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Bioefficacy of *Bacillus subtilis* against *Aspergillus flavus, the* cause of aflatoxin contamination in chilli

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Abstract
Bacillus subtilis a gram positive, endospore forming bacteria play a major role in biocontrol and PGPR activities. Thirty isolates of *B. subtilis* were obtained from different rhizosphere soil samples from different parts of North Eastern Karnataka region. All the isolates were rod shaped, positive for gram reaction, endospore, oxidase, catalase, starch hydrolysis, negative for indole, KOH test and green coloured colonies were grown on Hichrome *Bacillus* agar medium. All the isolates showed varied levels of antagonist activity *in vitro* against major pathogens of chilli. *In vitro* screening of *B. subtilis* (30 isolates) against *A. flavus* which causes the aflatoxin contamination. The varied levels of inhibition of mycelial growth of aflatoxin fungus was obtained. Among different isolates BS22 showed maximum 40.96 percent inhibition followed by BS5 was 38.88 percent and minimum was17.50 percent in case of BS29 as compared to control. The *B. subtilis* strains were isolated, identified and used in this present study is a promising natural bioagent which can be considered as an alternative to chemical pesticides in chilli disease management strategies and also used in integrated disease management.

Keywords: *B. subtilis*, biocontrol, PGPR, percent inhibition, *F. solani*, *R. solani*

Introduction
In modern days cultivation practices management of diseases of crop plants is difficult due to arrival of new races of pathogens. Use of is one of the options for management it hasa led to substantial pollution soil, air and water. Chemical residues have detrimental effects on human, plant and soil health and leads to development of new races resistant to chemicals (Gerhardson**,** 2002) [5]. Hence, an alternative control measure employing antagonistic bacterial agents is an attractive option (Han *et al*., 2005). Biocontrol is an important strategy to reduce the use of chemicals in disease management and burden on farmers. Recently, a considerable attention has been given to Rhizospheric microorganisms which have positive influence on the plant growth and health. These are known as Plant Growth Promoting Rhizobacteria (PGPR) (Schippers, 1992; Glick, 1995) [16, 6] such as *Azatobacter*, *Pseudomonas, Azospirullum*, *Bacillus* and *Brukholderia.* Among the PGPRs, the gram positive and endospore producing *Bacillus subtilis* which become more important tool safe guarde the plant health (Glick, 1995) [6]. The colony morphology of the isolates exhibit a range from flat to filamentous or branching (Wafula *et al*., 2014) [21], having either smooth or rough colony with colour ranging from white to cream. They grow well at pH ranging from 5 - 6.5 and temperature range of 25 to 35 ^{o}C commonly found situation in soil. *B. subtilis* is an endospore forming bacteria (Piggot and Hilbert, 2004) [14] which helps the organism to persist in the environment until conditions become favourable (Wafula *et al*., 2014) [21]. *B. subtilis* shows strong positive results in the methyl red test, oxidase test, litmus milk reactions and lipid hydrolysis test. The organism shows weakly positive for catalase test, gelatin hydrolysis test and negative results for citrate reduction, urease test, arginine hydrolysis and fluorescence in King's B medium (Montealegre *et al*., 2003) [12].

Plant growth promotion and bio control of plant pathogens by *Bacillus subtilis* through antibiosis, competition, mycoparasitism (Korsten and De Jager, 1995) [9] and induced systemic resistance in host plant (Lemessa and Zeller, 2007; Aliye *et al*., 2008; Ji *et al*., 2008) [10, 1, 8]. These mechanisms might act singly or in combinations by using extra-cellular lytic enzymes *viz,* chitinase, amylase, protease, lipase, xylanase and β 1, 3 glucanase which exhibit antagonistic property because of degradation of cell wall of fungi and bacteria (Ramyabharathi and Raguchander, 2013) [15], anti-microbial compounds such as HCN, H_2S and siderophore

(Dinesh Singh *et al.*, 2012) [4] and antibiotics such as subtilin, surfaction, iturin, biofilm, difficiden, bacilomycin, bacilycin and fengycin (Loeffler *et al*., 1990) [11] which is known to control a wide array of phytopathogens such as fungi, bacteria and nematodes. *B. subtilis* colonise rapidly, occupy all available niches, absorb nutrients and form biological screen around the root and prevents breeding, growth, invasion of harmful microorganisms. (Timmusk *et al*., 2005; Haggag and Timmusk, 2008) [19, 7].

However, the success of any biological control programme depends on our clear understanding about the biocontrol agent, their ecology, environments, biocontrol mechanisms and population dynamics in natural and autoclaved soil. The exact identity of strains to the species level is the first step in realizing the potential of any bio agent. Further, their study on the diversity regarding rhizosphere niche of different crops is a priority.

Materials and Methods

Bio efficacy of *B. subtilis* against chilli pathogens

The isolates of *B. subtilis* were evaluated *in vitro* for their antagonistic properties against major pathogen of chilli *Aspergillus flavus* using dual culture technique. The bio-agent and the pathogen were inoculated side by side in a single Petri plate containing solidified PDA medium. Three replications were maintained for each treatment with one control by maintaining only pathogen. The plates were incubated for 4 - 5 days at 28 ± 1 ºC. The mycelial diameter of pathogen was measured in two directions and average was recorded (Sumana and Devaki, 2013) [17]. Percent inhibition of growth of test pathogen was calculated using the following equation (Vincent, 1927).

$$I = \frac{C - T \times 100}{C}$$

Where;
I = Percent inhibition of mycelium
C = Growth of fungal mycelium in control.
T = Growth of fungal mycelium in treatment.

Results and Discussion

Bio efficacy of *B. subtilis* isolates against *A. flavus*

In vitro screening of *B. subtilis* (30 isolates) against *A. flavus* which causes the aflatoxin contamination. The varied levels of inhibition of mycelial growth of aflatoxin fungus was obtained. Among different isolates BS22 showed maximum 40.96 percent inhibition followed by BS5 was 38.88 percent and minimum was17.50 percent in case of BS29 as compared to control (Table 1). Singh *et al.* (2008) reported that the antagonistic activity of *T. harzianum* against *M. phaseolina* showed maximum inhibition was (75.5%) and least inhibition against *Aspergillus* spp. was 45.74 percent.

B. subtilis strains isolated, identified and used in this present study as a bioagent in the control of major fungal pathogens of chilli shows that it is a promising natural bioagent. It exhibited sufficient antibiosis capability due to its good inhibitory performance against *F. solani, R. solani, S. rolfsii*, *A. flavus* and *C. capsici in- vitro* in the laboratory. It can be considered as an alternative to chemical pesticides in disease management strategy and should be further studied under field condition and possibly scaled-up for the control of numerous phytopathogenic fungi causing diseases and great yield losses.

Table 1: *In vitro* bio efficacy of *B. subtilis* against *A. flavus*, the cause of aflatoxin contamination of chill**i**

Sl. No.	Isolate	Percent Inhibition	Remark
1	BS-1	30.44 (33.47)	M
2	BS-2	33.70 (35.47)	M
3	BS-3	34.38 (35.88)	M
4	BS-4	31.50 (34.12)	M
5	BS-5	38.88 (38.55)	M
6	BS-6	31.00 (33.81)	M
7	BS-7	29.33 (32.71)	M
8	BS-8	32.61 (34.80)	M
9	BS-9	37.16 (37.54)	M
10	BS-10	27.40 (31.55)	M
11	BS-11	30.27 (33.36)	M
12	BS-12	29.72 (33.02)	M
13	BS-13	26.30(30.84)	M
14	BS-14	32.27 (34.60)	M
15	BS-15	30.50 (33.50)	M
16	BS-16	29.44 (32.84)	M
17	BS-17	28.75 (32.41)	M
18	BS-18	28.90(32.50)	M
19	BS-19	26.38 (30.89)	M
20	BS-20	24.62 (29.73)	M
21	BS-21	25.66 (30.42)	M
22	BS-22	40.96 (39.77)	M
23	BS-23	33.11 (35.11)	M
24	BS-24	32.11 (34.50)	M
25	BS-25	36.42 (37.10)	M
26	BS-26	37.72 (37.87)	M
27	BS-27	25.05(30.02)	M
28	BS-28	27.11 (30.36)	M
29	BS-29	17.50 (24.71)	L
30	BS-30	32.22 (34.57)	M
32	Check	32.66 (34.84)	M
32	Control	00.00 (00)S	L
	S.E m ±	0.53	
	C.D at 1%	1.49	

>40%= High (H) =0, 20-40%=Moderate (M) =30, <20%=Low (L) =2

*Figures in the parentheses are arc sine values

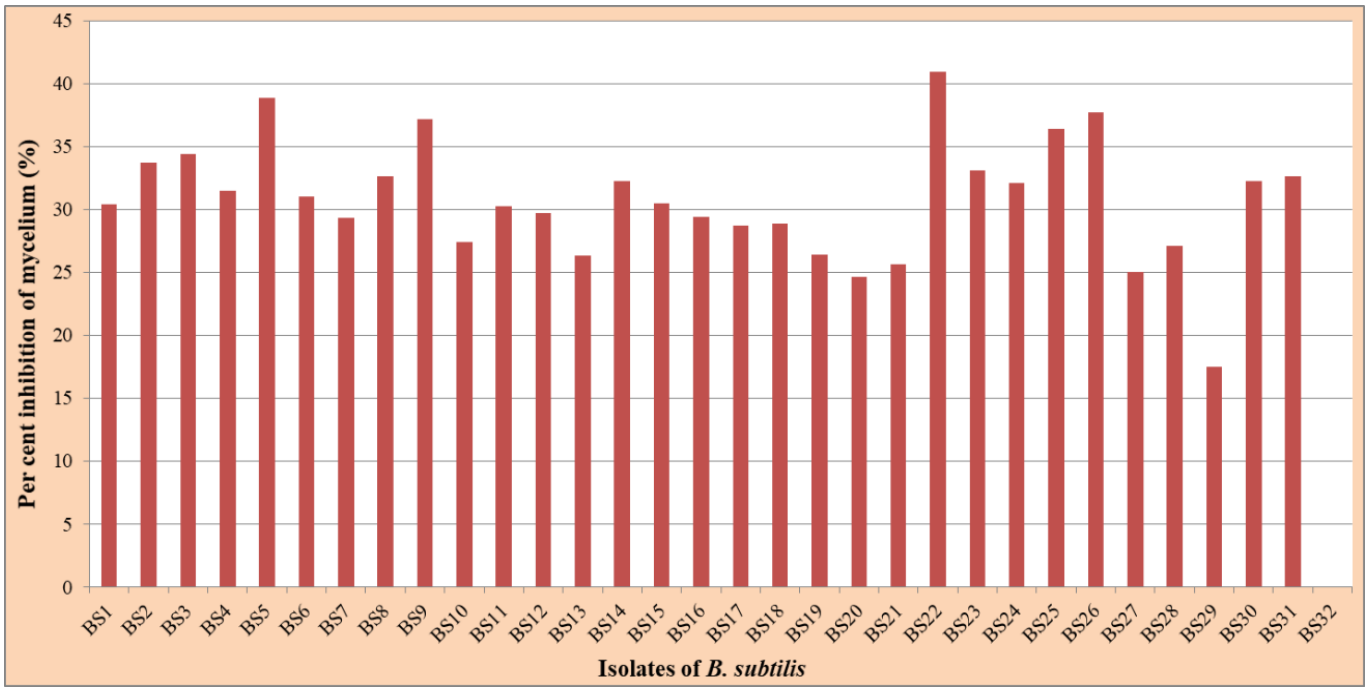

Fig 1: *In vitro* bio efficacy of *B. subtilis* against *A. flavus*, the cause of aflatoxin contamination in chilli

Plate 1: *Vitro* bio efficacy of *B. subtilis* isolates against *a flavus*, the cause of aflatoxin contamination in chilli

References

1. Aliye AN, Fininsa BC, Hiskias Y, Evaluation of rhizosphere bacterial antagonists for their potential to bioprotect potato (*Solanum tuberosum*) against bacterial wilt (*Ralstonia solanacearum*) Biol. Cont. 2008; 47:282-288.
2. Anand M, Naik MK, Ramegowda G, Devika Rani GS, Biocontrol and plant growth promotion activity of indigenous isolates of *Pseudomonas fluorescens*. J My copathol. Res. 2010; 48(1):45-50.
3. Ashwini N, Srividya S. Study of mycolytic enzymes of *Bacillus* spp. against *Colletotrichum gloeosporioides* causing anthracnose in Chilli. Acta Biologica Indica. 2012; 1(1):81-89.
4. Dinesh Singh, Yadav DK, Shweta S, Upadhyay BK, Utilization of plant growth promoting *Bacillus subtilis* isolates for the management of bacterial wilt incidence in tomato caused by *Ralstonia solanacearum* race 1 biovar 3. Indian Phytopath. 2012; 65(1):18-24.
5. Gerhardson B. Biological substitutes for pesticides: Trends in Biotechnology. 2002; 20:338-343.
6. Glick BR, The enhancement of plant growth promotion by free-living bacteria. Can. J. Microbiol. 1995; 41:9-17.
7. Haggag WM, Timmusk S. Colonization of peanut roots by biofilm-forming *Paenibacillus polymyxa* initiates biocontrol against crown rot disease. J Applied Microbiol. 2008; 104:961-969.
8. Ji X, Yingping L, Zheng IG, Zhimei M. Biological control against bacterial wilt and colonization of mulberry by an endophytic *Bacillus subtilis* strain. FEMS. Microbiol. Ecol. 2008; 65:565-573.
9. Korsten L, De Jager ES. Mode of action of *Bacillus subtilis* for control of avocado post-harvest pathogens. South African Avocado Growers' Association Yearbook. 1995; 18:124-130.
10. Lemessa F, Zeller W. Screening rhizobacteria for biological control of *Ralstonia solanacearum* in Ethiopia. Biological Control. 2007; 42:336-344.
11. Loeffler W, Kratzer W, Kremer S, Kugler M, Petersen F, Jung G, *et al*. Gegen pilze wirksame antibiotika der

Bacillus subtilis - Gruppe. Forum Mikrobiol. 1990; 3:156-163.
12. Montealegre JR, Reyes R, Pérez LM, Herrera R, Silva P, Besoain X. Selection of bio antagonistic bacteria to be used in biological control of *Rhizoctonia solani* in tomato. Electronic J. Biotechnol. 2003; 6:34.
13. Nakano M, Hulett M. Adaptation of *Bacillus subtilis* to oxygen limitation. *Microbiol.* 1997; 157(1):1-7.
14. Piggot P, Hilbert D. Sporulation of *Bacillus subtilis.* Current Opinion in Microbiol. 2004; 7(6):579-586.
15. Ramyabharathi SA, Raguchander T. Induction of defence enzymes and proteins in tomato plants by *Bacillus subtilis* EPCO16 against *Fusarium oxysporum f. sp. lycopersici*. Madra. Agric. J. 2013; 100(2):126-130.
16. Schippers B. Prospects for management of natural suppressiveness to control soilborne pathogenes. In: biological control of plant diseases, progress and challenges for the future. Life Sciences. Tjamos, E. C., Papavizas, G. C. and Cook, R. J. (Eds.). Plenum Press, New York, USA. 1992; 230:21-34.
17. Sumana K, Devaki NS. *In vitro* evaluation of some bioagents against tobacco wilt. J Biopest. 2013; 5(1):18-22.
18. Surendra Singh, Harichhand, Kishore Verma P. Screening of bioagents against root rot of mungbean caused by R. solani Legume res. 2008; 31(1)75-76.
19. Timmusk S, Grantcharova N, Wagner EGH. *Paenibacillus polymyxa* invades plant roots and forms biofilms. Appl. Environ. Microbiol. 2005; 11:7292-7300.
20. Vincent JM. Distortion of fungal hyphae in presence of certain inhibitors. Nature. 1927; 159:850.
21. Wafula EN, Johnson K, Daniel K, Anne M, Romano M. Isolation and characterization of *Bacillu*s species from soil in Ngere tea catchment area of Murang'a county, Kenya. Int. J Life Sci. Res. 2014; 2(3):27-35.