European Journal of Plant Pathology **103:** 25–33, 1997.

Biological control of cereal seed-borne diseases by seed bacterization with greenhouse-selected bacteria

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Accepted 7 July 1996

Key words: bacterial antagonism, *Drechslera teres, Microdochium nivale,* rhizobacteria, screening methods, *Tilletia caries*

Abstract

About 400 bacterial strains, isolated from roots of wild and cultivated plants, were screened for effects against diseases caused by *Drechslera teres* and/or *Microdochium nivale* in greenhouse tests and against common bunt caused by *Tilletia caries* in field tests. Four of the strains showed good biocontrol activity (>70% disease reduction) against *D. teres* and *T. caries* both in screenings and field tests. One *Pseudomonas* isolate, MA 342, strongly and reliably suppressed both *D. teres* and *T. caries* in the field, while effects against *M. nivale* were weaker. The effects could not be enhanced by varying pre-application or seed application procedures. This isolate could be stored as a suspension in a refrigerator, frozen or applied to seeds for at least one month without loosing its disease controlling ability.

Introduction

Seed bacterization has proven a successful method for enhancing biological nitrogen fixation in legumes (e.g., Stacey et al., 1992), and it has been widely tested also for other purposes, *viz*. for inducing growth promotion (Brown, 1974; Suslow and Schroth, 1982; Graystone et al., 1991) or biological control of plant diseases (Brown, 1974; Tahvonen, 1982; Pierson and Weller, 1994). In most cases single bacterial isolates of nitrogen fixers, plant growth-promoting bacteria or pathogen antagonists have been applied to seeds and seed pieces with the intention that they should proliferate, spread to roots and there exert their activity. It is well documented that nitrogen fixing *Rhizobium* and certain growth-promoting bacteria behave in this way (Kloepper, 1980; de Freitas and Germida, 1992; Wiehe et al., 1994). Also for some antagonistic organisms applied to seeds for combating specific plant diseases, seed bacterization seemingly gives rise to root colonization and in certain cases good disease control effect (Dileep Kumar and Dube, 1992; Toyoda et al., 1993; Kortemaa et al., 1994). However, several workers also report varying and inconsistent disease control results (Weller, 1988), failure to obtain effects (Hornby et al., 1993) or good greenhouse results that could not be repeated in the field (Duczek, 1994). One of the critical factors seemingly is the selection of suitable isolates for the climate, soil or growth medium, disease and crop combination tested and consequently various more or less laborious screening procedures have been applied (e.g., Leyns et al., 1990; de Bruyne et al., 1991). However, so far good and reliable general screening procedures have been difficult to envisage (Merriman and Russell, 1990) and new concepts here should lead to selection of antagonists with new characteristics.

In Swedish cereal production we are presently heavely dependent on chemical compounds for controlling seed-borne diseases and especially within organic farming alternative methods are eagerly required. Most of the chemical seed dressing is used for controlling *Drechslera teres* (Sacc.) Shoemaker, the causal agent of net blotch in barley, *Microdochium nivale* (Fr.) Samuels & Hallett, causing snow mould or other damages in wheat, rye, barley and oats and *Tilletia caries* (DC.) Tul. & C. Tul., causing common bunt in wheat.

This report presents results of a specific screening, developed to find new bacterial strains effective against diseases caused by seed-borne *D. teres*, *M. nivale* and *T. caries* after seed bacterization, and of field testing of one bacterial strain selected in the screening procedure.

Materials and methods

Bacterial isolation and maintenance

Bacterial strains were isolated from roots of wild and cultivated plants collected at various places in Sweden. Plants were dug up with roots and adhering soil and placed in plastic bags for transport to the laboratory, where the roots were washed vigorously in tap water to remove all soil. A young piece of root (2–3 cm long) was then excised and 5 to 10 small cuts were made in it with a flamed scalpel before it was rubbed against the surface of TSA 10 agar (10 g Tryptic Soy Broth (Difco Ltd.); 12 g Technical Agar (Oxoid Ltd.) in 1000 ml distilled water). The agar plates were incubated in 0°, 6° or 15 °C, observed for bacterial growth at least once a week and all morphologically distinct colonies from a Petri dish were pure-cultured on new TSA 10 plates for later use in the screenings. Strains were stored in small ampoules at −70 °C. As freeze support were used i) 10% glycerol in tap water (pH 7.15) and in ii) 20% (w/v) skim milk powder (Semper) in distilled water (autoclaved 5 min at 121 °C, pH adjusted to 6.45).

A few isolates were identified taxonomically, using fatty acid analysis (Microbial Identification System, MIDI Ltd., USA,), API 20 NE (API System Ltd., France) and Biolog GN MicroPlate (Biolog Inc., USA).

Screening procedures

Bacteria were screened for ability to suppress *D. teres* infections in barley (400 isolates), and/or *M. nivale* infections in winter wheat (309 isolates) in greenhouse screenings. They were also tested against *T. caries* infections in winter wheat (133 isolates) in field screenings. Normally, 30 isolates were tested in each screening experiment. Isolates showing over 70% disease reduction compared to control were retested. If the effect (>70% disease reduction) could be repeated, the isolates were selected for field experiments.

Isolates were cultured individually on TSA 10 agar plates, at 15 °C for 24–48 h. The bacterial lawn was then scraped from the agar surface and mixed with 40 ml of SNB nutrient broth (Gerhardson et al., 1985) and 40 ml of a 2% (w/v) solution of sodium-carboxymethyl cellulose (CMC) in sterile distilled water. This mixture was poured over seeds infected/infested with the target pathogen. After 20 min, excess liquid was poured off and the seeds were dried under a fan over night. Seeds treated with SNB and CMC without bacteria were used as control. In greenhouse screenings, the dried seeds treated with one isolate were sown in two pots (18 cm in diameter and 4 cm high), each with 50 seeds and in field screenings seeds from one treatment were sown in three to four rows, each 50 cm long. In the greenhouse, seeds were sown in an unsterilized, commercial peat mixture (Enhetsjord K Normal), mixed with 20% (v/v) sand. The field screenings were conducted on a well fertilized, cultivated soil (sandy loam) outside our institute.

For screening against *D. teres,* a spring barley (cv Golf) seed lot heavily infected with *D. teres* was used. After sowing the bacterial-treated seeds, the pots were watered, covered with glass lids and placed in the dark at 6 °C for seven days. The lids were then removed and the pots were placed in a greenhouse at about 20 °C for two weeks. The plants were watered daily and in the winter, extra light (Philips HPI-T mercury lamps, 400 W) was supplied to give a light period of at least 16 h. Disease was recorded as the number of plants with net blotch symptoms on the leaves and/or discoloured coleoptiles. The percent disease control was calculated by taking 100 – (100 × No. of diseased plants in the treatment/No. of diseased plants in the control).

For screenings against *M. nivale,* seeds of winter wheat (cv Kosack) were artificially infested with the pathogen prior to bacterial treatment. The fungus was cultivated for seven days in potato dextrose broth (24 g Potato Dextrose Broth, Difco Ltd., per 1000 ml distilled water) at about 20 °C on a rotary shaker in the dark. The resulting slurry was homogenized with a kitchen blender and poured over the seeds. After 30 min the surplus liquid was poured off and the seeds were left to dry under a fan over night. After a second treatment with bacteria, the seeds were sown and the pots were covered with glass lids and placed in the dark in 6 °C for five days. The lids were then removed and each pot was watered with 100 ml water and placed inside a five-litre plastic bag that was supported by two wooden sticks. The pots were placed in a greenhouse at 12–15 °C for eight days. The percent disease control was counted as above after recording the number of diseased and unemerged plants in treatments and controls.

In the field screenings against *T. caries*, seeds of winter wheat (cv Kosack) artificially infested by mixing 2 g crushed *T. caries* infected ears with 1 kg seed were used. Bacterial treatments and sowing was done at or somewhat later than at normal sowing time in the autumn. The wheat received no special treatment except weeding. In the late summer of the following year, the frequency of infected ears in each treatment was recorded and the percent disease control was counted as described above.

Field experiments

Two *Pseudomonas* isolates, MA 342 and MA 250, showing marked disease suppression in screenings, were tested in field experiments. MA 342 was tested against *D. teres, M. nivale* and *T. caries* and MA 250 was tested against *M. nivale*. Three other isolates (all *Pseudomonas* sp.) with good effects in the screenings were tested in one field experiment for effects against *D. teres* and in one field experiment against *T. caries*. Bacterial inocula were prepared by incubation for 48 h in half strength (15 g per litre) TSB (Tryptic soy broth, Difco) on a rotary shaker in the dark at 18–20 °C. In the experiments, seed lots (barley, cv Golf in the *D. teres* experiments, spring wheat cv Drabant and winter wheat cv Kosack in the *M. nivale* and *T. caries* experiments, respectively) infected/infested with the different pathogens as described for the screenings, were used. Seed was bacterized by mixing bacterial inoculum and seeds (300 ml inoculum per kg seed) in a plastic bag for 4 to 5 min. Seeds were then dried under a fan at room temperature for about 24 h and stored in room temperature for up to one week before sowing. Controls included i) untreated seeds and ii) seeds treated with the fungicide Panoctine Plus 400 (150 g guazatine + 10 g imazalil per litre; Rhône-Poulenc Ltd.) (*D. teres* experiments) or Panoctine 400 (150 g guazatine per litre; Rhône-Poulenc Ltd.) (*M. nivale* and *T. caries* experiments) at a dosage of 4 ml per kg. Treatments were in a randomized block design with four replications. Plot sizes varied from 8 m^2 (experiment with *T. caries*) and up to 25 m^2.

In experiments with *D. teres* and *M. nivale*, the number of germinated seeds were recorded by counting seedlings in 4 meters of row (corresponding to 0.5 m^2) per plot. The number of plants with leaf symptoms were counted in 8 meters of row (1 m^2) per plot in the 2–3 leaves stage (*D. teres*). In the *M. nivale* experiments, the plant stand was scored (0–100) in the spring. In one of the *M. nivale* experiments, 75 plants were dug up from each plot and examined for brown coleoptiles. At harvest the grain yield, the thousand kernel weight and hectolitre weight were recorded (*D. teres* and *M. nivale*). In experiments with *T. caries*, the frequency of infected ears at ripening was recorded.

Tests of bacterial handling and seed application procedures

Different formulations and application methods of isolate MA 342 were tested against *D. teres* in the greenhouse, using the same method as in the screenings. A shake culture grown in TSB, 50% for 48 h, was used as a standard (control). All liquid formulations were applied to seeds as described for the field trials.

Bacterial suspension and CMC: the liquid culture was mixed 1:1 (v/v) with 2% w/v aqueous solution of CMC.

Centrifuged bacterial suspension: the liquid culture was centrifuged (10,400 × g for 10 min) and the resulting pellet was resuspended in i) peptone water (5 g of bacteriological peptone, Oxoid, Ltd., per litre of tap water) or ii) in tap water to the original volume or iii) the supernatant was sterile filtrated (Minisart N, 0.2 um, Sartorius Inc.).

Freeze drying: after centrifugation the pellet was resuspended in a skim milk solution (200 g skim milk powder, Semper AB, per litre of sterile distilled water), shell frozen in glass jars and then freeze dried for about 48 h in a Hetosicc freeze drier (Heto Ltd., Denmark). The resulting powder was stored at 4 °C in plastic flasks with a screw cap. Seeds were treated with bacteria rehydrated in i) peptone water or ii) tap water, with or without 1% CMC or iii) dry powder was mixed with seeds by thoroughly shaking about 10 g powder per kg seed in a plastic container.

Pelleting: the shake culture was mixed 1:1 (v/v) with either 2% w/v aqueous solution of CMC or 50% w/v aqueous solution of gum arabic and the seeds were treated as described above. An excess amount of bentonite (Dresser Minerals Inc.) or talcum powder (Kebo Lab AB) was added to the plastic bag which was inflated and vigorously shaken for two minutes. The seeds were spread out on trays under a fan and allowed to dry at room temperature.

Effect of storage of bacterial suspensions and treated seeds

Bacterial suspensions of MA 342, grown in shake culture as described above, were stored in its liquid medium (TSB, 50%) at 4 °C and at −20 °C for two months and were then tested for effects against *D. teres* in the greenhouse test used for screenings. Small lots of the MA 342-treated, *D. teres*-infected seeds prepared for field trials were stored for 1, 3 and 6 weeks at 4 °C and at 20 °C and were then likewise tested in the greenhouse test.

Results

Isolation of bacteria

Both fast and slow growing bacterial colonies with a wide range of morphological characters were isolated. Only a few of the disease suppressing isolates were subjected to taxonomical identification. On the basis of biochemical (e.g., fatty acid analysis) and carbon source assimilation profiles (99 carbon sources tested) the isolates MA 342 and MA 250 were both identified as *Pseudomonas*, with MA 250 closest to *P. fluorescens* and MA 342 closest to *P. chlororaphis* (syn. *P. aureofaciens*).

Screening results

Figure 1 shows the distribution of bacterial isolates with different effects on the tested diseases in the screenings, from disease enhancement to disease suppression. In the *D. teres* screenings (Figure 1a), 4.5% of the 400 tested isolates reduced the disease incidence by more than 70%, compared to controls. In the *M. nivale* (Figure 1b) and the *T. caries* screenings (Figure 1c), the corresponding percentages were 12% out of 309 and 9% out of 133 isolates tested, respectively. In the *D. teres* screenings 0.5% and in the *T. caries* screenings 16% of the isolates increased disease incidence by more than 50%, while in the *M. nivale* screenings no isolates with pronounced disease enhancing effects were found.

Bacterial isolates originating from roots of monocotyledonous plants (49% of the tested isolates) did not differ significantly in disease affecting ability (disease suppression or enhancement by more than 50%) from those originating from dicotyledonous plants (51% of the tested isolates).

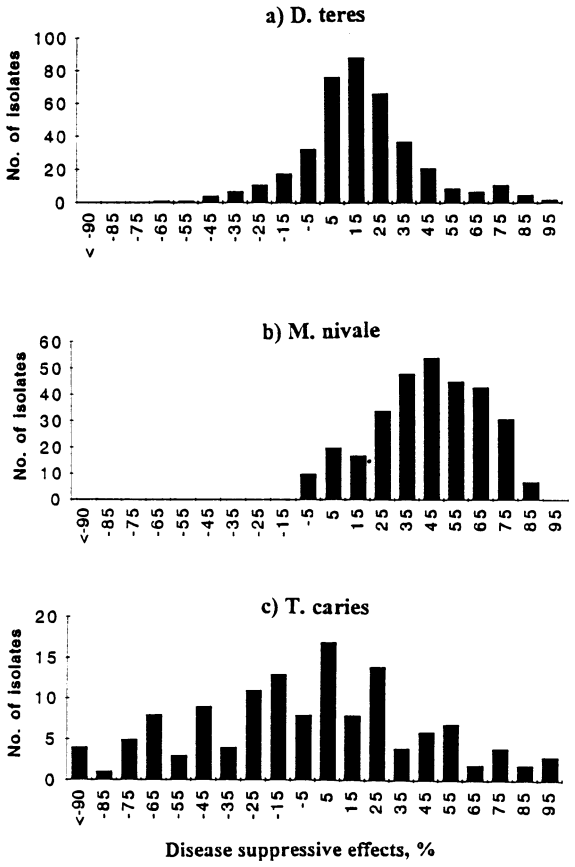

Figure 1. The number of bacterial isolates with different percentage of disease suppression, arranged in steps of ten percent, in screenings against a) *Drechslera teres*, b) *Microdochium nivale* and c) *Tilletia caries*.

Two bacterial strains, MA 342 and MA 250, showed a high degree of disease suppression in the earliest screenings and were selected for testing in field trials. MA 342 reduced disease incidence by 78% in the *D. teres* screening, 80% in the *M. nivale* screening and 100% in the *T. caries* screening. The corresponding figures for MA 250 were 13% in the *D. teres* screening, 79% in the *M. nivale* screening and −3.3% in the *T. caries* screening. MA 250 was, thus, included only in the *M. nivale* field trials.

Three other *Pseudomonas* strains also showed promising effects in the screenings where they reduced disease incidence by 76% to 89% in the *D. teres* screenings, 78% to 88% in the *M. nivale* screenings and 75% to 89% in the *T. caries* screenings. These three strains were tested in one *D. teres* and one *T. caries* field experiment.

Table 1. Results from four field experiments in 1991 and five field experiments in 1992, where *D. teres*-infected spring barley was bacterized with isolate MA 342. Figures are means from all the experiments conducted each year at various places in Sweden, from 59.9° to 55.6°

Treatment	Yield, kg ha^{-1}	No. of plants (m^2)$^{-1}$	No. of infect. plants (m^2)$^{-1}$	Hectolitre weight, kg[1)]	1000-kernel weight, g[1)]
			1991		
Control	4970 A[2)]	360 A	46.0 A	64.7 A	41.5 A
Panoctine Plus 400	5510 A	361 A	0.3 B	66.3 A	43.8 A
MA 342	5480 A	353 A	0.3 B	66.0 A	43.7 A
			1992		
Control	4290 A	358 A	48 A	67.9 A	51.7 A
Panoctine Plus 400	4380 A	378 A	1 B	67.8 A	50.5 A
MA 342	4300 A	380 A	1 B	68.3 A	52.6 A

[1)] The replicates for each treatment in every separate experiment were pooled before measurement.
[2)] Means with the same letter are not significantly different at $P = 0.05$ according to Duncan's multiple range test.

Table 2. Results from two field experiments with MA 342 and MA 250 treatment of spring wheat infected with *M. nivale*, conducted in 1992 at two different climate zones (59.9° and 55.6°)

Treatment	Yield, kg ha^{-1}[1)]	No. of plants (m^2)$^{-1}$[1)]	Plant stand, as scored (0–100)[1)]	% dark coleoptiles[2)]	Hectolitre weight, kg[3)]	1000-kernel weight, g[3)]
Control	4600 A[4)]	260 C	80.0 C	89 A	78.9	43.4
Panoctine 400	4520 A	491 A	96.8 A	8 C	79.5	43.4
MA 250	4240 A	428 AB	93.8 AB	40 B	79.9	44.5
MA 342	4310 A	395 B	91.2 B	29 BC	78.8	43.0

[1)] Figures are means of both field trials.
[2)] Only recorded in one of the field trials.
[3)] The replicates for each treatment were pooled before measurement. Only recorded in one of the field trials.
[4)] Means with the same letter are not significantly different at $P = 0.05$ according to Duncan's multiple range test.

Bacterial effects in field experiments

In 1991 four field experiments and in 1992 five field experiments with *D. teres*-infected seed were carried out at different localities in Sweden (Table 1). In these experiments, the effect of chemical treatment was compared to treatment with the bacterial isolate MA 342. In both years MA 342 reduced disease significantly compared to controls while no differences could be seen between bacterial and chemical treatment. No significant differences in yield between the treatments were obtained.

Table 2 shows the results from two field experiment with bacterial and chemical treatment of *M. nivale*-infested wheat seeds. Both bacterial and chemical treatment significantly increased the number of plants per m^2 and plant stand and significantly decreased plants with dark coleoptiles (only recorded in one of the field trials). MA 250 gave a better effect than MA 342 but was not as good as the chemical treatment. No significant differences in yield between the treatments were found.

The results presented in Table 3 show the control effect of the isolate MA 342 against seed-borne *T. caries*-infection in a field experiment conducted in Uppsala in 1991/92. Both MA 342 and the chemical Panoctine 400 significantly reduced the frequency of infected ears, compared to control.

Table 3. Effect of seed treatment with MA 342 against seed-borne *T. caries*-infection in a field experiment in winter wheat conducted in 1991/92 in the Uppsala area (59.9°)

Treatment	Infected ears, % (mean of four replicates)
Control	23 A[1)]
Panoctine 400	2 B
MA 342	0 B

1) Means with the same letter are not significantly different at $P = 0.05$ according to Duncan's multiple range test. The test was performed on arcsine transformed values.

Table 4. The influence of CMC, peptone water and tap water on the biocontrol effect of isolate MA 342 against *D. teres* in a greenhouse test. Disease incidence was recorded as percent plants with primary leaf symptoms

Treatment	Diseased plants, % (mean of four replicates)
Untreated, control	26.4 A[2)]
MA 342 in TSB	0.5 B
MA 342 in TSB, CMC, 1%	4.5 B
MA 342 in peptone water[1)]	5.0 B
MA 342 in tap water[1)]	3.5 B

1) The bacterial isolate was grown in TSB, centrifuged and resuspended in peptone water or tap water.
2) Means with the same letter are not significantly different at $P = 0.05$ according to Duncan's multiple range test. The test was performed on arcsine transformed values.

Effects of various seed application procedures for isolate MA 342

In the tests of different ways of handling the isolate MA 342 before seed treatment and of different ways of applying the isolate to the seeds, the biocontrol effects in greenhouse tests were in all cases better in the standard treatment (shake culture (TSB, 50%) used as described for the field trials). The result from one of these experiments is shown in Table 4. Seed treatment with sterile filtrated supernatant did not reduce the number of plants with symptoms of *D. teres* infection, compared to the nontreated control.

Effects of storage of bacterial suspensions and of treated seeds

Results of experiments carried out for testing effects of storage of bacterial suspension are presented in Table 5 and of effect of storing treated seeds in Figure 2.

Table 5. Disease suppression of *D. teres* by MA 342 after storing bacterial shake cultures in 4 °C and −20 °C for one and two months before treating diseased seeds. Disease incidence was recorded as percent plants with primary leaf symptoms. Figures show means of two replicates

Treatment	% diseased plants after storage at	
	1 month	2 months
Untreated, control	29	18
Fresh MA 342	n.t.[1)]	3
MA 342, 4 °C	5	18
MA 342, −20 °C	3	5

1) Not tested.

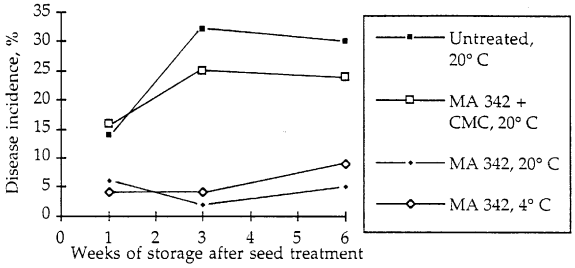

Figure 2. Disease suppression of *D. teres* by MA 342, applied as a seed treatment, after storing the treated seeds at 4 °C and 20 °C for different periods of time before sowing. Disease incidence was recorded as percent plants with brown coleoptiles.

Discussion

The field experiments performed showed that one isolate, MA 342, controlled diseases caused by *D. teres* and *T. caries* as reliably and effectively as the tested fungicides (Tables 1 and 3). These results together with the findings that this isolate is easily grown in shake culture, is simple to apply to seeds and is storable for prolonged times without loosing its activity (Table 5, Figure 2), suggest that it is suitable as a biological control agent and possibly also for commercial use. As MA 342 was only one of four isolates with significant disease control effect both in the *D. teres* screenings and in the field experiments, our screening procedure seemingly was effective. Few earlier attempts to control *D. teres* biologically (e.g., Abrahamsen, 1992; Ali Haimoud et al., 1993) have been reported, suggesting that this seed-borne pathogen may be especially easy to control by microbial agents. In our experience, however, several known antagonists (*Trichoderma* spp., *Serratia* spp., various strains of *Pseudomonas fluorescens*) are not effective against *D. teres* (Amunds-

son and Hökeberg, 1984), which makes such a statement less probable. More plausible reasons for finding isolates with good field performance among the rather limited number screened are that the isolation procedure adopted gave a specific selection of bacteria among which there are good agents, and secondly, that our *D. teres* screening procedure is highly relevant to field performance of screened organisms.

We have found only a few earlier reports dealing with biological control of *T. caries*. Kollmorgen and Jones (1975) found isolates of *Streptomyces* and *Bacillus* which reduced germination of *T. caries* and *T. foetida*. Kollmorgen (1976) showed that *Bacillus* reduced disease incidence under field conditions. Elsherif and Grossmann (1991) tested five *Pseudomonas* isolates in artificially infested winter wheat and 35 isolates in spring wheat. These authors grew the wheat plants in a peat/sand mixture in Mitscherlich vessels. One of the isolates tested in winter wheat controlled the disease to the same degree as the fungicide tested. However, to our knowledge, there are no reports on the field performance of this isolate. As the symptoms of this disease cannot be read before early ripening, the *in planta* screening is very time consuming. In the present study, four of the isolates with good biocontrol activity in the *D. teres* screenings also showed a high degree of disease control in the *T. caries* screenings. Since the biocontrol performance of these isolates was repeated in field experiments (data not shown), there appears to be a correlation between biocontrol activity against *D. teres* and *T. caries*. Thus, the *D. teres* screening, which takes three weeks from seed treatment to scoring, possibly can be used as a prescreen of isolates to be screened in the much slower *T. caries in planta* test.

The isolate MA 250 did not give full effect when tested in two field experiments with *M. nivale* (Table 2), even though it showed promising results in the *M. nivale* screening. This is in accordance with results from field testings of other high ranking isolates from the *M. nivale* screening (data not shown). Obviously the screening procedure used in this case was too artificial to select isolates with good field performance and we clearly met the well-known problem of choosing a screening regime that is time efficient enough to allow testing of a large number of isolates, but which at the same time selects for isolates that have all the different traits needed. The distribution of tested isolates according to disease control effects (Figure 1), showing most tested isolates to have good effects in the *M. nivale* screenings, also indicates that in this case the selection was not exclusive enough. In both the *D. teres* and the *T. caries* screenings a range of effects from strongly disease suppressing to strongly disease increasing isolates, and a majority of neutral isolates, were obtained. Probably this distribution mirrors an actual, field relevant situation for the specific select of bacteria tested: a few are effective disease antagonists, a few are strong disease synergists, even though they are not much investigated, and most are more or less disease neutral.

It is interesting to note from the screening results that bacterial origin (host plant and climate zone) was not a critical factor in finding effective disease antagonists. Thus, for effects toward the diseases tested here, we found no differences between monocotyledonous and dicotyledonous plants as sources for isolation. The original host plants, both wild and cultivated, were collected from a variety of climate zones, but we could not note any one as more favourable than any other. Several authors (e.g., Campbell, 1989; Deacon, 1991; Weller, 1988) state that in choosing sources for isolation of antagonists, it is important to look for environments that follow close to the conditions where the antagonist should act, that is under conditions where the target pathogen can cause disease. Our results contradict this statement.

The use of additives (Table 4) or different seed application methods did not increase the biocontrol activity of the isolate MA 342. The best result was obtained where bacteria in liquid growth medium without additives were shaken together with the seeds. This indicates that bacterial metabolites of importance for the disease suppression may be accumulated in the growth medium. These metabolites could then be inhibited by different additives such as CMC, bentonite and talcum and the biocontrol effect would show a decrease as in the treatments, MA 342 in peptone water and MA 342 in tap water, where the liquid growth medium is discarded (Table 4). However, the negative effects of resuspending centrifuged bacterial cells in peptone or tap water is not very strong and the hypothetical metabolites could, thus, be only partly inhibited or partly responsible for the effect. Actively metabolizing bacterial cells seem to be a prerequisite for the biocontrol as the sterile filtrated supernatant alone, where the concentration of the hypothetical metabolites probably is to low to decrease the number of infected plants, did not have any disease control effect.

The biocontrol activity after storing treated seeds could not be increased by adding CMC (Figure 2).

CMC and other additives have repeatedly been used to protect and adhere bacterial biocontrol agents to seed and seed pieces (Suslow and Schroth, 1978; Geels and Schippers, 1983; Kloepper et al., 1985; Weller and Cook 1986; Dileep Kumar and Dube, 1992) which again suggests that MA 342 may have a rather specific action mechanism. The surprisingly good shelf life on treated seeds (Figure 2) as well as the possibilities of storing MA 342 in its growth medium for up to one month or deep frozen for even longer without decrease in the biocontrol effect (Table 5), is of great advantage from a practical point of view. These characters and the availability of a reliable greenhouse biotest also is advantageous in the investigations for elucidating the action mechanism now under way.

Acknowledgements

We thank Annika Gustafsson, Tiiu Jerkeman and Britt-Marie Jingström for excellent technical assistance. This study was funded by Nordiskt Kontaktorgan för Jordbruksforskning and Stiftelsen Svensk Lantbruksforskning.

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