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In vitro Screening of *Bacillus subtilis* Isolates against *Sclerotium rolfsii* Cause for Collar Rot of Chilli

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A B S T R A C T

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. Thirty *B. subtilis* isolates were screened *in vitro* against *S. rolfsii.* The isolates showed different levels of inhibition of mycelia growth of *S. rolfsii*. Among different isolates BS16 inhibited maximum mycelial growth 64.04 per cent followed by BS 30 (11.98 %) and minimum inhibition of mycelial growth was observed in case of BS17 (11.98 %) compared to check isolate with 47 per cent inhibition of mycelial growth of *S. rolfsii*. 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.

Introduction

Modern agriculture is highly dependent on the use of chemical pesticides to control plant pathogens. Fungicides and fumigants commonly have drastic effects on the soil biota, as they are intentionally applied at much higher rates than herbicides and insecticides (Fraser, 1994). These methods pollute the atmosphere, and are environmentally harmful, as the chemicals build up in the soil (Nannipieri , 1994). Furthermore, the repeated use of such chemicals has encouraged the development of resistance among the target organisms (Goldman *et al.,* 1994). Therefore, control of plant pathogens using microbial bioinoculants has been considered as a potential control strategy in recent years. For instance, integration of biocontrol agents with reduced doses of chemical agents has the

potential to control plant pathogens with minimal impact on the environment (Chet and Inbar. 1994). Therefore, search for these biological agents is increasing.

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. 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).

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, 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

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).

In vitro screening of *Bacillus subtilis* isolates against *Sclerotium rolfsii*

The isolates of *B. subtilis* were evaluated *in vitro* for their antagonistic properties against major pathogens of chilli *Sclerotium rolfsii*, 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 screened *in vitro* against *S. rolfsii*. The isolates showed different levels of inhibition of mycelia growth of *S. rolfsii*. Among different isolates BS16 inhibited maximum mycelial growth 64.04 per cent followed by BS 30 (11.98 %) and minimum inhibition of mycelial growth was observed in case of BS17 (11.98 %) compared to check isolate with 47 per cent inhibition of mycelial growth of *S. rolfsii* (Table 1). Among 30 isolates of *B. subtilis*, fifteen isolates were high, eight isolates were showed moderate performance and seven isolates showed low performance (Plate 1) (Fig. 1). Bhatia *et al.*, (2005) reported ten isolates of fluorescent *Pseudomonas*, effectively involved in suppression of *S. rolfsii*.

Fig.1 *In vitro* bioefficacy of *B. subtilis* against *S. rolfsii*, the causal agent of collar rot of chilli

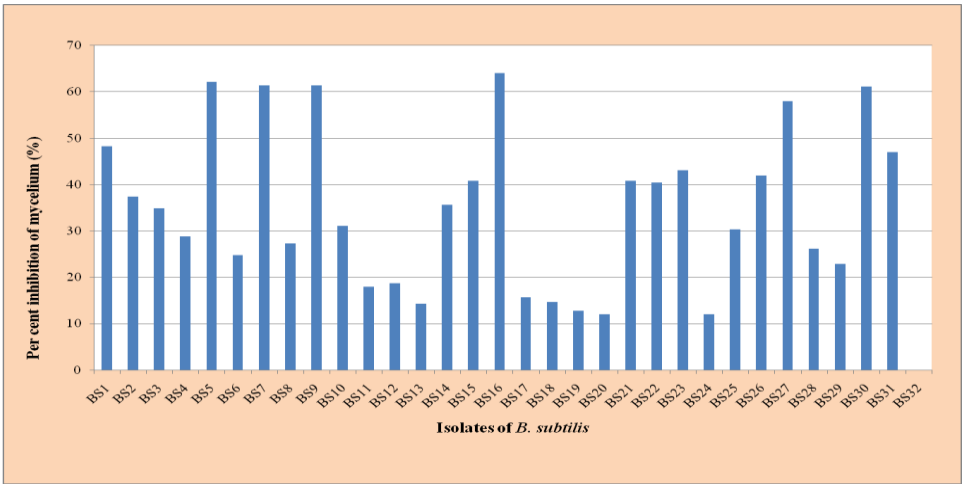

Plate.1 *In vitro* bioefficacy of *B. subtilis* isolates against *S. rolfsii*, the causal agent of collar rot of chill

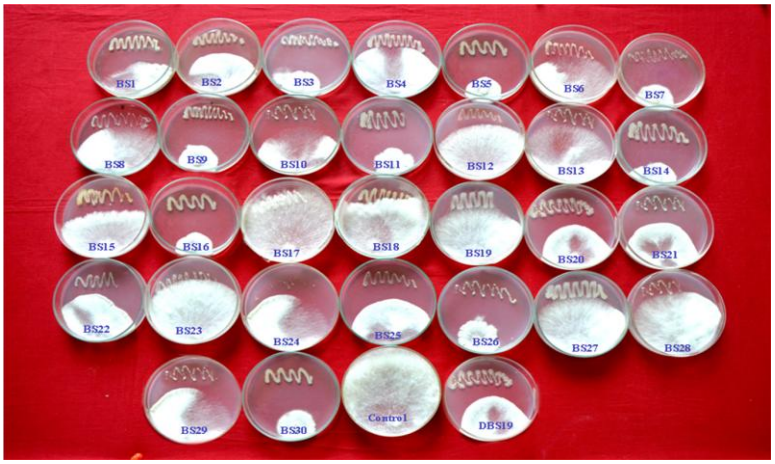

Table.1 *In vitro* bioefficacy of *B. subtilis* against *S. rolfsii*, the causal agent of collar rot of chilli

Sl. No.	Isolates	Per cent Inhibition	Remarks
1	BS-1	48.31(44.02)*	H
2	BS-2	37.45(37.72)	M
3	BS-3	34.83 (36.15)	M
4	BS-4	28.83(32.47)	M
5	BS-5	62.17 (52.02)	H
6	BS-6	24.71 (29.80)	M
7	BS-7	61.42 (51.58)	H
8	BS-8	27.34 (31.51)	M
9	BS-9	61.42 (51.58)	H
10	BS-10	31.08 (33.87)	M
11	BS-11	17.97(25.07)	L
12	BS-12	18.72 (25.63)	L
13	BS-13	14.23 (22.15)	L
14	BS-14	40.82 (39.70)	H
15	BS-15	35.58 (36.60)	M
16	BS-16	64.04 (53.14)	H
17	BS-17	11.98 (20.24)	L
18	BS-18	14.60 (22.45)	L
19	BS-19	12.73 (20.88)	L
20	BS-20	40.82 (39.70)	H
21	BS-21	40.44 (39.48)	H
22	BS-22	43.07 (41.00)	H
23	BS-23	11.98 (20.24)	L
24	BS-24	30.33 (33.41)	M
25	BS-25	41.94 (40.35)	H
26	BS-26	58.05 (49.61)	H
27	BS-27	26.21 (30.79)	M
28	BS-28	22.84 (28.54)	M
29	BS-29	15.73 (23.35)	L
30	BS-30	61.20 (51.20)	H
32	check	47 (43.26)	H
32	Control	00.00 (0.00)	L
	S.Em±	**0.34**	
	C.D at 1%	**0.95**	

>40%= High (H) =16; 20-40%=Moderate (M) = 8; <20%=Low (L) =8; *Figures in the parentheses are arc sine values

In conclusion, *B. subtilis* is the bioagent in the control of *Sclerotium rolfsii* (collar rot of chilli), recorded sufficient antagonism due to its good inhibitory performance. It can be considered as the one of the component of chilli disease management strategy.

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