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Bioefficacy of *Bacillus subtilis* against Major Pathogen of Chilli *Colletotrichum capsici* Causing Fruit Rot of Chilli

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A B S T R A C T

Bacillus subtilis is one of the potential PGPR and biocontrol agent against several plant pathogens of important crop plants. In the present study thirty isolates of *B. subtilis* were isolated from different rhizosphere soil samples from different parts of North Eastern Karnataka region. All the rhizospheric 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 thirty isolates of *B. subtilis* were tested for their efficacy against *C. capsici* under *in vitro*. All the isolates showed the varied level of inhibition of mycelial growth of *C. capsici*. Among different isolates, BS16 showed maximum 63.42 per cent followed by BS 30 (57.40 %) inhibition and minimum was 11.98 per cent in case of BS 24 compared to check (56 %). Biological control is by using antimicrobial agents an attractive option which reduces the use of chemicals in the management of diseases of chilli.

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). Hence, a biocontrol 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. 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) 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). *B. subtilis* is a gram positive, rod shaped bacteria with peritrichous flagella

(Nakano and Hulett, 1997). 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. 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) which helps the organism to persist in the environment until conditions become favourable (Wafula *et al*., 2014). *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).

Plant growth promotion and bio control of plant pathogens by *Bacillus* spp. are achieved by antibiosis, competition, mycoparasitism (Korsten and De Jager, 1995) and induced systemic resistance in host plant (Lemessa and Zeller, 2007; Aliye *et al*., 2008; Ji *et al*., 2008). 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), anti microbial compounds such as HCN, H_2S and siderophore (Dinesh Singh *et al.*, 2012) and antibiotics such as subtilin, surfaction, iturin, biofilm, difficiden, bacilomycin, bacilycin and fengycin (Loeffler *et al*., 1990) 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).

However, the success of any biological control programme depends on our clear understanding about the biocontrol agent, their ecology, environments, antagonistic mechanisms and population dynamics in the 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

Collection and isolation of *B. subtilis* isolates

Thirty isolates of *B. subtilis* were collected from different rhizosphere soil samples of chilli, chickpea, cotton, groundnut, onion, marigold, mustard, niger, pegionpea, paddy, sorghum, sunflower and wheat crops of Bagalkot, Ballari, Raichur and Koppal parts of North Eastern Karnataka agro ecosystem by serial dilution and plate count technique on nutrient agar medium and designated as BS-1 to BS-30. An isolate collected from UAS, Dharwad (DBS-19) was used for comparison to assess the biocontrol efficacy and PGPR activity (Pankaj Kumar *et al*., 2012).

Bioefficacy of *B. subtilis* isolates against *Colletotrichum capsici*

The isolates of *B. subtilis* were screened *in vitro* for their antimicrobial properties against major pathogen of chilli *Colletotrichum capsici* causing fruit rot/ anthracnose 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). Per cent

inhibition of growth of test pathogen was calculated using the following equation (Vincent, 1927).

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

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

Results and Discussion

The bacterium was isolated from soil collected from the rhizosphere soil of different crops serial dilution and plate count technique. The culture was morphologically identified based on characters such as shape, texture of colony, colony morphology and colour of colony.

Thirty *B. subtilis* isolates were evaluated *in vitro* against *C. capsici*. All the isolates recorded the varied level of inhibition of mycelial growth of *C. capsici*. Among different isolates, BS16 showed maximum 63.42 per cent followed by BS 30 (57.40 %) inhibition and minimum was 11.98 per cent in case of BS 24 compared to check (56 %) (Table 1). Among 30 isolates of *B. subtilis*, ten isolates were high; eleven isolates were showed moderate performance and seven isolates showed low performance (Plate 1) (Fig. 1). Anand *et al.*, (2010) reported that a significantly highest inhibition of mycelial growth of *C. gloeosporiods* causal agent of anthracnose of pomegranate was noticed by Pf4 isolate (81.43%) and least inhibition was noticed in Pf8 (47.02%). The bacterial antagonists such as *B. subtilis* isolate (BSCB4) *P. fluorescens* isolate (ENPf1) and *P. chlororaphis* isolate (PA23) were found to be effective in inhibiting the mycelial growth of *C. cassicola* causing stem blight of *P. amarus* (Mathiyazhagan *et al*., 2003). Ashwini and Shrividya (2012) 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%).

Fig.1 *In vitro* bioefficacy of *B. subtilis* against *C. capsici*, the causal agent of anthracnose of chilli

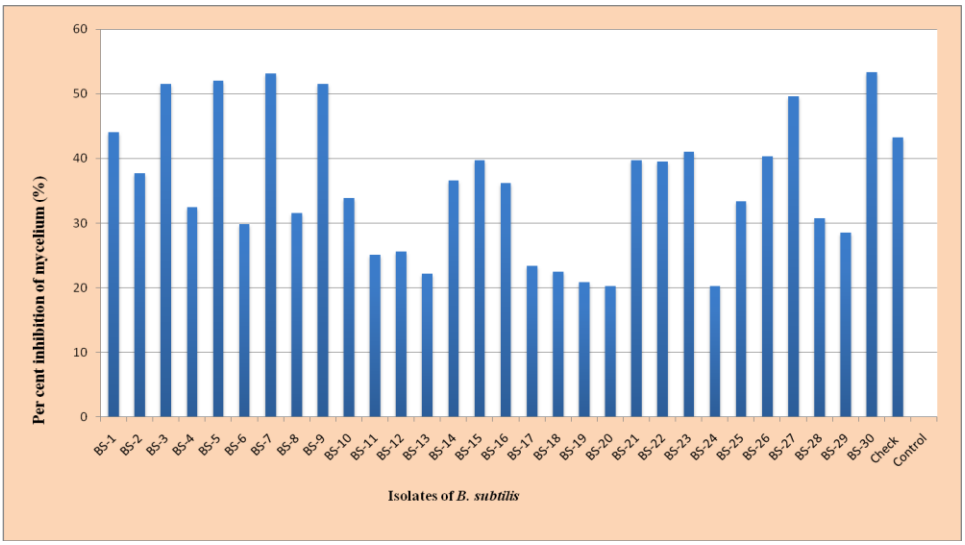

Plate.1 *In vitro* bioefficacy of *B. subtilis* isolates against *C. capcisi*, the causal agent of anthracnose of chilli

Table.1 *In vitro* bioefficacy of *B. subtilis* against *C. capsici*, the causal agent of anthracnose of chilli

Sl. No.	Isolates	Per cent Inhibition	Remarks
1	BS-1	40.82 (39.70)*	H
2	BS-2	27.34 (31.51)	M
3	BS-3	31.00 (33.00)	M
4	BS-4	40.44 (39.48)	H
5	BS-5	43.07 (41.00)	H
6	BS-6	11.98 (20.24)	L
7	BS-7	61.42 (51.58)	H
8	BS-8	31.08 (33.87)	M
9	BS-9	61.00 (51.00)	H
10	BS-10	18.72 (25.63)	L
11	BS-11	17.97(25.07)	L
12	BS-12	14.23 (22.15)	L
13	BS-13	35.34 (36.46)	M
14	BS-14	32.53 (34.76)	M
15	BS-15	18.83 (25.78)	L
16	BS-16	63.42 (52.77)	H
17	BS-17	14.05 (22.01)	L
18	BS-18	31.08 (33.87)	M
19	BS-19	61.20 (51.20)	H
20	BS-20	14.60 (22.45)	L
21	BS-21	12.73 (20.88)	L
22	BS-22	53.70 (47.11)	H
23	BS-23	26.21 (30.79)	M
24	BS-24	22.84 (28.54)	M
25	BS-25	18.87 (25.74)	L
26	BS-26	24.84 (32.54)	M
27	BS-27	56.48 (48.70)	H
28	BS-28	35.34 (36.46)	M
29	BS-29	32.08 (34.87)	M
30	BS-30	57.40 (49.24)	H
32	Check	56. 00 (48.00)	H
32	Control	00.00 (0.00)	L
	S.Em ±	**0.32**	
	C.D at 1 %	**0.91**	

>40%= High (H) = 11; 20-40%=Moderate (M =11

<20%=Low (L) =10; *Figures in the parentheses are arc sine values

In conclusion, *B. subtilis* exhibited sufficient antibiosis capability due to its good inhibitory performance against *C. capsici*. *B. subtilis* strains with good antimicrobial properties have been used 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.

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