Bacterization of Rice Plants for Control of Sheath Blight Caused by *Rhizoctonia solani*

T. W. Mew and A. M. Rosales

Plant pathologist and head and research assistant, respectively, the International Rice Research Institute, Los Banos, Laguna, Philippines.
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ABSTRACT

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Bacteria that produced fluorescent and nonfluorescent pigment on Kings Medium B and showed antagonism to *Rhizoctonia solani*, which causes rice sheath blight, were found in rice fields. Antagonists were isolated from sclerotia, rice field flood water, rhizosphere soils of upland and lowland fields, and diseased and healthy plants. Both fluorescent and nonfluorescent bacteria inhibited mycelial growth of the pathogen, affected sclerotial viability, and slightly promoted rice seed germination. When used in seed bacterization, the antagonists suppressed the disease and protected the plant from infection. Subsequent planting after the first crop, in which the seeds were treated with the bacteria, on the same soil also showed reduced disease severity. The bacteria appeared to establish in the soil after sowing of seeds treated with antagonistic bacteria.

Sheath blight, a disease caused by an aerial form of *Rhizoctonia solani* Kühn (*Thanatephorus cucumeris* (Frank.) Donk), occurs in rice (*Oryza sativa* L.) under various types of cultivation throughout the world. Since the introduction of modern high-yielding cultivars, sheath blight has increased in importance in the rice-growing countries in Asia. We speculate that this is due to changes in the microclimate and physiology of the crop. In general, these improved cultivars have a high tillering capacity and respond to an increased rate of nitrogen application. The disease also may be aggravated by such cultural practices as close spacing, increased crop intensity, and high seeding rate.

The disease can be controlled by fungicides or by planting resistant cultivars in combination with use of fungicides. Use of resistant cultivars alone has not achieved disease control, because the inherent level of resistance is low. Despite continuous efforts at the International Rice Research Institute (IRRI) and in national rice improvement programs, sheath blight has not been satisfactorily controlled by host resistance.

Inoculum of the pathogen (AG1) is carried over from one crop to the next, largely by sclerotia and infected weeds, straws, and stubble (4,12,20). Great numbers of sclerotia are produced in infected fields, in temperate regions (3), and in the tropics (T. W. Mew, *unpublished*).

Sclerotia are produced on diseased plants and usually remain attached to the plants. Because of physical forces such as rain splash or harvesting, sclerotia are deposited on the soil surface and are distributed over the fields by subsequent tillage. Chien et al (3) reported that 23% of the sclerotia fell on the soil around the rice straws during harvest.

At maturity, the sclerotia are at first nonbuoyant but become buoyant after 15–30 days, when the cells in the outer layer become empty (8). Sclerotia buried in submerged paddy soil were found nonviable after 14 days (14). This loss of viability was attributed either to anaerobic conditions or to microbial factors. Subsequently, bacteria were isolated that inhibited the mycelial growth of *R. solani* and affected sclerotia viability.

Here, we report our survey of fluorescent and nonfluorescent bacteria from paddy fields, their effect on rice growth, and their effect as biological control agents of rice sheath blight in greenhouse and screenhouse conditions. The antagonistic bacteria, for convenience, were designated as fluorescent and nonfluorescent on King's Medium B (KMB) for fluorescent pigment production (10). This area of research and disease management for sheath blight has not been attempted or explored in tropical rice producing countries. Preliminary results have been presented (15).

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MATERIALS AND METHODS

Survey and isolation of antagonistic bacteria from rice fields. To estimate the spatial distribution of antagonistic bacteria in rice fields, we took five soil samples (20 × 20 × 6 cm) per plot from irrigated and dryland rice plots at the IRRI experimental farm near Los Banos after the crop harvest. Soil samples were collected from 20 irrigated and 15 dryland plots. The intensity of sheath blight on the rice crop before harvest in these plots was recorded.

Sclerotia were collected by the wet-sieving flotation method (18), and enumerated based on the oven-dried weight of the soil. Sclerotia viability was determined by plating them on potato-dextrose agar (PDA) medium and counting germination after 2 days.

Bacteria were isolated from sclerotia when they were plated on PDA for the viability test, streaked on peptone-sucrose agar (PSA) plates, and single colonies were picked and maintained on PSA slants.

To isolate the bacteria from rice plants, more than 300 randomly selected healthy and sheath blight-infected plants (cultivar IR36) from the irrigated or dryland fields were sampled. For isolation, rice sheaths and culms or margins of advancing lesions in infected plants were cut into 5-mm pieces and washed in a flask by aeration for 30–60 min. The washing was streaked on KMB plates. Single colonies of fluorescent and nonfluorescent bacteria were picked and transferred to PSA slants. The isolates were tested for inhibition of mycelial growth of isolate LR-1 of *R. solani*. The tests were repeated and bacteria were lyophilized for maintenance.

Bacteria were isolated from paddy water by direct streaking on KMB plates, whereas isolation from the soil was by soil dilution. A 25-g oven-dried soil sample was placed in a graduated cylinder. Sterile water was added to a volume of 250 ml. The suspension was stirred and poured into a 500-ml Erlenmeyer flask, then shaken for 10 min. A serial dilution was prepared by adding 10 ml of the suspension to 90 ml of water until a 10^{-5} dilution was obtained. One milliliter of each dilution was pipetted onto KMB plates for bacterial plating and count.

To sample the bacteria from rhizosphere soils, plants were dug with a shovel and excess soil was removed from the roots by vigorous shaking. Ten grams of the soil closely adhering to the roots was used for serial dilution as described for paddy water.

Culture and preparation of inoculum of *R. solani*. The culture of *R. solani* was started from a single hyphal tip isolated from infected plants (cultivar IR36) grown in an irrigated field. The highly

virulent pathogen was grown on PDA and stored at 4 C. Fresh cultures were routinely isolated from infected plants to maintain virulence.

To assure adequate infection, inoculum was prepared by culturing the pathogen in a rice hull-rice grain medium. The medium contained three parts rice hull, one part rice grain, and 200 ml of water. The mixture was packed in 500-ml culture bottles (400 g per bottle) and autoclaved for sterilization. The following day, an agar disk from the periphery of a 5-day-old culture of *R. solani* on PDA was transferred aseptically to the mixture and incubated for 2 wk at room temperature (about 28–30 C).

For greenhouse experiments, the inoculum was grown on rice hull-rice grain mixture and added to steamed soil in seedboxes (28 × 11 × 11 cm) at a 1:20 (w/w) ratio.

Test of antagonism. All bacterial isolates were individually tested for ability to inhibit mycelial growth of *R. solani* LR-1 in vitro. Four isolates were spotted on the edge of an agar plate and a 5-mm-diameter agar disk of the *R. solani* from PDA was placed in the center. The fungus-bacteria dual culture was replicated three times and incubated at 28 C. Zones of inhibition were measured from the edge of the mycelium to the margin of each bacterial colony 5 days after incubation.

Several bacterial isolates based on in vitro tests were selected and used in greenhouse and screenhouse experiments to test their effect in prevention of sheath blight and growth of rice as described below.

Seed preparation and bacterial treatment. Rice seeds (cultivar IR36) were surface sterilized by being immersed for 3 min in a 2.5% calcium hypochlorite solution, rinsed three times in sterile distilled water, and dried overnight at room temperature.

Bacterial isolates used for seed treatment were grown on PSA for 24 hr at 28 C. The bacterial suspension prepared from these plates was then adjusted to a concentration of about 1×10^9 colony-forming units (cfu)/ml. Seeds were soaked in the suspension for 24 hr at a 1:10 (w/v) ratio and sown in seedboxes. For spray application of bacteria, the plants were grown in potted soil for 35 days and then sprayed with the bacterial suspension (about 1×10^6 cfu/ml) one day before inoculating them with sclerotia of *R. solani* LR-1.

Greenhouse and screenhouse tests. Selected isolates were tested for their ability to protect the rice plants grown in seedbox from sheath blight in greenhouse and in ground beds in the screenhouse. Bacteria-treated seeds were sown in two rows at 20 seeds per row in the seedbox filled with soil infested with pathogen inoculum (1:20 ratio of inoculum to soil). Seeds soaked in sterile distilled water without the bacterial suspensions were sown also in two rows in infested soil in the seedbox as a control. All treatments were replicated four times in a randomized complete block design. Sheath blight incidence and lesion size were measured 26 days after sowing. Plant height was measured at 35 days at termination of the experiment.

To test the residual effect of the bacteria on sheath blight control, rice seeds soaked in sterile distilled water were sown in the same seedboxes after the rice plants were removed from the first planting. Disease incidence and lesion size were estimated as in the first planting.

Bacterial numbers were estimated at the end of each experiment before planting a second rice crop. Fifty grams of soil samples were taken after the second rice planting. The soil was mixed in sterile distilled water, serially diluted, and plated on KMB to determine if fluorescent and nonfluorescent bacteria became established in the soil.

In a screenhouse (40% shade), five bacterial isolates were tested in soil (ground beds) that was naturally infested. Bacterial treatment of seeds was similar to the seedbox test in the greenhouse. The seeds were sown in steamed soil. At 14 days, the seedlings were pulled and transplanted into plots (1.2 × 1.5 m) in the screenhouse at a 20 × 20 cm spacing, with three plants per hill. A spray application of the bacterial suspension was made to plants 45 days after transplanting. Plant height was measured at weekly intervals and disease severity and yield were determined at harvest.

Detecting bacteria on the plant surface. To determine if the bacteria could become established on the surface of rice plants, a fluorescent bacterial isolate, In-b-150, was applied as a seed treatment. Some of the seeds were allowed to germinate in petri dishes with filter paper and others were grown in steamed soil in a seedbox. The seed and seedlings were examined under UV light at different periods from planting until 7 days after sowing; the whole seedlings were also firmly pressed on the KMB agar plates (13), and bacteria grown from the plant imprints were examined under UV light.

Plants grown in seedbox or screenhouse plots for 45 days were carefully pulled, and the soil removed by gently washing the plants in water. Plants were cut into different parts and each was pressed on KMB agar plates. Bacteria grown from the plant imprints of the different parts were examined under UV light 24 hr after incubation to trace the growth of the fluorescent bacteria. Plants grown from seeds soaked in sterile distilled water were used as a control.

RESULTS

Distribution of bacteria in rice fields. The bacteria appear to occur widely in rice fields (Table 1). They were isolated from rhizosphere soil in fields with high and low incidence of sheath blight. They were also isolated from sheath blight-infected and healthy rice plants. More were found on sclerotia collected in flooded than in dryland rice fields. Some of the sclerotia from which bacteria were isolated appeared to be dead, but others remained viable.

Similarly, some of the isolates produced blue fluorescent pigment on KMB, whereas others did not. In general, the number

TABLE 1. Source of bacteria that inhibit mycelial growth of *Rhizoctonia solani* isolated from rice plants, paddy water, and rhizosphere soil

Origin	Fluorescent		Nonfluorescent		Bacterial isolates obtained (total no.)	Isolates-inhibiting *R. solani* (%)
	Total no.	*R. solani*-inhibiting (no.)	Total no.	*R. solani*-inhibiting (no.)		
Rhizosphere						
Infected rice plant	4	3	126	33	130	22.7
Healthy rice plant	12	10	133	36	145	31.7
Sclerotia						
Dryland ricefield	5	5	103	26	108	28.7
Irrigated ricefield	1	1	116	64	117	55.6
Rice plants						
Sheath blight-infected	16	16	107	34	123	40.6
Healthy	10	10	178	50	188	31.9
Paddy water						
Total	56	51	869	285	925	
Percent		91.1		32.8		

of nonfluorescent bacteria associated with the rice system seemed higher than that of fluorescent bacteria. A high percentage (91%) of the fluorescent isolates inhibited mycelial growth of the pathogen, whereas only 33% of the nonfluorescent isolates inhibited the pathogen in vitro. The inhibition zone of mycelial growth on PDA ranged from 4 to 30 mm for the fluorescent bacteria and from 2 to 32 mm for the nonfluorescent bacteria in a routine test of 56 fluorescent and 231 nonfluorescent bacteria.

Bacteria establishment on the plant surface. The fluorescent isolate In-b-150 appeared to slightly affect the germination of rice seeds cultivar IR36 in petri dishes with moistened filter paper. Although the difference was not statistically significant, seeds treated with the bacterium had a higher germination percentage.

Fluorescent pigment was detected on all seeds soaked in a bacterial suspension of the fluorescent isolate and observed under UV light, from sowing to 7 days after germination in the petri dish. Plant imprints from 7-day-old seedlings pressed on KMB agar plates (after 7 days in the seedbox) showed that the fluorescent bacteria could be detected on the intact seed of the seedling, on the roots and the coleoptile, and on the first and second leaves.

Fluorescent bacteria grew from imprints of plant parts pressed on KMB (Fig. 1). The bacteria were detected from plants grown from seeds treated with the fluorescent isolate In-b-150 raised either in the screenhouse plots or greenhouse. In flooded (irrigated) plots fluorescent bacteria were detected only on portions of the leaf sheath above the waterline. No fluorescent bacteria were detected from plant imprints of checks, i.e., plants grown from seeds soaked in water.

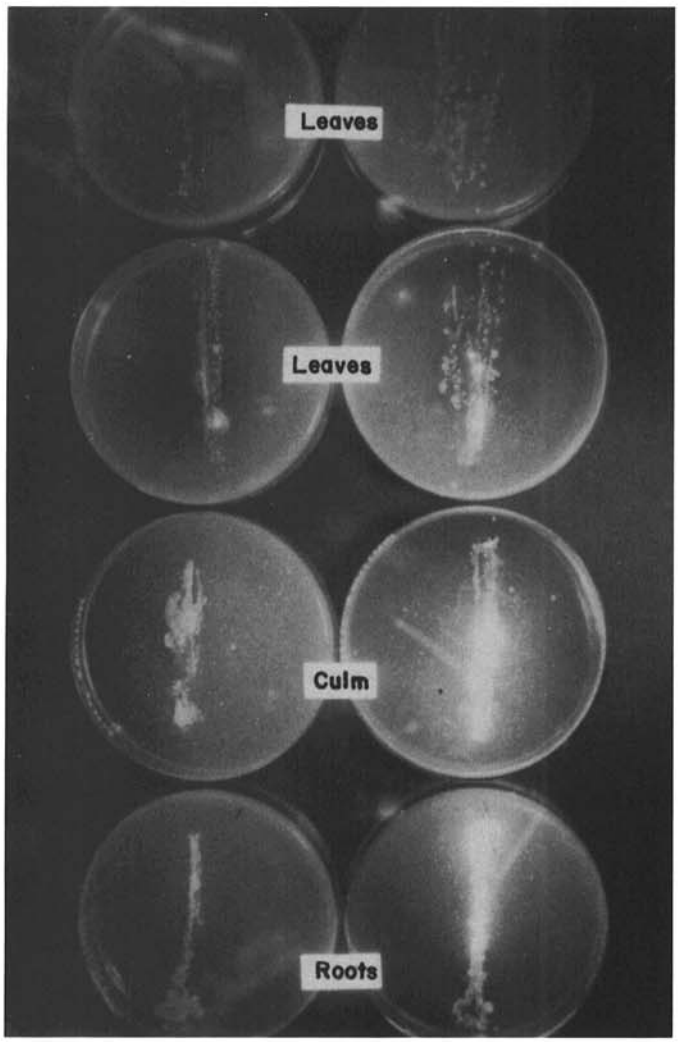

Fig. 1. Fluorescent bacteria grew from imprints of plant parts pressed on King's Medium B. **Right,** Plants grown from seed treated with fluorescent bacteria strain In-b-150. **Left,** Check, plant grown from untreated seed. Both plants were 45 days old, raised in screenhouse ground bed.

Greenhouse evaluation of the effect of fluorescent and nonfluorescent bacteria isolates on sheath blight. When sclerotia of *R. solani* produced in the laboratory were soaked for different periods (10 min; 12, 24, and 48 hr; and 1 wk) in the bacterial suspension, then allowed to infect rice plants grown to maximum tillering, disease incidence was reduced, compared with plants infected with sclerotia soaked in sterile distilled water. Isolate In-b-17, a nonfluorescent bacterium, seemed to have a greater effect than fluorescent isolates In-b-24 and In-b-150 or disease incidence. Lesions were smaller ($P = 0.05$) on rice plants infected with sclerotia soaked in the bacterial suspension for various periods (Fig. 2). The difference between the nonfluorescent and fluorescent bacteria was not significant.

When bacterial suspensions (about 10^6 cfu/ml) were sprayed on rice plants infected concomitantly with sclerotia inserted in the tillers, the difference in disease incidence and lesion size was significant ($P = 0.01$). Sheath blight incidence was 100, 30, and 75% on plants exposed to *R. solani*, and *R. solani* in combination with either isolate In-b-17 or In-b-24, respectively. Lesion size was 2.0, 0.6, and 1.3 cm. Again, the nonfluorescent isolate, In-b-17, appeared to protect the plants better from sheath blight infection than the fluorescent isolate In-b-24.

Effect of bacterial seed treatment on sheath blight in the greenhouse. Of the 336 isolates evaluated in vitro for effect on

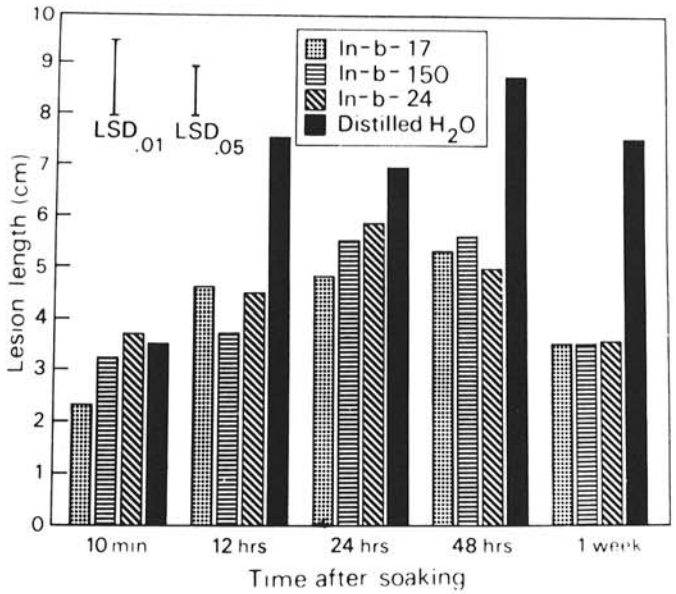

Fig. 2. Reduction of sheath blight lesions on rice plants (cultivar IR36) infected with sclerotia of *Rhizoctonia solani* presoaked in bacterial suspension.

Fig. 3. Suppression of rice sheath blight (*Rhizoctonia solani*) development after bacteria-seed treatment, then sown in **A,** pathogen-free soil; **B,** pathogen-infested soil; **C,** no bacteria-seed treatment pathogen-infested soil.

mycelial growth 23 suppressed sheath blight in an initial test. Nine of these, five fluorescent and four nonfluorescent, were further studied in seedboxes in the greenhouse for two consecutive plantings. In the first planting, seeds were treated with the bacteria. In the second, seeds were not treated and were sown in the same seedboxes infested with inoculum of *R. solani* LR-1.

Rice plants had significantly less sheath blight when grown from seed treated with either the fluorescent or nonfluorescent bacteria, than plants from nontreated seeds (Fig. 3). Plants from treated seeds had smaller lesions than plants from untreated seeds in both the first and second plantings (Table 2). Disease incidence, the number of rice plants showing sheath blight infections, was higher in the first planting of treated seeds than in the second planting. In either planting, plants from seeds treated with the bacteria were taller than those from the untreated seeds. The difference in plant height between fluorescent and nonfluorescent isolates was not significant. Disease severity in terms of lesion size and disease incidence, in plants from untreated seeds was not significantly different between plantings.

The total bacterial counts from soil samples at 35 days after planting were considerably higher in soil from seedboxes where seeds had been treated with the bacteria (Table 2). In all the seedboxes, both fluorescent and nonfluorescent bacteria were detected on KMB agar plates. It was obvious, however, that counts of fluorescent bacterial colonies were higher in seedboxes where seeds were treated with the fluorescent bacteria (Table 2). In contrast, the fluorescent bacteria were lower in seedboxes where nonfluorescent bacteria were applied to the seeds. But in the second planting, there was no difference in count between the two groups of bacteria. Total bacterial count for all treatments was less in the second planting.

Effect of bacterial seed treatment on sheath blight in the screenhouse. Throughout the crop season, plants grown from seeds treated with both fluorescent and nonfluorescent isolates grew faster and were taller and greener than the check (Fig. 4), before maximum tillering. At flowering, the difference between treatments was not readily apparent. The plants appeared to be taller when seeds were treated than when bacterial suspension was sprayed on the plants at 45 days after sowing (Table 3). Tiller number at maximum tillering also indicated seed bacterization produced an effect equal to spraying. Grain yield was higher in seed treatment than in spray application of bacteria. Incidence of sheath blight was lower when plants were grown either from seeds treated with or sprayed with bacteria. In-b-591, a nonfluorescent isolate, showed no effect on sheath blight suppression.

Fig. 4. Comparison of seed treatment with and without bacterization. Rice plants (cultivar IR36) on the left were grown from seed treated with bacterial strain In-b-590. Rice plants on the right were grown from untreated seeds in soil in a screenhouse.

TABLE 2. Effect of fluorescent and nonfluorescent bacteria applied to rice cultivar IR36 seeds on incidence and severity of sheath blight and bacterial count 35 days after planting in two consecutive plantings

Treatment	Incidence (%)		Lesion length (cm)		Plant height (cm)		CFU(log_{10})/g soil[y]			
	1st crop	2nd crop	1st crop	2nd crop	1st crop	2nd crop	Total	Nonfluorescent	Total	Nonfluorescent
Fluorescent + *Rhizoctonia solani*										
In-b-4	81 a[z]	56 b	5.4 bc	4.5 b	30 cd	29.7 f	9.9 a	9.7 a	8.4 d	8.3 c
In-b-20	81 a	50 b	4.1 bc	2.0 c	49 a	40.7 d	9.5 b	9.3 b	8.4 de	8.5 c
In-b-22	86 a	42 c	2.6 d	2.0 c	45 a	42.5 bcd	9.9 a	9.7 a	8.5 d	8.3 c
In-b-23	81 a	38 cd	3.6 bc	2.0 c	39 abcd	38.0 e	9.8 a	9.6 a	9.3 b	8.8 b
In-b-24	84 a	78 a	2.8 c	2.0 c	32 bcd	44.5 bc	9.9 a	9.6 a	9.6 a	9.2 a
Nonfluorescent + *R. solani*										
In-b-3	86 a	56 b	4.0 bc	2.0 c	48 a	48.2 a	9.8 a	9.7 a	8.9 c	8.8 b
In-b-9	80 a	56 b	3.7 bc	2.0 c	44 a	42 cd	9.8 a	9.7 a	8.2 e	8.4 c
In-b-17	60 b	28 d	1.7 c	1.2 c	40 abc	45 b	9.9 a	9.7 a	9.2 b	9.2 a
In-b-27	89 a	80 a	4.8 bc	4.0 b	41 ab	40.2 de	9.9 a	9.8 a	9.2 b	9.2 a
R. solani without bacteria	96 a	96 a	9.7 a	9.0 a	28 d	28 f	9.0 c	9.0 c	7.2 f	8.0 d

[y] Total bacterial count on oven-dried weight.
[z] Mean of four replications. Means followed by a common letter are not significantly different ($P = 0.05$) by Duncan's multiple range test.

TABLE 3. Effect of fluorescent (F) and nonfluorescent (NF) bacteria and spray application 45 days after transplanting on plant height, tiller number, and grain yield of rice cultivar IR36 and on sheath blight incidence in soil in a screenhouse

Bacterial isolate	Plant height[w] (cm)		Tiller[x] (no.)		Grain yield[y] (g)		Incidence (%)	
	ST[z]	S	ST	S	ST	S	ST	S
NF In-b-17	52.9 ab	49.9 b	10.0 a	7.6 abcd	107.5 a	87.0 bc	14.3 bcd	9.3 d
F In-b-24	55.7 ab	51.0 b	9.0 abc	6.7 cd	105.7 ab	84.5 c	13.0 cd	13.3 cd
F In-b-150	52.0 b	54.0 ab	8.0 abcd	9.3 ab	108.7 a	86.5 bc	12.3 d	23.3 abcd
NF In-b-590	59.5 a	53.5 ab	9.3 ab	9.0 abc	97.0 abc	93.0 ab	19.3 abcd	18.0 abcd
NF In-b-591	59.8 a	54.5 ab	9.0 abc	8.7 abcd	93.0 cd	95.0 a	32.3 a	28.7 ab
Check	51.0 b	49.4 b	6.3 d	7.3 bcd	88.0 cd	87.0 bc	27.6 abc	22.3 abcd

[w] Mean of six tillers measured 1 mo after transplanting.
[x] Mean of three replications, six hills per replicate measured at maximum tillering.
[y] Grain weight taken from six hills. Means followed by a common letter are not significantly different ($P = 0.05$) by Duncan's multiple range test.
[z] ST = seed treatment; S = spray application to plants 45 days after transplanting at about 10^9 cfu/ml.

DISCUSSION

This study demonstrates that the fluorescent and nonfluorescent bacteria were readily detected in the rice system and rice plants were protected from sheath blight infection when seeds were treated with the bacteria. Rice plants from seeds treated or sprayed with fluorescent or nonfluorescent bacteria had significantly less sheath blight. Fluorescent pseudomonads were found in the rhizosphere in cereal, fiber, and tuber crops (2,5,9,11,17,19). Strains of fluorescent pseudomonads, either individual or in combination, applied to wheat seeds suppressed take-all caused by *Gaeumannomyces graminis* (Sacc.) Oliver & Von Arx var. *tritici* (18). Treating tuber and root crop planting materials with fluorescent pseudomonads significantly increased yields (2,11). *Pseudomonas fluorescens* Migula applied to cotton seeds suppressed seedling damping-off caused by *R. solani* and *Pythium ultimum* Trow (9,16). The fluorescent pseudomonads appear to be widely distributed in temperate (6) as well as in tropical soil of dryland fields (13,14). The fluorescent bacteria used in this study were isolated from flooded and from dryland rice fields (Table 1). The nonfluorescent bacterial isolates also commonly isolated from the rice system sometimes gave better control of sheath blight than fluorescent isolates. Identification of the nonfluorescent isolates is not complete. Both groups of bacteria in the rice system seem to function as biological control agents. Although fewer fluorescent isolates were obtained, most of them inhibited mycelial growth and suppressed sheath blight. Both fluorescent and nonfluorescent bacterial isolates obtained from the rice system appear to promote crop growth during the early growth stage. Weller and Cook (19) presumed that siderophore and antibiotics were mechanisms related to bacterization of seeds by fluorescent pseudomonads. In this study, the effect on sheath blight control appears not related to in vitro inhibition of mycelial growth. Isolate In-b-17, a nonfluorescent bacterium demonstrated a strong effect both in vitro and in greenhouse and screenhouse experiments, whereas In-b-24, a fluorescent pseudomonad, was not effective in vitro but effective in vivo. Isolate In-b-590, a nonfluorescent isolate, was effective in both conditions. The two fluorescent isolates In-b-24 and In-b-150 were tentatively identified as *Pseudomonas aeruginosa* (Schroeter) Migula.

The ability of certain *Pseudomonas* spp. to suppress root disease is assumed to be due to their ability to colonize the root (1,6). In the flooded-rice system, sheath blight infection usually starts on the sheath near or slightly above the waterline. If the bacteria colonize only the root or the subterranean portion of the rice plant below the waterline after seed bacterization their efficiency cannot be very dependable. Our results indicate that after seed bacterization, the bacteria (fluorescent isolates for convenience of the study) were readily detected on aboveground plant parts even at 45 days after sowing (Fig. 1). The data suggest the bacteria could "migrate" and establish on plant surface as the plant grew.

The limiting factor to application of bacteria in the soil for biological control is the ability of the bacteria to survive under drought stress. Soil moisture status is the controlling factor in the death of most *Pseudomonas* spp. (7). *P. putida* is effective because it survives well in low matric potentials (6). In the flooded-rice system, the condition allows the *Pseudomonas* sp. and the nonfluorescent isolates to survive and reproduce.

Our survey shows the presence of large numbers of antagonistic bacteria in rice fields in the tropics, which provides a potential alternative to manage rice diseases. From the results, it appears the use of these bacteria as biological control agents for rice sheath blight in a flooded system is possible.

LITERATURE CITED

1. Brown, M. E. 1974. Seed and root bacterization. Annu. Rev. Phytopathol. 12:181-197.
2. Burr, T. J., Schroth, M. N., and Suslow, T. 1978. Increased potato yields by treatment of seedpieces with specific strains of *Pseudomonas fluorescens* and *P. putida*. Phytopathology 68:1377-1383.
3. Chien, C. C., Jong, S. C., and Liu, C. L. 1963. Studies on the number of sclerotia of rice sheath blight fungus dropped on the paddy fields and difference in germinability between natural and cultured ones. Int. Taiwan Agric. Res. 12:7-13. (In Chinese, English summary)
4. Chin, K. M., and Lim, W. C. 1975. The survival, spread and host range of *Rhizoctonia solani*, the causal organism of sheath blight disease of rice. MARDI Res. Bull., Malaysia 3(2):19-24.
5. Cook, R. J., and Rovira, A. D. 1976. The role of bacteria in the biological control of *Gaeumannomyces graminis* by suppressive soils. Soil Biol. Biochem. 8:269-273.
6. Dupler, M., and Baker, R. 1984. Survival of *Pseudomonas putida*, a biological control agent, in soil. Phytopathology 74:195-200.
7. Scher, F. M., and Baker, R. 1980. Mechanisms of biological control in a Fusarium-suppressive soil. Phytopathology 70:412-417.
8. Hashiba, T., Yamaguchi, T., and Mogi, S. 1972. Biological and ecological studies on the sclerotium of *Pellicularia sasakii* (Shirai) S. Ito. I. Floating on the water surface of sclerotium. Ann. Phytopathol. Soc. Jpn. 38:415-425.
9. Howell, C. R., and Stipanovic, R. D. 1979. Control of *Rhizoctonia solani* on cotton seedlings with *Pseudomonas fluorescens* and with an antibiotic produced by the bacterium. Phytopathology 69:480-482.
10. King, E. O., Ward, M. K., and Raney, D. E. 1954. Two simple media for the demonstration of pyocyanin and fluorescein. J. Lab. Clin. Med. 44:301-307.
11. Kloepper, J. W., Schroth, M. N., and Miller, T. D. 1980. Effects of rhizosphere colonization by plant growth-promoting rhizobacteria on potato plant development and yield. Phytopathology 70:1078-1082.
12. Kozaka, T. 1975. Sheath blight in rice plants and its control. Rev. Plant Prot. Res. 8:69-80.
13. Mew, T. W., Ho, W. C., and Chu, L. 1976. Infectivity and survival of soft-rot bacteria in Chinese cabbage. Phytopathology 66:1325-1327.
14. Mew, T. W., and Rosales, A. M. 1984. Bacterization of rice plants for sheath blight control. (Abstr.) Phytopathology 74:836.
15. Mew, T. W., and Rosales, A. M. 1984. Relationships of soil microorganisms to rice sheath blight development in irrigated and dryland rice cultures. Pages 147-157 in: Soil Borne Crop Diseases in Asia. FFTC, Book Series No. 26. Food and Fertilizer Technology Center for the Asian and Pacific Region, Taiwan.
16. Misaghi, I. J., Stowell, L. J., Grogan, R. G., and Spearman, L. C. 1982. Fungistatic activity of water-soluble fluorescent pigments of fluorescent pseudomonads. Phytopathology 72:33-36.
17. Smiley, R. W. 1979. Wheat rhizosphere pseudomonads as antagonists of *Gaeumannomyces graminis*. Soil Biol. Biochem. 11:371-376.
18. Ui, T., Naiki, T., and Akimoto, M. 1976. A sieving-flotation technique using hydrogen peroxide solution for determination of sclerotial population of *Rhizoctonia solani* Kuhn in soil. Ann. Phytopathol. Soc. Jpn. 42:46-48. (English summary)
19. Weller, D. M., and Cook, R. J. 1983. Suppression of take-all of wheat by seed treatments with fluorescent pseudomonas. Phytopathology 73:483-489.
20. Yamaguchi, T., Iwata, K., and Kuramoto, I. 1971. Study on forecasting the sheath blight of rice plant caused by *Pellicularia sasakii*. I. Relation between hibernated sclerotium and disease outbreak. Bull. Hokuriku Nat. Agric. Exp. Stn. 13:15-34. (In Japanese, English summary)