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Biocontrol of *Rhizoctonia solani* K1 by Iturin A Producer *Bacillus subtilis* RB14 Seed Treatment in Tomato Plants

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Abstract

Bacillus subtilis **RB14 was used as an antagonist against fungal pathogen *Rhizoctonia solani* K1 to control damping-off diseases in tomato plants. Tomato seeds were treated with *B. subtilis* RB14 culture. The concentration of bacterial cells for the treatment was about 10^8 cfu/ml. Treated tomato seeds showed 99% germination index similar to the untreated seeds. Scanning Electron Microscopic observations showed a clear evidence of the presence of *B. subtilis* RB14 on tomato seed surface. Clear inhibition zone was observed using treated seed in dual plate assay against *R. solani* K1. *B. subtilis* RB14 treated seed showed 80% reduction in disease incidence during *in vivo* plant experiments. *B. subtilis* RB14 produces lipopeptide antifungal antibiotic iturin A which could suppress *R. solani* K1. The phenomenon was supported by our observation where we found significant amount of iturin A from the root zone soil of the seed treated plants.**

Keywords

Bacillus subtilis **RB14, *Rhizoctonia solani* K1, Seed Treatment, Iturin A**

1. Introduction

Interest in utilization of biofertilizer and biological control of soil borne pathogens has increased considerably in last few decades worldwide. Biocontrol and elicitation of induced systemic resistance (ISR) by plant-associated bacteria was demonstrated earlier using *Pseudomonas* spp. and other gram negative bacteria. There were

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some previous reports about the potential of *Bacillus* spp. to control deferent plant pathogens [1]-[5]. *Bacillus* spp. have the characteristics of omnipresence in soils, rapid growth in liquid culture, high thermal tolerant spores, thus considered as a safe and stable biocontrol agent [6]. Recent advances in microbial and molecular techniques have significantly contributed to introducing many different bacterial genera into soils, onto seeds, roots, tubers or other planting materials to control disease and improve productivity. Seed treatment by *Bacillus* spp. [7]-[9] is one of the above promising techniques. Seed treatment is the on-seed delivery mechanism where ingredients (bacterial culture) wrapped around the seed to provide plant nutrients, bio-stimulants or bio-fungicides. During seed treatment, *Bacillus* species can offer several advantages over other bacteria because of their ability of producing highly resistant endospores and broad spectrum antibiotics [10]. Iturin A is a lipopeptide antibiotics produced by *B. subtilis* and its related strains as a secondary metabolite [11]-[13]. Iturin A is the most well studied antifungal cyclic lipopeptide. Iturin A not only shows significant antifungal activity against phytopathogens, it induces defense responses in plants and reduces disease severity.

In this study *B. subtilis* RB14 was used as an antagonist to control *Rhizoctonia solani* K1 which causes severe damping-off disease in various plants [14]. Damping off disease may occur before or after emergence of seeds. In pre-emergence damping off, the seeds fail to emerge after sowing. As a consequence of infection these seeds become soft, mushy and decompose. In post-emergence damping-off, the seedling emerges from the soil but dies shortly afterwards. The infected portions become pale brown, soft and thinner than non-affected tissue. Infected stems collapse. Symptoms may vary with age and stage of development of the tomato plant. Recent study shows that *B. subtilis* not only produce antibiotics they also produce different kinds of enzymes such as glucanase, chitinase, cellulase which also play a crucial role in antagonistic property through the enzyme mediated lytic mechanism [15]. The study aims to assess the effect of *B. subtilis* RB14 seed treatment to prevent damping-off disease caused by *R. solani* K1 in tomato plants.

2. Materials and Methods

2.1. Culture Medium Composition

For preculture and isolation of aliquots from submerged fermentation, modified LB-medium was used containing 10 g/L of Polypepton (Nihon Pharmaceutical, Tokyo, Japan), 5 g/L of yeast extract (Oriental Co., Tokyo), and 5 g/L of NaCl, adjusted to pH 7.0. *Bacillus* seed inoculum was collected from a medium containing 50 g/L of fish protein (provided by Kamaboko company, Odawara, Japan), 67 g/L of glucose, 5 g/L of KH_2PO_4, 0.5 g/L of $MgSO_4 \cdot 7H_2O$, 25 mg/L $FeSO_4 \cdot 7H_2O$, 22 mg/L $MnSO_4 \cdot 7H_2O$ and 184 mg/L $CaCl_2$. Agar plate containing LB medium with additional 1.5% agar (Shimizu Shokhin Kobushiki Co., Japan) was used for the colony-forming units (cfu) count. Pathogenic fungi *Rhizoctonia solani* K1 was cultivated in potato dextrose broth (PDB) medium containing 200 g/L of potato infusion, 20 g/L of glucose, and 10 g/L of Polypepton and the pH was adjusted to 5.6.

2.2. Cultivation of *B. subtilis* RB14 and *R. solani* K1

The strain *B. subtilis* RB14 [12] was transferred from culture stock (kept at −80˚C in 10% glycerol solution) into 5 ml of modified LB medium and incubated at 37˚C with 120 strokes per minute (spm) for 16 h for preculture. 400 μl of overnight cultured *B. subtilis* RB14 was inoculated into 40 ml of fish protein medium in 200 ml Erlenmeyer Flasks. The flasks were then incubated for 3 days at 30˚C with 120 spm for harvesting seed treatment aliquots. For harvesting fungal pathogen, at first *R. solani* K1 was grown on potato dextrose agar (PDA). A small mycelia disc of *R. solani* K1 from PDA plate was then inoculated into 40 ml of PDB and incubated in the dark at 28˚C for 7 days.

2.3. Soil Preparation

The soil used in this study was a commercially available black soil (Nittai Co., Tokyo, Japan), containing: 10.5% total carbon and 0.6% total nitrogen. It was mixed with vermiculite in a ratio of 4:1 (w/w) and the prepared soil was placed in polypropylene bag and autoclaved for 20 min at 121˚C three times at 12 h intervals. After sterilization the soil was amended with fertilizers: N: 0.04%, P: 0.09%, K: 0.06%, Ca: 0.06%, Mg: 0.06%, Fe: 0.001% and brought to approximately 60% of the maximum water-holding capacity by the addition of sterile distilled water.

2.4. Seed Surface Sterilization and Treating with *B. subtilis* RB14

Tomato seeds were disinfected with 70% ethanol and then with 0.5% sodium hypochlorite. Then the seeds were rinsed with sterile water several times. To prepare inoculum for seed treatment, two tubes containing 30 ml of a 3-day-old bacterial culture were centrifuged at 10,000× g for 10 min at 4˚C. The density of bacterial cells in the aliquots was about 10^8 - 10^9. The pellets were resuspended in 3 ml of sterilized distilled water. Sterilized tomato seeds were soaked into the inoculum for 15 min and the seeds were allowed to germinate on 2% agar plate at 30˚C for 3 days in the dark.

2.5. Estimation of Bacterial Concentration on the Seed and into the Soil

For the determination of the bacterial survivability on the seed, 10 coated seeds were taken in a sterilized polypropylene tube and after vortexing for 1 min the tube was serially diluted with 0.85% NaCl and spread on L-agar plate. The plates were incubated at 37˚C for 16 h and the number of colonies was counted. For the determination of viable cell number in soil, 3 g of soil containing RB14 was suspended in 8 ml of 0.85% NaCl solution (pH 7.0) in a 50-ml Erlenmeyer flask and shaken at 150 spm for 15 min. The suspension was used to determine the viable cell number by using L-agar plate similarly as described above.

2.6. *In Vitro* Fungal Growth Inhibition Assay

To investigate the biocontrol activity of the *B. subtilis* RB14 treated seeds, tests were performed on PDA plates. A small mat of fungal pathogen was placed at the centre then *B. subtilis* RB14 treated seed and control seeds were inoculated at either side of the fungal mat in multiple replicate plates. Plates were incubated in a static condition at 28˚C for 5 days. Clear zone of inhibition was observed in case of *B. subtilis* RB14 treated seeds but the control seed area was covered by the mycelia. Inhibition of fungal growth was assessed later by measuring the size of the inhibition zone (in mm). Growth of the test fungus was calculated in percentage basis (%) using the formula: $(a1 - a2/a1) \times 100$, where a1 represents the fungal growth (calculated as area from diameter measurements) without the antagonist (control), and a2 is fungal growth under bacterial challenge [16].

2.7. Investigation of Antagonist Attachment on the Seed Surface

In order to provide evidence that the antagonist *B.* subtilis RB14 is effectively adhered on the seeds, scanning electron microscopy (SEM) observation was performed. *B.* subtilis RB14 treated seeds and non treated seed samples were rinsed with sterile distilled water to remove loosely attached cells. The samples were fixed overnight in 2% glutaraldehyde in the 50 mM phosphate buffer saline (PBS) at 70% relative humidity. Samples were rinsed three times after 24 h in 50 mM phosphate buffer for 15 min each, followed by successive 15-min dehydrations in 50%, 70% and 90% ethanol and finally in 100% ethanol three times. Samples were examined by a scanning electronic microscope Hitachi S-5200 Nano SEM (Hitachi, Tokyo, Japan) operating at 1 kV.

2.8. *In Vivo* Plant Test

The sterilized soil (150 g) was placed in a plastic pot with a diameter of approximately 90 mm and a height of 80 mm. Each pot was sown with nine germinated seeds and placed in a growth chamber at 30˚C with 90% relative humidity under 16 h of light (about 6000 lx).

The soil was infested with the pathogenic fungus *R. solani* K1 for the positive control of infection. The mycelial mats of *R. solani* that formed on the surface of the PDB were homogenized by a homogenizer (ACE homogenizer, Nihonseiki, Tokyo, Japan) at 4000 rpm for 2 min in sterile water and inoculated into the soil 6 days before planting the germinated tomato seeds. The inoculum was introduced into the soil at a dosage of 1 g mycelium per 100 g of soil.

To check biocontrol activity, germinated *B.* subtilis RB14 treated and non-treated seeds were sown into the infected pots. For control experiment germinated non-treated seeds were sown into the pots in the presence and absence of any fungal pathogen. After 3 weeks, the percentage of diseased seedlings per pot was calculated. As well as the shoots were clipped off at the soil surface level and their length and dry weight (after overnight drying at 150˚C) were measured.

2.9. Determination of Iturin A from Soil

To check the release of iturin A in the soil, 3 g soil from each pot was suspended in 21 ml of solvent [acetonitrile and 3.8 mm trifluoroacetic acid (4:1 v/v)] in a 50-ml Erlenmeyer flask and kept in a shaker (140 spm) for 1 h at room temperature. Soil suspension was filtered through Whatman no. 2 filter paper (Advantec, Ltd, Tokyo, Japan), and the filtrate was dried by evaporation. The precipitate was dissolved in 2 ml of methanol and distributed to 1.5 ml Eppendorf tubes. The solution was centrifuged at 15,000× g for 2 min. The supernatant was filtered through a polytetrafluoroethylene (PTFE) filter of 0.2 µm pore size (Advantec, Ltd). The filtered solution was quantified by high performance liquid chromatography (HPLC) with ODS column (Chromolith Performance RP-18e 100 - 4.6, Merck KgaA, Darmstad, Germany). The system (LC-800 system, JASCO Co. Ltd, Tokyo, Japan) was operated at a flow rate of 2.0 ml/min and monitored at 205 nm with the eluent of acetonitrile: 10 mm ammonium acetate (35:65 v/v) for measurement of iturin A.

3. Results

3.1. Observation of Antagonist Attachment on the Seed Surface

When *B. subtilis* RB14 treated tomato seeds were placed on the PDA plate containing *R. solani* mat, clear zone of inhibition was observed (**Figure 1(A)**). About 28% pathogenic inhibition was observed by a single treated seed from the average value of multiple replicate plates. From the SEM image the bacterial attachment on the seed surface was further confirmed (**Figure 1(B)**). From cfu count it was found that the seed treating aliquot contained mainly spores. However, during the SEM examination presence of living cells on the seed surface was noticed. After seed treatment, the cells were able to re-germinate by using the seed surface nutrients as previously observed by Islam *et al*. 2005 [17]. It was also observed that seed treatment does not hamper the germination rate of tomato plants. Treated tomato seeds showed 99% germination index similar to the untreated seeds.

3.2. *In Vitro* Fungal Growth Observation

Comparative *in vitro* inhibition activity of *B. subtilis* RB14 treated and control seeds were shown in **Figure 1(A)** and **Figure 2**. As *B. subtilis* RB14 produces antifungal lipopeptide antibiotic iturin A, *R. solani* K1 cannot grow nearby as a result a large zone of inhibition was observed on the PDA plate. On PDA plate 72% fungal growth was observed in presence of a single *B. subtilis* RB14 treated seed whereas control seed showed 100% fungal growth as there was no growth inhibition.

3.3. *In Vivo* Plant Observation

In vivo fungal growth inhibition was observed when treated seeds were sown to the soil infested with *R. solani* K1 (**Figure 3**). When control seeds were sown into the *R. solani* K1 infested soil, 77% of tomato seedlings suffered

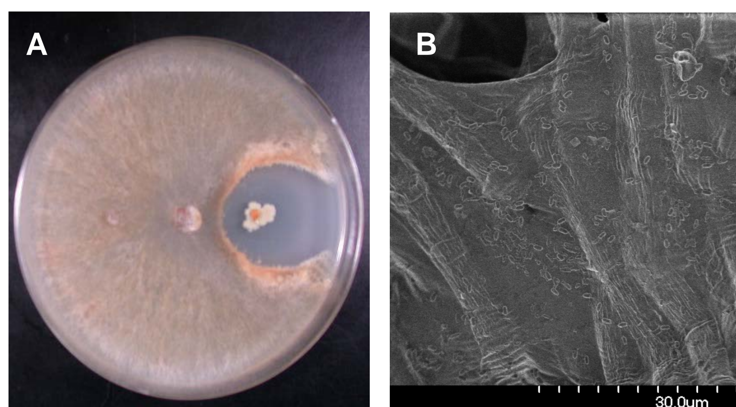

Figure 1. Observation of the presence of *B. subtilis* RB14 on tomato seeds. (A) Comparative antifungal activity of *B. subtilis* RB14 treated seed and non treated seed (showed by arrow) on PDA plate. (B) Confirmation of bacterial attachment on treated seed surface by scanning electron microscopy.

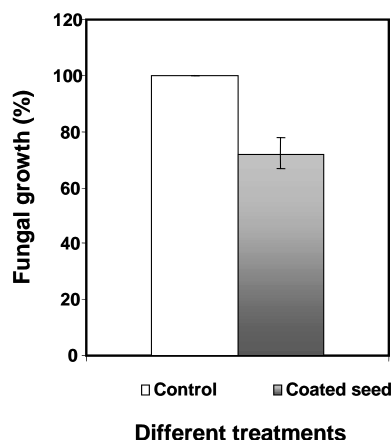

Figure 2. *In vitro* growth inhibition of *R. solani* K1 using *B. subtilis* RB14 treated seeds (■) and control seeds (□) on PDA plates. The results represent the average of multiple experiments (n ≥ 4).

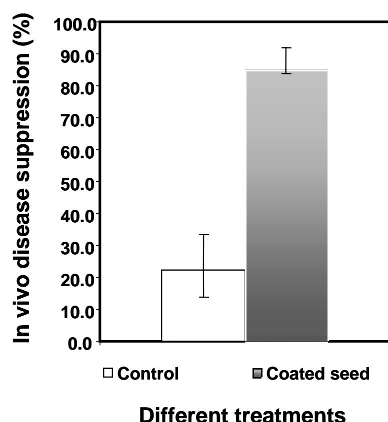

Figure 3. *In vivo* growth inhibition of *R. solani* K1 in tomato plants using *B. subtilis* RB 14 treated seeds (■) and control seeds (□); here n ≥ 4.

from damping-off disease. The *B. subtilis* RB14 treated seeds showed a sudden decrease in the disease occurrence which was found to be 17%. From the photographic image of the plants (**Figure 4**) the disease suppression ability was clearly noticed.

3.4. Presence of Iturin A in the Soil

B. subtilis RB14 is known for lipopeptide antibiotic iturin A production. Before seed sowing there was no iturin A in the soil whereas at the day of harvest significant amount of iturin A (about 4 μg/g dry soil) was isolated from the root region soil of the tomato plants treated with *B. subtilis* RB14. Reasonably iturin A was not found in the soil of the control plants.

4. Discussion

Previously it was thought that *Pseudomonas* antagonist were superior to *Bacillus* antagonist in respect of biocontrol [9]. In this study *B. subtilis* RB14 treated seeds can prevent *R. solani* K1 with superior potentiality. Successful seed surface attachment of *B. subtilis* RB14 was confirmed by *in vitro* investigation on PDA plate followed by SEM observation (**Figure 1(A)** and **Figure 1(B)**). In **Figure 2** comparative *in vitro* inhibition activity of *B. subtilis* RB14 treated and control seeds are presented. It was observed that single treated seed could allow 28% fungal growth inhibition on agar plate whereas control seed did not show any growth inhibition.

During the *in vivo* observation (**Figure 3**) significant reduction of damping off disease was observed in *B.*

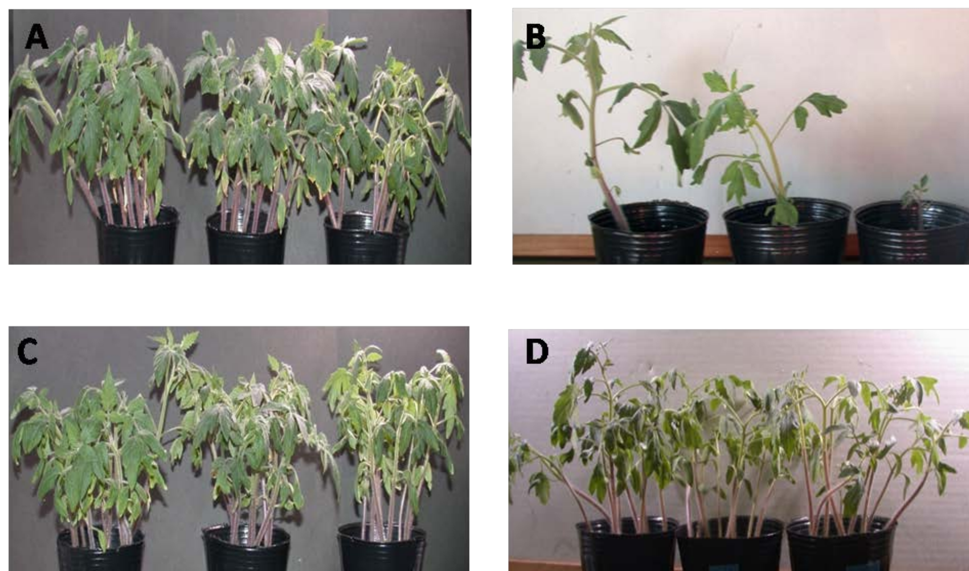

Figure 4. Photographic image of *in vivo* growth inhibition of *R. solani* K1 in tomato plants where (A) Positive control (plants without *R. solani* K1), (B) negative control (plants with *R. solani* K1) and (C) control for *B. subtilis* RB14 seed treatment and (D) *B. subtilis* RB14 seed treated plants in presence of *R. solani* K1.

subtilis RB14 treated seeds than control seeds. More than 80% disease was suppressed by *B. subtilis* RB14 treated seeds. From the photographic image of the plants as shown in **Figure 4**, the disease suppression ability was further confirmed. Clear difference was observed from the diseased plant and the seed treated plants. It was observed that each treated seed can hold about 10^8 cfu/ml *B. subtilis* cells. Treated tomato seeds showed 99% germination index similar to the untreated seeds. Plant health promotion was also observed (**Table 1**). As Plant growth promoting rhizo-bacteria (PGPR) (such as *Bacillus subtilis*) excrete phytohormone such as auxines, indole-3-acetic acid, cytokinins and gibberellines during seed germination which helps to improve seed germination and early development [18]. From a recent observation it was noticed that encapsulated microbial seed coating agent (ESCA) containing polyvinyl alcohol, sodium dodecyl sulfate, bentonite, and microencapsulated *Bacillus subtilis* SL-13 seedlings, 52.70%, 25.13%, 46.47%, and 33.21% plant height, root length, whole plant fresh weight, and whole plant dry weight was increased respectively [19]. In this study biocontrol of dumping off disease caused by *R. solani* K1 was found more prominent than plant health promotion. When the *B. subtilis* RB14 treated seeds were sown in the soil, gradually the cells were released into the soil from the seed surface. *B. subtilis* RB14 produces lipopeptide antifungal antibiotic iturin A in the soil which could suppress the disease. The phenomenon was supported by our observation; iturin A (about 4 μg/g dry soil) was identified from the root zone soil of the seed treated plants whereas control plants could not able to produce any iturin A. The release of the antibiotic started approximately 24 h after seed planting [20]. It is probable that young roots emerging from germinated seeds induced bacterial cells to produce iturin A. *B. subtilis* RB14 also produce surfactin and other lytic enzymes such as glucanase, chitinase, cellulase which also can play crucial role to defend fungal pathogen *R. solani* K1.

5. Conclusion

The selection of potential antagonistic organisms is the basic step in biological control. On the basis of these studies, it is concluded that the *B. subtilis* RB14 coated seed has a direct inhibitory effect on *R. solani* K1 growth and development, thereby these seeds are capable of suppressing damping-off diseases in tomato plants. Following the basic findings of applicability *B. subtilis* RB14 seed treatment against *R. solani* K1 in tomato plants, feasibility should be studied for other important plants and pathogens. In order to apply such seed inoculum for successful seed treatment, a greater understanding of their ecology is required. The safety and efficacy of the inoculant will be determined by the ecological success of the applied strain in the environment into which they are introduced. Greater knowledge of diversity, distribution and behavior of *Bacillus* spp. will be useful for

Table 1. Plant growth parameters in different trail run including control, *R. solani* K1 induced disease, *B. subtilis* RB14 treated seed and treated seed in presence of disease.

Combination	Height of the plant (cm)	Plant fresh weight (g)	Plant dry weight (g)
Control	20.10	3.9	0.52
R. solani K1	15.30	1.9	0.23
B. subtilis RB14 seed treatment	20.30	4.2	0.58
B. subtilis RB14 treatment in presence of *R. solani* K1	19.00	3.5	0.49

identification of new inoculants strain for effective biocontrol. Further research is essential to elucidate the mechanisms underlying sensing of the rhizoplane environment and the production of antibiotics and enzymes for successful biocontrol.

References

[1] Handelsman, J., Raffel, S., Mester, E.H., Wunderlich, L. and Grau, C.R. (1990) Biological Control of Damping-Off of Alfalfa Seedlings with *Bacillus cereus* UW85. *Applied and Environmental Microbiology*, **56**, 713-718.

[2] Brannen, P.M. and Kenney, D.S. (1997) Kodiak®—A Successful Biological Control Product for Suppression of Soil-Borne Plant Pathogens of Cotton. *Journal of Industrial Microbiology and Biotechnology*, **19**, 169-171. http://dx.doi.org/10.1038/sj.jim.2900439

[3] Knox, O.G.G., Killham, K. and Leifert, C. (2000) Effects of Increased Nitrate Availability on the Control of Plant Pathogenic Fungi by the Soil Bacterium *Bacillus subtilis*. *Applied Soil Ecology*, **15**, 227-231. http://dx.doi.org/10.1016/S0929-1393(00)00098-6

[4] Yu, G.Y., Sinclair, J.B., Hartman, G. and Bertagnolli, B.L. (2002) Production of Iturin A by *Bacillus amyloliquefaciens* Suppressing *Rhizoctonia solani*. *Soil Biology and Biochemistry*, **34**, 955-963. http://dx.doi.org/10.1016/S0038-0717(02)00027-5

[5] Collins, D.P. and Jacobsen, B.J. (2003) Optimizing a *Bacillus subtilis* Isolate for Biological Control of Sugar Beet Cercospora Leaf Spot. *Biological Control*, **26**, 153-161. http://dx.doi.org/10.1016/S1049-9644(02)00132-9

[6] Shoda, M. (2000) Bacterial Control of Plant Diseases. *Journal of Bioscience and Bioengineering*, **89**, 515-521. http://dx.doi.org/10.1016/S1389-1723(00)80049-3

[7] Podile, A.R. and Laxmi, V.D.V. (1998) Seed Bacterization with *Bacillus subtilis* AF 1 Increases Phenylalanine Ammonialyase and Reduces the Incidence of F*usarial* Wilt in Pigeonpea. *Journal of Phytopathology*, **146**, 255-259. http://dx.doi.org/10.1111/j.1439-0434.1998.tb04687.x

[8] Rydera, M.H., Yana, Z.N., Terrace, T.E., Rovira, A.D., Tang, W. and Correll, R.L. (1999) Use of Strains of *Bacillus* Isolated in China to Suppress Take-All and Rhizoctonia Root Rot, and Promote Seedling Growth of Glasshouse-Grown Wheat in Australian Soils. *Soil Biology and Biochemistry*, **31**, 19-29.

[9] Georgakopoulos, D.G., Fiddaman, P., Leifert, C. and Malathrakis, N.E. (2002) Biological Control of Cucumber and Sugar Beet Damping-Off Caused by *Pythium ultimum* with Bacterial and Fungal Antagonists. *Journal of Applied Microbiology*, **92**, 1078-1086. http://dx.doi.org/10.1046/j.1365-2672.2002.01658.x

[10] Cavaglieri, L., Orlando, J., Rodríguez, M.I., Chulze, S. and Etcheverry. M. (2005) Biocontrol of *Bacillus subtilis* against *Fusarium verticillioides in Vitro* and at the Maize Root Level. *Research in Microbiology*, **156**, 748-754. http://dx.doi.org/10.1016/j.resmic.2005.03.001

[11] Phae, C.G., Shoda, M. and Kubota, H. (1990) Suppressive Effect of *Bacillus subtilis* and Its Products on Phytopathogenic Microorganisms. *Journal of Fermentation and Bioengineering*, **69**, 1-7. http://dx.doi.org/10.1016/0922-338X(90)90155-P

[12] Hiraoka, H., Asaka, O., Ano, T. and Shoda, M. (1992) Characterization of *Bacillus subtilis* RB14, Coproducer of Peptide Antibiotics Iturin A and Surfactin. *The Journal of General and Applied Microbiology*, **38**, 635-640. http://dx.doi.org/10.2323/jgam.38.635

[13] Rahman, M.S., Ano, T. and Shoda, M. (2006) Second Stage Production of Iturin A by Induced Germination of *Bacillus subtilis* RB14. *Journal of Biotechnology*, **125**, 513-515. http://dx.doi.org/10.1016/j.jbiotec.2006.03.016

[14] Asaka, O. and Shoda, M. (1996) Biocontrol of *Rhizoctonia solani* Damping off of Tomato with *Bacillus subtilis* RB14. *Applied and Environmental Microbiology*, **62**, 4081-4085.

[15] Ashwini, N. and Srividya, S. (2014) Potentiality of *Bacillus subtilis* as Biocontrol Agent for Management of Anthracnose Disease of Chilli Caused by *Colletotrichum gloeosporioides* OGC1. 3 *Biotech*, **4**, 127-136. http://dx.doi.org/10.1007/s13205-013-0134-4

[16] Ortega-Morales, B.O., Ortega-Morales, F.N., Lara-Reyna, J., De la Rosa-García, S.C., Martínez-Hernández, A. and Jorge Montero, M. (2009) Antagonism of *Bacillus* spp. Isolated from Marine Biofilms Against Terrestrial Phytopathogenic Fungi. *Marine Biotechnology*, **11**, 375-383. http://dx.doi.org/10.1007/s10126-008-9152-3

[17] Islam, T.Md., Hashidoko, Y., Deora, A., Ito, T. and Tahara, S. (2005) Suppression of Damping-Off Disease in Host Plants by the Rhizoplane Bacterium *Lysobacter* sp. Strain SB-K88 Is Linked to Plant Colonization and Antibiosis against Soilborne Peronosporomycetes. *Applied and Environmental Microbiology*, 3786-3796. http://dx.doi.org/10.1128/AEM.71.7.3786-3796.2005

[18] Bakonyi, N., Bott, S., Gajdos, E., Szabó, A., Jakab, A., Toth, B., Makleit, P. and Veres, S.Z. (2013) Using Biofertilizer to Improve Seed Germination and Early Development of Maize. *Polish Journal of Environmental Studies*, **22**, 1595-1599.

[19] Tu, L., He, Y.H., Shan, C.H. and Wu, Z.S. (2016) Preparation of Microencapsulated *Bacillus subtilis* SL-13 Seed Coating Agents and Their Effects on the Growth of Cotton Seedlings. *BioMed Research International*, **2016**, Article ID: 3251357

[20] Szczech, M. and Shoda, M. (2006) The Effect of Mode of Application of *Bacillus subtilis* RB14-C on Its Efficacy as a Biocontrol Agent against *Rhizoctonia solani*. *Journal of Phytopathology*, **154**, 370-377. http://dx.doi.org/10.1111/j.1439-0434.2006.01107.x