate by way of example, *Bacillus velezensis ABN1001* was cultured for 72 hours in yeast extracts-sugar (20g/L yeast peptone, 15g/L molasses, 15g/L saccharose) media and stirred in an Erlenmeyer. A volume of the supernatant of the culture mixture (100µL) was extracted and tested in tubes (penicylinders) placed on Petri dishes inoculated with containing *Straptomyces scabies, Pseudomonas syringae, Clavibacter michiganensis, Xanthomonas campestris, Pseudomonas aeruginosa* and *Pectobacterium caravoterum*. The control strain, as in the previous example, was FZB24. The inhibition zone was measured and the results are summarized in Table 4.

Table 4: Results for the broad antibacterial activity of *Bacillus velezensis ABN1001*

Strains	Inhibition zone (average in mm)
FZB24	19.61
ABN1001	12.76

[0048] A significant inhibition zone was formed, therefore the strain of the present invention *Bacillus velezensis ABN1001* has broad antibacterial effects.

EXAMPLE 5

Sporulation and antifugal effect on Fusarium solani after fermentation

[0049] *Bacillus velezensis ABN1001* was fermented at maximal agitation rate and aeration in a bioreactor of 150 L. The fermentation lasted 48 hours in a media of Yeast Extracts-Sugar (20g/L yeast peptone, 15g/L molasses, 15g/L saccharose). The process was performed a second time in a bioreactor of 500 L. The spore count results are summarized in Table 5.

Table 5: Colony forming units (CFU) of *Bacillus velezensis ABN1001* after fermentation at maximal agitation rate and aeration

Time	Sample	CFU/mL for the 150L bioreactor	CFU/mL for the 500L bioreactor
30h	Total cells	5.95×10^9	1.05×10^{10}
	Spores	7×10^9	8.73×10^9
48h Not Pasteurized	Total cells	(not measured)	9.8×10^9
	Spores	(not measured)	9.77×10^9
48h Pasteurized	Spores without preservation agents	4.4×10^9	9.17×10^9
	Spores with preservation agents	3.13×10^9	5.3×10^9

[0050] A volume of the supernatant (not containing cells or spores) of the culture mixture (100µL) was extracted and tested in tubes placed on a surface containing *Fusarium solani* diluted at 1/100. In some tests, propionic acid as a preservative agent was added to the supernatant. The inhibition zone was measured and the results are summarized in Table 6.

Table 6: Results of the antifungal activity of *Bacillus velezensis ABN1001* against *Fusarium solani*

Time	Sample	Supernatant volume	Inhibition zone (mm) from 150L bioreactor	Inhibition zone (mm) from 500L bioreactor
48h Pasteurised	Supernatant only	100 µL	18.70	20.21
	Supernatant only	200 µL	20.27	22.14
	Supernatant with preservation agents	100 µL	37.12	37.22
	Supernatant with preservation agents	200 µL	43.66	48.18

[0051] The strain of the present invention *Bacillus velezensis ABN1001* has good sporulation efficiency and an antifungal effect on *Fusarium solani*.

EXAMPLE 6

Production of antimicrobial metabolites

[0052] *Bacillus velezensis ABN1001* was cultured for 72 hours in yeast extracts-sugar media and stirred in an Erlenmeyer. The supernatant was then analysed by liquid chromatography-mass spectrometry (LC-MS) to detect and quantify the lipopeptides surfactin, fengycin and iturin. These lipopeptides are antibacterial, antifungal, and reduce plant disease. Iturin and fengycin exhibit powerful antifungal activity and growth inhibition against other pathogens as well. Surfactins are not toxic for fungal pathogens but have a synergistic effect on the antifungal activity of Iturin. (Kim, Pyoung Il, et al. "Production of biosurfactant lipopeptides Iturin A, fengycin and surfactin A from Bacillus subtilis CMB32 for control of Colletotrichum gloeosporioides." *J Microbiol Biotechnol* 20.1 (2010): 138-145). The positive control, as in some of the previous examples, was *Bacillus amyloliquefaciens* FZB24. The results are summarized in Table 7 with the standard deviation (SD).

Table 7: Results of the secretion of antimicrobial metabolites by *Bacillus velezensis ABN1001*

Sample	Concentration of Surfactin (ppm) ± SD	Concentration of Fengycin (ppm) ± SD	Concentration of Iturins (ppm) ± SD
ABN1001	1388.61 ± 379.14	927.47 + 281.14	65.03 ± 5.90
FZB24	837.27 ± 142.71	583.65 ± 183.49	11.03 ± 2.43

[0053] The strain *Bacillus velezensis ABN1001* produces metabolites that are antimicrobial as can be seen from Table 7. The concentrations of the studied lipopeptides produced by the culture of strain *Bacillus velezensis ABN1001* are greater than the positive control.

EXAMPLE 7

Promoting growth of soya

[0054] Four assays were performed to assess the soya growth stimulation activity. In the first assay (#1), 12 soya seeds were placed in separate wells with Promix™ soil (Premier Horticulture LTD. Rivière du Loup, QC Canada). The culture was maintained for three weeks with regular watering containing 10^6 CFU/mL of *Bacillus velezensis ABN1001*. The control seeds were watered with tap water without adding *Bacillus velezensis ABN1001*. After three weeks, the plants were weighted and the growth of the roots was assessed visually. The results are summarized in Table 8.

[0055] In the second assay (#2), 12 soya seeds were placed in separate wells with Promix™ soil (Premier Horticulture LTD. Rivière du Loup, QC Canada). The culture was maintained for three weeks with regular watering containing 10^6 CFU/mL of *Bacillus velezensis ABN1001*. The control seeds were watered with tap water without adding *Bacillus velezensis ABN1001*. After three weeks, the plants were weighted and the growth of the roots was assessed visually. The results are summarized in Table 8.

[0056] In the third assay (#3), 20 soya seeds were placed in separate wells with half Miracle Grow potting soil, half sand. The culture was maintained for four weeks with watering every week containing 10^5 CFU/mL of *Bacillus velezensis ABN1001*. The control seeds were watered with tap water without adding *Bacillus velezensis ABN1001*. After four weeks, the plants were dried then the roots and leafs were weighted. The results are summarized in Table 8.

[0057] In the fourth assay (#4), 28 soya seeds were placed in separate wells with half Miracle Grow soil, half sand. The culture was maintained for four weeks with watering every week containing 10^5 CFU/mL *Bacillus velezensis ABN1001*. The control seeds were watered with tap water without adding *Bacillus velezensis ABN1001*. After four weeks, the plants were dried then the roots and leafs were weighted. The nodules were also counted. The results are summarized in Table 8.

Table 8: Results of the growth assay on soya

Assay number and sample	Average plant weight (g/plant)	Growth of roots	Average root weight (g/root)	Average leafs weight (g/leafs)	Average nodule per plant
#1 Tap water control	1.3	++			
#1 Water with ABN1001	1.63 Δ = +20%	+++			
#2 Tap water control	1.86	++			
#2 Water with ABN1001	1.99 Δ = +7%	+++			
#3 Tap water control			0.1469	0.59	
#3 Water with ABN1001			0.1738 Δ = +15%	0.74 Δ = +20%	
#4 Tap water control			0.1868	0.79	7
#4 Water with ABN1001			0.2709 Δ = +31%	1.01 Δ = +22%	15 Δ = +47%

[0058] As can be observed from the results, the strain of the present invention *Bacillus velezensis ABN1001* promotes growth.

EXAMPLE 8

Promoting growth of corn

[0059] To demonstrate by way of example, four assays were performed to assess the corn growth stimulation activity. In the first assay (#1), 12 corn seeds were placed in separate wells with Miracle Grow potting soil. The culture was maintained for three weeks

with regular watering containing 10^6 CFU/mL of *Bacillus velezensis ABN1001*. The control seeds were watered with tap water without adding *Bacillus velezensis ABN1001*. After three weeks, the plants were weighted and the growth of the roots was assessed visually. The results are summarized in Table 9.

[0060] In the second assay (#2), 9 corn seeds were placed in separate wells with Promix soil. The culture was maintained for four weeks with regular watering containing 10^5 CFU/mL of *Bacillus velezensis ABN1001*. The control seeds were watered with tap water without adding *Bacillus velezensis ABN1001*. After four weeks, the plants were weighted and the growth of the roots was assessed visually. The results are summarized in Table 9.

[0061] In the third assay (#3), 20 corn seeds were placed in separate wells with half Miracle Grow potting soil, half sand. The culture was maintained for four weeks with watering every week containing 10^5 CFU/mL of *Bacillus velezensis ABN1001*. The control seeds were watered with tap water without adding *Bacillus velezensis ABN1001*. After four weeks, the plants were dried then the roots and leafs were weighted. The results are summarized in Table 9.

[0062] In the fourth assay (#4), 25 corn seeds were placed in separate wells with half Miracle Grow soil, half sand. The culture was maintained for four weeks with watering every week containing 10^5 CFU/mL of *Bacillus velezensis ABN1001*. The control seeds were watered with tap water without adding *Bacillus velezensis ABN1001*. After four weeks, the plants were dried and the roots and leafs were then weighted. The results are summarized in Table 9.

Table 9: Results of the growth assay on corn

Assay number and sample	Average plant weight (g/plant)	Growth of roots	Average root weight (g/root)	Average leafs weight (g/leafs)
#1 Tap water control	3	++		
#1 Water with ABN1001	4.42 Δ = +32%	+++		
#2 Tap water control	1.39	++		
#2 Water with ABN1001	1.81 Δ = +23%	+++		
#3 Tap water control			0.2344	1.02
#3 Water with ABN1001			0.3464 Δ = +22%	1.04 Δ = +2%
#4 Tap water control			0.2105	0.74
#4 Water with ABN1001			0.2659 Δ = +21%	0.9 Δ = +18%

[0063] As demonstrated by the results, the strain of the present invention *Bacillus velezensis ABN1001* promotes growth.

EXAMPLE 9

Growth promoting activity on cucumber and tomato plants

[0064] In this assay, 25 seeds of each of Gusto cucumber and Sub Artic Plenty tomatoes (McKenzie Seeds, Brandon, Manitoba, Canada) were selected. The seeds were

incubated in a solution of 10^5 *Bacillus velezensis ABN1001* per mL for 3 minutes. Then the seeds were seeded in half Miracle Grow potting soil, half sand. The culture was maintained with watering containing 10^5 CFU/mL of *Bacillus velezensis ABN1001* three times per 5-10 days. The control seeds were not incubated in the solution and were watered with tap water without adding *Bacillus velezensis ABN1001*. The roots were harvested after 55-60 days in culture. The roots were then washed with tap water and left at room temperature for 24 hours. Finally, the roots were placed in brown paper bags inside an incubator at 60° C for one week and weighted at the end of the week. The results are summarized in Table 10.

Table 10: Results of the growth assay for cucumbers and tomatoes

Sample	**Average weight of the roots (g)**	
	Cucumber	**Tomato**
Water tap (control)	0.1391	0.2789
Water containing ABN1001	0.2037 Δ = +32%	0.3306 Δ = +16%

[0065] The results of Table 10 demonstrate that *Bacillus velezensis ABN1001* promotes growth.

Example 10

Strawberry field trial

[0066] *Bacillus velezensis ABN1001* in combination *Bacillus velezensis ABN110* was tested for activity on disease protection and yield improvement in strawberry field assays. A pasteurized fermentation spore solution was adjusted at a concentration of 2 x 10^9 cfu/ml per strain. The treatment consisted of weekly or every two weeks spraying of a solution at 1 liter per hectare. Eight (8) applications during the growing season were applied. Yield data collection was done every week. Disease control was measured at one month before the end of the season by counting powdery mildew affected leaves per plant. As seen in Table 11, results showed a significant improvement in yield and diseases control.

Table 11: Results on yield (Increase %) and disease control on strawberry plants

Yield (Sellable Fruits g/plant)			
Treatment	Control	Increase %	Signification
91	69	31	99%
Powdery Mildew Control (Nb Leaves Affected/plant)			
Treatment	Control	Decrease	Signification
0,8	2,95	-72	99%

CONCLUSION

[0067] The bacterial strain *Bacillus velezensis ABN1001* is a novel bacterial strain that exhibits protection against many plant diseases, broad antifungal activity, broad antibacterial activity, and promotes plant growth. Therefore, *Bacillus velezensis ABN1001* can be used to protect plants against disease, to treat plant disease, and to promote plant growth.

[0068] As can be seen therefore, the examples described above and illustrated are intended to be exemplary only. The scope is indicated by the appended claims.

[0069] The strain of the present invention was deposited at the National Microbiology Laboratory, International Depositary Authority of Canada (IDAC), 1015 Arlington Street, Winnipeg, Manitoba, Canada, R3E 3R2, as "*Bacillus velezensis ABN1001*" with Accession Number 040820-01 on August 4, 2020.

International Depositary Authority of Canada
National Microbiology Laboratory, Public Health Agency of Canada
1015 Arlington Street Tel: (204) 789-6030
Winnipeg, Manitoba Canada R3E 3R2 Fax:(204) 789-2018

International Form IDAC/BP/4

RECEIPT IN THE CASE OF AN ORIGINAL DEPOSIT
(issued pursuant to Rule 7.1 of the *Budapest Treaty* Regulations)

ATTACH COPIES OF THE ORIGINAL DEPOSIT CONTRACT AND VIABILITY STATEMENT

I. Depositor

Name:
ABNATURA INC.

Address:
1100 Place du Technoparc
Trois-Rivieres, QC
G9A 0A9

II. Identification of the Deposit

Identification reference given by the depositor:	Accession number assigned by this International Depositary Authority:
Bacillus velezensis ABN1001	040820-01

III. Scientific Description and/or Proposed Taxonomic Designation

The deposit identified under II above was accompanied by:
☒ a scientific description
☒ a proposed taxonomic designation
(mark with a cross where applicable)

IV. Receipt and Acceptance

This International Depositary Authority accepts the deposit identified under II above, which was received by it on August 4th, 2020 (Date of the original deposit).

V. International Depositary Authority of Canada:

Signature(s) of person(s) having the power to represent the International Depositary Authority of Canada:	Date:
	August 5th, 2020

Receipt in the Case of an Original Deposit 1/1 File #:211 (20)

International Depositary Authority of Canada
National Microbiology Laboratory, Public Health Agency of Canada
1015 Arlington Street Tel: (204) 789-6030
Winnipeg, Manitoba Canada R3E 3R2 Fax:(204) 789-2018

International Form IDAC/BP/9

STATEMENT OF VIABILITY
(Issued pursuant to Rule 10.2 of the *Budapest Treaty* Regulations)

I. Party to Whom This Viability Statement is Issued	
Name: Christian Cawthorn Norton Rose Fullbright Canada	Address: Norton Rose Fullbright Canada 1 Place Ville-Marie, suite 2500 Montreal, QC H3B 1R1
II. Depositor	**III. Identification of the Deposit**
Name: ABNATURA INC. Address: 1100 Place du Technoparc Trois-Rivieres, QC G9A 0A9	Accession number given by IDAC: 040820-01 Date of the deposit or of the transfer[1]: August 4th, 2020
IV. Viability Statement	
The viability of the deposit identified above was tested on (most recent test date): August 6th, 2020 On that date, the deposit was ☑ Viable ☐ No longer viable	
V. Conditions Under Which the Viability Test has Been Performed [2]	
Note: 2 separate tests were performed. One was done on a vial that arrived not frozen on ice, and one on a vial after freezing at -80C over night. Both grew extremely well.	
VI. International Depositary Authority of Canada	
Signature(s) of person(s) having the power to represent the International Depositary Authority of Canada: 	Date: August 6th, 2020

[1] Indicate the date of the original deposit or, where a new deposit or transfer has been made, the most recent relevant date (date of the new deposit or date of the transfer)
[2] To be filled in if the information has been requested and if the results of the test are negative.

Statement of Viability 1/1 File #: 211 (20)

WHAT IS CLAIMED IS:

1. A bacteria as deposited at IDAC under Accession No. 040820-01, strain "*Bacillus velezensis ABN1001*".

2. The bacteria according to claim 1, having plant disease protection activity.

3. The bacteria according to claim 1, having broad antifungal activity.

4. The bacteria according to claim 1, having broad antibacterial activity.

5. The bacteria according to claim 1, characterized in that it promotes plant growth.

6. A bacteria having all the identifying characteristics of *Bacillus velezensis ABN1001*, deposited at IDAC under Accession No.040820-01 and mutants thereof, said bacteria and mutants having at least one of plant disease protection activity, broad antifungal activity, broad antibacterial activity or plant growth promoting activity.

7. A composition comprising the bacteria according to any one of claims 1 to 6 and a carrier.

8. A composition comprising spores from the bacteria according to any one of claims 1 to 6 and a carrier.

9. The composition according to claim 7 or 8, wherein said carrier is a liquid carrier or a solid carrier.

10. The composition according to claim 7, comprising at least 10^5 CFU/mL of the bacteria of said composition.

11. The composition according to any one of claims 7 to 10, wherein the composition is formulated as a granule, fine powder, wettable powder, dry flowables, microencapsulation of agents, liquid formulation, solid formulation, whole broth culture, suspension concentrate or emulsifiable concentrate.

12. The composition according to any one of claims 7 to 12, further comprising a surfactant or a dispersant.

13. The composition according to any one of claims 7 to 13, further comprising at least one of a pesticide, fungicide or herbicide.

14. The composition according to any one of claims 7 to 14, further comprising at least one of plant growth modifier, fertilizer or manure.

15. A composition comprising a supernatant extracted from a culture of the bacteria according to claim 1 or 6.

16. A composition comprising metabolites extracted from a culture of the bacteria according to any one of claims 1 to 6.

17. The composition of any one of claims of 7-16, further comprising one or more plant growth-promoting bacteria.

18. The composition of claim 17, wherein the plant growth-promoting bacteria is *Bacillus amyloliquefaciens* strains D747, QST713, GB03, MBI600, FZB24, or FZB42, or *Bacillus pumilus* strains INR7 (also known as GB34) or QST2808.

19. A method for protecting plants against disease comprising administering to said plant a composition comprising a bacteria *Bacillus velezensis ABN1001*, deposited at IDAC under Accession No. 040820-01, and mutants thereof.

20. A method for treating plant disease comprising administering to said plant a composition comprising a bacteria *Bacillus velezensis ABN1001*, deposited at IDAC under Accession No. 040820-01 or mutants thereof.

21. A method for promoting plant growth comprising administering to said plant a composition comprising a bacteria *Bacillus velezensis ABN1001*, deposited at IDAC under Accession No. 040820-01 or mutants thereof.

22. The method according to claim 19 or 20, wherein said disease is caused by at least one of *Straptomyces scabies, Pseudomonas syringae, Clavibacter michiganensis, Xanthomonas campestris, Pseudomonas aeruginosa, Pectobacterium caravoterum, Fusarium solani, Botrytis cinerea, Pythium splendens, Colletotrichum acutatum, Rhizoctonia solani, Verticillium dahliae* or *Sclerotinia sclerotiorum*.

23. Use of bacteria *Bacillus velezensis ABN1001*, deposited at IDAC under Accession No. 040820-01 or a mutant thereof to protect a plant against a disease.

24. Use of bacteria *Bacillus velezensis ABN1001*, deposited at IDAC under Accession No. 040820-01 or a mutant thereof to treat a plant disease.

25. Use of bacteria *Bacillus velezensis ABN1001*, deposited at IDAC under Accession No. 040820-01 or a mutant thereof to promote plant growth.

26. The use of claim 23 or 24, wherein said disease is caused by at least one of *Straptomyces scabies, Pseudomonas syringae, Clavibacter michiganensis, Xanthomonas campestris, Pseudomonas aeruginosa, Pectobacterium caravoterum, Fusarium solani, Botrytis cinerea, Pythium splendens, Colletotrichum acutatum, Rhizoctonia solani, Verticillium dahliae* or *Sclerotinia sclerotiorum*.

27. Use of the composition of any one of claims 7-18 to protect a plant against a disease.

28. Use of the composition of any one of claims 7-18 to treat a plant disease.

29. Use of the composition of any one of claims 7-18 to promote plant growth.

30. The use of claim 27 or 28, wherein said disease is caused by at least one of *Straptomyces scabies, Pseudomonas syringae, Clavibacter michiganensis, Xanthomonas campestris, Pseudomonas aeruginosa, Pectobacterium caravoterum, Fusarium solani, Botrytis cinerea, Pythium splendens, Colletotrichum acutatum, Rhizoctonia solani, Verticillium dahliae* or *Sclerotinia sclerotiorum*.

INTERNATIONAL SEARCH REPORT

International application No.
PCT/CA2021/051105

A. CLASSIFICATION OF SUBJECT MATTER
IPC: ***C12N 1/20*** (2006.01) , ***A01N 63/22*** (2020.01) , ***A01P 1/00*** (2006.01) , ***A01P 21/00*** (2006.01) , ***A01P 3/00*** (2006.01) , ***C05F 11/08*** (2006.01)

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
Keyword search across whole IPC

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic database(s) consulted during the international search (name of database(s) and, where practicable, search terms used)
Questel-Orbit: FAMPAT, STN, SCOPUS

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	US 8404476 B2 (MARTINEZ et al.) 26 March 2013 (26-03-2013)	6 to 18 and 27 to 30
X	US 2019/0261633 A1 (LU et al.) 29 August 2019 (29-08-2019)	6 to 18 and 27 to 30
X	US 2020/0178540 A1 (DAGHER and DEZIEL) 11 June 2020 (11-06-2020)	6 to 18 and 27 to 30
X	ADENIJI et al. "Bacillus velezensis: phylogeny, useful applications, and avenues for exploitation" APPLIED MICROBIOLOGY AND BIOTECHNOLOGY, May 2019 (05-2019), vol. 103, no. 9, pages 3669-3682, ISSN: 0175-7598	6 to 18 and 27 to 30
X	YE et al. "Characteristics and Application of a Novel Species of Bacillus: Bacillus velezensis" ACS CHEM BIOL, 16 March 2018 (16-03-2018), vol. 13, no. 3, pages 500-505, ISSN: 1554-8929	6 to 18 and 27 to 30

☐ Further documents are listed in the continuation of Box C. ☒ See patent family annex.

*	Special categories of cited documents:	"T"	later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"A"	document defining the general state of the art which is not considered to be of particular relevance	"X"	document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"D"	document cited by the applicant in the international application	"Y"	document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"E"	earlier application or patent but published on or after the international filing date	"&"	document member of the same patent family
"L"	document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)		
"O"	document referring to an oral disclosure, use, exhibition or other means		
"P"	document published prior to the international filing date but later than the priority date claimed		

Date of the actual completion of the international search 04 October 2021 (04-10-2021)	Date of mailing of the international search report 28 October 2021 (28-10-2021)
Name and mailing address of the ISA/CA Canadian Intellectual Property Office Place du Portage I, C114 - 1st Floor, Box PCT 50 Victoria Street Gatineau, Quebec K1A 0C9 Facsimile No.: 819-953-2476	Authorized officer Cynthia Bruce-Payne (819) 639-7765

INTERNATIONAL SEARCH REPORT

International application No.
PCT/CA2021/051105

Box No. I **Nucleotide and/or amino acid sequence(s) (Continuation of item 1.c of the first sheet)**

1. With regard to any nucleotide and/or amino acid sequence disclosed in the international application, the international search was carried out on the basis of a sequence listing:

a. ☒ forming part of the international application as filed:

☒ in the form of an Annex C/ST.25 text file.

☐ on paper or in the form of an image file.

b. ☐ furnished together with the international application under PCT Rule 13*ter*.1(a) for the purposes of international search only in the form of an Annex C/ST.25 text file.

c. ☐ furnished subsequent to the international filing date for the purposes of international search only:

☐ in the form of an Annex C/ST.25 text file (Rule 13*ter*.1(a)).

☐ on paper or in the form of an image file (Rule 13*ter*.1(b) and Administrative Instructions, Section 713).

2. ☐ In addition, in the case that more than one version or copy of a sequence listing has been filed or furnished, the required statements that the information in the subsequent or additional copies is identical to that in the application as filed or does not go beyond the application as filed, as appropriate, were furnished.

3. Additional comments:

INTERNATIONAL SEARCH REPORT
Information on patent family members

International application No.
PCT/CA2021/051105

Patent Document Cited in Search Report	Publication Date	Patent Family Member(s)	Publication Date
US8404476B2	26 March 2013 (26-03-2013)	US2010179060A1	15 July 2010 (15-07-2010)
		BRPI0808780A2	30 October 2018 (30-10-2018)
		BRPI0808780A8	11 December 2018 (11-12-2018)
		CN101808525A	18 August 2010 (18-08-2010)
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