Control of Rice Sheath Blight by Phyllosphere Epiphytic Microbial Antagonists

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Epiphytic microorganisms on the phyllosphere of traditional and high-yielding rice varieties were isolated from different agroecological zones of Sri Lanka and screened for their *in vitro* and *in vivo* antagonism against *Rhizoctonia solani* Kühn AG-1 1A, the sheath blight pathogen of rice. Among a total of 196 bacterial and 91 fungal isolates, 12 bacterial and two fungal isolates which showed more than 50% growth inhibition of *R. solani* were tested for their *in vivo* antagonism. Among the 14 antagonists tested, six bacterial and one fungal isolate substantially reduced the incidence of sheath blight (by more than 82%) and severity (by more than 92%) of the rice varieties BG94-1 and IR8 grown in a pot experiment under open field conditions. Using five antagonists that showed the best *in vitro* antagonism, a pot experiment was conducted to determine whether the presence of indigenous microflora on the rice sheath had any effect on the effectiveness of antagonism. Three isolates (B4, GbB5 and HMWB4) controlled sheath blight incidence and severity equally well in the presence and absence of indigenous microflora. Two isolates (BG352B1 and BG300B1) were more effective when they were introduced into the rice sheath without indigenous microflora. Among the effective antagonists determined by the pot experiment, isolates B4, B16, BG94-1B5, GbB5, HMWB4 and BG379-F2 were tested under field conditions for two consecutive growing seasons. Under field conditions, severity of rice sheath blight was significantly reduced by the application of all the tested antagonists as a spray on rice sheath at a concentration of 10^8 cfu ml^{-1}, starting 3 days after the development of symptoms and continuing for three applications at 10-day intervals. Antagonistic performances were consistent in the two seasons under field conditions and *B. megatarium* A (isolate B16) and *Aspergillus niger* (isolate BG379-F2) performed as the most effective antagonists in both seasons. When disease severity was quantified as percentage sheath area covered by the disease lesions, the respective reductions in disease severity were greater than 50% and 61% by *B. megaterium* A (isolate B16) and *Aspergillus niger* (isolate BG379-F2), respectively, in both seasons.

KEY WORDS: Biological control; epiphytic microflora; *in vivo* antagonism; *Rhizoctonia solani.*

INTRODUCTION

Sheath blight, caused by *Rhizoctonia solani* Kühn AG-1 1A, is a disease which results in considerable economic losses in rice-producing countries worldwide (4). The yield losses are substantial especially under high-input cropping systems (16). Common cultural

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practices in modern rice cultivation such as increased cropping intensities, intense fertilizer use and high plant densities, accelerate sheath blight development (18).

Rice cultivars resistant to sheath blight are not currently available. Therefore, apart from the application of a few effective fungicides, strategies to control sheath blight are limited. Moreover, continuous use of fungicides such as Jingangmycin for the past 30 years in China, has led to reduced efficiency in disease control (16). Development of rice varieties with adequate resistance to sheath blight has been difficult because of the pathogen's wide host range and considerable variability in the host–pathogen–environment interactions (8,16). Even though control of sheath blight has centered on the use of foliar fungicides, their application is not advisable due to environmental pollution, health risks and emergence of resistant pathotypes. Therefore, the potential of various biological control methods has been investigated, with varying degrees of success (16).

Various components of the rice agroecosystem, including water in the paddies, soil, rice seeds and its plant parts, have been found to be rich sources of antagonists against pathogens of rice such as *Rhizoctonia solani, Magnaporthe grisea* and *Fusarium moniliforme*. Bacterization of rice seeds with antagonistic bacteria isolated from the rice agroecosystem has been highlighted as having potential for controlling rice diseases (16).

Sclerotia of *R. solani,* which are buoyant on the water in rice paddies, infect sheaths of the lower leaves at the late tillering or early internode elongation stages of rice. The phyllosphere (phylloplane) – the above ground surface of plants – is a habitat rich in bacteria, fungi, yeasts and algae (14), all epiphytic microorganisms (9). They play a wide range of roles as plant pathogens, natural antagonists of plant pathogens and plant growth promoters (15). Considering this nature of infection, the present study focused on screening potential antagonists for the sheath blight pathogen from the phyllosphere of different rice varieties grown at a number of locations in Sri Lanka and determining their efficiency in controlling sheath blight development under field conditions.

MATERIALS AND METHODS

Isolation of epiphytic microorganisms from the phyllosphere Eleven traditional (*i.e.*, long life cycles of 5–6 months, photoperiod-sensitive and taller plant type) and 15 newly improved, high-yielding rice varieties (*i.e.*, short life cycles of 3–4 months, photoperiod-insensitive and dwarf plant type) grown at six different locations in three different agroecological zones of Sri Lanka, were used for isolations. From field plots which contained plants showing typical sheath blight symptoms, specific plants that were not exhibiting symptoms were collected randomly for isolations.

From each variety, ten 1 cm^2 discs from the rice plant sheath were used for isolation of epiphytic microflora. All leaf discs of a given variety were vortexed together in sterile saline water for 5 min and serial dilutions were prepared (*i.e.*, 10^0, 10^{-1}, 10^{-2}). Small aliquots (0.25 μl) from the dilutions were plated on malt extract agar (MEA) and nutrient agar (NA) plates and incubated at 28°C for 5 days. The bacterial and fungal colonies that appeared on plates and with distinct morphological variations were maintained as pure cultures at 4°C. Bacterial cultures were stored in sterile distilled water and as slants in NA. Small discs of fungal isolates were stored in sterile distilled water and as slants in half-strength MEA. Eight independent isolations were done from the six different locations.

In vitro* screening of antagonists of *R. solani All morphologically different bacterial and fungal colonies isolated and stored were tested *in vitro* for their antagonistic ability against

R. solani by the dual culture plate technique described by Korsten *et al.* (12). *R. solani* was isolated from variety BG403 grown at the Rice Research and Development Institute, Batalagoda, Sri Lanka, and which was exhibiting typical sheath blight symptoms. Growth inhibition of *R. solani* was calculated after 7 days of incubation at 28°C according to the Skidmore formula (11) as $[(K_r - r_1/ K_r) \times 100]$, where K_r is the radius of the control pathogen growth and r_1 is the radius of the pathogen's growth towards the antagonist. Each bacterial/fungal isolate was replicated four times to test its *in vitro* antagonism. Mode of antagonism was determined by observing the growth pattern of the antagonist towards *R. solani*. Presence of an inhibition zone indicated chemical inhibition whereas colonization of the tested isolate over the pathogen in the culture plate indicated competitive antagonism.

Establishment of open-field pot experiment An open-field pot experiment to determine the *in vivo* efficiency of antagonists screened *in vitro* was conducted from November 2004 to March 2005 (known locally as the *maha* season) at the Dept. of Agricultural Biology, Faculty of Agriculture, University of Peradeniya, Sri Lanka in the mid-elevational (496 m above m.s.l.) humid zone (the Mid-Country Wet Zone) of Sri Lanka. Average daytime temperature ranged from 25–28°C and average seasonal rainfall was 1050 mm.

Rice seedlings of two varieties, BG94-1 and IR8, were established in 5-*l* plastic pots under open field conditions on paddy soil (Gleyic Luvisols, according to FAO/UNESCO classification) (17). Seedlings were transplanted 14 days after sowing, with five seedlings per pot. Fertilizer was applied as recommended by the Department of Agriculture. Mean day temperature at the site during the experimental period was 28°C. Plants were watered to maintain a standing water level of 3 cm until the ripening stage. No pesticides were applied for control of pests or diseases.

The experimental treatments were of a two-factor factorial design with two varieties and 14 isolates of antagonists. In addition, there was a control treatment for each variety, without any antagonist isolates. The treatment combinations were laid out as a completely randomized design with three replicates. Each pot with five hills was considered as a replicate.

Application of antagonists in the open-field pot experiment Fourteen isolates (12 bacterial and two fungal), which showed >50% growth inhibition of *R. solani in vitro*, based on the dual culture plate method, were selected as potential antagonists to be tested under pot experiment conditions. Bacterial and fungal isolates were cultured in nutrient broth and potato dextrose broth, respectively, to attain a concentration of 10^8 cfu ml^{-1}. Concentrations of the bacterial and fungal cultures were quantified based on the dilution plate technique (3) and optical density (A_{600}) of the culture at the time of quantification.

At 43 days after transplanting (DAT), the standing water of each plastic pot was mixed with 60 ml of broth culture of a given antagonist. At 45 DAT, rice plants were inoculated with *R. solani* which was cultured on boiled and sterilized rice seeds. Five rice seeds covered with the mycelium of *R. solani* were placed per hill, within the tillers, and these pots were covered with polythene bags for 2 days to maintain high humidity around the plants. At 47 DAT (*i.e.*, 2 days after inoculation with *R. solani*), 60 ml of broth cultures having the same concentration of antagonists as that applied to the water at 43 DAT, was sprayed along the sheaths of rice plants. In the control treatment, 60 ml of broth, but without antagonists, was applied to the standing water in the pot at 43 DAT. Thereafter, the rice plants were inoculated with *R. solani* at 45 DAT and the same volume of broth, but without antagonists, was sprayed at 47 DAT.

Application of antagonists in the presence and absence of indigenous microflora on the rice sheath When antagonists were applied on normal host plant surfaces, the resulting disease incidence and severity would indicate their ability to control sheath blight in the presence of other phyllosphere epiphytes (indigenous microorganisms). Conversely, when antagonists were introduced onto a rice sheath from which indigenous microflora had been removed, the resulting incidence and severity of the disease would indicate the efficiency of the antagonist in controlling the disease without any interactive effect from indigenous microflora. Therefore, a set of rice plants was used in an open-field pot experiment to determine the efficiency of five selected antagonists in controlling sheath blight in the presence and absence of indigenous microflora. The five antagonists which showed the highest *in vitro* antagonism were selected for the investigation. The rice plants were established similarly to the open-field pot experiment that was employed to determine the effect of 14 antagonists. Microorganisms dwelling on the sheath area of rice plants were removed by spraying 0.5% Clorox for 3 min and rinsing subsequently with sterile distilled water for 15 min. A previous study determined this method to be a successful and safe way of surface sterilization of rice sheath (6). Sheath areas of plants which were subjected to the removal of epiphytic microflora were sprayed separately with 60 ml of broth cultures of five different antagonists at a concentration of 10^8 cfu ml^{-1} at 43 DAT. These plants were then inoculated at 45 DAT with five rice seeds containing *R. solani* after which the rice sheaths were sprayed again with broth cultures (60 ml) of antagonists with the same concentration at 47 DAT. Rice plants from which epiphytic microflora were not removed, but inoculated with *R. solani* at 45 DAT, were also treated similarly with five antagonists at 43 and 47 DAT. Control treatments were maintained by spraying the rice sheaths with broth having no antagonists, similarly to the open-field pot experiment that was conducted to determine the effect of 14 antagonists. Each treatment was replicated three times according to a completely randomized design.

Field experiments Field experiments were conducted in two consecutive growing seasons, from May to Sept. 2005 (*yala* season) and from Nov. 2005 to March 2006 (*maha* season), at the Rice Research and Development Institute in Ibbagamuwa which is located within the low-elevation (141 m above m.s.l.) sub-humid zone (known as the Low-Country Intermediate Zone). During the first and second growing seasons, daily mean temperature at the site ranged from 23–28°C and from 24–32°C, respectively, and the seasonal rainfall was 453 mm and 707 mm, respectively. The soil was similar to that used in the open-field pot experiment.

Establishment of the field experiment A rice nursery was established with one of the varieties used in the pot experiment, BG94-1, in plastic trays with 424 holes and filled with sterilized sand. Sand culturing was done to minimize the colonization of microflora from the rhizosphere on roots of rice seedlings. Five seeds were placed in each hole and plastic trays were dipped in a water bath to provide moisture. Standard Hoagland solution was applied 10 days after seeding. Field plots of 135 cm × 135 cm were separated by aluminum sheets, with 15 cm of the sheet under soil level and 20 cm of the sheet above soil level. The individual plots were watered manually. Separation of individual plots by aluminum sheets and manual watering minimized the lateral spread of specific antagonists that were applied to different plots. Twenty-day-old seedlings were transplanted in the field at 15 cm × 15 cm spacing with three plants per hill according to a randomized complete block design with three replicates.

Application of antagonists in the field experiments Five bacterial antagonists (T1 = isolate BG94-1B5; T2 = isolate B16; T3 = isolate GbB5; T4 = isolate B4; T5 = isolate HMWB4) and one fungal isolate (T6 = isolate BG379-F2) were selected for use in the field experiment based on the findings of the open-field pot experiment. The broth cultures of antagonists at 10^8 cfu ml^{-1} were sprayed onto the sheath area of plants at maximum tillering stage (27 DAT). Ten days after the spraying of antagonists, rice plants were inoculated by placing parboiled rice seeds colonized with *R. solani* in the middle of the tillers at a concentration of 100 g of seeds per plot. Antagonists having the same concentration were sprayed onto the rice sheaths three times at intervals of 10 days after the inoculation of *R. solani.* In addition to the six different treatments of antagonists, a positive control (inoculated with *R. solani* but no antagonists) and a negative control (no application of *R. solani* or antagonists) were also maintained with three replicates according to a completely randomized block design.

Bacterial antagonists effective in controlling sheath blight under field conditions were identified by the Biolog microbial identification system according to manufacturer's instructions (Biolog Inc., Hayward, CA, USA). Furthermore, confirmation of the identity of the bacterial antagonist/s was done by biochemical tests such as Gram staining, 3% KOH test, spore test, and growth in selective media. Fungal antagonists successful under field conditions were identified by colony and spore morphology.

Collection and analysis of data In the open-field pot experiments, sheath blight incidence was measured as the percentage of tillers affected by the disease out of the total in each pot 7 days after inoculation of *R. solani.* The rate of disease development was quantified as the number of days taken for sheath blight symptoms to cover 50% of the rice sheath. Disease severity was measured as the percentage area of the sheath covered by symptoms, 5 days after the initial symptom development in the pot experiment. In the field experiments, disease severity was measured as percentage rice sheath area covered by the lesions at 7-day intervals from 50 DAT. In the field experiment, ten hills per plot were identified and used throughout the experimental period for quantification of disease severities. Significance of treatment effects on the above variables was determined by analysis of variance and mean separation was done by the least significant difference or Duncan's multiple range test. The degree of similarity of the treatments between the two field experiments was analyzed using a rank correlation technique. Briefly, for each parameter quantified, treatments were ranked separately for the two experiments based on the magnitude of the measured feature. In the case of perfect similarity of results between the two experiments, the treatment ranking for a given parameter would be the same, giving a perfect correlation in ranks, with a linear correlation coefficient of 1. However, as this is highly unlikely to occur in a real situation because of uncontrollable, random variation of measured parameters, the degree of similarity of results between the two experiments could be determined by examining the magnitude and statistical significance of the treatment rank correlation coefficient. The degree of similarity would be greater when the correlation coefficient approaches 1 and *vice versa.* Therefore, treatment rank correlation coefficient was used to determine whether the treatments used in the present study behaved similarly or not in the two field experiments.

RESULTS

Epiphytic microorganisms and *in vitro* antagonism A total of 196 bacterial and 91 fungal isolates which were morphologically different were isolated from the eight independent isolations and each was tested for *in vitro* antagonism against *R. solani.* Twelve bacterial isolates (6% of those isolated) and two fungal isolates (2% of those isolated) exhibited >50% growth inhibition of *R. solani in vitro.* Among these, all the bacterial isolates showed clear inhibition zones around the colony of *R. solani,* indicating a chemical inhibition. The two fungal isolates rapidly overgrew a *R. solani* colony, indicating a competitive growth. However, competition for nutrients by this fungal isolate could be excluded because PDA, which is a nutritionally rich medium, was used in the dual culture plate method.

Efficiency of antagonistic isolates *in vivo:* open-field pot experiment Disease incidence (% diseased tillers) and disease severity, as measured by the percentage sheath area affected, were significantly ($P<0.0001$) influenced by the antagonistic treatments in both varieties (Table 1). Furthermore, variety BG94-1 showed a significantly higher disease incidence and severity (% sheath area affected) than IR8, irrespective of the treatments applied.

The antagonistic isolates BG94-1B5, B16, HMWB4 and BG379-F2 evinced 100% control of disease incidence and disease severity (% sheath area affected) in both varieties (Table 1). In addition, isolate B4 also gave 100% control of disease incidence and disease severity (% sheath area affected) for var. BG94-1 with an appreciably high percentage control of the disease in var. IR8 also. For var. IR8, in addition to the above mentioned five antagonists, isolates BG357B3, MB40 and BG403-F1 gave 100% control of the disease incidence and severity (Table 1). Isolate GbB5 also gave very high control (>86%) of sheath blight incidence and severity in both varieties (Table 1).

All of the antagonistic isolates used showed a significant reduction of disease incidence relative to the control treatment in both varieties (Table 1). However, in both varieties, treatment with isolate BG352B1 resulted in a much higher disease severity in comparison with the control (*i.e.,* plants not treated with any antagonist).

Table 1 also presents the rate of disease development on vars. BG94-1 and IR8 when treated with the antagonistic isolates. In var. BG94-1, all antagonists except isolate BG403-F1 had a lower rate of disease development. In var. IR8 also, most antagonistic treatments resulted in a slower rate of disease development than the control. However, performances of the most effective antagonists differed for the two varieties. Some antagonistic treatments (indicated by an asterisk in the table) did not show a spread of disease symptoms up to 50% of the sheath even 3 weeks after inoculation.

Efficiency of controlling sheath blight by selected antagonists in the presence and absence of indigenous microflora Figures 1 and 2 show, respectively, the sheath blight incidence and severity on plants treated with five different antagonists in the presence and absence of indigenous microflora on the rice sheath. All five antagonists showed a lower disease incidence than the control treatment (*i.e.,* no application of antagonists) in both rice varieties. Although isolate BG352B1 showed a higher disease severity than the control treatment, the other antagonists gave effective control of disease severity (Fig. 2). Therefore, the results of this experiment confirmed the findings of the open-field pot experiment conducted with 14 antagonists. Effectiveness of isolates B4, GbB5 and HMWB4 did not change significantly with the presence or absence of indigenous

TABLE 1. Mean percentage sheath blight incidence and severity on rice varieties BG94-1 and IR8 treated with 14 different antagonistic isolates under open-field pot experiment conditions (means are averages of 15 hills)

Isolate	Percentage incidence (% diseased tillers)		Severity (% sheath area affected)		Severity (no. of days to cover 50% of leaf sheath)	
	BG94-1	IR8	BG94-1	IR8	BG94-1	IR8
No antagonist (Control)	36.40a[z]	30.87a	36.86c	44.04c	5.17b	4.96d
BG352B1	31.20ab	19.82b	57.40a	58.0a	5.82ab	6.00cd
BG300B1	11.78cde	8.28cde	8.65ef	7.17f	6.88a	7.16ab
GbB5	4.41de	4.15de	3.70fg	1.06g	6.00ab	7.66a
B15	28.40ab	12.29bcd	53.33ab	28.0d	6.0ab	6.90abc
BG94-1B5	0e	0e	0g	0g	*	*
MB4	26.90ab	15.27bc	59.47a	13.88e	6.37ab	6.0cd
BG450B11	23.12bc	16.87bc	50.88b	48.85b	6.23ab	6.23bc
BG403-F1	14.18cd	0e	26.0d	0g	5.10b	*
BG357B3	8.95de	0e	14.70e	0g	5.56ab	*
MB40	6.67de	0e	2.69fg	0g	5.50ab	*
B16	0e	0e	0g	0g	*	*
HMWB4	0e	0e	0g	0g	*	*
BG379-F2	0e	0e	0g	0g	*	*
B4	0e	1.24e	0g	0.60g	*	6.00cd

[z]Within columns, means followed by a common letter do not differ significantly at $P<0.05$.

*Indicates treatments which did not exhibit sheath blight lesions even 3 weeks after inoculation.

TABLE 2. Percentage sheath blight severity quantified as percentage diseased area on leaf sheath under six different antagonist treatments (T1–T6) and positive (T7) and negative (T8) controls during season 1 of the field experiment. Percentage disease control in comparison with T7 is given in parentheses. (Means are averages of 30 hills)

Days after transplanting	Treatment							
	T1	T2	T3	T4	T5	T6	T7	T8
50	5.5b[z] (64%)	5.3b (65%)	5.8b (62%)	5.0b (67%)	6.6b (57%)	4.6b (70%)	15.5a	0.4c
57	6.7c (62%)	5.5c (69%)	5.8c (67%)	6.4c (64%)	10.1b (43%)	5.8c (67%)	18.1a	0.2d
64	6.8b (55%)	4.8c (69%)	6.8b (56%)	6.1bc (60%)	7.9b (49%)	5.3bc (65%)	15.6a	0.6d
71	7.1b (59%)	3.9d (78%)	7.4b (58%)	6.6bc (63%)	6.8bc (61%)	5.1cd (71%)	17.9a	1e
78	5.7b (70%)	3.9b (79%)	4.5b (76%)	5.6b (71%)	5.1b (73%)	4.8b (75%)	19.3a	0.9c

[z]Within columns, means with a common letter do not differ significantly at P=0.05.

TABLE 3. Percentage sheath blight severity quantified as percentage diseased area on leaf sheath under six different antagonist treatments (T1–T6) and positive (T7) and negative (T8) controls during season 2 of the field experiment. Percentage disease control in comparison with T7 is given in parentheses. (Means are averages of 30 hills)

Days after transplanting	Treatment							
	T1	T2	T3	T4	T5	T6	T7	T8
50	12.85bc[z] (28%)	7.13d (60%)	14.5ab (18%)	10.46cd (41%)	8.16d (54%)	6.9d (61%)	17.73a	0.00e
57	17.25bc (48%)	9.43e (72%)	19.13b (42%)	12.73de (62%)	14.66cd (56%)	9.9e (70%)	33.13a	0.00f
64	24.65bc (45%)	13.10d (71%)	26.66b (40%)	20.93c (53%)	22.3bc (50%)	11.7d (74%)	44.66a	0.00e
71	31.95cd (39%)	26.13d (50%)	39.10b (25%)	34.73bc (33%)	29.63cd (43%)	17.93e (66%)	52.1a	0.00e
78	32.95bcd (38%)	26.7d (50%)	39.9b (26%)	35.2bc (34%)	30.7cd (43%)	18.03e (66%)	53.56a	0.00f

[z]Within columns, means with a common letter do not differ significantly at P=0.05.

microflora. However, in both rice varieties, antagonist BG352B1 gave much more effective control in the absence of indigenous microflora. Although BG300B1 also showed increased effectiveness when indigenous microflora were removed from the sheath, the increase was not so great as that of BG352B1 (Figs. 1, 2).

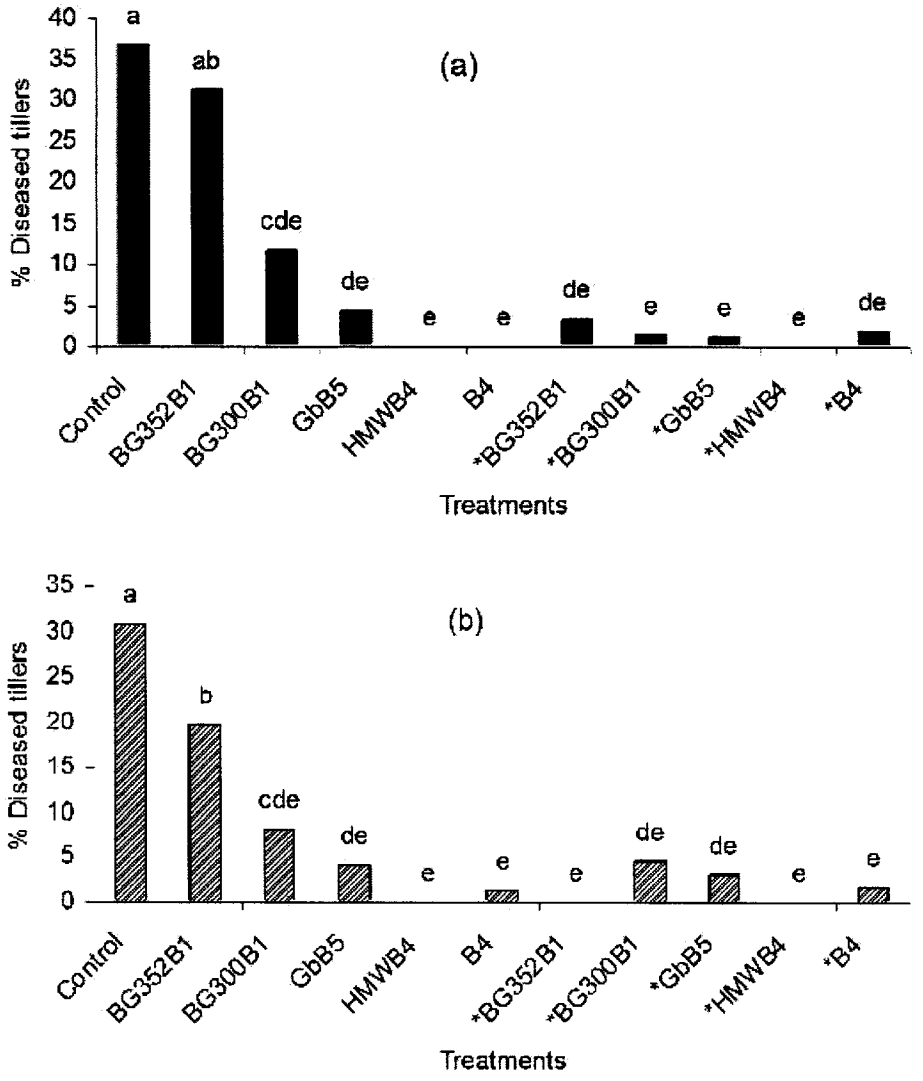

Fig. 1. Percentage disease incidence of varieties BG94-1 (a) and IR8 (b) treated with five different antagonists with and without indigenous microflora on the rice sheath. Treatments with asterisks indicate the absence of indigenous microflora. Each column represents the mean of 15 replicates. Columns with a common letter indicate means which do not differ significantly at P=0.05.

Control of sheath blight disease: Field experiments, season 1 As expected, T7 (*i.e.,* treatment without antagonists) showed the highest disease severity with respect to percentage diseased area on sheath throughout the data collection period for var. BG94-1 (Table 2). In contrast, T8, which did not have *R. solani* or any antagonist, had the lowest sheath blight severity. All other treatments containing the antagonists had a lower disease severity than T7. Treatment T7, which had no antagonist, showed an increasing trend of disease severity over the experimental period. Even though a slight increase

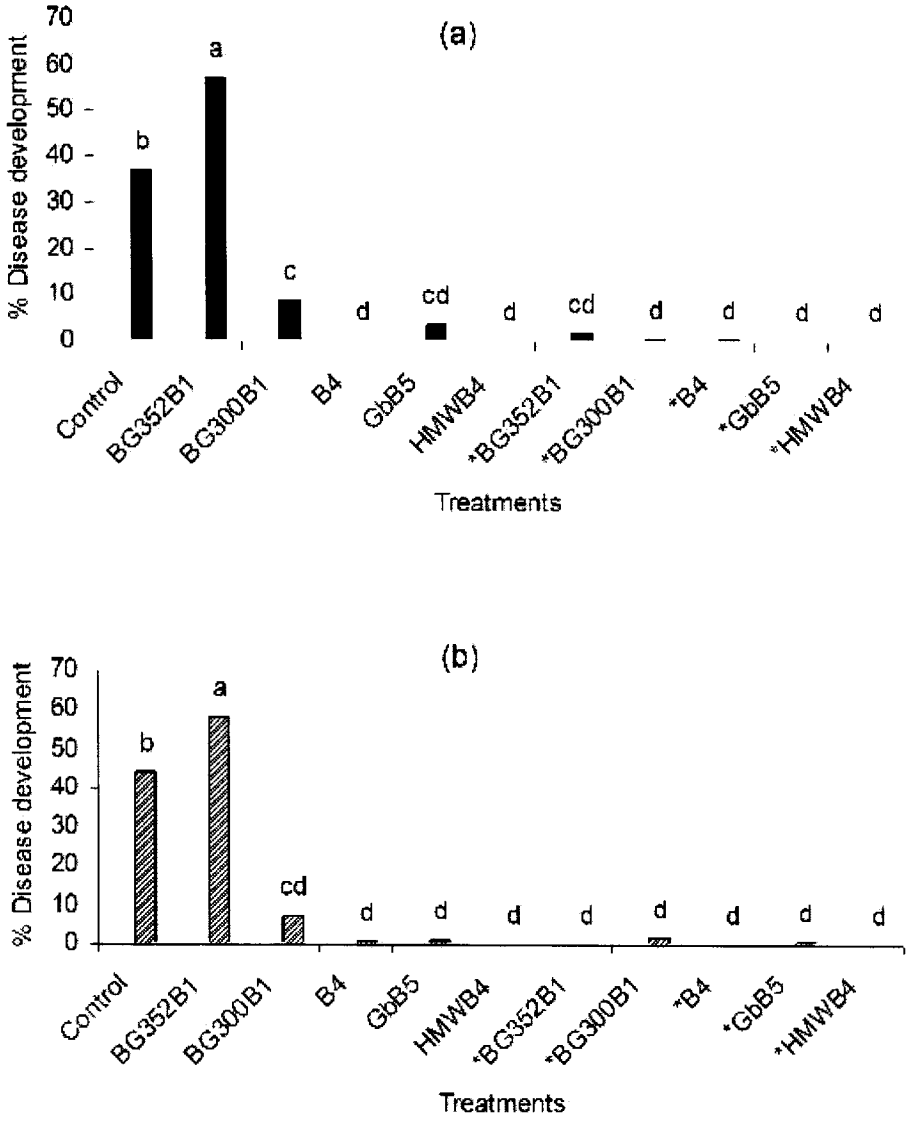

Fig. 2. Percentage disease severity with respect to area of lesion development of varieties BG94-1 (a) and IR8 (b) treated with five different antagonists with and without indigenous microflora on the rice sheath. Treatments with asterisks indicate the absence of indigenous microflora. Each column represents the mean of 15 replicates. Columns with a common letter indicate means which do not differ significantly at P=0.05.

in disease severity was apparent in the treatments with antagonists (*viz.*, T1–T6), in general all treatments showed a gradual decline in disease severity towards the end of the growing season. Treatments T2 (isolate B16) and T6 (isolate BG379-F2) gave the highest percentage control of disease severity among the antagonists, with more than 65% control with respect to diseased area developing on the sheath during the experimental

period (Table 2). All antagonists gave more than 43% sheath blight control with respect to area of lesion development at any given time of data collection.

Control of sheath blight disease: Field experiments, season 2 There was a significant variation among treatments in the control of sheath bight severity ($P<0.0001$) in the second growing season as well. Table 3 gives the mean percentage severity when quantified as percentage diseased area on the sheath. Season 2 experienced a higher disease severity than season 1 under all treatments. Similar to the first season, treatment T7 (no applied antagonist) showed the highest sheath blight severity while treatment T8 showed the lowest. In all treatments, there was a gradual increase in disease severity during the experimental period. However, all the antagonist treatments resulted in a much lower percentage disease severity than T7, the positive control (Table 3).

Similar to the first season of experimentation, treatments T2 (isolate B16) and T6 (isolate BG379-F2) provided the best control of sheath blight severity. The only difference was that in season 2, T6 gave slightly better control than T2 (Table 3). In growing season 2, treatments T6 and T2 gave >61% and >50% control, respectively, of the disease severity as determined by percentage area of lesion development. Moreover, in season 2, despite the higher disease severity at any given time of observation, all the antagonists provided >18% control of sheath blight severity than did T7 (Table 3). The ability of all antagonists to reduce disease severity with respect to percentage diseased area on sheath was consistent over the two growing seasons.

Bacterial isolates BG94-1B5 (T1), B16 (T2), GbB5 (T3) and B4 (T4) were identified as *Bacillus* spp. by Gram staining, 3% KOH test and spore test. Among the *Bacillus* spp., isolate B16 was identified as *B. megaterium* A by the BIOLOG method with a similarity index of 0.78. The identity of *B. megaterium* was confirmed by growing the bacterium on selective medium. Isolate HMWB4 (T5) was identified as *Dermococcus nishinomiyaensis* by the BIOLOG method with a similarity index of 0.74. The fungal isolate, BG379-F2 (T6), was identified as *Aspergillus niger* based on colony and spore morphology.

Rank correlation of treatments Comparison of results between the two seasons under field conditions using rank correlation showed a very strong correlation (0.81 at $P=0.01$) between comparative seasonal performances when it was measured as the percentage lesion area developed on rice sheath.

DISCUSSION

The present study confirmed that the rice phyllosphere harbors antagonistic bacterial and fungal isolates that can inhibit the growth of *R. solani*, the sheath blight pathogen of rice. Moreover, the study showed the *in vitro* and *in vivo* antagonistic efficiency of phyllosphere epiphytic microorganisms of rice in controlling sheath blight. It is the general belief in biological control that most of the antagonists that are effective *in vitro* are not so successful *in vivo* or *vice versa* (1). However, pot and field experiments repeated over several seasons showed that in the present study, antagonists selected *in vitro* are consistent in their effectiveness in controlling sheath blight *in vivo*. All antagonists tested in field experiments during the two seasons gave >18% control of disease severity with respect to percentage diseased area on the sheath, and *B. megaterium* A (isolate B16) and *A. niger* (isolate BG379-F2) provided >50%. This is an appreciable level of control that can be achieved by biological control agents under field conditions, especially when applied on aerial habitats.

Slight variations in the performance of antagonists could be due to variations in environmental conditions between the two seasons. Survival efficiency of epiphytic bacteria is heavily influenced by harsh environmental factors such as high solar radiation and low rainfall (2). Modifying the plant's microenvironment by agronomic practices and designing an appropriate formulation would mitigate such problems and help to increase the efficiency of the antagonist, irrespective of seasonal differences. However, better disease control under field conditions by treatments T2 and T6 could be due to their better survival efficiency as revealed by the results of the present study.

In both seasons, all antagonists had a similar comparative ranking in controlling sheath blight severity, thereby showing the consistent nature of antagonism across seasonal differences. This is a desirable attribute for biological control agents. Despite the fact that environmental conditions of aerial habitats are unfavorable for survival of microorganisms (2), antagonists identified and tested in the present study provided effective control even under the harsh environmental conditions in the aerial habitat.

In both seasons, typical sheath blight symptoms were observed 7 days after inoculation of the pathogen. The findings revealed that severity of rice sheath blight can be significantly reduced by the application of the tested antagonists as a spray on the rice sheath.

The present study demonstrated the effectiveness of some of the antagonists against the sheath blight pathogen irrespective of the presence or absence of indigenous microflora on the sheath. This is a desirable feature of antagonists and indicates that they could act effectively even in the presence of interaction by indigenous microflora. Indigenous bacterial epiphytes do not appear to inhibit the establishment of suppressive population sizes of the introduced bacterial antagonists (19).

In the pot experiment of the present study, there was a clear difference between the two varieties used, with IR8 showing a lower disease incidence and severity than BG94-1. Such variations in disease control by biological control agents have been reported by others (13) on chickpea cultivars when treated with different isolates of *Bacillus*. However, no information is available on sheath blight resistance of IR8. BG94-1 is a variety released by the Rice Research and Development Institute, Sri Lanka. Its parental lines are IR262 and Ld66 and the latter is a variety suitable for iron toxicity and acidic soils. As no records are available on sheath blight resistance of these lines, specific reasons for the difference between the two varieties cannot be determined without further investigations.

Many *Bacillus* species including *B. cepacia, B. subtilis* and *B. pumilus* isolated from rice seeds, paddy water and plant parts have shown an antagonistic effect against many rice pathogens including *R. solani, Fusarium moniliforme, Magnaporthe grisea, Sarocladium oryzae* (6,16). The ability to produce antimicrobial substances could be a possible reason for the antagonism of effective antagonists found in the present study. Inhibition zones produced by the antagonists around *R. solani* in dual culture tests *in vitro* indicated a possible chemical inhibition. Moreover, as explained by Landa *et al.* (13), production of antibiotics and/or antifungal substances by the antagonists could be supported by the following observations of the present study: (*a*) for all antagonistic isolates selected initially *in vitro,* there was no direct contact between the colony of *R. solani* and antagonistic colonies and hence the inhibition of fungal growth could be due to substances that diffused into the agar medium; (*b*) the PDA medium used for dual culture assay is rich in nutrients and thus competition might be excluded as a possible mode of antagonism; and (*c*) antibiosis is the general mode of antagonism observed for *Bacillus* spp. Production of

antifungal compounds by most of the *Bacillus* spp. has been reported (7). In addition, antimicrobial substances being produced by *Bacillus licheniformis* strain P40, *B. subtilis* and *B. amyloliquefaciens* have been reported (5,20). Among the antimicrobial chemicals, a Bacteriocin-like substance produced by *B. licheniformis* strain P40 and a wide variety of antifungal lipopeptide isomers belonging to the iturin, fengycin and surfactin families produced by *B. subtilis* GA1 have been identified (20,21). *Bacillus* spp. form spores that are resistant to unfavorable environmental conditions and thus can be adapted to formulation and application in the field (13). Therefore, the production of antibiotics and of spores of *Bacillus* spp. suggests that these species may be attractive biological control agents of plant diseases caused by phytopathogenic fungi (13).

Repeated *in vitro* and *in vivo* experiments in the present study demonstrated the efficiency of an isolate of *A. niger* in controlling rice sheath blight. *A. niger,* when applied to control sheath blight, has been reported to promote growth of rice (10). Despite the vast majority of strains of *A. niger* that are employed in industrial fermentation with a history of safe use, specific strains are reported to produce certain mycotoxins or may elicit allergic responses among workers. However, the US Environmental Protection Agency has identified *A. niger* as a non-significant human pathogen. Therefore, with proper characterization of the beneficial strains, *A. niger* could be used as a biological control agent of plant pathogens. Moreover, *B. megaterium* is an ubiquitous saprophyte in natural habitats such as soil, water and plant parts with widely distributed spores in those habitats. Based on the findings of the present study, *B. megaterium* A and *A. niger* could be considered as potential antagonist isolates of rice sheath blight under field conditions. Investigations on formulating these antagonists and on determination of the field efficacy of formulations must be conducted.

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