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# Biological Control of Damping-Off of Alfalfa Seedlings with *Bacillus cereus* UW85

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**We explored the potential of biological control of alfalfa (*Medicago sativa* L.) seedling damping-off caused by *Phytophthora megasperma* f. sp. *medicaginis* by screening root-associated bacteria for disease suppression activity in a laboratory bioassay. A total of 700 bacterial strains were isolated from the roots of field-grown alfalfa plants by using Trypticase soy agar. A simple, rapid assay was developed to screen the bacteria for the ability to reduce the mortality of Iroquois alfalfa seedlings that were inoculated with *P. megasperma* f. sp. *medicaginis* zoospores. Two-day-old seedlings were planted in culture tubes containing moist vermiculite, and each tube was inoculated with a different bacterial culture. Sufficient *P. megasperma* f. sp. *medicaginis* zoospores were added to each tube to result in 100% mortality of control seedlings. Of the 700 bacterial isolates tested, only 1, which was identified as *Bacillus cereus* and designated UW85, reduced seedling mortality to 0% in the initial screen and in two secondary screens. Both fully sporulated cultures containing predominantly released spores and sterile filtrates of these cultures of UW85 were effective in protecting seedlings from damping-off; filtrates of cultures containing predominantly vegetative cells or endospores inside the parent cell had low biocontrol activity. Cultures grown in two semidefined media had significantly greater biocontrol activities than cultures grown in the complex tryptic soy medium. In a small-scale trial in a field infested with *P. megasperma* f. sp. *medicaginis*, coating seeds with UW85 significantly increased the emergence of alfalfa. The results suggest that UW85 may have potential as a biocontrol agent for alfalfa damping-off, thus providing an alternative to current disease control strategies.**

*Phytophthora megasperma* f. sp. *medicaginis* (Drechs.) causes seedling and root disease of alfalfa (*Medicago sativa* L.) in poorly drained soils in many alfalfa-growing regions of the world (8, 17). The pathogen appears to depress yield by stunting and girdling the host roots (19). In the presence of the pathogen, resistant alfalfa cultivars frequently produce 40 to 50% more forage material than do fully susceptible cultivars (6). However, multiple lines of evidence suggest that additional control practices are needed to augment resistance to increase the probability of seedling survival. First, although some resistance to *P. megasperma* f. sp. *medicaginis* is expressed in 3- to 7-day-old seedlings (14, 21), optimum expression of seedling resistance is not observed until the seedlings are older; thus, even resistant seedlings may be vulnerable to damping-off in early stages of development. Second, due to the genetic diversity in alfalfa, resistant cultivars differ in the proportion of resistant plants in the population (11). Third, seedling survival and forage yield of alfalfa cultivars with resistance to *P. megasperma* f. sp. *medicaginis* can be increased by application of the fungicide metalaxyl to either the seed or soil (S. Nygaard, M.S. thesis, University of Wisconsin—Madison, 1985), suggesting that genetic resistance could be supplemented to provide additional protection from *P. megasperma* f. sp. *medicaginis* for optimum yield. Furthermore, although seed application of metalaxyl is an effective method for control of seedling damping-off caused by *P. megasperma* f. sp. *medicaginis*, Hunger et al. (13) found that a low frequency of isolates of *P. megasperma* are resistant to this fungicide, suggesting that its utility may ultimately be limited by selection for a resistant population of *P. megasperma* f. sp. *medicaginis*. Last, *P. megasperma* f. sp. *medicaginis* may be only one part of the disease complex, which can include *Pythium*, *Aphanomyces*, *Rhizoctonia*, and *Fusarium* spp. (4, 9, 10, 22). Multiple control measures may be required to obtain optimum yields in the presence of the pathogen complex.

Considering the limitations of metalaxyl and genetic resistance for controlling seedling damping-off caused by *P. megasperma* f. sp. *medicaginis*, it seems appropriate to search for supplemental control strategies. The potential of biological agents for control of *P. megasperma* f. sp. *medicaginis* on alfalfa has not been fully explored. Since certain bacteria (5, 18, 25) and fungi (5) have been shown to control other diseases caused by members of the genus *Phytophthora* on other host plants, biocontrol of *P. megasperma* f. sp. *medicaginis* appears to be worthy of investigation. In this paper, we report the isolation and characterization of a strain of *Bacillus cereus* that protects alfalfa from seedling disease caused by *P. megasperma* f. sp. *medicaginis*.

## MATERIALS AND METHODS

**Culture of bacteria and fungi.** Bacteria were cultured in tryptic soy broth (TSB), minimal medium C (Min 3C), and Casamino Acids (Difco Laboratories, Detroit, Mich.)-glucose broth (CB). Fungi were cultured on V8 medium. TSB contained 30.0 g of Trypticase soy broth (BBL Microbiology Systems, Cockeysville, Md.) per liter, and Trypticase soy agar (TSA) (BBL) contained an additional 15.0 g of agar per liter. Min 3C was a modification of that described by Thorne

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TABLE 1. Suppression of damping-off by filtrates of cultures of UW85 in various stages of sporulation and spore release[a]

| Culture medium and age (h) | Developmental stage | Seedling survival (%) |
|---|---|---|
| None | None | 6.0 a |
| TSB | | |
| 24 | >90% vegetative | 12.5 b |
| 48 | 50% unreleased, 50% released spores | 35.0 c |
| 72 | >90% released spores | 60.0 c |
| Min 3C | | |
| 24 | >90% vegetative | 1.0 a |
| 48 | 50% unreleased, 50% released spores | 24.0 b |
| 72 | >90% released spores | 44.0 c |

[a] Each value represents the mean percent of 72 seedlings; values followed by different letters differ significantly at $P = 0.05$. Each tube was inoculated with $10^4$ zoospores of *P. megasperma* f. sp. *medicaginis*; bacterial culture filtrate (0.3 ml) was added to each tube; seedling viability was scored 7 days after inoculation.

TABLE 2. Effects of bacterial culture media on the suppression of damping-off by UW85[a]

| Treatment | Seedling survival (%) |
|---|---|
| None | 0.6 a |
| UW85 in TSB | 45.8 b |
| UW85 in CB | 80.5 c |
| UW85 in Min 3C | 80.5 c |

[a] Each value represents the mean percentage of 72 seedlings; values followed by different letters differ significantly at $P = 0.01$. Each tube was inoculated with $10^4$ zoospores of *P. megasperma* f. sp. *medicaginis*. All seedlings that did not receive zoospores survived; all seedlings inoculated with bacteria alone survived; all seedlings inoculated with zoospores and sterile TSB, CB, or Min 3C died.

(24) and contained, per liter, 2.0 g of $(NH_4)_2SO_4$, 6.0 g of $KH_2PO_4$, 14.0 g of $K_2HPO_4$, 0.2 g of $MgSO_4 \cdot 7H_2O$, 0.25 mg of $MnSO_4 \cdot H_2O$, 1.0 g of trisodium citrate $\cdot 2H_2O$, 0.01 g of thiamine hydrochloride, 2.0 g of L-glutamic acid, and 5.0 g of acid-hydrolyzed casein (Sigma Chemical Co., St. Louis, Mo.). After autoclaving, 10 ml of a sterile 50% (wt/vol) glucose solution and 10 ml of sterile $FeCl_3 \cdot 6H_2O$ (4.0 mg/ml) were added.

CB medium contained, per liter, 10.0 g of acid-hydrolyzed casein (Sigma), 10 ml of 50% (wt/vol) glucose solution, and 1 ml of Spizizen salt solution (1). The Spizizen salt solution contained, per liter, 20.0 g of $(NH_4)_2SO_4$, 140 g of $K_2HPO_4$, 60.0 g of $KH_2PO_4$, 10.0 g of sodium citrate $\cdot 2H_2O$, and 2.0 g of $MgSO_4 \cdot 7H_2O$. The salt solution was adjusted to pH 7.0 with KOH. The Casamino acids, glucose, and Spizizen salt solutions were autoclaved separately. V8 medium contained, per liter, 800 ml of distilled water, 200 ml of V8 juice, 2.5 g of $CaCO_3$, and 15.0 g of agar.

All bacteriological tests for identification of UW85 were conducted by the methods of Gerhardt et al. (7). *B. cereus* 569 was the kind gift of C. B. Thorne (University of Massachusetts, Amherst, Mass.), and *B. subtilis* from Quantum 4000 was obtained from Gustafson Inc., Dallas, Tex.

**Isolation of bacterial collection.** A total of 20 2-year-old and 13 4-year-old alfalfa plants were removed from Wisconsin field sites at Cross Plains, Verona, Arlington, Marshfield, and Hancock. The first three sites have well-drained silt loam soils, Marshfield has a poorly drained silt loam soil, and Hancock has a sandy soil. The loosely adhering soil was removed from the roots by vigorous shaking, and a 1-cm segment 5 cm below the soil line was removed from the tap root of each plant. The root segment was placed in 10 ml of sterile distilled water and sonicated for 15 s with a 250-W Vibra-cell sonicator (Sonics and Materials, Danbury, Conn.) which operates at 20 KHz and was set at 20% output or 25 W. The sonicated liquid was then serially diluted in sterile distilled water, and the dilutions from $10^3$ to $10^6$ were plated on TSA.

After incubation at 30°C for 3 days, the plate with the greatest number of isolated colonies was selected for further processing. From each plate, one colony representing each morphological type was picked and streaked for purity on TSA. An average of 5 to 10 colonies were chosen from each plate. A stock culture was prepared from a single colony of each purified culture. Stock cultures were grown on TSA slants for 3 days at 30°C and then stored at 4°C.

**Bioassay for seedling damping-off.** Seeds of alfalfa cultivar Iroquois were immersed in 18 M sulfuric acid for 10 min and washed extensively with sterile distilled water. Approximately 1 g of seeds was placed in 10 ml of sterile distilled water in a 125-ml flask and shaken for 48 h at 30°C to encourage uniform imbibition and germination. During this time, the water was replaced once. The seed coats were removed with sterile forceps, and the seedlings were planted in culture tubes (12 by 75 mm) filled with moist vermiculite; three seedlings were planted in each tube. Immediately after this, 0.3 ml of a bacterial culture or culture filtrate was added. The bacterial cultures to be screened were prepared by inoculating 1 ml of TSB with a loopful of each stock culture. The cultures were maintained at a 45° angle from the shaker platform and were shaken vigorously for 3 days at 30°C, at which time most cultures contained between $10^8$ and $10^9$ CFU/ml.

Following 2 days of incubation of the seedlings at room temperature under standard laboratory illumination, $10^3$ zoospores of *P. megasperma* f. sp. *medicaginis* were added to each culture tube. In order to produce zoospores from strain I8, a plug of fungal mycelium was placed in the center of a V8 agar plate, the fungus was allowed to grow for approximately 7 days until it was within 1 cm of the periphery of the agar, and then the agar around the edge of the plate was removed. The plate was flooded with sterile distilled water and incubated at 16°C for 8 to 16 h, after which the water, which contained the zoospores, was decanted (14). The concentration of zoospores was determined with a hemacytometer, and dilutions were made with sterile distilled water. After addition of the zoospores, the seedlings were incubated at 24°C with a 12-h photoperiod at a light intensity of 244 microeinsteins/$m^2$ per s for 5 to 7 days and then scored for viability.

After the initial screening of the bacterial collection, subsequent experiments (Tables 1 and 2) were conducted in a modified bioassay as follows. The bacterial cultures or filtrates (0.3 ml) and zoospores were both added to 3-day-old seedlings. Each tube received $10^4$ zoospores, which were produced by flooding a 7-day-old culture grown on V8 agar with sterile distilled water and incubating the plate at 16°C for 8 to 16 h.

**Preparation of culture filtrate.** Cultures containing bacteria at various developmental stages were prepared by inoculating a loopful of bacteria into 40 ml of TSB or Min 3C and incubating the mixture at 28°C with vigorous shaking on a platform shaker at 170 rpm. The developmental stages of the cultures were determined by examining samples under

phase-contrast microscopy. Cultures were centrifuged at 8,000 × *g* for 20 min, and the supernatants were decanted. Each supernatant was passed through a 0.45-μm-pore-size filter and then through a 0.20-μm-pore-size filter and streaked on TSA plates to test for the presence of viable bacteria. Culture filtrates were stored at −20°C.

**Experimental design and statistical analysis of bioassay experiments.** The experimental design for the experiments reported in Tables 1 and 2 was a randomized complete block design with four blocks. Each treatment in each block was treated as an experimental unit which consisted of six culture tubes each containing three seedlings. Thus, an experimental unit consisted of a total of 18 seedlings. The experimental units within each block were randomized, and each treatment was represented once in each block. The arcsine square root of the proportion of the 18 seedlings that survived in each experimental unit was determined (23). The data were subjected to an analysis of variance and analyzed with the Statistical Analysis System (SAS Institute Inc., Cary, N.C.) statistical computer program. The data in Tables 1 and 2 are reported as the percent survival of 72 seedlings for each treatment.

**Field testing of UW85 in microplots.** To prepare the bacterial inoculum for seed treatment, 1 ml of an overnight culture of UW85 grown in TSB was spread on a TSA plate and incubated at 30°C for 3 days. The cells were scraped off the plate with a rubber spatula and suspended in 1 to 2 ml of sterile 1% methylcellulose in a 50-ml conical tube. Approximately 2.5 g of seeds was added, and the slurry was mixed vigorously with a vortex mixer until the seeds were uniformly coated. The seeds were spread in uncovered, sterile petri plates and dried in a laminar flow hood. Control seeds were coated with 1% methylcellulose and dried as described above.

To enumerate the bacteria on the dried seeds, three seeds were placed in each of separate tubes with 10 ml of sterile distilled water and sonicated for 15 s at the settings described above. Serial dilutions of the suspension were plated on TSA.

The fungicide metalaxyl [*N*-(2,6-dimethylphenyl)-*N*-(methoxyacetyl)-DL-alanine methyl ester] was applied by dusting alfalfa seeds with Apron 25W formulation (25% active ingredient; Gustafson). Approximately 100 seeds were placed in an envelope with 2.5 mg of Apron powder, and the seeds were shaken to obtain an even coating.

Field trials were conducted at Marshfield in a Withee silt loam soil known to be infested with *P. megasperma* f. sp. *medicaginis* (11). The subsoil had a high clay content, which restricted water flow, thus increasing the damping-off potential. A total of 100 seeds were spread by hand in a circular microplot 30 cm in diameter surrounded by a plastic border of standard garden edging. The seeds were covered with soil to a depth of 1 cm. Microplots were placed 1 m apart in a randomized complete block design with five replicates. Treatments included a seed coating of methylcellulose, methylcellulose and UW85, or metalaxyl. Emergence was determined by counting all of the seedlings in each microplot 3 weeks after planting.

## RESULTS

**Isolation and screening of bacterial collection.** We initiated a search for a biocontrol agent for alfalfa damping-off by screening bacteria that were associated with the roots of symptomless alfalfa plants. A total of 700 bacterial isolates were obtained from the roots of alfalfa grown in a variety of Wisconsin soils. The cultures were screened in a small-scale, rapid bioassay (Fig. 1) for the ability to reduce seedling mortality caused by *P. megasperma* f. sp. *medicaginis*. Each culture was tested in one tube containing three alfalfa seedlings inoculated with enough *P. megasperma* f. sp. *medicaginis* zoospores ($10^3$) to kill all of the seedlings in the absence of bacterial inoculation. The bacterial cultures that protected all three seedlings in a tube were retested in three tubes. The only culture that reduced mortality to 0% in the original test and in two subsequent tests was an isolate designated UW85, which was originally isolated from the root of a 2-year-old alfalfa plant collected at Arlington. Other isolates reduced mortality in the first test, but none consistently reduced mortality to 0% in two or three tests. In a harsher bioassay in which $10^4$ zoospores were added to each tube, UW85 treatment resulted in 40 to 90% seedling survival.

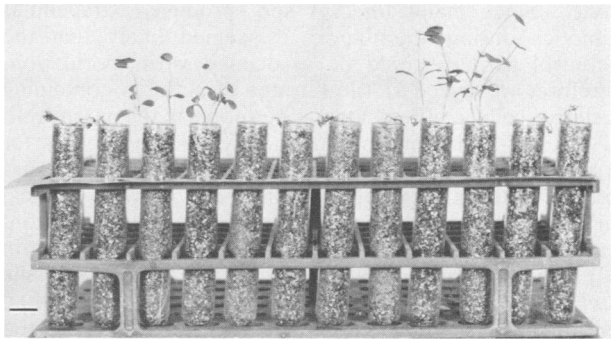

FIG. 1. Rapid bioassay system for alfalfa damping-off 7 days after inoculation. Dead seedlings were treated with $10^4$ zoospores of *P. megasperma* f. sp. *medicaginis*; live seedlings on the left were untreated, and live seedlings on the right were treated with zoospores and 0.3 ml of a culture of UW85 containing predominantly released spores ($5 \times 10^8$ CFU/ml). Bar = 1 cm.

**Identification of UW85 as *B. cereus*.** UW85 was identified as *B. cereus* on the basis of standard bacteriological criteria (3, 7). It is a facultatively anaerobic, endspore-forming rod in which the endospores are central and do not distend the parent cell. On TSA medium, UW85 forms irregular, wrinkled, off-white colonies. It produces acid and acetoin, but not gas, when grown on glucose. It clears egg yolk medium, hydrolyzes starch and casein, grows at 42°C, produces an extracellular hemolytic activity, and does not contain intracellular protein crystals. Furthermore, UW85 is lysed by phages such as CP-51 (24) and JO-1 (data not shown) that lyse known strains of *B. cereus*.

In order to determine whether the suppression of damping-off was due to a common product among *Bacillus* strains, we tested other strains of *B. cereus* and *B. subtilis*. These strains included approximately 100 *Bacillus* isolates in our bacterial collection, from alfalfa roots; *B. subtilis* isolated from Quantum 4000; and *B. cereus* 569. We tested these strains under more severe conditions than in the original bioassay, by increasing the zoospore inoculum to $10^4$ per tube. Under these conditions, almost all (98%) of the *P. megasperma* f. sp. *medicaginis* control seedlings died, UW85 increased seedling survival to 46%, and none of the other *Bacillus* strains had detectable biocontrol activity.

**Appearance of extracellular biocontrol activity.** Sterile filtrates of 3-day-old cultures reduced seedling mortality as effectively as did the entire culture. This suggested that an extracellular product was responsible for the biocontrol

activity. Since many *Bacillus* spp. produce extracellular antibiotics during sporulation, it seemed likely that the biocontrol activity could be associated with sporulation. Therefore, we tested sterile filtrates of cultures containing vegetative cells, cultures containing spores in the parent cell, and cultures containing predominantly released spores for the ability to suppress damping-off in the alfalfa bioassay. We found little activity in the filtrates of vegetative cultures, significantly more activity in filtrates of cultures that contained some released spores and some spores remaining in the parent cell, and much biocontrol activity in both the culture and filtrate of fully released spore cultures (Table 1). Tests of cultures with less than 10% free spores showed little activity (data not shown). Efforts to detect intracellular activity in the vegetative and endspore cultures have been unsuccessful.

Since many other *Bacillus* strains have been tested and UW85 was the only one that consistently suppressed damping-off in our bioassay, the activity appeared to be fairly unusual or unique to UW85. Our results suggest that the material produced by UW85 that is responsible for its biocontrol activity was released into the culture medium concurrently with the release of spores but that the activity was not due to a product of sporulation (such as dipicolinic acid) that is common among bacilli.

**Effect of bacterial culture medium on biocontrol activity of UW85 cultures.** UW85 was grown in various media, and the biocontrol activity of the resulting cultures was tested. We tested minimal media containing sucrose, lactose, or mannitol as the carbon source, we varied the concentration of the salts in the medium, and we tested complex media containing oatmeal, yeast extract, and potatoes as the nutrient base. We found that all of the sporulated cultures grown on these media had some biocontrol activity and none of the nonsporulated cultures had activity. Cultures grown in TSB had higher activities than those grown in any other medium except for the semidefined media Min 3C and CB (Table 2). None of the uninoculated media, including TSB, CB, and Min 3C, suppressed damping-off, although TSB appeared to increase slightly the rate at which the seedlings died.

The results presented in Table 2 are representative of many similar experiments. We have conducted the bioassay over 100 times under the conditions described, and although there is some variability in the frequency of seedling infection, we consistently measure significant disease suppression with UW85. Although a formal statistical comparison of separate experiments must be approached with caution since the experiments were conducted at different times and each treatment is not represented in all of the experiments, examination of the mean percent survival and standard errors of the means of each treatment across all of our experiments is instructive about the consistency of disease suppression. For example, the seedling survival in the *P. megasperma* f. sp. *medicaginis* zoospore control treatment averaged from 112 separate experiments ($n$ = 112) was 32.6% ± 3.1% (standard error of the mean [SE]), whereas the mean survival of seedlings treated with zoospores and UW85 grown in TSB was 65.0% ($n$ = 85, SE = 3.0%). Treatment of seedlings with UW85 grown in CB medium resulted in a mean survival of 87.7% ($n$ = 23, SE = 2.8), and in Min 3C survival was 83.8% ($n$ = 10, SE = 5.3). In separate two-sample T tests, we compared each UW85 treatment with the control, and all three UW85 treatments differed significantly from the zoospore control ($P \ll 0.001$). These comparisons demonstrate that the bioassay results are reproducible in a large number of experiments, which is important since a consistent bioassay is critical for a further analysis of the mechanism of biocontrol by UW85.

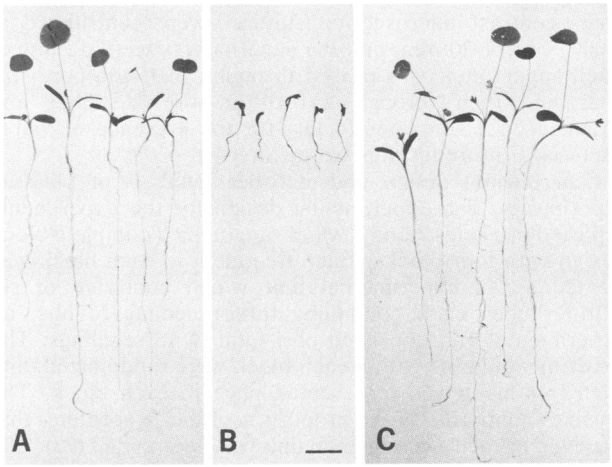


FIG. 2. Symptoms of alfalfa seedlings 7 days after inoculation. (A) Untreated; (B) inoculated with $10^4$ zoospores of *P. megasperma* f. sp. *medicaginis*; (C) inoculated with $10^4$ zoospores of *P. megasperma* f. sp. *medicaginis* and 0.3 ml of UW85 culture grown in Trypticase soy broth for 3 days. Bar = 1 cm.

**Emergence of alfalfa seedlings from UW85-coated seeds.** Alfalfa seeds were planted in early spring in a poorly drained field containing a Withee silt loam with a high clay content in the subsoil, which was responsible for restricting water flow. A combination of factors contributing to a high damping-off potential, including heavy rain followed by extremely dry weather, resulted in overall poor emergence. However, the seed treatments of both metalaxyl formulated as Apron and UW85 in methylcellulose substantially increased emergence. Emergence in the control plots, in which the seeds were coated only with the carrier methylcellulose, was 18%. Emergences of the UW85- and Apron-treated seeds were 30% and 29%, respectively. The values for the seed treatments differed significantly from the control value, as determined by an analysis of variance and a least significant difference test ($P$ = 0.05).

## DISCUSSION

We proposed that an appropriate place to search for a biocontrol strain is at the site of infection by the pathogen. Thus, to obtain a biocontrol agent for alfalfa damping-off, we developed a collection of root-associated bacteria, which contained representatives of the aerobic and facultatively anaerobic populations that grew on TSA. Two requirements were considered in developing a screening assay for biocontrol organisms. First, it had to be rapid and simple in order to screen many potential biocontrol agents, and second, it needed to have at least some relevance to natural infection in order to identify organisms that may be useful for disease suppression in the field. The assay that we developed partially satisfied both of these requirements. It is simple and reproducible, so that one person could easily screen 100 to 200 organisms per week, and it requires little space (Fig. 1). Since damping-off symptoms are quite obvious (Fig. 2), dead and viable seedlings can be easily distinguished and the assay can be scored rapidly.

Although the conditions of the assay were artificial and certainly not representative of field soil conditions, some of

the aspects of the assay were appropriate for the study of alfalfa damping-off. First, the assay involved screening directly for disease suppression. This seemed preferable to screening for inhibition of the pathogen in vitro, since there might be no relationship between in vitro antibiosis and disease suppression. Interestingly, UW85 does not inhibit growth of *P. megasperma* f. sp. *medicaginis* on plates, thus we would not have identified it had we screened the bacteria in a plate assay. Second, the *P. megasperma* f. sp. *medicaginis* inoculum was applied as zoospores, which have been implicated as the major infectious propagule for *Phytophthora* spp. in soil. In addition, the plants were kept moist for the duration of the assay to approximate soil conditions that are conducive to *P. megasperma* f. sp. *medicaginis* infection. With this screening assay, we identified a bacterium that suppressed disease not only in the artificial conditions of the laboratory bioassay, but also in a small-scale field experiment.

The mechanism by which UW85 suppresses damping-off remains to be determined. Our results suggests that the disease-suppressive material is released from the parent cell concurrently with spore release. This pattern is typical of secondary metabolite production by many sporulating bacteria that release antibiotics and bioactive agents during sporulation (2, 12, 16, 20). It will be interesting to determine whether the material produced by UW85 that has biocontrol activity resembles known antifungal agents. Other *B. cereus* strains have been shown to produce antifungal agents such as mycocerein and tunicamycin, but these compounds are active against fungi that are not inhibited by UW85, suggesting that the active material produced by UW85 is not one of these previously described antibiotics (15, 26).

UW85 does not inhibit mycelial growth of the pathogen, but culture filtrates from the bacterium lyse zoospores of oomycete fungi (G. S. Gilbert, J. L. Parke, and J. Handelsman, Exp. Mycol., in press). However, zoospore lysis is not the only mechanism of biocontrol since the biocontrol activity is unique to UW85 and many other bacilli lyse zoospores. Thus, zoospore lysis does not provide a sufficient explanation for the biocontrol activity exhibited by UW85.

UW85 has two particularly attractive characteristics that may contribute to its potential success as a biocontrol agent. First, it is a facultative anaerobe, which might enable it to grow under the saturated soil conditions that are conducive to damping-off. However, it remains to be determined whether UW85 grows on roots and produces disease-suppressive material under anaerobic conditions. Second, its ability to sporulate might enable UW85 to survive on seeds during storage in the soil and under many adverse conditions. Survival will be of critical importance to the success of any biocontrol agent that is expected to suppress disease that develops after the initial application of the agent. In particular, it remains to be determined whether UW85 can suppress *P. megasperma* f. sp. *medicaginis* infections on mature alfalfa roots during stand establishment and in subsequent years. Although many questions remain to be addressed regarding its consistency of efficacy in the field, mechanism of action, and the genetic regulation of biocontrol activity, UW85 appears to have potential as a biocontrol agent for alfalfa damping-off.

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## LITERATURE CITED

1. **Anagnostopoulos, C., and J. Spizizen.** 1961. Requirements for transformation in *Bacillus subtilis*. J. Bacteriol. **81**:741–746.
2. **Banerjee, A. B., and S. K. Bose.** 1964. Biosynthesis of mycobacillin, a new antifungal peptide. I. Role of nucleic acid. J. Bacteriol. **87**:1397–1406.
3. **Claus, D., and R. C. W. Berkeley.** 1986. Genus *Bacillus* Cohn 1872, 174, p. 1104–1139. *In* P. H. A. Sneath, N. S. Mair, M. E. Sharpe, and J. G. Holt (ed.), Bergey's manual of systematic bacteriology, vol. 2. The Williams & Wilkins Co., Baltimore.
4. **Delwiche, P. A., C. R. Grau, E. B. Holub, and J. B. Perry.** 1987. Characterization of *Aphanomyces euteiches* isolates recovered from alfalfa in Wisconsin. Plant Dis. **71**:155–161.
5. **Filonow, A. B., and J. L. Lockwood.** 1985. Evaluation of several actinomycetes and the fungus *Hyphochytrium catenoides* as biocontrol agents for *Phytophthora* root rot of soybean. Plant Dis. **69**:1033–1036.
6. **Frosheiser, F. I.** 1980. Conquering *Phytophthora* root rot with resistant alfalfa cultivars. Plant Dis. **64**:909–912.
7. **Gerhardt, P., R. G. E. Murray, R. N. Costilow, E. W. Nester, W. A. Wood, N. R. Krieg, and G. B. Phillips (ed.).** 1981. Manual of methods for general bacteriology. American Society for Microbiology, Washington, D.C.
8. **Graham, J. H., F. I. Frosheiser, D. L. Stuteville, and D. C. Erwin.** 1980. A compendium of alfalfa diseases. American Phytopathological Society, St. Paul, Minn.
9. **Halpin, J. E., E. W. Hanson, and J. G. Dickson.** 1954. Studies on the pathogenicity of seven species of *Pythium* on alfalfa, sweetclover, and ladino clover seedlings. Phytopathology **44**:572–574.
10. **Hancock, J. G.** 1983. Seedling diseases of alfalfa in California. Plant Dis. **67**:1203–1208.
11. **Havey, M. J., and C. R. Grau.** 1985. Decline of established alfalfa in soils naturally infested with *Phytophthora megasperma* f. sp. *medicaginis* and level of correlation by seedling assay. Plant Dis. **69**:221–224.
12. **Hodgson, B.** 1970. Possible roles for antibiotics and other biologically active peptides at specific stages during sporulation of Bacillaceae. J. Theor. Biol. **30**:111–119.
13. **Hunger, R. M., P. B. Hamm, C. E. Horner, and E. M. Hansen.** 1982. Tolerance of *Phytophthora megasperma* isolates to metalaxyl. Plant Dis. **66**:645–649.
14. **Irwin, J. A. G., S. A. Miller, and D. P. Maxwell.** 1979. Alfalfa seedling resistance to *Phytophthora megasperma*. Phytopathology **69**:1051–1052.
15. **Kamogashira, T., S. Takegata, and K. Sugiura.** 1988. Isolation of tunicamycin produced by *Bacillus cereus* K-279. Agric. Biol. Chem. **52**:859–861.
16. **Katz, E., and A. L. Demain.** 1977. The peptide antibiotics of *Bacillus*: chemistry, biogenesis, and possible functions. Bacteriol. Rev. **41**:449–474.
17. **Kuan, T. L., and D. C. Erwin.** 1980. Formae speciales differentiation of *Phytophthora megasperma* isolates from soybeans and alfalfa. Phytopathology **70**:333–338.
18. **Lifshitz, R., C. Simonson, F. M. Scher, J. W. Kloepper, C. Rodrick-Semple, and I. Zaleska.** 1986. Effect of rhizobacteria on the severity of phytophthora root rot of soybean. Can. J. Plant Pathol. **8**:102–106.
19. **Marks, G. C., and J. E. Mitchell.** 1971. Penetration and infection of alfalfa roots by *Phytophthora megasperma* and the pathological anatomy of infected roots. Can. J. Bot. **49**:63–67.
20. **Paulus, H., and E. Gray.** 1964. The biosynthesis of polymyxin B by growing cultures of *Bacillus polymyxa*. J. Biol. Chem. **239**:865–871.
21. **Pratt, R. G., J. E. Mitchell, and D. A. Willis.** 1975. Resistance and susceptibility to *Phytophthora megasperma* expressed in alfalfa cotyledons. Phytopathology **65**:365–369.

22. **Schmitthenner, A. F.** 1964. Prevalence and virulence of Phytophthora, Aphanomyces, Pythium, Rhizoctonia, and Fusarium isolated from diseased alfalfa seedlings. Phytopathology **54:** 1012–1018.
23. **Snedecor, G. W., and W. G. Cochran.** 1980. Statistical methods, 7th ed. Iowa State University Press, Ames, Iowa.
24. **Thorne, C. B.** 1978. Transduction in *Bacillus thuringiensis*. Appl. Environ. Microbiol. **35:**1109–1115.
25. **Utkhede, R. S.** 1984. Effect of bacterial antagonist on *Phytophthora cactorum* and apple crown rot. Phytopathol. Z. **109:** 169–175.
26. **Wakayama, S., F. Ishikawa, and K. Oishi.** 1984. Mycocerein, a novel antifungal peptide antibiotic produced by *Bacillus cereus*. Antimicrob. Agents Chemother. **26:**939–940.