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EFFECTIVENESS OF *Bacillus subtilis* (EHRENBERG) COHN AGAINST *Rhizoctonia solani* KUHN *IN VITRO*

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ABSTRAK

Penelitian ini dilakukan di Department of Plant Pathology, College of Agriculture, University of the Philippines Los Baños (UPLB-CA), College, Laguna. Tujuan penelitian adalah untuk memilih strain *Bacillus subtilis* sebagai agen pengendali hayati *Rhizoctonia solani* pada tanaman jagung. Sebanyak 41 strain isolat *Bacillus* diisolasi dari akar tanaman jagung yang dikumpulkan dari berbagai tempat di Filipina diskrin secara *in vitro* terhadap isolat *R. solani*, penyebab penyakit busuk batang pada tanaman jagung. Dari ke 41 isolat *Bacillus* tersebut, dua strain *B. subtiolis* yakni BR23 dan BS100 ditemukan sangat efektif baik di laboratorium maupun di rumah kaca dalam menekan tujuh isolat *R. solani* virulen yang juga dikoleksi dari berbagai tempat di Filipina.

Kata kunci : Pengendalian hayati, Banded Leaf and Sheath Blight, *Bacillus subtilis. Rhizoctonia solani*..

ABSTRACT

The study was conducted at the Department of Plant Pathology, College of Agriculture, University of the Philippines Los Baños (UPLB-CA), College, Laguna. The aim of this study was to select *Bacillus subtilis* strains for use as a biological control of *Rhizoctonia solani* in corn. Forty one strains of *Bacillus* isolates of corn roots were collected from different parts of the Philippines and these were screened *in vitro* against *R. solani* isolates that cause banded leaf and sheath blight (BLSB) in corn. These isolates were also collected from all over the country. Of these, two *Bacillus subtilis* strains BR23 and BS100 were found to be very effective against seven virulent *R. solani* isolates, both in laboratory and greenhouse screening tests.

Key words : Biological control, Banded Leaf and Sheath Blight, *Bacillus subtilis Rhizoctonia solani*.

I. INTRODUCTION

Banded leaf and sheath blight (BLSB), also called banded leaf and sheath spot in corn is caused by *Rhizoctonia solani* Kuhn. It is the main disease in several countries of Asia and other parts of the world (Sharma *et al*., 2002). Report on yield loss of corn due to BLSB is very limited. In Indonesia, Sudjono (1995) reported that it caused a yield loss of up to 100 percent. Dela Vega and Silvestre (2003) reported that as the disease intensities increase, the yield loss and yield reduction also enhance with a directly proportional relationship.

The fungus is favored by hot and humid conditions. It causes seed rot and seedling blight. According to Sweets and Wrather (2000), seed rot occurs before germination; seeds are soft and brown and may be overgrown with other fungi. Seedling blight may be either pre-emergence, in which the seed germinates but seedling is killed before it emerges from the soil, or post-emergence, in which the seedling emerges through the soil surface before developing symptoms. With the pre-emergence seedling blight, the coleoptile and developing root system tend to turn brown and have a wet, rotted appearance, while with post-emergence seedling blight, the seedling tend to yellow, wilt, and die.

Bacillus subtilis is a ubiquitous bacterium commonly recovered from water, soil, air, and decomposing plant residue. *B. subtilis* produces a variety of proteases and other enzymes that enable it to degrade a variety of natural substrates and contribute to nutrient cycling (EPA, 2003a). *B. subtilis* GBO3 is a spore-forming bacterium which, when applied to seeds, colonizes the developing

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root system of the plants. The bacterium competes with and thereby suppresses plant disease fungal organisms such as *Rhizoctonia*, *Fusarium*, *Aspergillus*, and others. The bacteria continue to live on the root system and provide protection throughout the growing season (EPA, 2003b). The objective of this study was to select a *B. subtilis* strain for the biological control of *R. solani* in corn.

II. MATERIAL AND METHODS

2.1 Isolation and Preservation of Putative *Bacillus* spp.

Putative *Bacillus* spp. were isolated from corn roots collected from different parts of the Philippines as follows: Root samples were shaken vigorously to remove soils sticking onto roots and the roots were air-dried for two days. Five grams of each sample were added with 15 ml SDW, and macerated thoroughly using sterile mortar and pestle. The suspension was collected and heated at 80^0C for 30 minutes. A serial dilution up to 10^{-3} was prepared. A 0.1-ml aliquot from each dilution was plated onto potato dextrose peptone agar (PDPA) plate and spread using a sterile glass rod hockey. Culture plates were incubated for 48 hours at room temperature. The developing discrete bacterial colonies were transferred to new PDPA slants, labeled properly and kept in the refrigerator (10^0C). Another set of cultures were preserved on PDPA slants in screw-cap test tubes covered with mineral oil, kept at room condition.

2.2 In Vitro Evaluation of Prospective Bacillus Antagonists for Biocontrol

a. Test for diffusible toxic metabolite

Agar co-cultivation test was conducted as follows: five millimeter agar mycelial disc of *R. solani* was seeded at the center of a 9-cm PDPA plate. Four to six putative *Bacillus* isolates were placed at equidistant sites of 1 cm from plate periphery. The plates were sealed with parafilm and incubated at room temperature for two days. Radial growth of the fungus away from or towards to bacterial antagonists was measured.

b. Test for volatile toxic compounds

The putative *Bacillus* isolate was streaked on PDPA plates and incubated for 24 h at room condition. A bottom plate with PDA inoculated at the center with a mycelial plug of *R. solani* was placed on top of the bottom plate with the 24 h old *Bacillus* isolate. The two bottom plates were sealed together with parafilm and incubated at room condition. For the control, the plate with *R. solani* mycelial plug was placed on top of the bottom plate containing PDPA only (no bacteria). Two days after incubation, the radial growth of the fungus was measured.

2.3 Growth of *Bacillus* Antagonist on Corn Root Surface

a. Filter paper method

Selected *Bacillus* isolates from the above screening were used. Each isolate was grown on 2 PDPA plates and incubated for 36 h. After incubation, the cells were suspended in SDW (1 ml/plate); to obtain uniform suspension, the bacterial biomass was ground with sterile mortar and pestle.

Corn seeds were immersed into the bacterial suspension for 2 h, and then air dried at room condition. Six seeds were arranged at equidistant position 1 inch from the top of a wet filter paper (25.5 cm long x 28.5 cm wide). Then, the filter paper was folded covering each seed separately. The filter paper set-up was placed in standing position on a tray with sterile water. Each isolate had three replicates.

The root length, shoot length, root weight, and number of bacterial colonies from the base, middle, and tip of the roots were determined. To measure the number of bacterial colonies from different parts of the roots, the root was divided into three parts (base, middle, and tip);1 g of root was added with 3 ml SDW and macerated thoroughly using mortar and pestle. The suspension was collected and heated at 80^0C for 30 minutes (pasteurization method). A serial dilution up to 10^{-2} was prepared. A 0.1-ml aliquot from each dilution was added to a PDPA plate and spread using a sterile glass rod hockey. Culture plates were incubated for 48 hours at room temperature and numbers of colony forming unit (cfu) of the bacteria were counted.

b. Baked soil method

Two *Bacillus subtilis,* BR23 and BS100, were selected from the above screening tests and further evaluated. The bacterial inoculums were prepared and the corn seeds were treated with the bacteria as described above.

The seeds were sown in plastic bags (30 cm x 15.5 cm) containing baked and non-baked field soils. Uncoated seeds were used as control. Five seeds per treatment were sown per bag and replicated five times. The bags were arranged in complete randomized design (CRD). Seedling height, seedling weight, and root length were recorded at 10 DAS.

The population of *B. subtilis* BR23 and BS100 were checked on the roots (rhizosphere and rhizoplane) as described in the filter paper method.

III. RESULTS AND DISCUSSION

3.1 *In Vitro* Evaluation of Putative *Bacillus* Antagonists for Biocontrol

a. Test for diffusible toxic metabolite

Thirty six of putative *Bacillus* strains were isolated from healthy corn roots. These isolates plus five *B. subtilis* strains (Table 1) were evaluated for their antagonistic activity against the *R. solani* isolates in agar co-cultivation tests. Only 12 of these isolates (BSC4, BSC5, BSC6, BSC8, BSC14, BSC16, BSC17, BSC18, BSC19, BSC20, BSC21, and BSC22) did not inhibit the growth of the seven test *R. solani* isolates (Table 2). Mycelial growth inhibition of *R. solani* isolates by *Bacillus* BSC2, BSC12, *B. subtilis* BR23 and BS100 are shown on Figure 1.

The presence of inhibition zones observed after 48 h incubation suggested the presence of fungistatic metabolites secreted by the bacteria.

Table 1. Origin and Code of *Bacillus* Isolates Used in Agar Co-Cultivation Test Against *R. solani*.

ORIGIN	CODE	
	PUTATIVE *BACILLUS* ISOLATES	*Bacillus subtilis* ISOLATES
Dr. Arcadio J. Quimio isolates		BS10, BS100, BR1, BR23 and CA30.
UPLB-CA	BSC1, BSC2, and BSC3	
Rosales, Pangasinan	BSC4, BSC5, BSC6, BSC7, and BSC8	
Pontevedra, Capiz	BSC9, BSC10, and BSC15	
Kulaman, Sultan Kudarat	BSC11 and BSC12	
Malungon, Sarangani	BSC13 and BSC14	
Miag-Gao, Iloilo	BSC16, BSC17, BSC18, BSC19, BSC20, and BSC21	
Musuan, Bukidnon	BSC22, BSC23, BSC24, BSC25, and BSC26	
Tiaong, Quezon	BSC27, BSC28, BSC29, BSC30, and BSC31	
Tuguegarao, Cagayan	BSC32, BSC33, BSC34, BSC35, and BSC36	

B. subtilis can secrete several antifungal metabolites such as subtiline, bacitracin, bacillin and bacillomycin, lipopeptide iturin A which belong to the iturin family (Alippi and Mónaco, 1994 *in* Montealegre *et al*. 2003; Feignier *et al*., 1996; Tsuge *et al*., 2001).

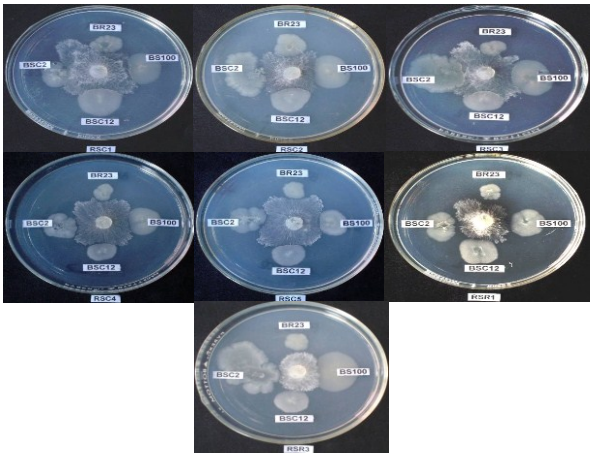

Figure 1. Mycelial Growth Inhibition of *Rhizoctonia solani* Isolates by *Bacillus* BSC2, BSC12, *B. subtilis* BR23 and *B. subtilis* BS100 in Agar Co-Cultivation est After 48 h of Incubation.

Table 2. Mycelial Zone Inhibition of *Rhizoctonia solani* Isolates by *Bacillus* spp. Solates in Agar Co-Cultivation Test After 48 h of Incubation[1].

Bacillus spp. ISOLATES	ZONE OF INHIBITION (mm)						
	RSC1	RSC2	RSC3	RSC4	RSC5	RSR1	RSR3
BSC1	1.0 a-c	2.0 a	0.7 c	1.7 ab	0.5 h-j	0.8 c-g	1.0 b-e
BSC2	1.0 a-c	1.0 b-d	0.5 cd	1.2 b-d	1.3 c-e	0.8 c-g	1.0 b-e
BSC3	1.3 a	2.0 a	1.0 b	0.8 d-f	1.2 d-f	0.8 c-g	0.8 c-e
BSC4	0.0 e	0.0 f	0.0 g	0.0 h	0.0 k	0.0 g	0.0 e
BSC5	0.0 e	0.0 f	0.0 g	0.0 h	0.0 k	0.0 g	0.0 e
BSC6	0.0 e	0.0 f	0.0 g	0.0 h	0.0 k	0.0 g	0.0 e
BSC7	1.0 a-c	1.0 b-d	0.5 cd	0.5 e-h	1.0 e-g	1.0 c-f	0.8 c-e
BSC8	0.0 e	0.0 f	0.0 g	0.0 h	0.0 k	0.0 g	0.0 e
BSC9	1.0 a-c	1.0 b-d	1.0 b	1.0 c-e	2.7 a	0.7 d-g	0.5 e
BSC10	1.0 a-c	1.0 b-d	1.0 b	1.0 c-e	1.0 e-g	0.7 d-g	0.8 c-e
BSC11	1.2 ab	0.7 de	0.7 c	1.5 a-c	2.0 b	3.0 a	2.2 b
BSC12	1.2 ab	0.7 de	1.0 b	2.0 a	2.0 b	1.7 bc	3.5 a
BSC13	0.5 c-e	1.0 b-d	0.3 de	0.5 e-h	1.5 cd	1.5 b-d	2.0 bc
BSC14	0.0 e	0.0 f	0.0 g	0.0 h	0.0 k	0.0 g	0.0 e
BSC15	1.0 a-c	1.3 b	0.5 cd	0.5 e-h	1.2 d-f	0.5 e-g	1.8 c-e
BSC16	0.0 e	0.5 e	0.0 g	0.0 h	0.0 k	0.0 g	0.0 e
BSC17	0.0 e	0.0 f	0.0 g	0.0 h	0.0 k	0.0 g	0.0 e
BSC18	0.0 e	0.0 f	0.0 g	0.0 h	0.0 k	0.0 g	0.0 e
BSC19	0.0 e	0.0 f	0.0 g	0.0 h	0.0 k	0.0 g	0.0 e
BSC20	0.0 e	0.0 f	0.0 g	0.0 h	0.0 k	0.0 g	0.0 e
BSC21	0.0 e	0.0 f	0.0 g	0.0 h	0.0 k	0.0 g	0.0 e
BSC22	0.0 e	0.0 f	0.0 g	0.0 h	0.0 k	0.0 g	0.0 e
BSC23	1.0 a-c	0.7 de	0.2 ef	1.2 b-d	0.7 g-i	0.1 fg	0.3 e
BSC24	1.0 a-c	0.5 e	0.2 ef	0.7 d-g	0.7 g-i	0.1 fg	0.3 e
BSC25	1.0 a-c	0.5 e	0.1 fg	0.2 gh	0.5 h-j	0.1 fg	0.3 e
BSC26	1.0 a-c	0.5 e	0.1 fg	0.0 h	0.5 h-j	0.1 fg	0.1 e
BSC27	0.5 c-e	0.5 e	0.1 fg	0.2 gh	1.3 c-e	0.3 fg	0.1 e
BSC28	0.5 c-e	0.5 e	0.0 g	0.4 e-h	1.2 d-f	0.3 fg	0.1 e
BSC29	0.5 c-e	1.0 b-d	0.1 fg	0.5 e-h	1.2 d-f	0.3 fg	0.1 e
BSC30	0.2 de	0.8 c-e	0.1 fg	1.0 c-e	1.0 e-g	0.3 fg	0.1 e
BSC31	0.0 e	0.7 de	0.1 fg	0.5 e-h	1.0 e-g	0.4 e-g	0.1 e
BSC32	0.0 e	0.8 c-e	0.1 fg	0.1 gh	0.1 jk	0.1 fg	0.4 e
BSC33	0.0 e	1.0 b-d	0.1 fg	0.1 gh	0.1 jk	0.0 g	0.0 e
BSC34	1.0 a-c	1.0 b-d	0.1 fg	0.5 e-h	0.5 h-j	0.2 fg	0.3 e
BSC35	1.0 a-c	1.0 b-d	0.1 fg	0.5 e-h	0.5 h-j	0.1 fg	0.3 e
BSC36	0.5 c-e	1.0 b-d	0.1 fg	1.0 c-e	0.3 i-k	0.1 fg	0.2 e
BS10[2]	1.0 a-c	1.0 b-d	1.0 b	1.0 c-e	1.7 bc	0.7 d-g	0.8 c-e
BS100[2]	0.5 c-e	0.5 e	0.5 cd	0.2 gh	1.0 e-g	1.3 b-e	2.0 bc
BR1[2]	1.0 a-c	1.0 b-d	1.0 b	1.0 c-e	0.8 f-h	1.5 b-d	0.7 de
BR23[2]	1.2 ab	1.2 bc	1.7 a	1.0 c-e	1.0 e-g	1.0 c-f	1.0 c-e
CA30[2]	1.3 a	1.3 b	1.0 b	0.5 e-h	0.8 f-h	2.0 b	0.5 e
5% LSD	0.6	0.5	0.2	0.6	0.5	1.0	1.3
CV (%)	65.8	44.8	36.0	77.9	113.0	121.8	145.2

[1] Data are average of three plates.
In a column, means followed by a common letter are not significantly different at the 5% level by LSD.
[2] *Bacillus subtilis*.

b. Test for volatile toxic metabolites

The presence of antifungal volatile metabolites from 29 *Bacillus* antagonists was determined based on the radial growth of *R. solani* after 48 h of incubation at room condition. Significant reduction of mycelial growth of the *R. solani* isolates was observed (Table 3; Fig. 2). The results are summarized as follows: RSC1 (BR23 < BSC2 < BS100 < BSC12 < BSC10 < BSC11), RSC2 (BSC12 < BSC13 = BR23 < BSC3 < BS100), RSC3 (BS100 = BSC12 = BSC2 = BR23 = BSC13 < BSC15), RSC4 (BS100 = BSC12 < BR23 < BR1 <BSC7 < BSC15), RSC5 (BR23 = CA30 < BS100 = BR1 = BS10 = BSC2), RSR1 (BR1 = BSC1 < CA30 < BSC9 = BSC12< BSC11 < BS100 = BSC2), dan RSR3 (BS100 < BR23 < BSC7 = BSC1 = BSC2 = BSC3).

Table 3. Effect of Antifungal Volatiles Produced by *Bacillus* Isolates on Radial Growth of *Rhizoctonia solani* after 48 h of Incubation[1)].

Bacillus spp. ISOLATES	RADIAL GROWTH (cm) OF *R. solani* ISOLATES						
	RSC1	RSC 2	RSC 3	RSC4	RSC5	RSR1	RSR3
BSC1	3.0 d-i	3.0 f-i	3.3 g	3.0 g-i	2.3 i-k	1.6 l	2.3 j-l
BSC2	2.0 hi	3.2 f-i	2.0 i	2.4 h-k	2.0 jk	2.7 h-l	2.3 j-l
BSC3	2.6 e-i	2.3 h-j	2.3 hi	2.4 h-k	2.5 h-k	2.7 h-l	2.3 j-l
BSC7	2.7 e-i	8.5 a	3.5 g	2.3 i-l	2.9 g-j	3.6 e-j	2.1 j-l
BSC9	2.8 e-i	3.4 f-h	3.5 g	3.4 fg	3.5 d-h	2.5 j-l	3.8e-j
BSC10	2.4 f-i	3.0 f-i	3.3 g	3.1 f-h	3.3 e-i	3.6 e-j	3.1 g-l
BSC11	2.5 f-i	3.0 f-i	3.1 gh	2.6 h-k	2.9 g-j	2.6 i-l	5.0 c-f
BSC12	2.3 f-i	1.5 j	1.7 i	1.6 l	3.2 f-i	2.5 j-l	2.8 h-l
BSC13	2.7 e-i	2.0 ij	2.0 i	2.7 g-j	2.4 i-k	3.8 c-i	2.8 h-l
BSC15	2.7 e-i	3.1 f-i	2.3 hi	2.4 h-k	5.9 b	5.0 a-c	5.0 c-f
BSC23	4.2 c-h	4.0 c-f	3.8 d-g	4.3 de	3.8 d-g	4.2 a-g	5.8 b-d
BSC24	5.5 bc	4.7 b-e	3.7 fg	3.4 fg	4.2 c-f	3.7 c-i	5.9 b-d
BSC25	4.3 b-f	3.5 f-h	3.6 g	3.8 ef	3.3 e-i	3.8 b-h	4.3 d-h
BSC26	4.5 b-f	3.1 f-i	4.8 c	4.8 cd	4.2 c-f	4.5 a-e	3.5 f-h
BSC27	3.9 c-h	4.2 c-f	4.6 c-e	3.8 ef	3.7 d-g	3.2 f-k	3.7 e-j
BSC28	6.7 ab	4.9 bc	4.6 c-e	4.6 de	5.1 bc	4.6 a-e	5.0 c-f
BSC29	3.7 c-i	3.6 e-h	4.8 c	5.8 b	3.6 d-g	5.1 ab	4.2 d-i
BSC30	3.8 c-i	3.9 c-f	4.7 cd	4.4 de	3.6 d-g	4.7 a-e	3.7 e-j
BSC31	5.5 bc	4.7 b-e	5.4 c	4.4 de	4.3 c-e	4.7 a-e	6.7 a-c
BSC32	3.4 c-i	3.6 d-g	4.8 c	4.8 cd	4.4 cd	4.6 a-e	3.6 f-k
BSC33	3.5 c-i	3.5 e-h	3.8 e-g	3.4 fg	3.6 d-g	4.3 a-f	4.5 d-h
BSC34	4.5 b-f	4.9 bc	4.8 c	4.3 de	3.7 d-g	4.6 a-e	4.1 d-i
BSC35	5.2 b-e	5.5 b	4.8 c	5.5 bc	4.3 c-e	4.5 a-e	5.4 b-e
BSC36	4.8 c-f	5.0 bc	4.6 c-f	3.5 fg	3.8 d-g	3.6 d-j	4.7 d-g
BS10[2)]	3.0 d-i	3.0 f-i	3.2 g	3.1 f-h	2.0 jk	4.8 a-d	3.1 g-l
BS100[2)]	2.1 g-i	2.4 g-j	1.6 i	1.5 l	1.9 jk	2.7 h-l	1.5 l
BR1[2)]	8.5 a	3.9 c-f	8.3 ab	2.1 j-l	1.9 jk	1.5 l	6.8 ab
BR23[2)]	1.6 i	2.1 ij	2.0 i	1.8 kl	1.5 k	3.0 g-k	1.8 kl
CA30[2)]	3.0 d-i	4.8 b-d	7.5 b	7.5 a	1.7 k	2.2 kl	2.5 i-l
Control	8.5 a	8.1 a	9.0 a	8.0 a	9.0 a	5.4 a	7.7 a
5% LSD	2.3	1.2	0.9	0.8	1.0	1.3	1.8
CV (%)	35.7	20.6	13.5	13.1	18.0	20.9	27.4

[1)] Data are average of three plates.
In a column, means followed by a common letter are not significantly different at the 5% level by LSD.
[2)] *Bacillus subtilis*.

Table 4. Population of *Bacillus* Isolates on Roots of Corn (IPB Supersweet var.) Seedlings from Bacteria-Coated Seeds 5 Days after Germination on Sterile Filter Paper[1)].

ISOLATES	NUMBER OF BACTERIAL COLONIES (x 10^4 CFU/g ROOT)		
	BASE	MIDDLE	TIP
Bacillus BSC2	7.19 a	2.81 b	2.43 b
Bacillus BSC12	4.84 b	2.99 b	2.29 b
B. subtilis BS100	3.70 b	2.32 b	2.29 b
B. subtilis BR23	6.37 a	5.70 a	2.83 a
Control	0.00 c	0.00 c	0.00 c
5% LSD	1.22	1.21	1.29
CV (%)	15.2	24.0	32.9

[1)] Data are averages of three plates.
In a column, means followed by a common letter are not significantly different at the 5% level by LSD.

3.2 Growth of *Bacillus* Antagonist on Corn Root Surface

a. Filter paper method

Four *Bacillus* isolates (BSC2, BSC12, BS100, and BR23) applied as seed treatment, were used to determine if they can multiply on seedling roots on filter paper. The result showed that the roots of seedlings from seeds treated with *B. subtilis* BR23 had the highest total bacterial count. More bacteria were found at the base and the least was at the tip of the roots (Table 4). The data indicated that the bacteria multiplied and spread from the seed to the roots of the growing seedling.

Seedlings from the *Bacillus* BSC12 treatment had the longest roots, followed by those treated with BR23 and BS100. However, they were not significantly different from those of the control. The shoots length of seedlings from seeds\treated with *Bacillus* BR23, BS100, and BSC12 were not significantly different from that of the control. The heaviest roots were found on seedlings of BS100 treatment; it was significantly heavier than that of the other treatments (Table 5; Figure. 3).

Table 5. Effect of *Bacillus* Isolates on Root Length (cm), Shoot Length (cm), and Root Weight (g) of Corn (IPB Supersweet var.) Seedlings from Bacteria-Coated Seeds Germinated on Sterile Filter Paper[1)].

ISOLATES	ROOT LENGTH (cm)	SHOOT LENGTH (cm)	ROOT WEIGHT (g)
Bacillus BSC2	7.38 b	2.10 b	0.05 b
Bacillus BSC12	9.95 a	2.89 ab	0.06 b
B. subtilis BS100	8.90 ab	3.30 a	0.09 a
B. subtilis BR23	8.95 ab	3.74 a	0.07 b
Control	8.07 ab	3.52 a	0.06 b
5% LSD	2.13	1.15	0.02
CV (%)	51.0	33.9	41.5

[1)] Data are averages of 15 seedlings, 5 days after germination.In a column, means followed by a common letter are not significantly different at the 5% level by LSD.

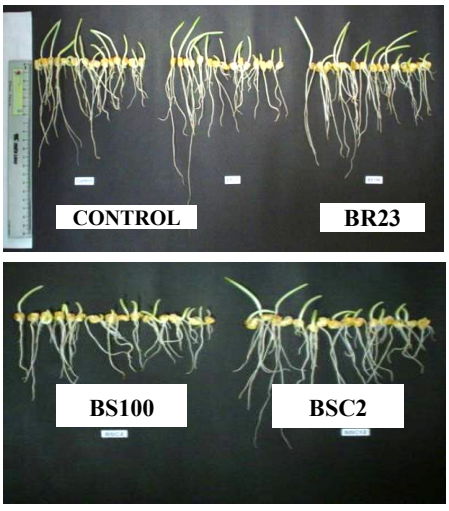

Figure 3. Effect of *Bacillus* Antagonists on Shoot and Root Length of Corn (IPB super sweet var.) Grown for 5 Days on Sterile Filter Paper.

b. Baked soil method

The *B. subtilis* BR23 has been written on page 12 and BS100 isolates coated-corn seeds sown in plastic bags containing baked and non-baked field soil showed no significant differences in terms of seedling height, seedling weight and root length (Tables 6 and 8). However, more bacteria were found on roots of seedlings from the seeds coated with *B. subtilis* BR23 on every portion of the root compared with that of BS100 and control (Tables 7 and 9).

Table 6. Effect of *Bacillus subtilis* BR23 and BS100 on Corn (IPB Supersweet var.) Seedlings from Bacteria-Coated Seeds Grown in Bake d Field Soil Under Screenhouse Condition (10 DAS)[1].

Bacillus subtilis ISOLATES	SEEDLING HEIGHT (cm)	FRESH SEEDLING WEIGHT (g)	ROOT LENGTH (cm)
BR23	30.40	1.43	15.90
BS100	30.50	1.40	15.20
Control	29.02	1.27	13.00
5% LSD	4.62	0.23	2.50
CV (%)	8.8	21.4	40.1

[1] Data are averages of five replicates; no significant differences were bserved. Corn seeds were grown in plastic bags with baked soil, 5 seeds/bag.

Table 7. Population of *Bacillus* Isolates on Roots of Corn (IPB Supersweet var.) Seedlings from Bacteria-Coated Seeds Grown in Baked Field S Soil Under Screenhouse Condition (10 DAS)[1].

Bacillus subtilis ISOLATES	NUMBER df OF BACTERIAL COLONIES[2] (x 10^4 CFU/g ROOT)		
	BASE	MIDDLE	TIP
B. subtilis BR23	91.67 a	27.00 a	17.67 a
B. subtilis BS100	45.33 b	13.33 b	9.00 b
Control	0.00 c	0.00 c	0.00 c
5% LSD	13.38	9.53	0.66
CV (%)	14.7	35.5	3.8

[1] Data are averages of five replicates.g
In a column, means followed by a common letter are not significantly different at the 5% level by LSD. Corn seeds were grown in plastic bags with baked soil, 5 seeds/bag.

Table 8. Effect of *Bacillus subtilis* BR23 and BS100 on Corn (IPB Supersweet var.) Seedlings from Bacteria-Coated Seeds Grown in Non-Baked Yield Soil Under Screenhouse Condition (10 DAS)[1].

Bacillus subtilis ISOLATES	SEEDLING HEIGHT (cm)	FRESH SEEDLING WEIGHT (g)	ROOT LENGTH (cm)
BR23	33.80	1.46	23.50
BS100	31.40	1.31	20.50
Control	32.30	1.59	20.40
5% LSD	4.69	0.27	4.46
CV (%)	11.0	40.2	20.6

[1] Data are averages of five replicates; no significant differences were observed. Corn seeds were grown in plastic bags with non-baked field soil, 5 seeds/bag.

Table 9. Population of *Bacillus* isolates on roots of corn (IPB Supersweet var.) seedlings from bacteria-coated seeds grown in non-baked field soil under screenhouse condition (10 DAS)[1].

Bacillus subtilis ISOLATES	NUMBER OF BACTERIAL COLONIES (x 10^4 CFU/g ROOT)		
	BASE	MIDDLE	TIP
B. subtilis BR23	196.67 a	74.14 a	80.00 a
B. subtilis BS100	59.33 b	36.66 b	25.66 b
Control	0.00 c	0.00 c	0.00 c
5% LSD	13.38	9.53	0.66
CV (%)	19.9	23.4	12.7

[1] Data are averages of three plates; figures corrected for background bacteria in control.
In a column, means followed by a common letter are not significantly different at the 5% level by LSD.
Corn seeds were grown in plastic bags with non-baked field soil, 5 seeds/bag.

Likewise, the number of bacteria found on the root of seedlings from the seeds coated with BS100 was higher than that of control. This result confirmed the results of the filter paper test, wherein the number of bacteria on seedling roots from the seeds coated with *B. subtilis* BR23 was higher than those coated with BS100. The results also showed that more bacteria colonize the base of the roots compared with the middle and tip in both baked and non-baked field soils (Tables 7 and 9).

IV. CONCLUSION

B. subtilis strains BR23 and BS100 were found very effective against *R. solani* RSC3 in both laboratory and greenhouse screening tests. The studies showed the potential of *B. subtilis* BR23 for commercialization as a seed treatment or spray formulation for the control of *R. solani*.

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