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BIOLOGICAL CONTROL OF BANDED LEAF AND SHEATH BLIGHT DISEASE (*Rhizoctonia solani* KUHN) IN CORN WITH FORMULATED *Bacillus subtilis* BR23

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ABSTRACT

Rhizoctonia solani Kuhn. causing banded leaf and sheath blight diseases is one of the important fungi of corn world wide. The fungus is commonly controlled by using fungicide because no resistant variety available. The objective of the study was to develop a seed treatment formulation of the selected *Bacillus subtilis* to control *R. solani* in corn. The study was conducted in the Department of Plant Pathology, College of Agriculture, University of the Philippines Los Bañòs, College, Laguna from May 2004 to August 2005, using sweet corn var. IPB Supersweet as test plant. Corn seeds were surface sterilized for 10 minutes in 1% sodium hypochlorite solution and 5% ethanol, washed thrice with sterile distilled water and air-dried. The seeds were coated with formulated *B. subtilis* BR23 and used for several experiments, such as evaluation for their germination and growth in the laboratory, effectively on *R. solani* in the baked and nonbaked field soil under greenhouse condition, and in the microplots artificially infested with *R. solani*. The treatment was compared with other standard seed treatment of synthetic fungicides such as captan (10 g per kg seeds) and metalaxyl (10 g per kg seeds). The experiments were designed in a completely random design with three replications. Parameters observed were seed germination, plant height, disease scores, and plant yield. Laboratory formulated *B. subtilis* BR23 used as seed treatment had no detrimental effects on seed germination and seedling vigor. In microplots artificially infested with a selected highly virulent *R. solani*, seed treatment with the same formulation increased grain yield by 27% compared to that of the control captan seed treatment with 14.4%. The studies showed the potential of *B. subtilis* BR23 for commercialization as a seed treatment for the control of banded leaf and sheath blight disease (*R. solani*) in corn.

[***Keywords***: *Zea mays*, biological control, *Bacillus subtilis*, *Rhizoctonia solani*]

INTRODUCTION

Corn is one of the most important food crops worldwide. In some Asian countries, including Indonesia and the Philippines, corn is the second most important cereal crop after rice. Corn is also an important staple food crop in Central and South American countries, Africa, and Asia (Shurtleff 1980).

In Indonesia, the demand for corn as food and animal feed is steadily increasing (Swastika *et al*. 2001). More than 70% of the corn produced in the country is consumed as food (CBS 1999).

Corn plant is susceptible to a wide variety of pests and diseases that can reduce the yield and quality significantly. One of the important diseases that became serious in recent years is banded leaf and sheath blight (BLSB) caused by *Rhizoctonia solani* Kuhn. The disease causes severe loss in several countries of Asia (Sharma *et al.* 2002). The occurrence of the disease has also been reported from other parts of the world.

The use of chemicals to manage BLSB in corn is limited and has adverse ecological implication, while the use of resistant varieties has not progressed much because of the limited host genetic variability for resistance to *R. solani* (Sharma *et al.* 2002). In recent years, several studies on biological control of *R. solani* with *Bacillus subtilis* in soybean (Liu and Sinclair 1987), potato, mungbean, and rice (Tschen 1987), peanut (Turner and Backman 1991), wheat (Kim *et al.* 1997), and sugarbeet (Moussa 2002) indicated that *B. subtilis* has good potential for controlling *R. solani*. Different commercial formulations of *B. subtilis* are available and have been recommended for various diseases (APS 2002). The objective of the study was to develop and evaluate potential application of a seed treatment formulation of selected *B. subtilis* against *R. solani* in corn.

MATERIALS AND METHODS

The study was conducted in the Department of Plant Pathology, College of Agriculture, University of the Philippines Los Bañòs (UPLB-CA), College, Laguna from May 2004 to August 2005. Sweet corn var. IPB Supersweet obtained from the Institute of Plant Breeding UPLB-CA was used as the test material. The seeds were surface sterilized for 10 minutes in 1% sodium hypochlorite solution and 5% ethanol,

washed thrice with sterile distilled water (SDW) and air-dried before used.

Preparation of *B. subtilis* Formulation

B. subtilis BR23 was cultured in aqueous medium (1 g brown sugar and 0.25% yeast extract in 1000 ml of SDW) by taking 20 ml (10%) of the bacterial suspension (10^8 cfu ml^{-1}) and added to 180 ml of fresh aqueous medium in 1-L E-flask. The culture was shaken for 36 hours at room temperature. The bacterial cells were harvested by centrifugation at 5000 rpm for 6 minutes. The centrifuged cells (20 ml) were mixed with sterilized talc added in 0.25% yeast extract and 1% (25 g) carboxymethyl cellulose (CMC). Talc was sterilized in autoclave for 15 minutes at 15 psi. The mixture was air-dried in a laminar flow chamber for 2 days. The formulated *B. subtilis* BR23 was then placed in sterile E-flask and kept in the refrigerator (10°C) until further use.

For nonformulated *B. subtilis* BR23 treatment, the culture was grown on potato dextrose peptone agar (PDPA; 250 g potato, 20 g dextrose, 20 g agar, and 10 g peptone in 1000 ml distilled water) plates and incubated at room temperature for 36 hours. After incubation, the cells were suspended in SDW (1 ml per plate) and then grinded with sterile mortar to homogenize bacterial suspension.

Evaluation of Formulated *B. subtilis* on Seed Germination and Seedling Vigor

Corn seeds were coated with formulated *B. subtilis* BR23. The treated seeds were grown in a baked field soil under greenhouse condition. Baked soil was prepared by heating the soil at 120°C for 2 hours to kill all microorganisms in the soil. The treatments were as follows: (A) talc + 1% CMC, (B) formulated *B. subtilis* BR23 (talc + 1% CMC + 0.25% yeast extract + *B. subtilis* BR23), (C) talc + 1% CMC + 0.5% gum Arabic, (D) formulated BR23 + 0.5% gum Arabic, (E) *B. subtilis* suspension (nonformulated), and (F) noninoculated control.

For treatments A, B, C, and D, 1 g of each treatment material was added to 100 g of corn seeds wetted with 1 ml SDW in a sterile plastic bag. The mixture was shaken until the seeds were coated evenly. For treatment E, corn seeds were coated with 36 hour-old *B. subtilis* suspension on PDPA cultures. The treated corn seeds were placed in plastic trays (24 cm x 32 cm x 8 cm) containing baked field soil (50 seeds per tray).

The treatments were arranged in a completely random design (CRD), and replicated three times. Parameters evaluated were seed germination at 7 days after sowing (DAS), seedling height at 7 and 14 DAS, and fresh weight of 20 seedlings randomly selected from each replicate at 14 DAS.

Evaluation of Formulated *B. subtilis* Against *R. solani* Under Screenhouse

R. solani RSC3 isolate was cultured in a rice hull-rice grain (RHRG) substrate packed in 1-L dextrose bottles. RHRG substrate consisted of 80 g rice hull, 130 g rice grain, and 150 ml tap water. The substrate was sterilized for 2 hours at 15 psi. After sterilization, the substrate was inoculated with a 3-mm agar disk taken from the margins of actively growing cultures of *R. solani*. The inoculated substrate was then incubated for 2 weeks at room temperature.

One part of the inoculated RHRG *R. solani* substrate was mixed thoroughly with 10 parts of soil (baked and nonbaked field soil). The mixture was placed in plastic trays and incubated for 14 days before used. Noninoculated control was tray containing a mixture of RHRG (without *R. solani*) and soil. The following treatments arranged in CRD were evaluated: (A) seeds treated with *B. subtilis* BR23 suspension, (B) seeds treated with formulated *B. subtilis* BR23 (10 g per kg seeds) + 0.5% gum Arabic, (C) seeds treated with captan (10 g per kg seeds), (D) seeds treated with metalaxyl (10 g per kg seeds), (E) seeds treated with captan + metalaxyl, (F) inoculated control, and (G) noninoculated control.

Seed treatment application followed the methods described previously. Each treatment was replicated in three plastic trays with 50 seeds per tray. Parameters recorded were seed germination at 7 DAS, seedling vigor and seedling height at 14 DAS, number of damping-off seedlings at 14 DAS, and fresh weight of 20 seedlings randomly selected from each replicate at 14 DAS.

Evaluation of Formulated *B. subtilis* in Microplots Artificially Infested with *R. solani*

The experiment was conducted in 1 m x 4 m microplots. Twelve microplots were used in this experiment; nine were infested with RHRG *R. solani* inoculum, and three served as noninoculated control. The inoculated microplots were leaved for 2 weeks before used.

Corn seeds were planted in a hole at a distance of 20 cm x 40 cm (one seed per hole; 50 hill population per plot). Standard cultural practices were followed, e.g. application of carbofuran 17 kg ha^{-1} by putting in the hole together with the corn seeds at planting time to control insect pest, and N-P-K fertilizer application at a rate of N 150 kg, P_2O_5 150 kg, and K_2O 100 kg ha^{-1}.

The treatments were arranged in RCBD and replicated three times. The treatments were as follows: (A) seeds treated with formulated *B. subtilis* (10 g per kg seeds) + 0.5% gum Arabic, (B) seeds treated with captan (10 g per kg seeds), (C) inoculated control, and (D) noninoculated control.

Procedures for treatments A and B followed as described before. Parameters observed were seed germination at 7 DAS, disease scores at 30, 45, and 60 DAS, plant height at 30, 45, and 60 DAS, and grain yield. Disease scores were assessed by using the 1-9 scale of Ahuja and Payak (1983) as follows:

Scale 1 : Disease on one leaf sheath only; few small, noncoalescent lesions present.
Scale 2 : Disease on two sheaths; lesions large and coalescent.
Scale 3 : Disease up to four sheaths, lesions many and always coalescent.
Scale 4 : As in scale 3 + rind discolored with small lesions.
Scale 5 : Disease on all sheaths except two internodes below the ear.
Scale 6 : Disease up to one internode below the ear shoot; rind discoloration on many internodes with large depressed lesions.
Scale 7 : Disease up to internode bearing the ear shoot but shank not affected.
Scale 8 : Disease on the ear; husk leaves show bleaching, bands and caking among them-selves as also of silk fibers; abundant fungal growth between and on kernel rows; kernel formation normal except their being lusterless; ear size less than normal; some plant prematurely dead.
Scale 9 : In addition to scale 8, shrinkage of stalk; reduced ear dimensions; wet rot and disorganization of ear; kernel formation absent or rudimentary; premature dead plants common; abundant sclerotia production on husk leaves, kernels, ear tips or silk.

The scores reading were transformed to percent disease severity by using formula:

$$P = \frac{\Sigma (n \times v)}{Z \times N} \times 100\%$$

P = disease severity
n = number of sample in each category
v = numerical value of each category
Z = the highest numerical value of scale
N = total number of sample.

Yield was counted by using formula:

$$Y = \frac{10{,}000\ m^2}{\text{plot size } (m^2)} \times \frac{100 - mc}{100 - 15} \times W \times 0.80$$

Y = yield
mc = moister content when weighing
W = wet ear weight.

Percent increase of yield was counted by using formula:

$$I = \frac{T - IC}{NC} \times 100\%$$

I = percent increase of yield
T = treatment
IC = inoculated control
NC = noninoculated control.

RESULTS AND DISCUSSION

Effect of *B. subtilis* Formulation on Seed Germination and Seedling Vigor

The formulated *B. subtilis* BR23 was prepared by mixing 2 ml of *B. subtilis* BR23 suspension obtained from *B. subtilis* BR23 cultured in brown sugar + yeast extract medium and mixed with sterilized talc, 0.25% yeast extract, and 1% CMC then air-dried in laminar flow chamber. The formulation was evaluated as seed treatment material in comparison with the same formulation plus 0.5% gum Arabic. The results showed no significant differences on seed germination and seedling height at 7 DAS (Table 1). The data indicated that seed treatment with the formulation BR23 biomass + yeast extract + CMC + gum Arabic did not adversely affect seed germination and seedling vigor.

At 14 DAS, seedling height was not significantly different between bacterial formulations, but both were significantly higher than the control (Table 1). Kilian *et al.* (2000) reported that colonization of the root and of the rhizosphere was influenced by a number of factors including the application technique. Canaday and Ownley (1999) found that populations of *B. subtilis* on roots were proportional to the rate of *B. subtilis* applied.

Table 1. Effect of seed treatment with formulated *Bacillus subtilis* BR23[1] on the seed germination (7 DAS), seedling height and seedling weight (14 DAS) of corn var. IPB Supersweet sown in baked field soil[2].

Treatments	Germination (%)	Seedling height (cm)	Fresh seedling weight (g)
Talc + 1% CMC	97.33a	43.70c	1.68c
Formulated *B. subtilis* BR23	95.33a	46.83ab	1.96ab
Talc + 1% CMC + 0.5% gum Arabic (GA)	96.00a	44.00c	1.81bc
Formulated *B. subtilis* BR23 + 5% GA	93.33a	46.93a	2.09a
B. subtilis BR23 suspension	93.33a	45.27bc	1.83bc
Noninoculated control	97.33a	43.93c	1.70c
5% LSD	6.27	1.61	0.19
CV (%)	3.70	2.00	5.70

[1]Formulated *B. subtilis* BR23: Talc + BR23 biomass + 0.25% yeast extract + 1% carboxymethyl cellulose.
[2]Data are averages of three tray replicates. Corn seeds were coated with each treatment before sowing in the plastic trays; 50 seeds per tray.
In a column, means followed by a common letter are not significantly different at the 5% level by LSD.

Evaluation of Formulated *B. subtilis* Against *R. solani* under Screenhouse Condition

In baked field soil, the lowest percent pre-emergence damping-off was observed in seeds treated with captan + metalaxyl, but this was not significantly different from those treated with captan, formulated *B. subtilis* BR23, *B. subtilis* BR23 suspension, metalaxyl, and the non-inoculated control treatments (Table 2). The highest percent level of control was shown by captan + metalaxyl treatment, but it was comparable to *B. subtilis* BR23 in both formulated and suspension treatments. The same results were shown in nonbaked field soil (Table 3).

Post-emergence damping-off at 14 DAS in baked field soil was significantly higher in the inoculated control than that of formulated *B. subtilis* BR23, captan, and captan + metalaxyl treatments (Table 2). In nonbaked field soil, the lowest post-emergence damping-off was shown in the captan and metalaxyl treatments (Table 3).

Seedling height at 14 DAS in baked field soil was significantly higher in formulated *B. subtilis* BR23 than that of metalaxyl treatment (Table 2). In nonbaked field soil, the tallest seedlings were shown from seeds treated with *B. subtilis* BR23 suspension and this was significantly higher than that of seedlings grown in soil with captan + metalaxyl treatment (Table 3).

At 14 DAS in baked field soil, seedling weight from the product-coated seeds were not significantly different from those of *B. subtilis* BR23 suspension, captan, metalaxyl, and captan + metalaxyl seed treatments (Table 2). However, in nonbaked field soil, seedling weight from the product-coated seeds was significantly heavier than that of captan, metalaxyl, and captan + metalaxyl seed treatments (Table 3). There were different results between baked and nonbaked soil. This might be because the role of microorganisms in nonbaked soil is more than that in baked soil.

The ability of *B. subtilis* BR23 to suppress damping off might be due to antibiotic produced by the bacteria. As reported by US EPA (2003), *B. subtilis* strain QST 713 control the growth of certain fungi, presumably by competing for nutrients, growth sites on plants, and by directly colonizing and attaching to fungal pathogens.

Evaluation of *B. subtilis* BR23 in Microplots Artificially Infested with *R. solani*

Results showed that the seeds from all plots had 100% germination. BLSB incidence at 30 and 45 DAS in formulated *B. subtilis* BR23 plots were significantly lower than that of inoculated control plots and comparable with captan and noninoculated control plots (Table 4).

BLSB severity increased with time and the most severely infected plants were on the inoculated control plots. Severities were not significantly different between formulated *B. subtilis* BR23 and the captan seed treatment, but both were lower than that of inoculated control (Table 4). However, all plots showed low BLSB severity. This might be due to unfavorable condition for *R. solani* to grow where the experiment was conducted during dry season.

The plant height at 30 DAS in formulated *B. subtilis* BR23 plots was significantly higher than that of noninoculated and inoculated control plots (Table 5), while at 45 DAS only plants in captan and inoculated control plots showed significant differences. At 60 DAS, no significant differences were seen in the plant height in all treatments.

Table 2. Effect of seed treatment with formulated *Bacillus subtilis* BR23[1)] on the pre-emergence damping-off (7 DAS), post-emergence damping-off (14 DAS), seedling height (14 DAS), and fresh seedling weight (14 DAS) of corn var. IPB Supersweet sown in baked field soil artificially infested with *R.* solani under screenhouse condition[2)].

Treatments	Pre-emergence damping-off (%)	Level of control (%)	Post-emergence damping-off (%)	Seedling height (cm)	Fresh seedling weight (g)
Noninoculated control	6.67b	-	0.00c	49.20ab	2.67bc
B. subtilis BR23 suspension	13.00b	43.57	6.67b	51.93ab	3.10a
Formulated *B. subtilis* BR23	9.67b	45.82	2.67bc	53.33a	2.77abc
Captan	8.00b	46.88	3.33bc	50.47ab	2.63c
Metalaxyl	18.00b	39.82	6.67b	48.80b	2.83abc
Captan + metalaxyl	6.00b	48.09	4.67bc	50.53ab	3.05ab
Inoculated control	48.00a	0.00	14.67a	38.67c	1.92d
5% LSD	13.18		5.92	4.17	0.40
CV (%)	27.10		33.50	4.90	8.50

[1)]Formulated *B. subtilis* BR23: Talc + BR23 biomass + 0.25% yeast extract + 1% carboxymethyl cellulose.
[2)]Data are averages of three replicate trays; 50 seeds per tray.
In a column, means followed by a common letter are not significantly different at the 5% level by LSD.
Noninoculated control was the soil without *R. solani.*

Table 3. Effect of seed treatment with formulated *Bacillus subtilis* BR23[1)] on the pre-emergence damping-off (7 DAS), post-emergence damping-off (14 DAS), seedling height (14 DAS), and fresh seedling weight (14 DAS) of corn var. IPB Supersweet sown in non-baked field soil artificially infested with *R.* solani under screenhouse condition[2)].

Treatments	Pre-emergence damping-off (%)	Level of control (%)	Post-emergence damping-off (%)	Seedling height (cm)	Fresh seedling weight (g)
Noninoculated control	6.00c	-	0.00c	47.20ab	2.10b
B. subtilis BR23 suspension	26.00b	91.18	3.33a	48.87a	2.67a
Formulated *B. subtilis* BR23	17.33bc	92.17	2.00a	48.67a	2.60a
Captan	17.33bc	92.17	0.67a	47.77ab	2.25b
Metalaxyl	16.00bc	92.31	0.67a	47.13ab	2.22b
Captan + metalaxyl	16.00bc	92.31	4.00a	46.53b	2.19b
Inoculated control	88.00a	0.00	3.33a	23.93c	1.17c
5% LSD	15.36		4.04	1.89	0.35
CV (%)	17.40		89.30	2.40	9.10

[1)]Formulated *B. subtilis* BR23: Talc + BR23 biomass + 0.25% yeast extract + 1% carboxymethyl cellulose.
[2)]Data are averages of three replicate trays; 50 seeds per tray.
In a column, means followed by a common letter are not significantly different at the 5% level by LSD.
Noninoculated control was the soil without *R. solani.*

The higher yields were obtained from plants in noninoculated control plots (4.81 t ha^{-1}) and formulated *B. subtilis* BR23 plots (4.42 t ha^{-1}) (Table 5). The lowest yield was obtained in inoculated control plots due to heavy BLSB infection that inhibited the filling and growing of ears. The formulated *B. subtilis* BR23 increased grain yield by 27.03% compared to that of the control fungicide captan which increased yield by 14.35%.

The data showed that seed germination, plant height, BLSB incidence and severity were not significantly different between formulated *B. subtilis* BR23 and the captan seed treatment. The grain yields were also not significantly different between the treatments. However, the grain yield of the formulated *B. subtilis* BR23 was significantly higher than that of inoculated control whereas captan seed treatment was not. This is probably due to higher weight of plants from the product-coated seeds. There was no data recorded on top weight, but screenhouse test result showed that seedling weights from the product-coated seeds were significantly heavier than that of captan seed treatment (Table 3).

Several biocontrol studies using *B. subtilis* as seed treatment material have shown its effectiveness against *R. solani* causing root rot of soybean (Liu and Sinclair 1987), potato black scurf, damping-off of mungbean, and rice sheath blight (Tschen 1987), root

Table 4. Effect of seed treatment with formulated *Bacillus subtilis* BR23[1)] on germination, BLSB incidence and severity in corn var. IPB Supersweet sown in microplots artificially infested with *Rhizoctonia solani* RSC3[2)].

Treatments	Germination (%)	BLSB incidence (%)		BLSB severity (%)[3)]				
		30 DAS	45 DAS	30 DAS	45 DAS	60 DAS	75 DAS	90 DAS
Noninoculated control	100	0.00b	0.00c	0.00b	0.00c	0.00c	0.00c	0.00c
Formulated *B. subtilis* BR23	100	6.95b	16.67bc	1.54b	5.09bc	6.93bc	8.08b	8.74b
Captan	100	16.67ab	27.78ab	3.70ab	9.26ab	11.27b	12.56b	13.37b
Inoculated control	100	44.44a	50.00a	9.46a	16.12a	20.31a	24.07a	25.35a
5% LSD		28.15	23.04	6.49	8.41	7.13	8.04	7.66
CV (%)		82.90	48.90	88.40	55.30	37.10	36.00	32.30

[1)]Formulated *B. subtilis* BR23: Talc + BR23 biomass + 0.25% yeast extract + 1% carboxymethyl cellulose.
[2)]Data are averages of three replicates, each replicate with 24 plants.
In a column, means followed by a common letter are not significantly different at the 5% level by LSD.
[3)]Disease severity computed as:

$$P = \frac{\Sigma (n \times v)}{Z \times N} \times 100\%$$

P = disease severity
n = number of sample in each category
v = numerical value of each category
Z = the highest numerical value of scale
N = total number of sample.

Table 5. Effect of seed treatment with formulated *Bacillus subtilis* BR23[1)] on plant height and yield of corn var. IPB Supersweet sown in microplots artificially infested with *Rhizoctonia solani* RSC3[2)].

Treatments	Plant height (cm)[3)]			Yield (t ha^{-1})	Percent increase of yield
	30 DAS	45 DAS	60 DAS		
Noninoculated control	123.33b	228.13ab	277.77	4.81a	–
Formulated *B. subtilis* BR23	132.00a	234.57ab	274.93	4.42ab	27.03
Captan	126.33ab	240.63a	271.90	3.81bc	14.35
Inoculated control	120.87b	222.00b	270.97	3.12c	–
5% LSD	5.99	14.53	13.09	0.91	
CV (%)	2.40	3.10	2.4	3.80	

[1)]Formulated *B. subtilis* BR23: Talc + BR23 biomass + 0.25% yeast extract + 1% carboxymethyl cellulose.
[2)]Data are averages of three replicates.
In a column, means followed by a common letter are not significantly different at the 5% level by LSD.
Inoculated control: soil was not inoculated with *R. solani.*
[3)]Ten plants randomly selected in each replicate were measured.

cancer of peanut (Turner and Backman 1991), and root rot of sugarbeet (Moussa 2002). Certain strains also used as seed treatments are effective against other pathogens such as *Gibberella zeae* causing Fusarium head blight on wheat (Schisler *et al*. 2002), *Fusarium moniliforme* causing seedling damping-off of jack pine (Hwang *et al.* 1995), and *Sclerospora graminicola* causing downy mildew of pearl millet (Raj *et al*. 2003).

Merriman *et al*. (1974) observed that *B. subtilis* significantly reduced the Rhizoctonia disease (*R. solani*) and increased grain yield and dry matter of wheat. According to McMullen and Lamey (2000), *B. subtilis* used as seed treatment colonize the developing root system, suppressing disease organisms such as *Fusarium* and *Rhizoctonia*. As the root system develops, the bacteria grow with the roots extending the protection throughout the growing season. As a result, a vigorous root system is established by the plant, which often results in more uniform stands and greater yields.

The results of experiment indicated that formulated *B. subtilis* BR23 can be used to substitute chemical fungicides especially in controlling soil borne diseases like *R. solani*. Moreover, the biological control agent safe to environment because there is no residual effect to the soil.

CONCLUSION

Formulated *Bacillus subtilis* BR23 used as seed treatment had no detrimental effects on corn seed germination and seedling vigor. Seed treatment with the same formulation suppressed *Rhizoctonia solani* in microplots and increased grain yield by 27% compared to that of the control captan seed treatment with 14.4%. *B. subtilis* BR23 has a potential for commercialization as a seed treatment for the control of banded leaf and sheath blight disease (*R. solani*) in corn.

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