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73 Īpašnieks(i):
VENSKA LANTMÄNNEN, RIKSFÖRBUND EK.
FÖR.; Box 30192, S-104 25 Stockholm, SE

72 Izgudrotājs(i):
Berndt GERHARDSON (SE),
Annika GUSTAFSSON (SE),
Tiiu JERKEMAN (SE),
Britt-Marie JINGSRTÖM (SE),
Lennart JOHNSON (SE),
Margareta HÖKEBERG (SE)

74 Pilnvarotais vai pārstāvis:
Gunārs ROTBERGS,
Aģentūra "FORAL",
Raiņa bulv. 19, Rīga LV-1159, LV

54 Virsraksts: Sastāvs un paņēmieni augu slimību apkarošanai

57 Kopsavilkums: Izgudrojums piedāvā jaunu *Pseudomonas chlororaphis* celmu MA 342, kas deponēts kolekcijā The National Collections of Industrial and Marine Bacteria Limited, Scotland ar numuru NCIMB 40616, vai tā bioloģiski tīras kultūras, kuras spēj ražot antipatogēniski aktīvus metabolītus vai to mutantus, kuriem būtībā ir tādi paši raksturojumi kā izejas celmam, un to izmantošanu sastāvos patogēnisko sēnīšu izsaukto augu slimību apkarošanai, kā arī paņēmienus minēto augu slimību apkarošanai.

IZGUDROJUMA FORMULA

1. *Pseudomonas chlororaphis* celms, kas deponēts kolekcijā "The National Collections of Industrial and Marine Bacteria Limited, Aberdeen, Scotland" ar NCIMB iestājnumuru 40616, vai tā bioloģiski tīras kultūras, kuras spēj ražot antipatogeniski aktīvus metabolītus vai to mutantus, kuriem būtībā ir tie paši raksturojumi kā izejas celmam.

2. Sastāvs patogēnisko sēņu izsaukto augu slimību apkarošanai, kurš kā aktīvo sastāvdaļu satur 1. punktā noteikto mikroorganismu vai tā buljona kultūru, kas satur tā antipatogeniski aktīvus metabolītus.

3. Sastāvs saskaņā ar 2. punktu, kas atšķiras ar to, ka patogēnais mikroorganisms ir sēne Drechslera teres.

4. Sastāvs saskaņā ar 2. punktu, kas atšķiras ar to, ka patogēnais mikroorganisms ir sēne Drechslera graminea.

5. Sastāvs saskaņā ar 2. punktu, kas atšķiras ar to, ka patogēnais mikroorganisms ir sēne Drechslera avenae.

6. Sastāvs saskaņā ar 2. punktu, kas atšķiras ar to, ka patogēnais mikroorganisms ir sēne Microdochium nivale.

7. Sastāvs saskaņā ar 2. punktu, kas atšķiras ar to, ka patogēnais mikroorganisms ir sēne Tilletia caries.

8. Sastāvs saskaņā ar 2. punktu, kas atšķiras ar to, ka patogēnais mikroorganisms ir sēne Ustilago avenae.

9. Sastāvs saskaņā ar no 2. līdz 8. punktiem, kas atšķiras ar to, ka aktīvā sastāvdaļa samaisīta ar nesējsastāvu, kas pieņemams agrokultūru praksē.

10. Sastāvs saskaņā ar 9. punktu, kas atšķiras ar to, ka aktīvā sastāvdaļa samaisīta ar šķidru nesēju.

11. Sastāvs saskaņā ar 9. punktu, kas atšķiras ar to, ka aktīvā sastāvdaļa impregnēta cietā porainā materiālā.

12. Sastāvs saskaņā ar 9. punktu, kas atšķiras ar to, ka turpmāk satur piedevas, kas kalpo kā saistītāji.

13. Sastāvs saskaņā ar 9. punktu, kas atšķiras ar to, ka turpmāk satur barojošu avotu.

14. Paņēmiens patogēno sēņu izsaukto augu slimību apkarošanai un ietverošs aktīvās sastāvdaļas efektīvas devas ievadišanu apkārtējā vidē, kurā jāiznīdē slimību izsaucošais aģents, atšķiras ar to, ka aktīvās

sastāvdaļas ir mikroorganisms saskaņā ar 1. punktu vai tā buljona kultūra, kas satur antipatogeniski aktīvus metabolītus vai to atvasinājumus.

15. Paņēmiens saskaņā ar 14. punktu, kas atšķiras ar to, ka aktīvo sastāvdaļu pielieto graudiem.

16. Paņēmiens saskaņā ar 14. punktu, kas atšķiras ar to, ka aktīvo sastāvdaļu pielieto augu veģetatīvās vairošanās daļām.

17. Paņēmiens saskaņā ar 14. punktu, kas atšķiras ar to, ka aktīvo sastāvdaļu pielieto augiem.

18. Paņēmiens saskaņā ar 14. punktu, kas atšķiras ar to, ka aktīvo sastāvdaļu pielieto augšanas videi, kurā augi aug vai ir jāaudzē.

Composition and method for controlling plant diseases

Field of the Invention

The present invention relates to plant protection products. More specifically, the invention relates to a novel strain of the bacterial species Pseudomonas chlororaphis and the use of compositions containing this bacterial strain or
5 antibiotic substances produced by this strain in plant production in order to protect plants against attacks by phytopathogenic microbial agents.

Background of the Invention

Several agents of microbial nature with the ability to induce plant diseases
10 cause considerable damages, and accordingly economic losses, in crop plants. Many of them attack leaves and/or other aerial plant parts and then usually reach new uninfected crops by airborne spores. Others are transmitted from one crop generation to the next by being seedborne and several economically important disease-inducing agents are soilborne and reside more or less inactive in the
15 soil until a susceptible crop is grown.

Procedures exerted for controlling microbial disease-inducing agents in crop production are often costly, but in most crop growing systems economically necessary. One widely used method is treatment with biostatic or biocidal chemicals. They are in most cases applied as sprays on growing crops, as seed or
20 root treatments or as soil disinfectants. Other standard methods are breeding for resistance and management of the cropping system itself.

These standard control methods all have some drawbacks. Managing of the cropping system is effective or convenient only for certain disease problems. Also the breeding of crop plants for resistance is possible or suitable only in certain
25 cases, may take long time and the resistance obtained may be broken after some time by the appearance of new strains of the pathogen. Chemical compounds often are highly effective, but they may give unwanted effects in the environment, require careful handling as most are risky for human health and they also may become ineffective where resistant pathogen strains develop.

30 The use of biological control agents or biopesticides may be more effective or more preferable than the use of other control methods and, thus, such agents have been extensively tried. Several bacterial and fungal strains are known to inhibit growth of various microbial disease-inducing agents. To be effective and usable they have to be stable, give reproducible results in the field and there

must be possibilities to apply them under field conditions. To date few have fulfilled these requirements and, thus, have been used as commercial products.

Brief Description of the Invention

The present invention provides a biological control agent useful and effective for controlling plant pathogen attacks in commercial plant growings. A novel strain (MA 342) of the bacteria Pseudomonas chlororaphis showing the desired characteristics is provided. The isolate was deposited at the National Collections of Industrial and Marine Bacteria Limited (NCIMB), Aberdeen, Scotland on 14th February 1994 under the terms of the Budapest Treaty and has received NCIMB Accession No. 40616.

The invention also provides a plant disease controlling composition containing as active ingredient the novel strain MA 342 or mutants thereof with essentially the same characteristics or antipathogenically active metabolites or derivatives thereof. Further, the invention provides a method of controlling plant diseases using the novel strain MA 342 or mutants thereof with essentially the same characteristics or antipathogenically active metabolites or derivatives thereof.

Brief Description of the Drawing

Figure 1: This figure shows the fatty acid profile obtained with the Microbial Identification System (MIDI, Newark Ltd., USA) of the bacterial isolate MA 342.

Detailed Description of the Invention

Below follows a characterization of the novel bacterial strain and a description of preferred methods for strain proliferation and for formulations and applications in the field or in greenhouses. Several examples are offered to further illustrate, but not to limit, the method and composition of the invention.

Characterization of the novel bacterial strain MA 342

Morphological characteristics: Colony morphology on TSA 10 (10 g Tryptic Soy Broth (Difco Ltd.); 12 g Technical Agar (Oxoid Ltd.) in 1000 ml distilled water) is round, white, moderately convex colonies that form well visible hyaline crystals in the agar at high cell densities. It is a Gram negative rod that shows a bright yellow fluorescence on Kings medium B (1.5 g K_2HPO_4 ; 1.5 g $MgSO_4 \cdot 7 H_2O$; 20 g Proteose Peptone (Difco Ltd.); 10 ml glycerol, 15 g Bacto Agar (Difco Ltd.) in

1000 ml distilled water).

Fatty acid analysis: The fatty acid profile of the bacterium is shown in figure
This analysis was performed using the Microbial Identification System (MII
Ltd., Newark, USA), version 3.7. According to this test program, MA 342 is mo
5 similar to Pseudomonas chlororaphis, with a matching index of 0.705.

Biochemical characteristics:

Characteristics tested in API 20 NE* rapid test		Reaction of isolate MA 342
10	Nitrate reduction	-
	Indole production	-
	Acid from glucose	-
	Arginine dihydrolase	+
	Urease	-
15	Esculin hydrolysis	-
	Gelatin hydrolysis	+
	B-galactosidase	-
	Glucose assimilation	+
	Arabinose assimilation	-
20	Mannose assimilation	-?
	Mannitol assimilation	+
	N-acetyl-glucosamine assimilation	-
	Maltose assimilation	-
	Gluconate assimilation	+
25	Caprate assimilation	-
	Adipate assimilation	-
	Malate assimilation	+
	Citrate assimilation	+
	Phenyl-acetate assimilation	-
30	Cytochrome oxidase	+

*API System Ltd., France

Characteristics tested
in additional tests

	Levan production	-
5	Xylose assimilation	-?
	Sorbitol assimilation	+?

**Preferred methods for strain proliferation and for formulations
and applications in the field or in greenhouses**

10 Quantities of the active strain is best obtained by a fermentation process
that comprises inoculating a sample of a pure culture of the strain into a liquid
shake culture or in a fermentor containing a suitable fermentation medium. The
strain may also be grown on a sterile surface, e. g. an agar surface, and when
grown out, the cells may be suspended in water or other liquid media known in
15 the art. Growing media may in principle be any bacterial growth medium known
in the art. The fermentation is carried out until a sufficient concentration of cells,
e. g. about $5 \cdot 10^9$ cfu (colony forming units)/ml for liquid cultures, is obtained. The
so obtained fermentation broth or bacterial suspension may be employed as such
for use in plant protection, or they may be treated or formulated before being
20 used.

In one type of treatment the bacterial cells in the fermentation broth may
be killed, e. g. by heating, or centrifuged down and the resulting broth or
supernatant, containing bacterial metabolites, may be used for plant protection
purposes, with or without prior purification and/or concentration. Bacterial
25 suspensions and fermentation broths may also be homogeneously mixed with one
or more compounds or groups of compounds known in the art, provided such
compounds are compatible with the bacterial strains or its antipathogenically
active metabolites or derivates of these. Suitable compounds may be powdery
additives or solid carriers, such as talcum, kaolin, bentonite or montmorillonite,
30 wettable powders known in the art, carbon source nutrients (such as glucose,
sucrose and fructose) or complex bacterial nutrients (such as yeast extract,
bacteriological peptone and tryptone), metal salts, salts from fatty acids, fatty acid
esters, ionic or non-ionic surfactants, plant nutrients, plant growth regulators,
fungicides, insecticides, bactericides and the like. Bacterial suspensions and

fermentation broths may also be dried or freeze-dried prior to or after being mixed with suitable compounds and the resulting product used for plant protection. A suitable way of drying is for example air drying of vermiculite supplied with bacterial fermentation broth.

5 Bacterial and metabolite preparations may be applied in any manner known for treating seeds, vegetative propagation units, plants and soil with bacterial strains. Spraying, atomizing, dusting, scattering, pelleting, dipping or pouring may be chosen in accordance with the intended objective and the prevailing circumstances. Advantageous rates of application for seed treatment
10 are normally from 10^{11} to 10^{12} cfu/ha and for spraying 10^{12} to 10^{14} cfu/ha or a corresponding amount of bacterial metabolites.

Example 1

Isolation of the Microorganism MA 342

The dug up roots of the plant Empetrum nigrum were washed in sterile tap
15 water to remove adhering soil. From a young root a piece, 2-3 cm long, was cut out and handled under sterile conditions. The piece was taken from the region above the root tip area. Small cuts were made in the root piece with a flamed scalpel. The root piece was then rubbed against the surface of TSA 10 agar. After bacteria had grown out, MA 342 was picked and was pure cultured on to TSA 10.

20

Example 2

Preservation of the Microorganism MA 342

The Pure culture was deep frozen in small ampoules at -70°C . As freeze protecting agent were used 10% glycerol in tap water, pH adjusted to 7.15 after autoclaving. After freezing at -70°C , the ampoules were stored at -20°C .

25 For long term preservation the isolate was freeze dried. After growing for 48 hours on TSA 10 agar, the bacterial lawn was scraped off the agar surface, mixed with a freeze drying protecting agent (50 g Dextran T 70 (Pharmacia Fine Chemicals Ltd.); 50 g Na-L-glutamate (Kebo AB) in 1000 ml of distilled water), poured into small ampoules (20 ml) and put in a Hetosicc freeze drier (Heto Ltd.,
30 Denmark) for 24 hours. After freeze drying the ampoules were gas tightly sealed with rubber stoppers and stored at 4°C .

Example 3

Effect of MA 342 against Microdochium nivale in primary greenhouse screenings

The bacterium was applied to the seeds of wheat as follows: 24 hours old
 5 cultures on TSA 10, grown at 15°C, were scraped off from the agar surface of a
 9 cm Petri dish and mixed with 40 ml of nutrient broth (SNB: 18 g sucrose; 5 g
 bacterial peptone (Oxoid Ltd.); 2 g yeast extract (Oxoid Ltd.); 0.5 g K₂HPO₄ and
 0.25 g MgSO₄·7 H₂O in 1000 ml distilled water and pH adjusted to 7.2-7.4) and
 40 ml of a 2% (w/v) solution of sodium carboxymethyl cellulose (CMC) in sterile
 10 distilled water. This mixture was poured over the seeds. After 20 minutes the
 excess mixture was poured off and the seeds were dried under a fan overnight.

For each treatment in this greenhouse screening two pots were sown with
 50 seeds in each pot. The pots were 18 cm in diameter and 4 cm high and filled
 to two thirds with an unsterilized commercial peat mixture (Enhetsjord K
 15 Normal), mixed with 20% (v/v) sand.

The winter wheat seeds (cv. "Kosack") were artificially infested with M.
nivale prior to the treatment with bacteria. The pathogen was cultivated for
 seven days in potato dextrose broth (24 g Potato Dextrose Broth (Difco Ltd.) per
 1000 ml distilled water) at room temperature on a rotary shaker. The resulting
 20 slurry was homogenized with a kitchen blender and poured over the seeds. After
 30 minutes the liquid was poured off and the seeds were left to dry under a fan
 over night. Seeds thus infested were then treated with MA 342 and sown in pots
 as described above.

After sowing, the pots were covered with glass lids and placed in the dark
 25 at 6°C. After five days the lids were removed, each pot watered with 100 ml of
 water and placed inside a five litres plastic bag that was supported by two wooden
 sticks. The pots were then placed in a greenhouse at 12-15°C for eight days.

The following treatments of seeds were tested:

1. P. chlororaphis strain MA 342, mixed with SNB/CMC, on M. nivale-
 30 infested seeds
2. M. nivale-infested seeds, as disease control
3. Untreated seeds as healthy control

The disease suppressive effect was recorded as the percentages of emerged,
 healthy (=without mycelia) plants out of those sown. Results from a typical M.

nivale primary screening is shown in table 1.

Table 1. Effect of MA 342 on emergence and disease development in winter wheat, raised from M. nivale-infested seeds.

5	Treatment	Percentage (%) emergence	Percentage (%) healthy plants out of those sown
	1. MA 342	87	81
	2. Disease control	13	7
10	3. Untreated control	90	89

Example 4

Effect of MA 342 against Drechslera teres in secondary greenhouse screenings

15 For these screenings the isolate MA 342 was grown for 48 hours in half strength (15 g/l) Tryptic soy broth (Difco Ltd.) on a rotary shaker in the dark at 18-20°C. Seeds of the barley cultivar "Golf", naturally infected with D. teres, were then mixed with 300 ml of the resulting bacterial suspension per kg seed in a plastic bag and, after mixing, the bag was shaken for about 4 minutes. Seeds thus
20 treated were dried under a fan at room temperature for one day and then sown in pots as described in Example 3.

The sown pots, three per treatment, were placed first in the dark at 6°C for seven days and then in a greenhouse at about 20°C as described in Example 3. For reading treatment effects the frequency of germinated plants and the
25 frequency of plants with primary attack on the first leaf were counted. The bacterial effect was related to an untreated control and seeds treated with the fungicide Panocrine Plus 400 (guazatine 150 g/l + imazalil 10 g/l), Rhône-Poulenc Ltd., in a dosage of 4 ml per kg seed.

Table 2. Effect of MA 342 and of Panoctine Plus 400 against D. teres in barley in a typical greenhouse secondary screening.

Treatment	Per cent germinated plants	Per cent plants with attack on the first leaf
Control	92.5	13.0
Panoctine Plus 400, 4 ml/kg	95.5	0.0
MA 342, 300 ml/kg	96.1	0.0

Example 5

Effect of MA 342 on plant pathogens in field experiments

Field experiments, designed as randomized blocks and with three to eight repetitions, had plot sizes varying between experiments from 0.15 m² (one T. caries experiment) to about 15 m² (most experiments). The experiments were placed at different localities in Sweden and in most cases on loamy soils with about 3 per cent humus content.

Treatments of seeds with bacteria and with Panoctine Plus 400 were done as described in Example 4 above. After the treated seeds had been dried with a fan they were stored at room temperature for various times before they were sown in field plots. All seeds, except those infested with T. caries, were naturally infested or infected with the various diseases tested. Seeds of winter wheat (cv. "Kosack") were artificially infested with spores of T. caries by mixing 2 g crushed T. caries-ears with 1 kg wheat seeds.

Effect of MA 342 on Tilletia caries

The effect was read as the frequency of infected ears at time of ripening. Results obtained in two trials in 1991/92 and two trials in 1992/93 are shown in Table 3. The difference between bacterial treatment and the fungicide treatment is significant in 1992/93.

Table 3. Effect of MA 342 bacterial suspension against seed borne Tilletia caries infection.

Treatment	Per cent infected ears	
	1991/92	1992/93
Control	23	65
Panact. 400*, 4 ml/kg	2	24
MA 342, 300 mg/kg	0	9

* Panactine 400 (guazatine 150 g/l), Rhône-Poulenc Ltd.

Effect of MA 342 on Drechslera teres, D. Graminea, D. avenae
and Ustilago avenae

In the field experiments with these pathogens the number of germinated plants per m² and the number of infected plants per m² were measured and, in addition, in most of the experiments also i) grain yield ii) thousand kernel weight and iii) weight per hectolitre.

Effect on Drechslera teres: Results from field experiments conducted in 1991-1993 and where effects against D. teres-infection in barley were tested are shown in tables 4, 5 and 6.

Table 4. Results from four field experiments in barley infected with Drechslera teres in 1991. Means from four experiments conducted at Alnarp, Lanna, Strängnäs and Ultuna.

Treatment	Yield kg/ha	No. of plants/m ²	Infected plants/m ²	Hectolitre weight, kg	1000-kernel weight, g
Control	4970	361	47	64.7	41.5
Pan. Plus 400, 4 ml	5390	353	1	66.3	43.8
MA 342, 300 ml/kg	5480	353	1	66.0	43.7

Table 5 Results from five fields experiments in barley infected with Drechslera teres in 1992. Means from experiments conducted at Svalöf, Nygård, Kölbäck, Ultuna and Röbbäcksdalen.

5	Treatment	Yield kg/ha	No. of plants/m ²	Infected plants/m ²	Hectolitre weight, kg	1000-kernel weight, g
	Control	4290	358	48	67.9	51.7
	Pan. Plus 400, 4 ml	4380	378	1	67.8	50.5
10	MA 342, 300 ml/kg	4300	380	1	68.3	52.6

Table 6. Results from two field experiments in barley infected with Drechslera teres in 1993. Means from the experiments conducted at Kölbäck and Ultuna.

15	Treatment	Yield kg/ha	Infected plants/m ²
	Control	6310	74
20	Pan. Plus 400, 4 ml	6730	1
	MA 342, 200 ml/kg	6720	5

Effect on Drechslera graminea: The results from experiments with D. graminea-infected seeds conducted in 1991-1993 are shown in tables 7, 8 and 9.

25 Table 7. Results from one field experiment in 1991 conducted in Uppsala in barley infected with D. graminea.

30	Treatment	Yield kg/ha	Infected plants/m ²
	Control	3440	31
	Pan. Plus 400, 4 ml	4160	2
	MA 342, 300 ml/kg	4390	1

Table 8. Results from five field experiments conducted in 1992 in barley infected with D. graminea. Means from experiments conducted at Svalöv, Nygård, Kölbäck, Ultuna and Röbbäcksdalen.

5	Treatment	Yield kg/ha	No. of plants/m ²	Infected plants/m ²	Hectolitre weight, kg	1000-kernel weight, g
	Control	2590	383	101	66.2	40.2
	Pan. Plus 400, 4 ml	3470	381	5	66.6	39.9
10	MA 342, 300 ml/kg	3460	368	7	66.2	39.8

Table 9. Results from two field experiments in 1993 in barley infected with D. graminea. Means from experiments conducted at Kölbäck and Ultuna.

15	Treatment	Yield kg/ha	Infected plants/m ²
	Control	2810	46
20	Pan. Plus 400, 4 ml	4160	1
	MA 342, 200 ml/kg	3990	8

Effect on Drechslera avenae: The results from experiments with D. avenae-infected oats seeds conducted in 1991-1993 are shown in tables 10, 11 and 12.

25 Table 10. Results from one field experiment in 1991 conducted in Uppsala in oats ("Puhti") infected with D. avenae.

30	Treatment	Yield kg/ha	Infected plants/m ²
	Control	4940	74
	Pan. Plus 400, 4 ml	4860	32
	MA 342, 300 ml/kg	5090	17

Table 11. Results from four field experiments in 1992 in oats ("Puhti" and "Vital") infected with D. avenae. Means from experiments conducted at Svalöv and Ultuna.

5	Treatment	Yield kg/ha	No. of plants/m ²	Infected plants/m ²	Hectolitre weight, kg	1000-kernel weight, g
	Control	3990	428	22	57.5	35.4
	Pan. Plus 400, 4 ml	4080	456	13	57.8	35.5
10	MA 342, 300 ml/kg	4000	445	3	57.4	35.0

Table 12. Results from a field experiment in 1993 conducted in Uppsala in oats ("Vital") infected with D. avenae.

15	Treatment	Yield kg/ha	Infected plants/m ²
	Control	7570	79
	Pan. Plus 400, 4 ml	7870	14
20	MA 342, 200 ml/kg	7680	27

Effect on Ustilago avenae: In the field experiments with U. avenae bunt ears per m² or percentage of bunt ears were read at the time of ripening. Grain yield was not measured. Results from three experiments conducted 1991-1993 are shown in

25 Table 13.

Table 13. Results from three field experiments conducted in 1991-1993 in Uppsala in oats infected with *Ustilago avenae*.

5	Treatment	1991	1992	1993
		Bunt ears/m ²	% infected ears	Bunt ears/m ²
	Control	7	10,6	95
	Panoctine Plus 400, 4 ml	3	8,7	not tested
10	MA 342, 300 ¹⁾ ml/kg	1	1,7	15

¹⁾ 300 ml 1991 and 1992; 200 ml in 1993.

Example 6

Application of MA 342 to seeds and other plant parts

15 Applying aqueous mixtures containing MA 342 to seeds. Bacterial suspensions produced as described in example 3 or as described in example 4 above were mixed with each of the following substances or compounds:

- Talcum powder (Kebo Lab AB), 48 g/l of bacterial suspension
- Bacteriological peptone (Oxoid, Ltd.), 5 g/l of bacterial suspension
- 20 Tween 20 (Merck Ltd.), 20 ml/litre of bacterial suspension
- Metocel (Cellulose ether, Sveda Kemi AB), 12 g per litre of bacterial suspension
- Lissapol (ICI Agrochemicals Ltd.), 1 g per litre of bacterial suspension
- Bond (Newman Agrochemicals Ltd.), 1 g per litre of bacterial suspension

25 In other experiments the bacterial suspensions were centrifuged at 10,000 x g for about 10 min. and the resulting pellets were resuspended in either 0.1 M MgSO₄ or in peptone water (5 g of bacteriological peptone (Oxoid, Ltd.) per litre of tap water).

After thoroughly mixing, the resulting suspensions were applied to seeds as described in example 4 for unmixed bacterial suspensions.

30 Applying freeze-dried bacteria to plant seeds. MA 342 bacteria, grown in a shake culture as described in example 4 above, were centrifuged and the resulting pellet was resuspended in a skim milk solution (200 g skim milk powder, Semper AB,

Sweden, per litre of sterile distilled water) as a freeze drying protecting agent. The mixture was shell frozen in glass jars and then freeze dried in these jars for about 48 hours in a Hetosicc freeze drier (Heto Ltd., Denmark). The resulting powder was stored at 4°C in plastic bags or in plastic flasks with a screw cap until used. For seed application the powder was either mixed in water or in other aqueous solutions and then applied to seeds as described in example 4 for bacterial suspensions, or it was mixed with seeds in a dry condition by thoroughly shaking the powder and the seeds (about 10 g powder per kg seed) in a plastic container.

- 10 Pelleting seeds treated with MA 342. MA 342 bacteria, grown in a shake culture as described in example 4 above, were mixed in volumes 1:1 with an adherent (2% w/v aqueous solution of sodium carboxy-methyl cellulose or 50% w/v aqueous solution of gummi arabicum). The seeds were treated with this mixture as in example 4 above. An excess amount of bentonite (Dresser Minerals Inc.) or talcum powder (Kebo Lab AB) was then added to the plastic bag, the bag was inflated and vigorously shaken for a couple of minutes. After this the seeds were spread out on big trays under a fan and allowed to dry in room temperature.

- 20 Spraying plant shoots with bacterial suspensions. MA 342 bacteria, grown in a shake culture as described in example 4 above, were filled in plastic hand sprayers or in a powered sprayer and then sprayed on to plant leaves and shoots. In other treatments the bacteria were first centrifuged at about 10.000 x g for ten minutes, the pellet was resuspended in tap water and this resulting bacterial suspension was used for the spraying of plant leaves and shoots.

Example 7

- 25 Effects of purified metabolites from MA 342 against diseases caused by Drechslera teres in greenhouse experiments.

- 30 The isolate MA 342 was grown for 48 hours in half strength (15 g/l) Tryptic soy broth (Difco Ltd.) on a rotary shaker in the dark at 18-20°C. The resulting bacterial suspension was centrifuged at 48,000 g for 30 min. and metabolites in the supernatant was then further purified with Sep-pak C 18 cartridges (Waters Associates) as follows:

1. 80 ml of supernatant was added to a Sep-pak activated with 10 ml methanol.
2. The Sep-pak was washed first with 5 ml 30% ethanol and then with 5 ml 40% ethanol.
- 5 3. The metabolites were eluted by 5 ml 70 % ethanol.
4. The 70% ethanol-eluate was evaporated in a rotation-evaporator until about 1.5 ml water solution was left. It was then diluted with tap water up to a volume of 6.5 ml.

Seeds of the barley cultivar "Golf", naturally infected with D. teres, were sub-
 10 merged in this 6.5 ml water solution of the metabolites for 30 minutes and were
 then sown in pots with 50 seeds per pot. The pots were covered with glass lids and
 placed in the dark at 6°C. After nine days the lids were removed and the pots
 were placed in a greenhouse at 15-22°C for about two weeks. Germinated plants
 and disease attacks were then read as described in Example 4 above. As controls
 15 were used 1) untreated seeds and 2) seeds treated with supernatant not purified
 with Sep-pac and containing MA 342 cells.

Table 14. Effect of purified metabolites from MA 342 and supernatant con-
 taining MA 342 cells against D. teres in barley in a typical green-
 house testing.

Treatment	Per cent germinated plants	Per cent plants with attack on the first leaf
Untreated control	97,0	21,6
25 Supernatant with MA 342 cells	99,0	1,0
Evaporated cell-free eluate	99,0	0,0

Example 8

Results of comparative tests of MA 342 isolate and 11 other isolates
 30 of Pseudomonas chlororaphis received from different culture collections

Eleven different non-Swedish isolates of Pseudomonas chlororaphis having the
 designations stated in Table 1 were tested, together with MA 342 isolate, for
 effect against leaf spot disease in barley and for inducing reactions in biochemical

tests according to the test system API 20 NE. In addition, the isolates were compared for colony appearance and crystal formation on agar plates. The 11 non-Swedish isolates were from different countries and are deposited in four different well-reputed culture collections (Table 1).

5 **Test of disease-inhibiting ability in greenhouse tests**

The tests were conducted with Drechslera teres-infected barley, as described in Example 4, and all isolates were tested simultaneously in order to achieve an adequate comparison. As is evident from Table 1, the results show that MA 342 is clearly unique in the sense that no one of the other isolates tested herein has the property like MA 342 in this kind of test, viz. the ability to inhibit infection by Drechslera teres.

Table 1. Designations, country of origin and effects of MA 342 and 11 other P. chlororaphis isolates against D. teres-infection in barley in greenhouse tests.

Isolate designation	Country of	Effect on leaf spot in greenhouse tests. Percentage of infected plants after treatment with the isolate in question
MA 342	Sweden	9
USDA B2075	Czechoslovakia	53
USDA B1869	New Zealand	60
USDA B14874	USA, Colorado	56
25 USDA B14869	USA, Illinois	58
USDA B1854	USA, Louisiana	43
NCTC 10686	England	54
NCTC 7357	England	58
DSM 6508	Germany	56
30 ATCC 9446	USA	61
ATCC 17414	USA	50
ATCC 17811	USA	58
Untreated control		71

Induction of reactions in biochemical tests according to
test system API 20 NE

The tests were carried out as described above. The results are shown in Table 2. They show that MA 342 also in this respect is unique can be differentiated from the other 11 isolates tested. Some of the isolates tested may not, according to this test, be considered to be central within the species Pseudomonas chlororaphis.

Table 2. Induced reactions by the different isolates tested in a number of biochemical tests according to the test system API 20 NE. A "+" represents a positive reaction and a "-" no/negative reaction.

10

Property tested in API 20 NE test	Isolate tested											
	MA 342	B 2075	B 1869	B 14874	B 14869	B 1854	NCTC 10686	NCTC 7357	DSM 6508	ATCC 9446	ATCC 17414	ATCC 17811
Nitrate reduction	-	-	+	-	-	+	+	+	+	+	+	+
Indole production	-	-	-	-	-	-	-	-	-	-	-	-
Acid from glucose	-	-	-	-	-	-	-	-	-	-	-	-
Arginine dihydrolase	+	+	+	+	-	+	+	+	+	+	+	-
Urease	-	-	-	-	-	-	-	-	-	-	-	-
Esculin hydrolysis	-	-	-	-	-	-	-	-	-	-	-	-
Gelatin hydrolysis	+	+	-	-	-	+	+	+	-	+	+	+
B-galactosidase	-	-	-	-	-	-	-	-	-	-	-	-
Glucose assimilation	+	+	+	+	+	+	+	+	+	+	+	+
Arabinose assimil.	-	+	+	-	-	-	+	-	+	-	-	-
Mannose assimilation	-	+	+	-	+	+	+	+	+	+	+	-
Mannitol assimilation	+	+	+	-	-	+	+	+	+	+	+	-
Glucosamine assimil.	-	+	+	-	-	+	+	+	+	+	+	-
Maltose assimilation	-	-	-	-	-	-	-	-	-	-	-	-
Gluconate assimilation	+	+	+	+	+	+	+	+	+	+	+	-
Caprate assimilation	-	+	+	+	+	+	+	+	+	+	+	-
Adipate assimilation	-	-	-	-	-	+	-	-	-	-	-	-
Malate assimilation	+	+	+	+	+	+	+	+	+	+	+	+
Citrate assimilation	+	+	+	+	+	+	+	+	+	+	+	+
Phenyl-acetate assimil.	-	-	+	+	+	+	+	+	+	-	-	+
Cytochrome oxidase	+	-	+	+	+	+	-	+	+	+	+	+

Comparison of colony appearance and crystal formation on agar plates

The isolates were cultured in Petri dishes on TSA 10, as described above. We observed small differences in colony appearance between all different isolates but all isolates could not be differentiated in this way. However, the MA 342 isolate
5 was the only isolate forming typical hyaline crystals in the agar and could therefore, by this property, be differentiated from all other isolates.

CLAIMS

1. A Pseudomonas chlororaphis strain deposited at The National Collections of Industrial and Marine Bacteria Limited, Aberdeen, Scotland with the NCIMB accession number 40616 or biologically pure cultures thereof having the ability
5 to produce antipathogenically active metabolites, or mutants thereof having essentially the same characteristics as the parent strain.
2. A composition for controlling plant diseases caused by pathogenic fungi, characterized by containing as active ingredient the microorganism claimed in claim 1 or culture broth thereof containing antipathogenically active metabolites
10 thereof.
3. The composition according to claim 2, characterized in that the pathogen is the fungus Drechslera teres.
4. The composition according to claim 2, characterized in that the pathogen is the fungus Drechslera graminea.
- 15 5. The composition according to claim 2, characterized in that the pathogen is the fungus Drechslera avenae.
6. The composition according to claim 2, characterized in that the pathogen is the fungus Microdochium nivale.
7. The composition according to claim 2, characterized in that the pathogen
20 is the fungus Tilletia caries.
8. The composition according to claim 2, characterized in that the pathogen is the fungus Ustilago avenae.
9. The composition according to claims 2-8, characterized in that the active ingredient is in admixture with a carrier composition acceptable in agricultural
25 practice.
10. The composition according to claim 9, characterized in that the active ingredient is in admixture with a liquid carrier.

11. The composition according to claim 9, characterized in that the active ingredient is impregnated in a solid, porous material.
12. The composition according to claim 9, characterized by further comprising additives that serve as adherents.
- 5 13. The composition according to claim 9, characterized by further comprising a nutrient source.
14. A method of controlling plant diseases caused by pathogenic fungi and comprising the introduction of an effective dose of an active ingredient into the environment where the disease-inducing agent is to be inhibited, characterized
10 in that the active ingredients is the microorganism claimed in claim 1 or culture broth thereof containing antipathogenically active metabolites or derivatives thereof.
15. The method according to claim 14, characterized in that the active ingredient is applied to seeds.
- 15 16. The method according to claim 14, characterized in that the active ingredient is applied to plant vegetative propagation units.
17. The method according to claim 14, characterized in that the active ingredient is applied to plants.
18. The method according to claim 14, characterized in that the active ingredient
20 is applied to a growing medium in which a plant is growing or is to be grown.

STATEMENT UNDER ARTICLE 19

In order to define distinctly the claimed scope of invention claim 1 has been amended by combination of the subject matter of the original claim 1 with the subject matter of the original claim 2. Further, claim 2, original claim 3, has been supplemented with a statement that the composition also may contain a culture broth of the microorganism containing antipathogenically active metabolites, with reference to Example 7.

It is respectfully submitted that the amended claims refer to a patentable invention in relation to the prior art cited in the Search Report, in view of the unambiguous results presented in Example 8 providing results of comparative tests.

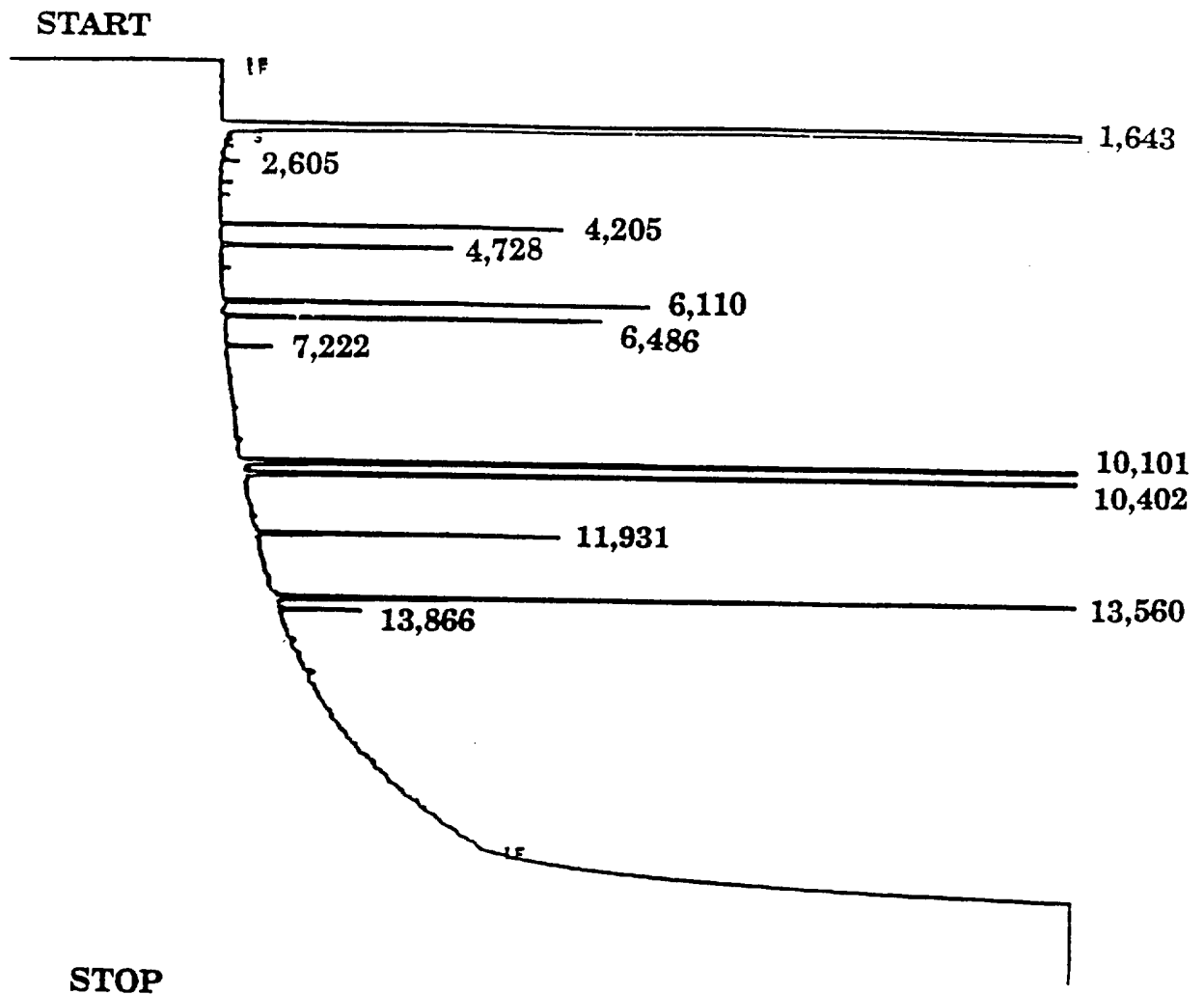


FIGURE 1