International Journal of Chemical Studies

P-ISSN: 2349–8528
E-ISSN: 2321–4902
IJCS 2018; 6(3): 3389-3392

Received: 19-03-2018
Accepted: 22-04-2018

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Bioefficacy of *Bacillus subtilis* isolates against *Fusarium solani*, the causal agent of wilt of chilli

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Abstract
Biological control is an important strategy to reduce the use of chemicals in disease management. Recently, a considerable attention has been given to the plant growth rhizobacteria which positive influence on plant growth and health. Among the PGPR microbes, *Bacillus subtilis* play a major role in plant growth promotion and biocontrol. 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 biocontrol and PGPR activities *in vitro* against *Fusarium solani* is the causal agent of wilt disease in chilli. Thirty isolates of *B. subtilis* were recorded the varied levels of inhibition of mycelial growth of *F. solani*. Among different isolates, BS16 showed maximum inhibition of 65.27 per cent which differed significantly from others followed by BS 30 with 63.42 and least in case of BS17 isolate (38.88 %) compared to check isolate which showed 41.94 per cent inhibition of mycelial growth of *F. solani. B. Subtilis* BS16 is one the potential bioagent which can be used in chilli ecosystem for integrated disease management.

Keywords: *B. subtilis*, Biocontrol, PGPR, Per cent inhibition, *F. solani*.

Introduction
Management of diseases of crop plants is difficult due to arrival of new races of pathogens. Chemical control is one of the options for management but bears risk of soil and water pollution. Pesticide residues have detrimental effects on human, plant and soil health and leads to development of mutant resistant to pesticides (Gerhardson**,** 2002) [4]. Hence, a biocontrol measure employing antagonistic bacterial agents is an attractive option.

Biocontrol is an important strategy to reduce the use of chemicals in disease management. Soil has enormous potential antagonistic microorganisms which are helpful in reducing the pathogen population through different mode of actions such as competition for food and space (Martin 1971, Lynch 1983), mycoparasitism, antibiosis, production of PGPR compounds and production of enzymes (Janisiewicz *et al*. 2000). In recent years several microbes with potential biocontrol properties have come to light. Microbes such as bacteria, fungi, viruses, protozoa and nematodes that are known to produce an array of metabolites, form the basis for antimicrobial compounds. The microbial strains with good antimicrobial properties have been used in plant disease management.

Among the microbes, the endospore forming, *Bacillus subtilis* is the one which plays a major role in plant growth promotion and biocontrol of pathogens (Glick, 1995) [5]. *B. subtilis* is a gram positive, rod shaped bacteria with peritrichous flagella (Nakano and Hulett, 1997) [13]. The colony morphology of the isolates exhibit a range from flat to filamentous or branching (Wafula *et al*., 2014) [20], 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 ºC 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) [20].

Plant growth promotion and bio control of plant pathogens by *Bacillus* spp. are achieved by 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, 7].

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], antimicrobial compounds such as HCN, H_2S and siderophore (Dinesh Singh *et al.*, 2012) [3] 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* multiply 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) [6, 18].

However, the success of any biological control programme depends on our clear understanding about the 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.

Materials and Methods

Isolation of *B. subtilis* by serial dilution and plate count technique

The isolates of bacterium were isolated from soil collected from different rhizosphere soil samples from different parts of North Eastern Karnataka region 1g of soil sample was added to a glass tube containing 9 ml sterilized distilled water, heat the soil suspension to 80 °C for 20 min. From this 1ml of the suspension was transfered to the 1st test tube, 1ml of the suspension from 1st test tube was transferred to a 2nd test tube containing 9ml of distilled water and same process was continued until the last 6th test tube. 0.1ml of the suspension from test tubes 5 and 6 will be transferred to plates, then nutrient agar (NA) media or LB agar is poured by using pour plate technique, and incubated at 30 °C for 48 h (Pankaj Kumar *et al*., 2012). The culture was identified based on characters such as shape, texture of colony, colony morphology and colour of colony.

Bioefficacy of *B. subtilis* against *Fusarium solani* (wilt of chilli)

The isolates of *B. subtilis* were tested *in vitro* for their biocontrol and PGPR activities against major pathogen of chilli such as *Fusarium solani* (wilt of chilli) by 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 ^{o}C. The mycelial diameter of pathogen was measured in two directions and average was recorded (Sumana and Devaki, 2013) [17]. Per cent inhibition of growth of test pathogen was calculated using the following equation (Vincent, 1927) [19].

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

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

Results and Discussion

F. solani is the causal agent of wilt disease in chilli. Thirty isolates of *B. subtilis* were tested *in vitro* against *F. solani;* all the isolates recorded the varied levels of inhibition of mycelial growth of *F. solani*. Among different isolates, BS16 showed maximum inhibition of 65.27 per cent which differed significantly from others followed by BS 30 with 63.42 and least in case of BS17 isolate (38.88 %) compared to check isolate which showed 41.94 per cent inhibition of mycelial growth of *F. solani* (Table 1). Among 30 isolates of *B. subtilis*, twenty nine isolates were showed more than 40 per cent inhibition comes under group high performance (Plate 1) (Fig. 1). Yahia *et al*. (1981) [21] found that under laboratory conditions, *T. viride, Streptomysis griseus* and *B. subtilis* inhibited the linear growth of *F. solani*, fababean rot pathogen.

Karimi *et al*. (2012) [8] screened the antagonistic effects of 6 isolates of each of *Pseudomonas* and *Bacillus* genera isolated from rhizosphere of chickpea against *F. oxysporum* f. sp. *ciceris* (*Fusarium* wilt of chickpea) *in vitro* and *in vivo* in which *P. aeuroginosa* (P10 and P12), *B. subtilis* (B1, B6, B28 and B99) and *P. aeuroginosa* (P12 and B28) provided better control ($P \leq 0.05$) than untreated control (15.8-44.8 %). The results indicated that PGPR improved the growth parameters in the plant and helped in the biocontrol of pathogen.

Ramyabharathi and Raguchander (2013) [15] evaluated 15 strains of *B. subtilis* against *F. oxysporum* f. sp. *lycopersici* causing *Fusarium* wilt of tomato, under *in vitro* conditions. *B. subtilis* strain, EPCO16 has greater inhibition to *F. oxysporum* f. sp. *lycopersici*. Based on results, *B. subtilis* strain, EPCO16 was regarded as best bio control and growth promoting agent.

Sumana and Devaki (2013) [17] assessed the possible use of bio control agents. *T. viride, P. fluorescens* and *B. subtilis* were evaluated for their antagonistic activity against *F. oxysporum* f. sp. *nicotianae* (*Fusarium* wilt of tobacco) under *in vitro* conditions. Different biocontrol agents showed varying degrees of antagonism. *T. viride, T. harizianum and B. subtilis* showed 70, 40 and 36 per cent inhibition of radial growth of *F. oxysporum* f. sp. *nicotianae* respectively. The bacterial and fungal antagonists were not mutually antagonistic each to other.

Tables and Figures

Table 1: Bioefficacy of *B. subtilis* against *F. solani*, the causal agent of wilt of chilli

Sl. No.	Isolate	Per cent Inhibition	Remarks
1	BS-1	57.40 (49.24)*	H
2	BS-2	56.48 (48.70)	H
3	BS-3	52.77 (46.57)	H
4	BS-4	55.09 (47.90)	H
5	BS-5	53.70 (47.11)	H
6	BS-6	57.40 (49.24)	H
7	BS-7	56.48 (48.70)	H
8	BS-8	56.48 (48.70)	H
9	BS-9	56.01 (48.44)	H

10	BS-10	62.03 (51.94)	H
11	BS-11	58.33 (49.78)	H
12	BS-12	52.31 (46.31)	H
13	BS-13	56.48 (48.70)	H
14	BS-14	58.79 (50.05)	H
15	BS-15	60.18 (50.86)	H
16	BS-16	65.27 (53.88)	H
17	BS-17	38.88 (38.56)	M
18	BS-18	58.79 (50.05)	H
19	BS-19	55.55 (48.17)	H
20	BS-20	62.50 (52.22)	H
21	BS-21	52.31 (46.31)	H
22	BS-22	51.38 (45.78)	H
23	BS-23	59.72 (50.59)	H
24	BS-24	55.55 (48.17)	H
25	BS-25	60.64 (51.13)	H
26	BS-26	58.33 (49.78)	H
27	BS-27	55.55 (48.17)	H
28	BS-28	56.94 (48.97)	H
29	BS-29	61.57 (51.67)	H
30	BS-30	63.42 (52.77)	H
31	Check	41.94 (40.35)	H
32	Control	00.00 (00)	L
S.Em±	**-**	**0.35**	
C.D at 1%	**-**	**0.99**	

>40%= High (H) =30
20-40%=Moderate (M) =1
<20%=Low (L) =1 *Figures in the parentheses are arc sine values

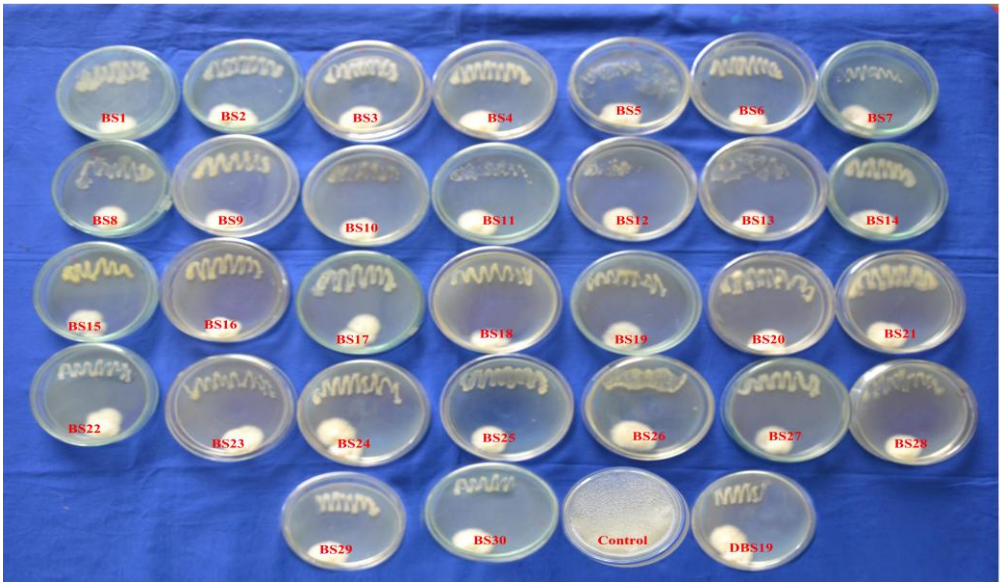

Plate 1: Bioefficacy of *B. subtilis* isolates against *F. solani*, the causal agent of wilt of chilli

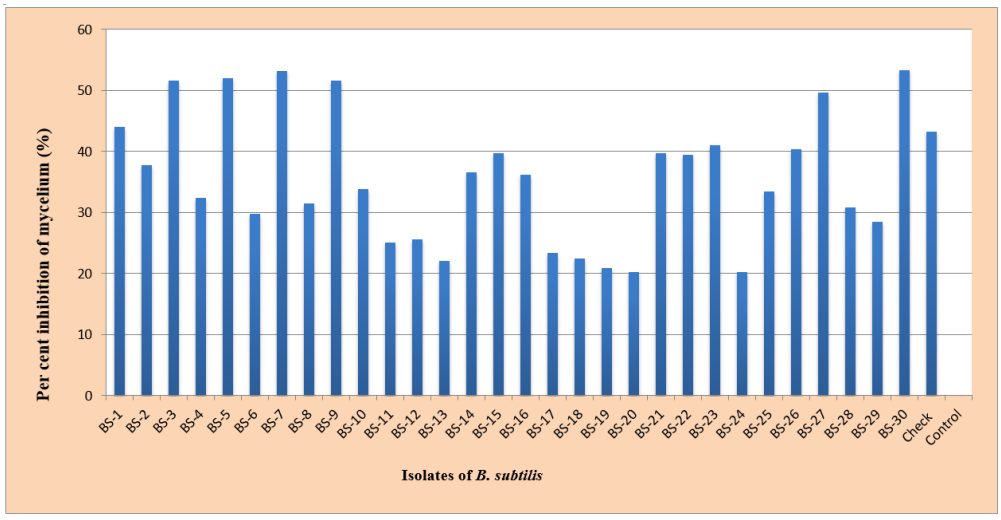

Fig 1: Bioefficacy of *B. subtilis* against *F. solani*, the causal agent of wilt of chilli

Conclusion

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.

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. 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.
3. 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.
4. Gerhardson B. Biological substitutes for pesticides: Trends in Biotechnology. 2002; 20:338-343.
5. Glick BR. The enhancement of plant growth promotion by free-living bacteria. Can. J Microbiol. 1995; 41:9-17.
6. 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.
7. 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.
8. Karimi K, Amini J, Harighi B, Bahramnejad B. Evaluation of biocontrol potential of *Pseudomonas* and *Bacillus* spp. against *Fusarium* wilt of chickpea. Auster. J Crop Sci. 2012; 6(4):695-703.
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 GC, Cook RJ. (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. Timmusk S, Grantcharova N, Wagner EGH. *Paenibacillus polymyxa* invades plant roots and forms biofilms. Appl. Environ. Microbiol. 2005; 11:7292-7300.
19. Vincent JM. Distortion of fungal hyphae in presence of certain inhibitors. Nature, 1927, 159:850.
20. 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.
21. Yahia AH, El Hassan SA, Ismail FK. Studies on damping off disease of tomato seedlings and its biological control. Mesapotamia J Agric. 1981; 16:115-125.