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Efficacy of novel formulations of Bacillus megaterium in suppressing sheath blight of rice caused by Rhizoctonia solani

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abstract

Efficacy of novel formulations of Bacillus megaterium in suppressing sheath blight of rice caused by Rhizoctonia solani

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Abstract. Bacterial antagonists in pellet and granule formulations, produced using Bacillus megaterium with pharmaceutical technology, were tested under both greenhouse and field conditions. When used by broadcasting to rice plants, the pellet formulation [containing B. megaterium (No. 16)] performed as good as a fungicide (Iprodione) in suppressing sheath blight in the pot tests under the greenhouse condition. When sprayed onto rice plants, the granule formulation [containing B. megaterium (No. 16)] was as effective as a fungicide (Iprodione) and was more effective than the pellet formulation in the field test.

Key words: Bacillus megaterium, biological control, Oryza sativa, pellet and granule formulations, Rhizoctonia solani, rice sheath blight

Introduction

Rice sheath blight, caused by Rhizoctonia solani Kuhn, is one of the most destructive rice diseases worldwide (Ou, 1985). This fungal disease is second in importance only to rice blast disease, caused by Magnaporthe grisea (anam. Pyricularia oryzae) (Rush and Lee, 1992; Thurston, 1995; Dehne and Oerke, 1998). Yield reductions of up to 20% may occur if the disease develops and reaches the uppermost leaves (Teng et al., 1990). Management of sheath blight disease of rice has been directed toward the integration of cultural practices with chemical control (Chin and Bhandhufalck, 1990; Damicone et al., 1993). However,

chemical control using effective fungicides has various undesirable effects, such as being phytotoxic to rice plants (Groth et al., 1990) and the requirement for critical timing of fungicide application may hinder its usage (Lee and Rush, 1983). Alternatively, using rice plants resistant to sheath blight disease has been considered as a control option (Rush and Lee, 1992).

Biological control has also become a prominent option for controlling various plant diseases (Cook, 1993; Larkin et al., 1998). Rice sheath blight is one of the plant diseases which has been controlled using a biological control approach (Mew and Rosales, 1986; Vasantha Devi et al., 1989; Kanjanamaneesathian et al., 1998; Pengnoo et al., 2000). However, fresh cells of potential antagonists have been used for sheath blight control testings in most of these studies (Mew and Rosales, 1986; Vansantha Devi et al., 1989; Gnanamanickam and Mew, 1990; Gnanamanickam et al., 1992). Although effective and suitable for research purposes, fresh cells of antagonists may not be suitable for use in the rice field by the farmers (Pengnoo et al., 2000). Preliminary testing of bacterial formulations to control sheath blight of rice has been investigated in pot testings in the greenhouse (Kanjanamaneesathian et al., 1998) and in the field conditions (Pengnoo et al., 2000). Although these formulations demonstrate the desired characteristics and provide quite satisfactory protection for rice plants from R. solani infection in both tests (Kanjanamaneesathian et al., 1998; Kusongwiriyawong et al., 1999; Pengnoo et al., 2000), they have a comparatively short shelf life, and the numbers of bacterial antagonists in the formulations greatly decline during 6 months storage (Kanjanamaneesathian et al., 2000). This undesirable characteristic of the formulation makes it unsuitable for large scale production and commercialisation. For this reason, further research and development regarding improved formulations are required.

Research in the area of improved formulations and delivery systems is critical for further development and implementation of effective biological control (Lewis, 1991; Lumsden et al., 1995; Larkin et al., 1998). Novel formulations of bacterial antagonists have been developed and used for sheath blight disease control trials in both greenhouse and field conditions (Wiwattanapatapee et al., 2003). In the preliminary tests, these pellet formulations containing Bacillus megaterium show good floating properties and gradually release their bacterium load over time. After production, they also contain a high level of B. megaterium after storage for 6 months at room temperature. In a greenhouse test, this formulation showed promising results in suppression of the development of sheath blight (Wiwattanapatapee et al., 2003).

The objectives of this research were (a) to test the efficacy of developed bacterial formulations, and (b) to compare the efficacy of various application methods in suppressing sheath blight disease development in both greenhouse and field conditions using improved formulations of B. megaterium.

Materials and Methods

Formulations and B. megaterium (No. 16) used in the experiment

Two novel formulations were produced using pharmaceutical technology for use in both greenhouse and field trials to control sheath blight disease in this experiment. The pellet formulation was prepared using an extrusion-spheronization process as described by Wiwattanapatapee et al. (2003) and applied by broadcasting to rice plants in both greenhouse and field tests. The pellet formulation used in these tests contained 109 cfu/g of B. megaterium (No. 16).

A second granule formulation was prepared using the wet granulation method and applied by spraying on the rice plants in both greenhouse and field tests. This formulation was composed of monohydrate lactose (Veghel, The Netherlands) 800 g, polyvinyl pyrrolidone K 30 (supplied by Vidhyasom, Thailand) 100 g, sodium alginate (Sigma-Aldrich, USA) 100 g, and bacterial suspension 200 mL. All ingredients were mixed with the bacterial suspension in a planetary mixer until they became a damp mass. This mass was then passed through a sieve (1.6 x 1.6 mm pore size) and dried in an incubator at 65?C for 2 h. In the greenhouse test, the granule formulation containing bacteria at 109 cfu/g was dissolved in tap water (at 0.3:10, w/v) and sprayed on the rice plants with a hand-held sprayer. In the field test, this same formulation was dissolved in tap water (at 1:10, w/v) and sprayed on the rice plants with a knapsack sprayer.

One isolate of B. megaterium (No. 16) was used as the active ingredient in the formulation. This bacterium was isolated and selected for use in this experiment based upon its ability to inhibit mycelial growth and sclerotial germination of R. solani and suppress sheath blight lesion on rice tissue in vitro (Kanjanamaneesathian et al., 1998). This bacterium was identified using biochemical and physiological tests as described by Pengnoo et al. (2000). The mode of action of B. megaterium (No. 16) in inhibiting R. solani was through the production of an unidentified heat stable antibiotic substance (Pengnoo et al., 2000).

Testing the efficacy of novel formulations of B. megaterium under greenhouse conditions

Testing location

The efficacy of the formulations against sheath blight was investigated under greenhouse conditions between September 2002 and January 2003 at two locations, (1) the greenhouse at Prince of Songkla University, Surat Thani campus, Surat Thani, and (2) the Central Laboratory and Greenhouse Complex, Faculty of Natural Resources, Prince of Songkla University, Hat Yai campus, Songkhla, Thailand.

Experimental design and treatments

In the first experiment, the aims of this preliminary greenhouse tests were two-fold. The first objective was to verify the physical characteristics of the formulations produced in the laboratory (Wiwattanapatapee et al., 2003), and the second objective was to evaluate the efficacy of these formulations in suppressing sheath blight disease.

At the Surat Thani campus, there were six treatments, which consisted of rice plants (cv. Khao Dawk Mali 105) inoculated with either pellets containing B. megaterium, pellets (blank) (for broadcast application), granules containing B. megaterium, granule (blank) (for spray application), or fungicide (Iprodione). Each treatment consisted of three replications (four rice seedlings per replication). Rice plants inoculated only with sorghum seeds infested with R. solani was used as a control treatment. The experiment was arranged in a Completely Randomised Design (CRD).

In the second experiment, the aim was primarily to evaluate the efficacy of formulations which had been slightly modified, particularly in the case of

granule formulation. This experiment was conducted at the Central Laboratory and Greenhouse Complex, Faculty of Natural Resources, Prince of Songkla University, Hat Yai campus, Songkhla. There were eight treatments, consisting of rice plants (cv. Hom Mali 105) inoculated with either pellets containing B. megaterium, pellets (Blank), granules containing B. megaterium, granule (Blank), or fungicide (Iprodione). Each treatment consisted of three replications (ten rice seedlings per replication). Rice plants inoculated only with R. solani were used as a control treatment. The experiment was arranged in a Complete Randomised Design (CRD).

Pot preparation

Paddy rice field soil from Phetchaburi [clay texture (32.4% sand, 18.9% silt, 48.7% clay), pH 7.1, 3.0% organic matter, 0.2% total N, 23.7 mg/kg available P and 0.6 mg/kg available K] was used in the pot test in the Surat Thani trial, while paddy rice field soil from Songkhla [clay texture (2.5% sand, 48.0% silt, 49.5% clay), pH 4.9, 2.1% organic matter, 0.03% total N, 9.6 mg/kg available P and 129.0 mg/kg available K] was used in the pot test in the Hat Yai trial. These soil samples were loaded in plastic pots (21 cm in diameter and 18 cm in height) and the pot was filled with tap water until the soil was soaked. The water level was maintained above the soil level. After 72 hours, the soils were agitated manually to break up aggregates and excess water was drained. Soil level in the plastic pots was adjusted to a height of 13 cm so that 5 cm depth of water was retained in each plastic pot.

Pathogen inoculation

Twenty g of sterile rice seeds infested with R. solani were placed in the centre of each plastic pot 25 days after sowing, 1 day prior to formulation application. The sterile rice seed was dispersed with a sterile spatula so that the pathogen inoculum made contact with all rice plants. The water level in the plastic pot was maintained at the same level throughout the experiment.

Formulation application

Either pellet or granule formulations were applied to the rice seedlings in the plastic pots. Pellet formulation of either 15 g/pot (in the first experiment) or 30 g/pot (in the second experiment) was placed at the centre of each plastic pot, on top of the inoculum of R. solani. Granule formulation at 150 mL/pot (at 0.3:10, w/v) was sprayed on the rice seedlings in the plastic pots using a hand-held sprayer in both the first and the second experiment. Rice seedlings sprayed with fungicide (Iprodione 1 g/L water; at 150 mL/pot) was used as a benchmark to compare the efficacy of the formulations. Rice seedlings in each plastic pot inoculated with only 20 g of sterile rice seeds infested with R. solani were used as a control treatment.

Disease assessment

Sheath blight disease assessment in the greenhouse tests was carried out once, at 10 days, in the first experiment and twice, at 10 and 20 days, in the second experiment, after formulation application. Disease was assessed by counting the number of rice seedlings which showed sheath blight symptoms.

Testing the efficacy of pellet and granule formulations under field conditions

Testing location

The efficacy of the formulations against sheath blight was investigated under field conditions from March to July 2003 at Muang District, Phetchaburi Province, Thailand. This site was chosen for the field trial because it is located in the central region of Thailand where sheath blight disease is a threat to the farmers.

Experimental design and treatments

There were eight treatments in the field trial. Treatment consisted of a rice plant (cv. RD-23) inoculated with either a pellet (3 levels) or granule (3 concentrations) formulation, or a chemical fungicide (Iprodione) one day after pathogen inoculation. Rice plants inoculated only with R. solani were used as a control treatment. Each treatment consisted of six replications, with twelve rice hills/one replication. The experiment was arranged in a Randomised Complete Block Design (RCBD).

Rice field preparation

The rice plot had clay texture (32.4 % sand, 18.9 % silt and 48.7 % clay), pH 7.1, 3.0 % organic matter, 0.2 % total N, 23.7 mg/Kg available P and 0.6 mg/Kg available K. It was flooded with water and ploughed until any soil aggregates were broken up. Excess water was drained 2 days later and the field partitioned into 6 blocks. Each block was further partitioned into 8 sub-plots (2 x 2 m) by earth embankments 30 cm wide to prevent water movement among the sub plots. The rice field was flooded again and the water level was maintained by opening or closing a small gate on each sub-plot embankment.

Rice seedling and rice plant preparation

Rice seedlings (cv. RD-23) were raised in the seedling bed of a farmer's field in Muang District, Phetchaburi Province, Thailand. Rice (cv. RD-23) was selected for this field trial because it is very susceptible to sheath blight disease. After 14 days, these rice seedlings were transplanted into the rice field with 20 cm spacing between and within rows in each 2 x 2 m sub plot. Eight rows and eight columns of rice seedlings were planted in each sub-plot, with two rice seedlings planted at each hill site. The two rows and two columns of rice plants planted adjacent to the earth embankments in each sub plot were used only as guard rows. The four rice plants in the innermost rows and columns were used for disease assessment, while the eight other adjacent rice plants (in the cross configuration surrounding these four innermost rice plants) were used for yield assessment.

Pathogen inoculation

Only twelve rice hills in the centre of each sub plot were inoculated with the pathogen in the efficacy test in the field trial. Twenty g of sterile rice seeds infested with R. solani were placed in the centre of each rice hill 45 days after rice transplanting in the field experiment, 1 day prior to formulation application. The water level in the sub plot was maintained at the same level throughout the experiment.

Formulation application

Twelve rice hills were applied with either pellet or granule formulations. Pellet formulation (either 10, 20, or 30 g) was placed at the centre of each rice hill, on top of the inoculum of R. solani. Granule formulation (either 1.2 L, 2.4 L or 3.6 L/12 rice hills; at 1:10, w/v) was sprayéd to the rice hills

using a knapsack sprayer. Rice plant sprayed with fungicide (Iprodione 1 g/L water; at 3.0 L/12 rice hills) were used as a benchmark to compare the efficacy of the formulations. Rice plants inoculated with only 20 g of sterile rice seeds infested with R. solani were used as a control treatment.

Disease and yield assessment

For sheath blight disease assessment, only the 4 innermost rice hills were uprooted, and sheath blight symptoms were assessed 7 days after formulation application. Roots of the uprooted rice plants were washed to eliminate excessive soils. These roots were later cut and discarded and the above-ground portions of the rice plants were used for sheath blight disease assessment. Disease was assessed by counting the number of tillers which showed sheath blight symptoms. The entire length of the lesion on each rice tiller which had sheath blight symptoms was also measured. Fresh and dry weight of the inoculated rice plants were also assessed after disease measurement in the field test.

For rice yield assessment, rice plants were harvested from the remaining eight hills at the end of the experiment, 110 days after transplanting. Panicles were cut at the base of the uppermost internode and the weight of these panicles was assessed.

Statistical analysis

Data from both greenhouse and field tests were subjected to standard analysis of variance procedures for a completely randomised design using the Statistical Package for Social Science (SPSS/PC+) computer software package. One-way analysis of variance was carried out on the percent of rice seedlings (in the greenhouse trials) and rice tillers (in the field trials) which showed sheath blight symptoms. One-way analysis of variance was also done on the length of the lesions on each rice tiller (from the field trial) which had sheath blight symptoms. Data was compared with Duncan's Multiple Range Test (DMRT) at $P < 0.05$.

Enumeration of viable bacteria in pellet and granule formulations

Viable bacteria in one gram of the formulations used in all trials were counted using the drop plate method (Zuberer, 1994). The plates were incubated at room temperature (26-30?C) for 2 days after which colony-forming units were counted. CFU/g of viable bacteria was the average of six replications per dilution.

Results

Pot tests in the greenhouse

Fungicide (Iprodione) performed best in suppressing sheath blight disease in the pot test (at Surat Thani). A pellet of B. megaterium (No. 16) was as effective as Iprodione in sheath blight suppression. Both pellet and granule formulations containing B. megaterium (No. 16) were effective in sheath blight suppression. Blank formulations (both pellet and granule), without B. megaterium (No. 16) did not control sheath blight in the pot test (Table 1).

Biocontrol of sheath blight was evident in the assessment of % tillers with sheath blight symptoms where treatment with pellet formulation containing B. megaterium (No. 16) had the lowest % infection (Table 2). However, this was not significantly different from those with granule formulation containing B. megaterium (No. 16), blank formulations (both pellet and granule) or fungicide

(Iprodione) when disease assessment was carried out at both 10 and 20 days after formulation application (Table 2).

Field test

Granule formulation, containing B. megaterium (No. 16), at 3.6L/sub plot, was as effective as the fungicide (Iprodione) at 3.3L/sub plot in sheath blight suppression in the rice field at Phetchaburi when % tillers with sheath blight symptom was assessed (Table 3). However, the fungicide (Iprodione) performed better than granule formulation (at 3.6L/sub plot) when lesion lengths were compared (Table 3).

Rice plants applied with either pellet (20g) or granule (2.4L) formulations, both containing B. megaterium (No. 16), had quite high fresh, dry and panicle weights. These, however, were not significantly different from those with 10 or 30g pellet, 1.2 or 3.6L granules, fungicide (Iprodione) or control (inoculated only with R. solani) (Table 4).

Discussion

Sheath blight disease of rice has been controlled with either fresh cells (Mew and Rosales, 1986; Vasantha Devi et al., 1989; Gnanamanickam and Mew, 1990; Gnanamanickam et al., 1992) and formulation of antagonists (Kanjanamaneesathian et al., 1998; Kusongwiriyawong et al., 1999; Pengnoo et al., 2000; Wiwattanapatapee et al., 2003). Research to devise effective formulations is essential because farmers will be more likely to accept biological control measures when they are familiar with their handling properties and can use conventional equipment to apply them (McIntyre and Press, 1991). In a previous study, pellet and granule formulations were successfully produced and their efficacy successfully demonstrated in both greenhouse and field experiments (Wiwattanapatapee et al., 2003). The pellet formulation has better viability of the bacteria and bacterial release than formulations produced for sheath blight control in previous studies (Kanjanamaneesathian et al., 1998; Pengnoo et al., 2000).

In the pot test under greenhouse conditions, pellet formulation (broadcast) was more effective than granule formulation and as effective as the fungicide (Iprodione) in sheath blight suppression, particularly at the Surat Thani site (Tables 1 and 2). In the pot test, the pellet formulation containing B. megaterium (No. 16) inhibited R. solani by producing an unidentified heat stable antibiotic (Pengnoo et al., 2000). Possibly the bacterium in the pellet formulation produces an antibiotic which may be concentrated enough within the closed system to inhibit R. solani. More research should be carried out to identify of this antibiotic, which may lead to the discovery of new fungicides with a microbial origin, such as in the case of the Polyoxins and Validamycin A (Yamaguchi, 1998). Fungicides of microbial origin would be an ideal alternative for plant disease control because they are susceptible to microbial degradation and have quite low toxicity to mammals, particularly in the case of Validamycin A (Yamaguchi, 1998). In this regard, they are safer and are considered more environmentally friendly than synthetic fungicides.

In the field test, granule formulation (at 3.6 L/subplot) containing B. megaterium (No. 16) was as effective as the fungicide (Iprodione), and more effective than pellet formulations in sheath blight suppression (Table 3). In the field, the concentration of antibiotic from the bacteria in the pellet formulation may be reduced when this type of formulation is used in a comparatively open system. This may have contributed to the failure of the pellet formulation to control sheath blight in the field test.

However, the fact that granule formulation (when sprayed at high concentration) is very effective in reducing % rice tillers with sheath blight

symptoms indicated that granule formulation sprayed on rice plants is more effective than pellet formulation broadcast in the centre of the rice tillers for sheath blight control in field conditions (Table 3). This is likely because sheath blight disease is usually most severe during the tillering stage and disease spread (both horizontally and vertically) occurs very rapidly when the rice canopy is thick and relative humidity is very high (Reissig et al., 1986). When sprayed, the bacteria in the granule formulation may be deposited on various rice plant tissues (such as leaf sheath and leaf blade), and this bacteria may reduce infection and deter disease spread both horizontally and vertically. This may partially explain the effectiveness of granule formulations in reducing both % rice tillers with sheath blight symptoms and lesion length (Table 3). However, at the end of the trials, rice plants tested with granule formulation containing B. megaterium (No. 16) were not significantly different from those tested with pellet formulations, fungicide (Iprodione), or the control (inoculated only with R. solani), particularly with respect to panicle weight (Table 4). Thus, it may be necessary to apply granule formulation containing B. megaterium (No. 16) several times during the reproductive and grain filling stages so that a reasonable yield can be obtained. The high inoculum load of R. solani (at 20 g/rice plant) used in this study may also have contributed to the severity of sheath blight disease, particularly when the disease was assessed at the maturation stage (Table 4). This high inoculum load of pathogen is not likely to exist in fields where sheath blight disease occurs naturally. Thus, the quantity of bacterial formulation applied to effectively control sheath blight disease in the field where disease occurs naturally should be less than that used in the experiment. More studies in actual rice fields in areas where sheath blight disease is problematic are required to compare the effective doses of granule formulations with recommended fungicide doses, for instance Benomyl, Edifenphos, Futolanil, Iprodione, Pencycuron, or Validamycin.

Acknowledgments

We wish to thank the Department of Earth Sciences, Faculty of Natural Resources, and the Department of Pharmaceutical Technology, Faculty of Pharmaceutical Sciences, Prince of Songkla University (PSU), Hat Yai Campus, Thailand, for laboratory facilities. We are grateful to Mr. David Patterson, an English consultant at PSU, for his invaluable comments on the original manuscript. We also would like to thank Miss Amornrat Chumthong for her help in both laboratory and field work. The National Research Council of Thailand, through the Faculty of Pharmaceutical Sciences, Prince of Songkla University, provided funding for this research.

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Table 2 Efficacy of novel formulations in suppressing the development of sheath blight disease in the pot test (at Hat Yai, Songkhla)

Biocontrol	% of tillers with sheath blight symptoms*	
	10 days**	20 days**
Pellet with B. megaterium	43.1b	69.6b***
Pellet (Blank)	62.2ab	77.8ab
Granule with B. megaterium	54.6ab	82.1ab
Granule (Blank)	57.9ab	79.3ab
Fungicide (Iprodione)	57.1ab	72.9b
Control (only R. solani)	72.2a	93.8a

*Percentage of infected rice tillers = all infected rice tillers/total rice tillers x 100

**Days after formulation application

***Means followed by the same letter are not significantly different at the 5% level by Duncan's Multiple Range Test.

Table 3 Efficacy of novel formulations in suppressing the development of sheath blight disease in the field
(at Phetchaburi)

Biocontrol*	% tillers with sheath blight symptoms**	lesion length (cm)
Pellet (10g/rice hill)	82.3b	16.6b***
Pellet (20g/rice hill)	84.5ab	16.2b
Pellet (30g/rice hill)	79.9b	15.1c
Granule (1.2L/sub plot)	80.1b	13.8d
Granule (2.4L/sub plot)	70.6c	13.6d
Granule (3.6L/sub plot)	50.6d	12.9d
Iprodione (3.0L/sub plot)	55.7d	8.5e
Control (only R. solani)	92.5a	18.7a

*All types of formulations contained B. megaterium

**Percentage of infected rice tillers = all infected rice tillers/total rice tillers x 100

***Means followed by the same letter are not significantly different at the 5% level by Duncan's Multiple Range Test.

Table 4 Effect of novel formulations on fresh and dry weight of rice plants and weight of rice panicles

Biocontrol	fresh weight (g)*	dry weight (g)**	panicle weight (g)***
Pellet (10g)	73.8ab	58.0ab	182.8ab****
Pellet (20g)	85.4a	66.1a	218.9a
Pellet (30g)	57.9b	43.3b	158.5b
Granule (1.2L)	73.3ab	56.2ab	190.8ab
Granule (2.4L)	84.6a	64.6a	217.3a
Granule (3.6L)	69.8ab	55.4ab	190.6ab
Iprodione	74.6ab	60.2ab	202.6ab
Control (R. solani)	77.5ab	55.9ab	186.2ab

*,**Fresh and dry weights are the average of four rice hills which were inoculated with novel formulations and R. solani inoculum

***Panicle weight is the average of eight rice hills which were inoculated with novel formulations and R. solani inoculum

****Means followed by the same letter are not significantly different at the 5% level by Duncan's Multiple Range Test