

Biocontrol and Other Beneficial Activities of *Bacillus subtilis* Strains Isolated From Cow Dung, Soil Compost and Soil Rhizosphere Microflora

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Abstract

Background: Biocontrol is process by which naturally occurring microorganisms inhibits or suppress the growth of many pathogens which affects plant growth. Biocontrol activities of these microbes reduce the use of chemical fertilizers which harms the environment. Besides biocontrol activity these naturally occurring microbes also possess other beneficial activities like enhancing plant growth and producing some beneficial enzymes.

Objective: To evaluate the *in vitro* biocontrol activity and other beneficial activities of *Bacillus subtilis* isolated from cow dung, soil compost and rhizosphere soil of plant roots, by studying the antagonistic activity of the isolated *Bacillus subtilis* strains against *Fusarium moniliforme* NCIM 1099 and test their phosphate solubilizing activity and amylase activity of isolated *Bacillus subtilis* strains.

Materials and methods: Samples were collected from various rhizosphere soils of plant roots, cow dung and soil compost and strains of *Bacillus subtilis* were isolated from these samples. The isolated strains were identified and were tested for *in vitro* biocontrol activity against the fungi *Fusarium moniliforme* NCIM1099.

Results: All the tested strains of isolated bacteria significantly inhibited the fungal growth. The inhibitory activity was high as 61.2% from the *Bacillus subtilis* isolated from rhizosphere soil. The strains were also tested for its other beneficial activities such as phosphate solubilization and amylase activity and observed to be positive.

Conclusion: These biocontrol and beneficial activities make the bacterium a suitable candidate as an industrially important microbe.

Keywords: Biocontrol; *Bacillus subtilis*; *Fusarium*

Abbreviations: PGPR: Plant growth promoting rhizobacteria; ATCC: American Type Culture Collection; NCIM: National Collection of Industrial Microorganisms

Introduction

Biocontrol is process by which naturally occurring microorganisms inhibits or suppress the growth of many pathogens which affects plant growth. Disease suppression by biocontrol agents is the sustained manifestation of interactions among the plant, the pathogen, the biocontrol agent, the microbial community on and around the plant, and the physical environment [1,2]. Biological control of fungal diseases of plants is eco-friendly and is a potential component of integrated disease management [3]. There has been a steady increase in commercially prepared and marketed bacterial bio products based on rhizobacteria such as *Bacillus*, *Pseudomonas*, *Streptomyces*, *Rhizobium*, and *Agrobacterium* species.

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The large scale application of plant growth promoting rhizobacteria (PGPR) to crops as inoculum would substantially reduce the use of chemical fertilizers and pesticides which pollute environment [2]. Cow dung is normally used as an organic fertilizer for enhancing soil fertility, as a source of fuel, for dressing seeds, plastering cut ends of vegetatively propagated sugarcane, dressing plant wounds, sprinkling diluted suspension of cow dung on plant surface, etc. Microorganisms isolated from cow dung are found to inhibit plant pathogens and also enhances plant vitality [4]. Cow dung treated seeds also evade pathogenic fungal and bacterial attack, since, bacteria in cow dung play a significant role by colonizing the surface area of the treated seeds [5]. *Bacillus subtilis* strains isolated from cow dung had several beneficial attributes, which include biocontrol, plant growth promotion, phosphorus solubilization and production of industrially important enzymes like amylases [5].

Bacillus species offer several advantages over other bacteria for protection against root pathogens because of their ability to form endospores, having high thermal tolerance, being omnipresent and because of the broad-spectrum antibiotic activity. *Bacillus* species including *Bacillus subtilis*, *Bacillus pumilis*, *Bacillus magetarium*, *Bacillus polymyxa*, have been known to be effectively inhibiting many soil borne fungal diseases to plants by means of antagonistic activities [6].

Because *Bacillus* sp. occupies the identical ecological niche within the plant, it is considered an ecological homologue to plant pathogenic fungi like *Fusarium moniliforme* and the inhibitory mechanism, regardless of the mode of action, operates on the competitive exclusion principle [7].

Hence, we evaluated the *in vitro* biocontrol activity and other beneficial activities of *Bacillus subtilis* isolated from cow dung, soil compost and rhizosphere soil of plant roots. We studied the antagonistic activity of the isolated *Bacillus subtilis* strains against *Fusarium moniliforme* NCIM 1099 and also tested the phosphate solubilizing activity and amylase activity of isolated *Bacillus subtilis* strains.

Materials and Methods

Collection and processing of samples

During the period between January and July 2009, 4 samples of rhizosphere soil, 2 cow dung samples and 2 soil compost samples were collected from various villages around Ponneri (13.3200°N, 80.2000°E) and transported to laboratory in a polythene bag at ambient temperature. Further processing was performed at Department of Microbiology, at Pachaiyappa's college, Chennai. Ten grams of each sample were weighed and homogenized with 90 mL of sterile distilled water to make a dilution of 10^{-1} . Further dilutions ranging from 10^{-2} to 10^{-5} were made by serial dilution method. An aliquot of 1 mL of the diluents from each dilution was subjected to pour plate technique in appropriate media and incubated at 37°C for 18-24 hours. The isolated bacteria were then maintained in nutrient agar slants and nutrient broth (Himedia Labs, Mumbai).

Morphological and phenotypic characterization of isolated *Bacillus* strains

Parameters investigated included cell and spore morphology, production of enzymes (catalase, oxidase, and urease), growth characteristics (growth in the presence of NaCl), carbon source utilization (glucose, mannitol, fructose and lactose), utilization of citrate, indole formation, Methyl red and Voges-Proskauer reaction [8].

Screening for antifungal activity

The isolated and identified bacterial strains were screened for their antifungal activity against the fungus *Fusarium moniliforme* NCIM1099 and for their other beneficial activities. Antagonism study by dual plate technique was performed using the pure culture of *Fusarium moniliforme* following the method described earlier [5,9]. Each experiment was run in triplicates. Results were expressed in percent inhibition which was calculated as follows [10]:

$$\text{Percent inhibition} = 1 - \left[\frac{\text{Fungal growth (in cm)}}{\text{Control growth (in cm)}} \right] \times 100$$

The ability of the isolated strains of *Bacillus* to solubilize phosphate and to produce amylase enzyme was determined by the methods described elsewhere [5, 11, 12].

Statistical analysis

Arithmetic mean and standard deviation were calculated for each triplicate experimental and control measurements. One way ANOVA followed by Tukey’s test was used for multiple comparisons of means.

Results and Discussion

Eight *Bacillus subtilis* strains were isolated from the collected samples on nutrient agar by pour plate technique. Results of the microscopic, morphological and biochemical identification of *Bacillus subtilis* are listed in Table 1. These results were compared with that of reference strain of *Bacillus subtilis* ATCC 6633 for confirmation of species.

S.No	Test performed	<i>Bacillus subtilis</i> (Isolates)	<i>Bacillus subtilis</i> (ATCC 6633)
1.	Gram staining	+	+
2.	Cellular morphology	Rods	Rods
3.	Motility Test	+	+
4.	Spore Test	+	+
5.	Catalase	+	+
6.	Oxidase	+	+
7.	Indole	+	+
8.	Methyl Red	-	-
9.	Voges Proskauer	-	-
10.	Citrate	+	+
11.	Urease	+	+
12.	Glucose	+	+
13.	Lactose	-	-
14.	Sucrose	+	+
15.	Mannitol	+	+

Table 1: Phenotypic and biochemical characterization of isolates of *Bacillus subtilis* as compared with the reference strain.

Table 2 represents the results of in vitro biocontrol activity of *Bacillus subtilis* strains against *Fusarium moniliforme* NCIM 1099 as expressed by the percentage of inhibition. Figure 1 shows the graphical representation of percent inhibition. The obtained result was subjected to statistical analysis by using one way ANOVA and Tukey’s test for multiple comparisons of means. The results suggested that each isolate demonstrated unique inhibitory activity towards the fungal pathogen, which was significantly different from each other at 95% confidence interval with a pooled standard deviation of 1.043.

TriPLICATE Number	<i>Bacillus subtilis</i> isolates							
	BS 1	BS 2	BS 3	BS 4	BS 5	BS 6	BS 7	BS 8
1	37.8	40	61.2	56.7	45.6	28.9	8.9	16.7
2	35.6	42.3	60	57.8	43.4	27.8	8.9	15.6
3	36.7	41.1	62.3	58.9	46.7	28.9	9	16.7
Mean	36.7	41.1	61.1	57.8	45.2	28.5	8.9	16.3
SD	0.9	0.9	0.9	0.9	1.4	0.5	0.1	0.5

Table 2: Percent inhibition of *Fusarium moniliforme* by *Bacillus subtilis* isolates.

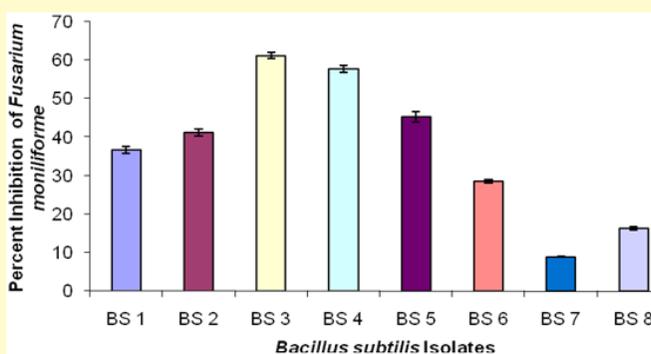


Figure 1: Graphical representation of percent inhibition of *Fusarium moniliforme* (Mean ± SD) by *Bacillus subtilis* isolates.

Testing of phosphate solubilization activity of the isolated strains revealed that, all the *Bacillus subtilis* strains showed phosphate solubilization activity on bromophenol blue containing Pikovasky agar medium by producing yellow coloured halo zone around the streak line of the bacteria. Also, all the isolated strains demonstrated positive amylase activity on starch containing medium by producing characteristically distinct halo zone around the streak line of bacteria.

Cow dung, rhizosphere soil and soil compost have been known to be rich sources of several known microorganisms such as bacteria and fungi as well as some unidentified microorganisms [2,5,13]. *Bacillus subtilis* strains isolated from cow dung samples have been reported to inhibit fungal plant pathogens *Fusarium oxysporum* and *Botryodiplodia theobromae*. Similarly *Bacillus subtilis* strains isolated from rhizosphere soil inhibited *Rhizoctonia solani* [10]. Similarly, in this study, all the *Bacillus subtilis* strains isolated from cow dung, rhizosphere soil and soil compost inhibited the fungal plant pathogen *Fusarium moniliforme*. But the degree of inhibitory activity varied with each of the isolated strains. In this study, *Bacillus subtilis* strains isolated from cow dung, rhizosphere soil and soil compost inhibited *Fusarium moniliforme* growth *in vitro* up to a maximum of about 41.1%, 61.2% and 16.3% respectively. *Bacillus subtilis* from soil compost was earlier found to inhibit the growth of *Fusarium oxysporum* to the extent of 62% [13]. The same species from cow dung was found to inhibit the growth of *Fusarium oxysporum* by 35% [5]. The percentage of inhibition of *Rhizoctonia solani* by *Bacillus subtilis* from rhizosphere soil of tomato plants was about 48.15 [10]. Phosphate solubilization and mobilization through soil microorganisms converts insoluble and fixed forms of phosphorus into soluble phosphorus is an important aspect of increasing soil phosphorus availability [14]. In this study, the isolated *Bacillus subtilis* strains from various sources were demonstrate to possess the ability to solubilize phosphate, suggesting its use as a plant vitality agent.

Microorganisms are the main sources of amylase which hydrolyses starch into simple carbohydrates. This conversion makes the easy availability of carbon sources for plants. Moreover, *Bacillus subtilis* are high thermo-tolerant bacteria it may be used for the

production industrially important amylase. In this study, all the isolated strains of *Bacillus subtilis* showed amylase activity suggesting the possibility of these strains to be used for the industrial production of amylase enzyme.

Conclusion

In conclusion, the bacteria *Bacillus subtilis* may be used as biocontrol agent for controlling plant disease caused by fungal plant pathogen *Fusarium moniliforme* and also for other beneficial activities such as phosphate solubilization and amylase production.

Bibliography

1. Handelsman J and EV Stabb. "Biocontrol of Soilborne Plant Pathogens". *The Plant Cell* 8.10 (1996): 1855–1869.
2. Kilian M., et al. "FZB24® *Bacillus Subtilis* - Mode of Action of a Microbial Agent Enhancing Plant Vitality." *Pflanzenschutz-Nachrichten Bayer* 53.1 (2000): 72–93.
3. Kishore G Krishna., et al. "Biological Control of Collar Rot Disease with Broad-Spectrum Antifungal Bacteria Associated with Groundnut". *Canadian Journal of Microbiology* 51.2 (2005): 123–132.
4. Swain Manas R., et al. "Indole-3-Acetic Acid Production and Effect on Sprouting of Yam (*Dioscorea Rotundata* L.) Minisetts by *Bacillus Subtilis* Isolated from Culturable Cowdung Microflora". *Polish Journal of Microbiology* 56.2 (2007): 103–110.
5. Swain MR and RC Ray. "Biocontrol and Other Beneficial Activities of *Bacillus Subtilis* Isolated from Cowdung Microflora". *Microbiological Research* 164.2 (2009): 121–130.
6. Shoda Makoto. "Bacterial Control of Plant Diseases". *Journal of bioscience and bioengineering* 89.6 (2000): 515–21.
7. Bacon CW., et al. "Biological Control of *Fusarium Moniliforme* in Maize". *Environmental Health Perspectives* 109.2 (2001): 325–332.
8. Idriss ElSORRA E., et al. "Extracellular Phytase Activity of *Bacillus Amyloliquefaciens* FZB45 Contributes to Its Plant-Growth-Promoting Effect". *Microbiology* 148.Pt 7 (2002): 2097–2109.
9. Sadfi N., et al. "Evaluation of Bacterial Isolates from Salty Soils and *Bacillus thuringiensis* Strains for the Biocontrol of *Fusarium* Dry Rot of Potato Tubers". *Journal of Plant Pathology* 83.2 (2001): 101–117.
10. Montealegre Jaime R., et al. "Selection of Bioantagonistic Bacteria to Be Used in Biological Control of *Rhizoctonia Solani* in Tomato". *Electronic Journal of Biotechnology* 6.2 (2003).
11. Mehta S and CS Nautiyal. "An Efficient Method for Qualitative Screening of Phosphate-Solubilizing Bacteria". *Current Microbiology* 43.1 (2001): 51–56.
12. Sridevi M and KV Mallaiah. "Phosphate Solubilization by *Rhizobium* Strains". *Indian Journal of Microbiology* 49.1 (2009): 98–102.
13. Muhammad S and NA Amusa. "In-Vitro Inhibition of Growth of Some Seedling Blight Inducing Pathogens by Compost-Inhabiting Microbes". *African Journal of Biotechnology* 2.6 (2003): 161–164.
14. Henri Fankem., et al. "Solubilization of Inorganic Phosphates and Plant Growth Promotion by Strains of *Pseudomonas Fluorescens* Isolated from Acidic Soils of Cameroon". *African Journal of Microbiology Research* 2.7 (2008): 171–178.

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