International Journal of Chemical Studies

P-ISSN: 2349–8528
E-ISSN: 2321–4902
IJCS 2018; 6(3): 3446-3449

Received: 18-03-2018
Accepted: 20-04-2018

Rajkumar K
Department of Plant Pathology, University of Agricultural Sciences, Raichur, India

Naik MK
Department of Plant Pathology, University of Agricultural and Horticultural Sciences, Shivamogga, India

Amaresh YS
Department of Plant Pathology, University of Agricultural Sciences, Raichur, India

Chennappa G
Department of Plant Pathology, University of Agricultural Sciences, Raichur, India

Ravikiran
Department of Plant Pathology, University of Agricultural Sciences, Raichur, India

Correspondence
Rajkumar K
Department of Plant Pathology, University of Agricultural Sciences, Raichur, India.

In vitro screening of *Bacillus subtilis* isolates against *Rhizoctonia solani*, the causal agent of root rot of chilli

Rajkumar K, Naik MK, Amaresh YS, Chennappa G and Ravikiran

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, green coloured colonies were grown on Hichrome *Bacillus* agar medium based on these tests isolates were confermed as *Bacillus subtilis*. All the isolates showed varied levels of antagonist activity *in vitro* against *Rhizoctonia solani*, the causal agent of root rot of chilli. Among different isolates, BS16 showed highest 46.58 per cent inhibition followed by BS 30 (44.97 %) and least inhibition of mycelial growth of 12.85 per cent was observed in case of BS17 compared to check isolate with 38.55 per cent inhibition of mycelial growth of *R. Solani*. The *B. subtilis* strains were isolated, identified and used as a promising natural bioagent in chilli disease management strategies.

Keywords: *B. subtilis*, Biocontrol, PGPR, Per cent inhibition, *R. 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) [3]. 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. Recently, a considerable attention has been given to some of the rhizobacteria which have positive influence on the plant growth and health. These are referred as Plant Growth Promoting Rhizobacteria (PGPR) (Schippers, 1992; Glick, 1995) [10, 4] such as *Azatobacter*, *Pseudomonas*, *Azospirullum*, *Bacillus* and *Brukholderia.* Among the PGPRs, the endospore forming, *Bacillus subtilis* is the one which plays a major role in plant growth promotion and biocontrol of pathogens (Glick, 1995) [4]. *B. subtilis* is a gram positive, rod shaped bacteria with peritrichous flagella (Nakano and Hulett, 1997) [8]. The colony morphology of the isolates exhibit a range from flat to filamentous or branching (Wafula *et al*., 2014), having either smooth or rough colony with colour ranging from white to cream. *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) [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
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) [9]. The culture was identified based on characters such as shape, texture of colony, colony morphology and colour of colony.

***In vitro* screening of *B. subtilis* against *Rhizoctonia solani*, the causal agent of root rot of chilli**

The isolates of *B. subtilis* were evaluated *in vitro* for their antagonistic properties against *Rhizoctonia solani***,** the causal agent of root rot 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 Potato Dextrose Agar (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) [11]. Per cent inhibition of growth of test pathogen was calculated using the following equation (Vincent, 1927) [12].

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

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

Results and Discussion

In vitro evaluation of *B. subtilis* isolates against *R. solani* was carried by using dual culture technique. All the 30 isolates exhibited the varying degrees of per cent inhibition of mycelial growth of *R. solani.* Among different isolates, BS16 showed highest 46.58 per cent inhibition followed by BS 30 (44.97 %) and least inhibition of mycelial growth of 12.85 per cent was observed in case of BS17 compared to check isolate with 38.55 per cent inhibition of mycelial growth of *R. solani* (Table 1). Among 30 isolates of *B. subtilis,* fourteen isolates were showed moderate performance and eighteen isolates showed low performance (Plate. 1) (Fig. 1). Matar *et al*. (2009) [6] *B. subtilis* (B7) showed effectiveness in reducing disease incidence and severity levels of tomato plants when added to the *F*. *oxysporum* and *R. solani* infested soil. Also, it stimulated the growth of tomato plants compared to the other. Anand *et al*. (2010) [1] studied the *in vitro* evaluation of *R. solani* (root rot of cotton) by using *P. fluorescens* and the results revealed that Pf4 showed maximum inhibition of mycelial growth of *R. solani* (84.37 %) and minimum inhibition was noticed in Pf12 (55.56 %). Majumdar *et al*. (2011) [5] isolated *B. subtilis* from rhizosphere soil of a healthy rice plant. *Bacillus* spp. KM 5 showed highest antagonist activity *in vitro* against *R. solani* causing sheath blight of rice. It has been observed that KM 5 was able to inhibit more than 80 per cent growth of *R. solani.* Ashwini and Shreevidya (2012) [2] reported that the strains of *Bacillus* spp. from 15 chilli rhizosphere soil were screened for chitinolysis on chitin amended plates and their involvement in the suppression of few pathogens was determined. The selected isolate showed broad spectrum antagonism against *Alternaria* spp. (55 %), *C. gloeosporioides* (57 %), *P. capsici* (62 %), *R. solani* (42 %), *F. solani* (42 %), *F. oxysporum* (40 %) and *Verticillium* spp. (36 %).

Table 1: *In vitro* bioefficacy of *B. subtilis* against *R. solani*, the causal agent of root rot of chilli.

Sl. No.	Isolates	Per cent Inhibition	Remarks
1	BS-1	29.71 (33.02) *	M
2	BS-2	18.87 (25.74)	L
3	BS-3	17.67 (24.84)	L
4	BS-4	14.05 (22.01)	L
5	BS-5	36.94 (37.42)	M
6	BS-6	25.30 (30.18)	M
7	BS-7	20.08 (26.61)	M
8	BS-8	23.29 (28.84)	M
9	BS-9	40.96 (39.78)	M
10	BS-10	27.71 (31.75)	M
11	BS-11	19.67 (26.32)	L
12	BS-12	17.67 (24.84)	L
13	BS-13	19.27 (26.03)	L
14	BS-14	24.89 (29.92)	M
15	BS-15	29.71 (33.02)	M
16	BS-16	46.58 (43.03)	H
17	BS-17	12.85 (20.99)	L
18	BS-18	16.86 (24.23)	L
19	BS-19	35.34 (36.46)	M
20	BS-20	32.53 (34.76)	M
21	BS-21	18.83 (25.78)	L
22	BS-22	14.05 (22.01)	L
23	BS-23	18.87 (25.74)	L
24	BS-24	15.66 (23.29)	L
25	BS-25	18.47 (25.44)	L
26	BS-26	14.85 (22.66)	L
27	BS-27	12.44 (20.65)	L
28	BS-28	18.47 (25.44)	L
29	BS-29	14.05 (22.01)	L
30	BS-30	44.97 (42.03)	M
31	Check	38.55 (38.43)	M
32	Control	0.00 (0.00)	L
	S.Em±	0.33	
	C.D at 1%	0.92	

>40%= High (H) = 0
20-40%=Moderate (M) =14
<20%=Low (L) =18
* Figures in the parentheses are arc sine values

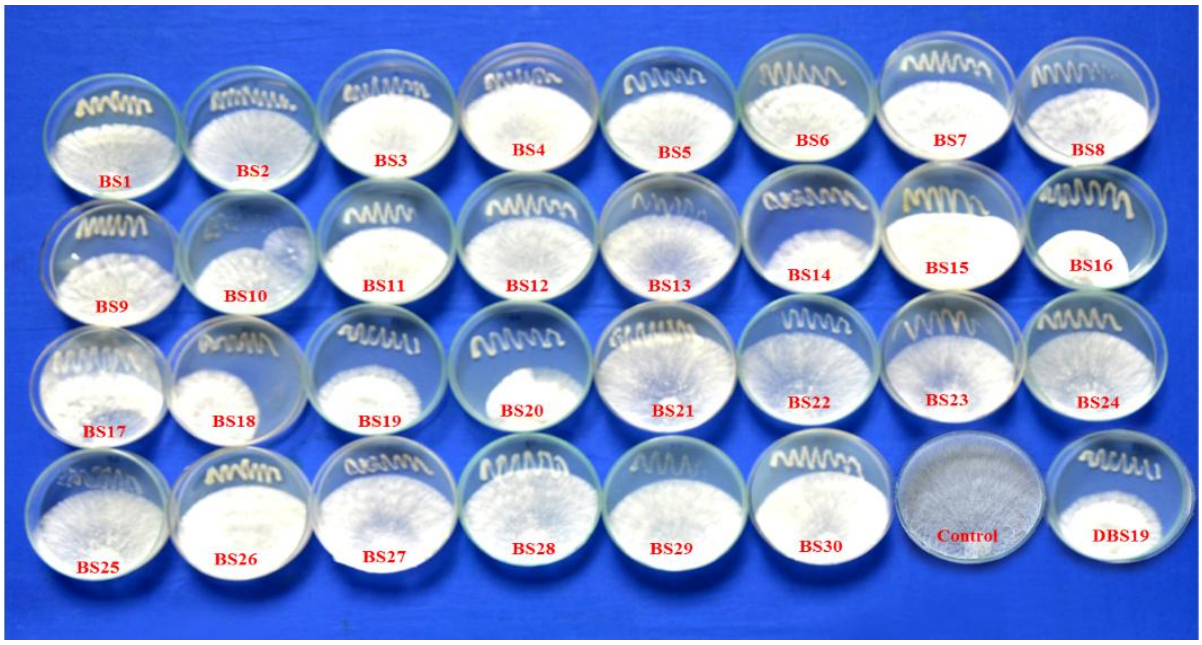

Plate 1: *In vitro* bioefficacy of *B. subtilis* against *R. solani*, the causal agent of root rot of chilli

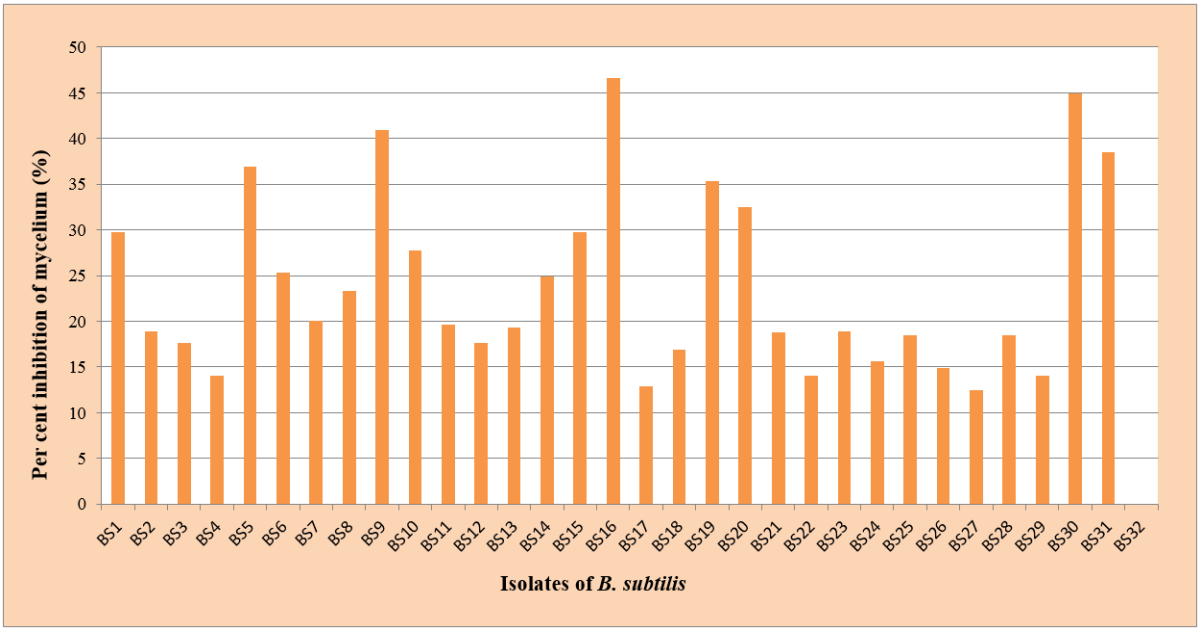

Fig 1: *In vitro* bioefficacy of *B. subtilis* against *R. solani*, the causal agent of root rot of chilli

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. Anand M, Naik MK, Ramegowda G, Devika Rani GS. Biocontrol and plant growth promotion activity of indigenous isolates of *Pseudomonas fluorescens*. *J Mycopathol. Res.* 2010; 48(1):45-50.
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. Gerhardson B. Biological substitutes for pesticides: *Trends in Biotechnology*. 2002; 20:338-343.
4. Glick BR. The enhancement of plant growth promotion by free-living bacteria. Can. J Microbiol. 1995; 41:9-17.
5. Majumdar K, Razdana M, Agarwala N, Muralib KK, Bhattacharyab RC, Durejaa P. Isolation and characterization of a potential biocontrol agent *Bacillus* KM5 from rhizosphere soil of a rice plant. *Archi. Phytopathol.* and *Pl. Prote.* 2011; 44(12):27-35.
6. Matar SM, Elkazzaz SA, Wagih EE, Eldiwany AI, Moustafa HE, AboZaid GA, AbdElsalam HE, Hafez EE. Antagonistic and inhibitory effect of *Bacillus subtilis* against certain plant pathogenic fungi, *Indian Biotechnol.* 2009; 8:53-61.
7. 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.
8. Nakano M, Hulett M. Adaptation of *Bacillus subtilis* to oxygen limitation. Microbiol. 1997; 157(1):1-7.

9. Pankaj Kumar, Dubey RC, Maheshwari DK. *Bacillus* strains isolated from rhizosphere showed plant growth promoting and antagonistic activity against phytopathogens. Asain J Boil. Life sci. 2012; 2(2):56- 67.
10. 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.
11. Sumana K, Devaki NS. *In vitro* evaluation of some bioagents against tobacco wilt. J Biopest. 2013; 5(1):18-22.
12. Vincent JM. Distortion of fungal hyphae in presence of certain inhibitors. *Nature* 1927; 159:850.