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Invitro Bioefficacy of *B. subtilis* Isolates against *Ralstonia solanacearum* Causing Bacterial Wilt of Brinjal

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A B S T R A C T

Bacterial wilt caused by *Ralstonia solanacearum*, a genetically diverse soil-borne pathogen with a wide host range, is a devastating plant vascular disease (Hayward, 1991). No effective chemical product is available for Ralstonia-induced wilt. Alternative methods such as biological control agents (BCAs) have shown effectiveness. *Bacillus subtilis* is a non-pathogenic bacterium that lives in soil, often in association with roots of higher plants and it also produces a variety of biologically active compounds with a broad spectrum of activities towards phytopathogens and that are able to induce host systemic resistance. Thirty *Bacillus subtilis* isolates were isolated from rhizospheric soil of healthy brinjal plants from Hyderabad Karnataka region. These isolates were evaluated by screening their antagonistic ability to reduce incidence of bacterial wilt of brinjal caused by *Ralstonia solanacearum in vitro*. All the isolates showed the varied level of inhibition of *Ralstonia solanacearum*. Among different isolates, BS6 showed highest inhibition zone of 19.5mm diameter followed by BS-10 showed 18.16 mm and least zone of inhibition was produced by an isolate BS-11 of 5.5 mm diameter.

Introduction

Soil has enormous potential of antagonistic microorganisms, which are beneficial in reducing the pathogen population through different modes of action. Certain bacterial species associated with or livings in the rhizosphere improve the plant growth and were termed as plant growth-promoting rhizobacteria (PGPR) by Kloepper *et al.*, (1980). Among the PGPR microbes, *B. subtilis* is the one which plays a major role in plant growth promotion (Glick, 1995) and biocontrol of plant pathogens. *Bacillus subtilis* is a gram positive, rod shaped having 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 the temperature between 25 to 35 °C commonly associated with soil. It is an endospore forming bacteria (Piggot and Hilbert, 2004) which support to withstand extreme temperatures as well as dry environments. This endospore helps the organism to persist in the environment until conditions become favorable (Wafula *et al*., 2014). *Bacillus subtilis* (Cohn, 1872), is an adept rhizobacterium and has gained global attention as a biopesticide (Edgecomb and Manker, 2006) for the control of several plant diseases. One of the major factors limiting the cultivation of brinjal crop is the incidence of bacterial wilt caused by *Ralstonia solanacearum*. The soil-borne pathogen causes substantial economic loss to the crops.

Bacterial wilt of brinjal and other solanaceous vegetables caused by *Ralstonia solanacearum* (Smith) Yabuchi *et al*., (1995) is a devastating disease of crops (Hayward, 1991). It occurs widely in tropical and subtropical regions of the world (Kelman, 1998) causing severe losses in yield. The disease affects crops such as tomato, eggplant, potato, tobacco and pepper as well as other important crops like banana, peanut and ginger

Ralstonia solanacearum is an aerobic non-sporeforming, rod shaped, non-capsulated, Gram-negative, plant pathogenic bacterium. The organism is motile with tuft of polar flagella. It colonises the xylem tissues, causing bacterial wilt in wide range of potential crop plants. Bacterial wilt of tomato, pepper, eggplant and Irish potato diseases, first time proved to be invaded by Erwin Frank Smith. They appeared dull white with light pink colored center on TZC medium and the colonies were highly fluidal producing copious slime. The bacterium was positive for nitrate reduction test and negative for starch hydrolysis and gelatin liquification. Because of its devastating lethality, *R. solanacearum* is now more intensively studied phytopathogenic bacterium and bacterial wilt of tomato is a model system for investigating the mechanisms of pathogenesis. *Ralstonia* is synonymous to *Pseudomonas* with a similarity in most of the aspects except, it does not produce fluorescent pigment. It belongs to the Kingdom: Bacteria; Phylum: Proteobacteria; Class: Beta-Proteobacteria Order: Burkholderiales; Family: Burkholderiaceae and Genus: *Ralstonia*. With this background information, the present investigation was undertaken to study the *in vitro* bioefficacy of *B. subtilis* isolates against *Ralstonia solanacearum* causing bacterial wilt of brinjal

Materials and Methods

Collection and isolation of *B. subtilis* isolates

The *Bacillus subtilis* were isolated from the soil sample collected during survey from rhizosphere of brinjal from five different districts *viz.*, Raichur, Kalburgi, Yadgir, Bidar, Koppal of Hyderabad Karnataka region. Isolation of *Bacillus subtilis* was carried out by serial dilution and plate count technique on nutrient agar medium and isolates obtained were designated as BS-1 to BS-30. Thereafter *In vitro* experiments to assess antagonistic effect of *B. subtilis* isolates against *Ralstonia solanacearum* were also conducted.

Bioefficacy of *B. subtilis* isolates against *Ralstonia solanacearum*

The thirty isolates of *B. subtilis* were evaluated for their ability to inhibit the growth of *R. solanacearum* by following the dual culture assay (Ganesan and Gnanamanickum, 1987). A luxuriant lawn of *R. solanacearum*

was prepared on nutrient agar plates by spreading 1000 µl of 24hrs old *R. solanacearum* multiplied in nutrient broth. Ten µl of each isolate of *B. subtilis* grown in nutrient broth overnight was spotted on to the lawn of pathogen. The inoculated plates were incubated at 30 ˚C for 48 hours. Three replications were maintained for each treatment. Observations were recorded for the zone of inhibition produced by an antagonist around the growth of the pathogen. Control was maintained without inoculating antagonist.

Results and Discussion

Each of the thirty *Bacillus subtilis* isolates were tested for inhibition of *R. solanacearum* by dual culture technique on nutrient agar medium. The isolates exhibited great variation in inhibition of the pathogen, ranging from 5.5 mm to 19.5 mm diameter. The isolate BS-6 produced the highest inhibition zone of 19.5mm diameter, which significantly inhibited the pathogen and that was followed by BS-10 (18.16 mm), BS-3 (17.83 mm), BS-4 (17.16 mm), BS-29 (17.16 mm), BS-26 (17.16 mm) and BS-15 (16.00 mm) and least zone of inhibition was produced by an isolate BS-11 of 5.5 mm diameter. Out of 30 *B. subtilis* isolates tested, 8 isolates produced a zone of inhibition of ≥15 mm diameter. Furthermore, remaining 22 isolates indicated a zone of inhibition of <15 mm diameter (Table 1). There was a significant difference in the formation of inhibition zone by the isolates of *B. subtilis* against *R. solanacearum* (Plate 1). Further, irrespective of isolates, 48 h old culture of *B. subtilis* produced more zone of inhibition than 24 h old culture probably due to the secretion of ample quantity of antibiotics due to the prolonged incubation. Growth inhibition of pathogen may be due to the secondary metabolites specially the antibiotics secreted by the bacterial biocontrol agents on the agar media, which have broad spectrum activity (Fig. 1).

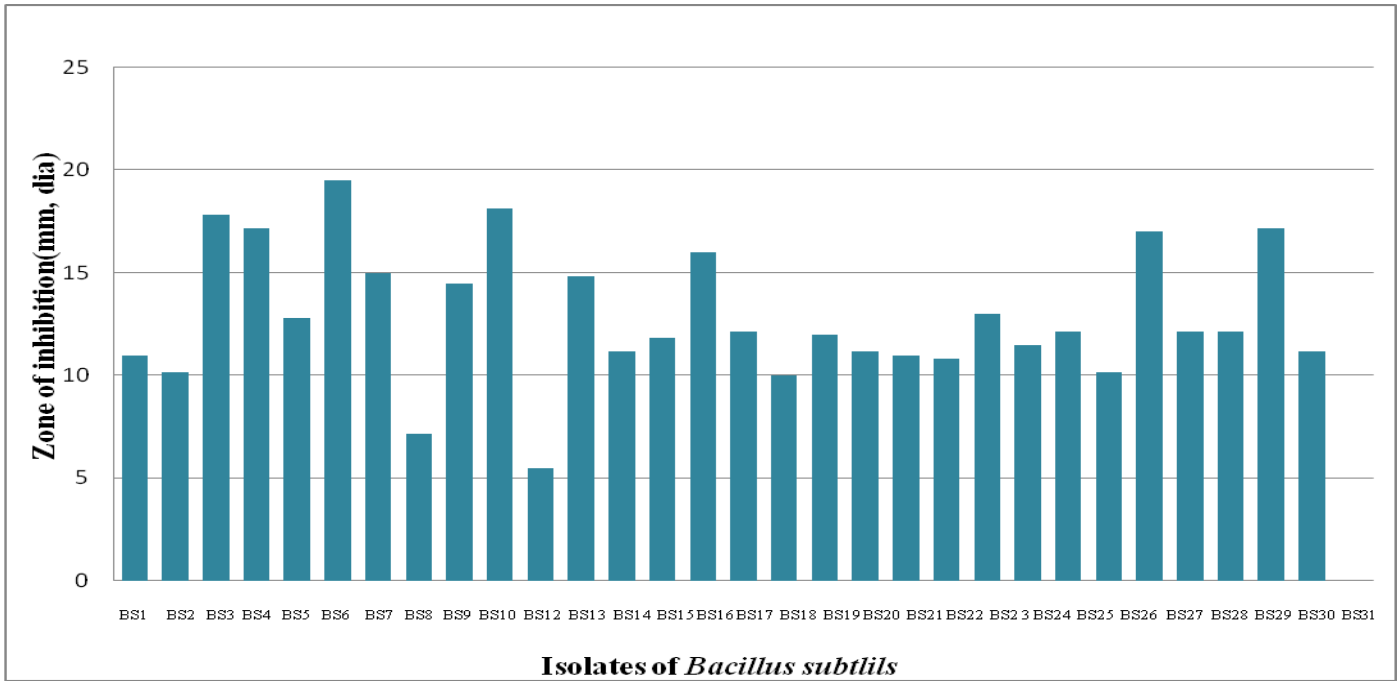

Plate.1 *Invitro* bioefficacy of *B. subtilis* isolates against *Ralstonia solanacearum*

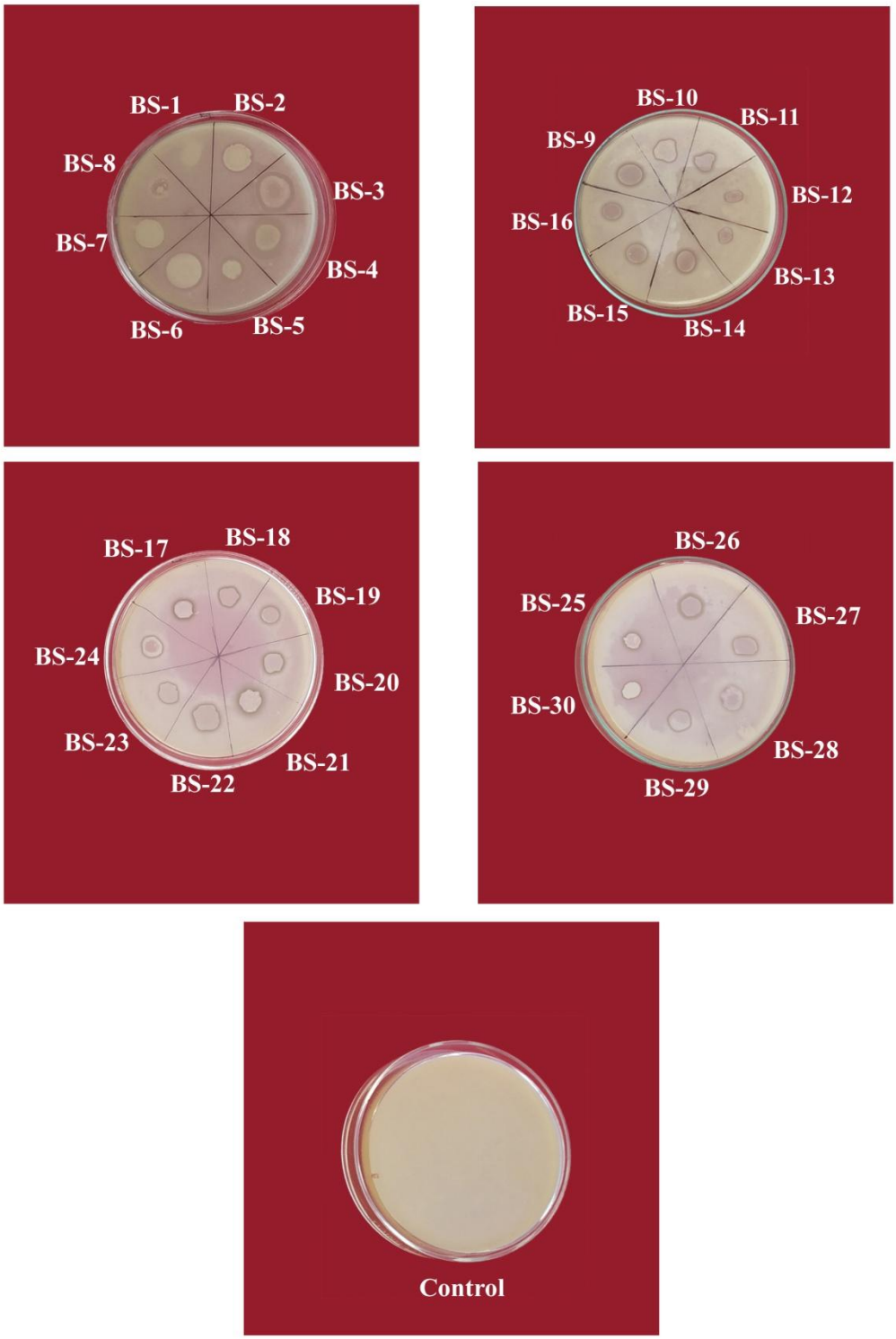

Fig.1 *Invitro* bioefficacy of *B. subtilis* isolates against *Ralstonia solanacearum*

Table.1 *Invitro* bioefficacy of *B. subtilis* isolates against *Ralstonia solanacearum*

Sl. No.	*B. subtilis* Isolates	Zone of inhibition (mm, dia)
1	BS-1	11.00
2	BS-2	10.16
3	BS-3	17.83
4	BS-4	17.16
5	BS-5	12.83
6	BS-6	19.50
7	BS-7	15.00
8	BS-8	07.16
9	BS-9	14.50
10	BS-10	18.16
11	BS-11	05.50
12	BS-12	14.83
13	BS-13	11.16
14	BS-14	11.83
15	BS-15	16.00
16	BS-16	12.16
17	BS-17	10.00
18	BS-18	12.00
19	BS-19	11.16
20	BS-20	11.00
21	BS-21	10.83
22	BS-22	13.00
23	BS-23	11.50
24	BS-24	12.16
25	BS-25	10.16
26	BS-26	17.00
27	BS-27	12.16
28	BS-28	12.16
29	BS-29	17.16
30	BS-30	11.16
31	Control	00.00
S.Em±	**-**	**0.48**
C.D at 1%	**-**	**1.82**

Chen *et al.,* (2013) screened 60 strains of *B. subtilis* obtained from the rhizosphere soil of tomato at various locations across China against *R. solanacearum* (bacterial wilt of tomato) Of these, six strains exhibited 50 per cent inhibition of radial growth of *R. solanacearum* under *in vitro* conditions. Antagonistic activity of *P. fluorescens, P. putida, B. subtilis* and *Enterobacter aerogenes* both by *in vitro* and *in vivo*

conditions against *R. solanacearum* was performed by Seleim *et al.*, (2011) and they reported the highest bacterial wilt reduction by *P. putida* followed by *B. subtilis*. Basha *et al.*, (2017), tested the antagonistic efficiency of bacterium *P. fluorescens*, *P. aeroginosa*, *B. subtilis* and *B. megatherium* against *R. solanacearum* causing bacterial wilt of tomato by *in vitro* and found that *B. subtilis* was most effective in inhibiting the growth of the pathogen followed by *P. fluorescens*, *B. megatherium* and *P. aeroginosa* were found to be least effective.

In conclusion, *B. subtilis* exhibited sufficient antibiosis capability due to its good inhibitory performance against *Ralstonia solanacearum*. *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 diseases and great yield losses.

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