Biocontrol of Bean Rust by *Bacillus subtilis* Under Field Conditions

C. JACYN BAKER and J. R. STAVELY, Research Plant Pathologists, USDA, ARS, Plant Pathology Laboratory, Beltsville, MD 20705; and NORTON MOCK, Senior Laboratory Technician, University of Delaware, Newark 19717

ABSTRACT

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In field tests at Beltsville, MD, bean rust severity was reduced at least 75% in 1982 and 1983 with three applications per week of *Bacillus subtilis*. Two isolates of *B. subtilis* were tested. Isolate PPL-3 had an inhibitory effect on yield and a stimulatory effect on plant growth. Isolate APPL-1 had no apparent effect on plant growth. In some tests, treatments with *B. subtilis* were more effective than the weekly application of the fungicide mancozeb.

Rust caused by *Uromyces appendiculatus* (Pers. ex Pers.) Unger, also known as *U. phaseoli*, is a major disease problem on snap and dry beans (*Phaseolus vulgaris* L.) worldwide. In 1981, yield losses of 54 and 78% were reported in Michigan and North Dakota (8,17), respectively. In the United States, estimated losses approach a quarter of a billion dollars. *U. appendiculatus* is a highly variable pathogen with more than 50 races having been described over the past 40 yr (11,16). Resistance to this pathogen is available in bean germ plasm (11,12). Certain fungicides are used to help control bean rust; however, they are not always economically practical (7). Furthermore, general concern about possible adverse effects of chemical pesticides makes it desirable to find alternative control measures.

We previously reported that cultures of *Bacillus subtilis* (Ehrenberg) Cohn and cellfree filtrates thereof provided control of bean rust under greenhouse and coldframe conditions (3). These bacterial cultures and filtrates prevented germination of the urediniospores and were effective against all major races of bean rust on all bean cultivars tested. The objective of this study was to determine if cultures of *B. subtilis* or cellfree filtrates reduce bean rust severity under field conditions. Initial results were published previously (1,2).

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MATERIALS AND METHODS

Bacterial cultures. All bacterial cultures were maintained on nutrient agar at 30 C. *B. subtilis* isolate APPL-1, obtained from M. J. O'Brien, and isolate PPL-3, obtained from A. W. Saettler, were tested in this study. Each isolate was transferred from nutrient agar to 1.5 L of Eugon broth (Difco Laboratories, Detroit, MI) in a 2.8-L Fernbach flask and incubated on a circulatory shaker at 100 rpm in the dark at 30 C. The culture age at which maximum inhibitor concentration was reached was determined by removing samples periodically from the flask. A spore germination bioassay was used to determine the potency of inhibitors produced (3). The bacterial concentration was determined using a dilution plate technique and expressed as colony-forming units (cfu) per milliliter.

Bioassays. Procedures for spore preparation and for the germination bioassay have been described previously (3). Spores were germinated on serially diluted test solutions. The activity of a particular test solution was expressed as the inverse of the lowest dilution that inhibited germination to less than 10% of the control. For example, the activity of a culture filtrate that inhibited germination at a dilution of one part in 800 would be 800 activity units.

The greenhouse bioassay for rust control has also been described previously (3). Greenhouse plants with primary leaves partially expanded were sprayed with test solutions before inoculation with rust spores. Rust inhibition was expressed as percent reduction in the number of rust uredinia from the number that occurred on untreated plants.

Preparation of field test solutions.

Solutions were prepared within 2 hr of application and were prepared from 7-day-old shake cultures unless otherwise stated. Three liters of culture of both isolates were diluted with equal volumes of water. In some treatments culture filtrate was prepared by passing the culture through a Pellicon Cassette system fitted with a 0.2-μ filter (Millipore Corporation, Bedford, MA). Autoclaved supernatants were prepared from 7-day-old cultures centrifuged at 3,000 *g* for 30 min; the supernatants were then autoclaved for 20 min and diluted with equal volumes of water. Uninoculated sterile Eugon broth diluted with an equal volume of water was a control treatment. Mancozeb (Dithane M-45), currently used for controlling bean rust, was prepared at a concentration of 2.4 g/L (1,920 ppm a.i.). This product contained 16% manganese, 2% zinc, and 62% ethylene bisdithiocarbamate.

Field tests. Field tests were conducted during the late summer and early fall of 1982 and 1983. Bean cultivars Lake Shasta and Pinto 111, which are among the most rust-susceptible snap and dry beans, respectively, were used to assay the effects of various treatments on disease severity. Lake Shasta is highly susceptible to the common races 38 and 39 that are virulent on snap beans in the eastern United States (11). This cultivar, however, is highly resistant to race 40 in that only small necrotic spots develop and only moderately susceptible to races 41 and 43, resulting in moderate size uredinia (J. R. Stavely, *unpublished*). Pinto 111 is highly susceptible to all available races except 38 and 39, to which it is resistant, resulting in small uredinia.

Test solutions were applied to three replicate 2-m rows of beans each containing 1 m of each cultivar. A randomized complete block design was used in which each treatment row was adjacent to a rust-spreader row of beans that contained an approximately equal mixture of the cultivars Bountiful, Lake Shasta, Pinto 111, Mountaineer White Half Runner, and Aurora. The treated rows and spreader rows were all seeded on the same day in late July. Along both side borders of the plot were five rows of the mixture of cultivars. Three of these border rows were seeded 4 wk before the treated rows. The plants in these border rows were artificially inoculated at 1-wk intervals from emergence until they were 5 wk old. A suspension containing equal portions of urediniospores of races 38, 39, 40, 41, and 43 with an overall concentration of about 50,000 urediniospores per milliliter was sprayed onto the border rows.

Disease severity was recorded 9 wk after seeding. A modified Cobb scale, developed to estimate rust intensity of bean leaves (13), was used to assign a numerical score. The scale for the numerical score has seven categories for degrees of rust intensity, where 1 = only an occasional uredinium or about 2% of the leaf area covered by rust, 2 = 5%, 3 = 10%, 4 = 25%, 5 = 40%, 6 = 65%, and 7 = maximum potential surface area occupied by uredinia. The scale is based only on the amount of leaf area occupied by uredinia. Telia were assessed the same as uredinia.

In preliminary tests, optimal control was obtained with application of the most effective treatments three times per week, so this schedule was used unless otherwise stated. Test solutions were sprayed onto plants with a 2-gal hand-pumped compression sprayer (Hudson Manufacturing Co., Chicago, IL).

Nine weeks after planting, all pods from Lake Shasta plants were collected, counted, and weighed collectively for each replicate per treatment. Plants were severed at ground level, counted, and weighed collectively for each replicate.

RESULTS

Cultures of *B. subtilis* were monitored over a 10-day period to determine when they were most inhibitory. During log phase growth, colony-forming units increased to about 1×10^7/ml after 20 hr and the inhibitor increased to about 40 activity units (Fig. 1). During stationary growth phase, the colony-forming units fluctuated, whereas maximum activity occurred after 7 days at about 1,600 units. Seven-day-old cultures used for the field study contained about 10^7 cfu/ml with an inhibitor activity between 800 and 1,600 units.

Field tests in 1982 were limited to the APPL-1 isolate. There was less rust on plants treated with cultures of isolate APPL-1 based on statistical analysis of the disease severity data (Table 1). Plants of Pinto 111 that were treated with water or not treated developed the highest disease severity rating of 7. Test solutions containing either 1- or 7-day-old cultures significantly reduced bean rust on both cultivars. The 7-day-old culture was more effective on Pinto 111 than the 1-day-old culture. Disease severity was reduced to a rating of 3.2, which corresponded to about 86% less surface area covered with rust than in the water treatment. Supernatant that had been autoclaved to ensure no further bacterial growth also significantly reduced bean rust severity. Sterile media reduced disease severity on Lake Shasta but not significantly on Pinto 111. Plants treated with fungicide three times a week had no rust.

Field experiments in 1983 included treatments with culture filtrates of isolates APPL-1 and PPL-3. Both isolates provided control of bean rust (Table 2). When applied three times per week, PPL-3 culture filtrate controlled rust on Pinto 111 significantly better than mancozeb applied once per week.

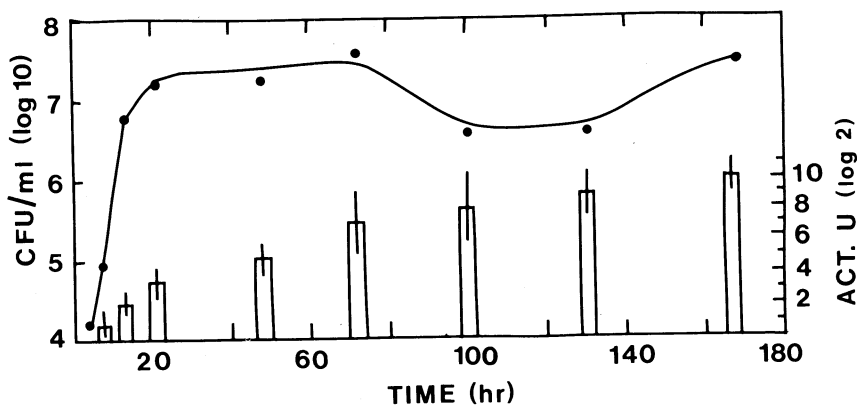

Fig. 1. *Bacillus subtilis* culture age and inhibitor production. Shake cultures were grown in Eugon broth at 30 C. Colony-forming units (solid line) were determined by dilution plating. Inhibitor activity is expressed as the inverse of the lowest dilution at which urediniospore germination is inhibited. Bar represents two standard deviations.

Table 1. Effect of isolate APPL-1 of *Bacillus subtilis* on bean rust under field conditions in 1982

Treatment[y]	Disease severity rating[z] (approximate % area covered with rust)	
	Lake Shasta	Pinto 111
APPL-1 (1-day-old)	2.27 cd (6)	3.8 bc (22)
APPL-1 (7-day-old)	2.20 cd (6)	3.2 c (13)
Autoclaved supernatant	2.00 d (5)	4.2 b (28)
Water	4.80 a (38)	7.0 a (99)
No treatment	5.00 a (40)	7.0 a (99)
Medium	3.27 b (13)	6.3 a (76)
Mancozeb	0.00 e (0)	0.0 d (0)

[y] All test solutions were applied three times per week to three replicate 2-m rows of beans each containing 1 m of each cultivar.

[z] Based on a scale of 1–7, where 1 = only an occasional uredinium and 7 = maximum potential leaf area covered with uredinia. Any two means followed by the same letter are not significantly different according to the Duncan-Waller test ($P = 0.05$).

Table 2. Comparison of the effects of culture filtrates (CF) of isolates PPL-3 and APPL-1 of *Bacillus subtilis* on bean rust under field conditions in 1983

Treatment[y]	Frequency (applications/week)	Disease severity rating[z] (approximate % area covered with rust)	
		Lake Shasta	Pinto 111
PPL-3 CF	3	2.5 de (8)	3.2 d (13)
APPL-1 CF	3	2.7 cde (9)	4.7 bc (36)
PPL-3 CF	1	3.5 bc (18)	5.8 a (60)
Water	3	4.5 a (32)	6.2 a (72)
No treatment	...	5.2 a (45)	6.2 a (72)
Mancozeb	1	1.8 e (4)	4.2 c (28)

[y] Test solutions were applied to three replicate 2-m rows of beans each containing 1 m of each cultivar.
[z] Based on a scale of 1–7, where 1 = only an occasional uredinium and 7 = maximum potential leaf area covered with uredinia. Any two means followed by the same letter are not significantly different according to the Duncan-Waller test ($P = 0.05$).

Table 3. Comparison of the effects of culture filtrates (CF) of two isolates of *Bacillus subtilis* and mancozeb on development of bean cultivar Lake Shasta under field conditions[z]

Treatment	Pod wt (g) per plant	Pod no. per plant	Wt (g) per plant
APPL-1 CF	44.8 a	7.2 a	36.3 ab
PPL-3 CF	7.5 b	1.7 b	59.1 a
Water	45.3 a	7.6 a	29.3 b
Mancozeb	71.0 a	9.4 a	56.7 ab

[z] Test solutions were applied three times per week to three replicate 1-m rows of Lake Shasta. Any two means in each category followed by the same letter are not significantly different according to the Duncan-Waller test ($P = 0.05$).

However, isolate PPL-3 noticeably affected plant growth. Parameters related to plant growth and yield were measured with Lake Shasta (Table 3). Isolate PPL-3 significantly reduced pod production per plant.

DISCUSSION

In previous work, *B. subtilis* provided control of bean rust in the greenhouse. In the present work, frequent applications of *B. subtilis* provided control of bean rust under field conditions. One application per week of isolate PPL-3 was not sufficient to control rust, thus the bacterium either did not survive well on the foliage or the inhibitory material was not produced under these conditions. Autoclaved culture filtrates significantly reduced bean rust, thus the control may be due to a preformed component in the culture filtrate rather than the bacterium per se.

Earlier work on biological control of plant disease with *B. subtilis* under field conditions concentrated on soilborne diseases (5,6,9,14,15). The bacterial culture was applied as a seed treatment.

Recently, Pusey and Wilson (10) reported the postharvest control of stone fruit brown rot by *B. subtilis*. They suggested that a heat-stable antibiotic was interfering with spore germination or early germ tube development. This would be similar to what we have previously reported for the effect of *B. subtilis* on *U. appendiculatus* (*U. phaseoli*) (3).

Isolate PPL-3 of *B. subtilis* had a significant effect on plant development under field conditions. Plants treated with this particular isolate three times per week appeared much greener and more succulent and had fewer flowers throughout the growing season and into the fall beyond the time when most plants had become senescent. This interfered with fruit production. Effects of certain isolates of *B. subtilis* on plant growth have been noted previously. Stimulation of plant growth has been reported in cereals and carrots (9), corn (6) and pepper, snapdragon, and tomato seedlings (5). The mancozeb fungicide used in our work also seems to stimulate plant growth, perhaps by supplying such limiting nutrients as zinc or manganese; however, it did not inhibit fruit production (Table 3). The use of biological control on aerial plant surfaces is much less developed than in the soil/rhizoplane environment (4). This may be partially due to the rapidly changing conditions on the leaf surface, such as daily weather fluctuations, other microflora, and plant metabolites.

Isolate PPL-3 applied three times per week provided control of bean rust at a level equivalent to that of the fungicide applied once per week (Table 2). This demonstrates the potential use of *B. subtilis* for controlling bean rust. An isolate similar to APPL-1 needs to be obtained, through selection or genetic manipulation, that will provide better rust control without undesirable side-effects on plant development. Purification and identification of the inhibitor from *B. subtilis* are necessary to understand fully the mechanism of control expressed in this system. Once this is accomplished, modification of the structure could improve its durability and lead to a more environmentally safe method of control.

ACKNOWLEDGMENT

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