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Root priming with *Bacillus* spp. against bacterial wilt disease of tomato caused by *Ralstonia solanacearum*

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ABSTRACT

Bacillus spp. have long been used as biological control organisms against plant bacterial diseases but the mechanisms by which the bacteria confer protection against the pathogens are not properly understood. Among nine strains of *Bacillus* spp. three of them, viz. NBAII 63 (*B. megaterium*), NBAII 71 (*B. cereus*) and NBAII 65 (*B. megaterium*) were found highly inhibitory against *R. solanacearum*. These strains of *Bacillus* spp. produced indole acetic acid (IAA) and siderophore and solubilized the phosphorous. High amount of IAA (174.2 µg/ml) and siderophore (1.32 µg/ml) production followed by the highest phosphorous solubilization (53.3 µg/ml) by the strain NBAII 63 were found. These three potential *Bacillus* strains showed the increased activity of defense related enzymes, viz. peroxidase (PO), polyphenoloxidase (PPO), phenylalanine ammonia-lyase (PAL) and total phenols in pre treated tomato plants challenged with *R. solanacearum*. Significant activities of PO, PPO, PAL were observed at 8 days after the treatment of antagonist and declined gradually afterwards. The maximum phenol content (185 µg/g of plant tissue) was observed in the tomato plants whose roots were treated with *Bacillus* strain 63 at 8 days after inoculation treatment. These *Bacillus* strains could be used as potential biocontrol agent for the management of bacterial wilt disease of tomato.

Key words: *Bacillus* spp., Bacterial wilt, *Ralstonia solanacearum*, Root dipping, Tomato

Bacterial wilt disease caused by *Ralstonia solanacearum* is one of the most devastating soil borne disease of South East Asia (Burgess *et al.* 2008), limiting the production of solanaceous vegetable crops, and affects more than 450 plant species across the world. There is a typical browning of vascular tissues in roots, stems and tubers. In extreme cases the loss in yield due to the disease in egg plant and tomato has been reported to be as high as 80 and 90 per cent respectively (Sivakumar *et al*. 2011). As the disease is widely distributed, it has a wide host range and is mainly soil borne; it is difficult to control with chemicals and cultural practices (Grimault *et al*. 1993). Plant growth-promoting rhizobacteria (PGPR) improve plant health through mechanisms like antagonism effect against plant pathogens, improving host nutrition and stimulating plant host defense mechanisms (Choudhary and Johri 2009). Plant possess a range of active defense compounds which act against invading pathogens and utilization of plant's own defense mechanism is the subject of current interest in management of plant diseases. Induced systemic resistance is the enhancement of the plant's defense response by plant growth promoting rhizobacteria (PGPR) and systemic acquired resistance (SAR) is the defense response of plant against pathogen attack and other elicitors (Choudhary *et al.* 2007). Many *Bacillus* species like *B. amyloliquefaciens, B. subtilis, B. pasteurii, B. cereus*, etc. can also induce defense response and reduce disease incidence in different host-pathogen combinations (Kloepper *et al*. 2004). These bacteria can activate plant's defence mechanisms by enhancing the levels of defense related enzymes like peroxidase (PO), polyphenol oxidase (PPO), phenylalanine ammonia-lyase (PAL) and phenolic compounds and makes the plant resistant to pathogen. Phenols have been known to occur in all plants investigated so far. Some of them occur constitutively, whereas others are formed in response to pathogen ingress and associated as part of an active defense response in the host (Nicholson *et al.* 1992, Lattanzio *et al.* 2006). The constitutive phenolics are known to confer resistance either directly or indirectly through activation of post infection responses in the hosts (Nicholson *et al.* 1992, Lattanzio *et al.* 2006). In the present study, the efficacy of root dipping of *Bacillus* strains were tested for their ability to induce defense related enzymes and phenolic content in tomato plants against bacterial wilt

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pathogen *Ralstonia solanacearum*.

MATERIALS AND METHODS

Isolation and characterization of Bacillus spp. from crop rhizosphere: Rhizosphere colonizing *Bacillus* spp. were isolated from tomato grown in Coimbatore, Hyderabad, Varanasi, Jorhat, Devikulam, Guntur, Bengaluru and Dindigul of India. Fresh roots (1 g) were suspended in 10 mL of sterile water and vortexed for five minutes. The suspension was held at 75^0C for 20 min to kill all vegetative microbial cells. The suspension was then plated on to Nutrient Agar plates and incubated at 37 ^{0}C for 24 h. The growths of bacterial colonies were recorded. The bacterial colonies were characterized based on their colony morphology, gram reaction and endospore staining for presence of spores and also growth under aerobic and anaerobic conditions (Norris *et al*. 1981, Sneath 1986, Schaad *et al.* 2001). Molecular characterization was done using bacterial genomic DNA which was isolated (Graves and Swaminathan 1993, Wattiau *et al.* 2001) from cultures grown in nutrient broth 153 for 16 h at 28±2°C. Amplification of 16S rRNA gene was performed from the genomic DNA of bacteria using universal primers fD1 5′-GAGTTTGATCCTGGCTCA-3′ and rP2 5′-ACGGCTACCTTGTTACGACTT-3′. Each PCR mixture consisted of 0.25U of *Taq* DNA polymerase, 10X *Taq* buffer, 2.5mM of $MgCl_2$, 2.5mM of each of four dNTPs and 2µl each of forward and reverse primers in final volume of 50µl. PCR reactions were performed under the following conditions: 94°C for 3 min, 35 cycles of 94°C for 1 min, 48°C for 1 min and 72°C for 2 min, a final extension of 72°C for 10 min, and completion at 4°C. Purified PCR products were sequenced; homology search of the 16S rRNA sequences was done using the BLAST function of NCBI GenBank (Higgins *et al*. 1992). The nucleotide sequences of 16S rRNA were deposited in GenBank, NCBI and obtained the accession numbers of the nine *Bacillus* strains.

Isolation of pathogen and pathogenicity test: Bacterial wilt pathogen of brinjal, *R. solanacearum* was isolated from infected tissue of tomato plants using triphenyl-tetrazolium chloride (TTC) medium (Kelman 1954). The collar portion of infected plants were cut into two or three pieces and put into sterile water. The bacterial ooze coming out of the cut end was streaked on to TTC medium. The growth of the pathogen was observed after 48 hours. The pathogenicity of the bacterial isolates was confirmed by root dipped inoculation method (Winstead and Kelman 1952) on the 20-day-old tomato seedlings. The roots of the seedlings were dipped in 10^7 cfu/ml concentration of *R. solanacearum* suspension. Inoculated seedlings were transplanted in 10 cm pots filled with sterilized soil and placed at 30ºC in the green house. Symptom developments were observed at 14 days after inoculation. The inoculated brinjal plants were showing drooping of the leaves followed by sudden wilting and complete collapse of the plants.

Testing the antagonism of Bacillus spp. against R. solanacearum*:* Nine characterized strains of *Bacillus* spp. were screened against *R. solanacearum* under *in vitro* by filter disc method. *R. solanacearum* suspension was first inoculated on to Nutrient Agar plates by spread plate. *Bacillus* spp. was supplied as filter paper discs of 5 mm in diameter which were soaked with 20 µl of the antagonist suspension. The antagonistic culture suspension (10^8cfu/ml) was prepared from 48 h old grown culture. The discs were placed in the petriplates immediately after soaking. Inhibition zone around the filter discs was measured 48 h after incubation. The experiment was conducted using completely randomized design with four replications. A Control treatment was also maintained without the *Bacillus* strain.

Plant growth promotion properties of Bacillus spp.: The plant growth promoting traits, *viz*. phosphate solubilization, siderophore production and indole acetic acid (IAA) production by the promising *Bacillus* strains (NBAII 63, 65, 71) were measured. Quantitative estimation of phosphate solubilization was done as described by Mehta and Nautiyal (2001). Indole acetic acid production was assayed calorimetrically using ferric chloride-perchloric acid reagent (Vikram *et al*. 2007). Siderophore production was quantified using standard procedure described by Schwyn and Neilands (1987).

Evaluation of antagonistic and plant growth promoting abilities of Bacillus spp. under glasshouse condition: The growth promotion and antagonistic properties of three promising *Bacillus* strains were evaluated under glass house conditions during 2012-13 based on its performance under *in vitro* condition. Tomato seeds (cv: Pusa Ruby) were initially surface sterilized with 1% sodium hypochlorite followed by five washings with sterile water. Seeds were sown in the pots containing sterile potting mixture having river sand, soil and farmyard manure in the ratio of 1:1:1. The potting mixture was sterilized (121°C and 15psi) for 1 hr on two consecutive days. After 20 days, the seedlings were uprooted carefully from the pots and roots were dipped for 20 minutes in the aqueous suspension (10^8 cfu/ml) of *Bacillus* strains which were prepared from 24 h old pure culture grown on NA medium. The treated seedlings were transplanted (3 seedlings per pot) in the pots containing sterile potting mixture. Then the culture suspension of *R. solanacearum* (2×10^8 cfu/ml) was drenched evenly @ 30 ml/pot 2 days after transplanting. Five replications were maintained for each treatment and were arranged in completely randomized design. Tomato seedlings inoculated only with *R. solanacearum* was used as positive control. Wilt incidence was observed by counting the proportion of wilted plants in total plants per pot using 0-5 scale (Winstead and Kelman 1952) and calculated wilt incidence as mentioned by Schaad *et al*. (2001).

Analysis of defense-related enzymes and total phenols: The promising strains of *Bacillus* spp. was tested for their efficacy to induce defense related enzymes like peroxidase (PO), polyphenol oxidase (PPO), phenylalanine ammonia-lyase (PAL) and total phenolic compounds against bacterial wilt pathogen *R. solanacearum*. Shoot and leaf samples were collected at different time intervals (0, 2, 4, 6, 8, 10 and 12 days after pathogen inoculation) for enzyme assays.

One gram of sample from each treatment was homogenized with 2 ml of 0.1 M sodium phosphate buffer (pH 7.0) in a pre-chilled mortar and pestle under ice cold condition. The homogenate was centrifuged for 15 min at 10000 rpm. The supernatant was used as a crude enzyme extract for assaying peroxidase, polyphenol oxidase, phenylalanine ammonia lyase activity and 80% ethanol extracts were used for assaying total phenolic content (modified from Anand *et al*. 2010).

Assay of total phenols: An aliquot of 0.1 ml of ethanol extract was evaporated in hot water bath. After complete evaporation of ethanol, 6 ml water was added and shaken well before addition of 0.5ml Folin- Ciacalteau reagent (1 N). After 5 min, 2 ml of 20% sodium carbonate solution was added and incubated for 30 min in dark condition at room temperature. Absorbance was recorded at 660 nm in spectrophotometer and the phenol content in the sample was calculated using pyrocatechol as the standard. The quantity of total phenols was expressed in µg/g of fresh plant weight (Malik and Singh 1980).

Assay of peroxidase (PO): The reaction mixture consisted 100 µl enzyme extract, 1.5 ml of 0.05M pyrogallol in 0.1M sodium phosphate buffer (pH 6.5) and 0.5 ml of 1% hydrogen peroxide. Boiled enzyme preparation served as blank. The changes in absorbance at 420 nm were recorded at 30 sec interval for 3 min in spectrophotometer. The enzyme activity was expressed as change in the absorbance of the reaction mixture/min/g of fresh plant weight at 420 nm (Hammerschmidt *et al*. 1982).

Assay of polyphenol oxidase (PPO): Polyphenol oxidase activity was assayed by the change in color intensity of catechol oxidation products. The reaction mixture consisted of 100 µl of enzyme extract and 1.5 ml of 0.1 M sodium phosphate buffer (pH 7.0), the reaction started when 200µl of 0.01 M catechol was added. The change in the absorbance was recorded at 30 sec interval for 3 min at 495nm and the enzyme activity was expressed as changes in absorbance at 495 nm/min/g of fresh plant weight (Mayer *et al*. 1965).

Assay of phenyl alanine ammonia lyase (PAL): PAL activity assay was determined based on the production of trans-cinnamic acid. The reaction mixture consists of 0.4 ml of enzyme extract made up to 1.5 ml by addition of 0.1 M sodium borate buffer (pH 8.8) and 0.5 ml of 12m M L phenylalanine was added. The reaction mixture was incubated for 1 hr in light at 25°C and reaction was stopped by incubating at 47°C for 10 min. The absorbance was recorded at 290 nm and the amount of trans-cinnamic acid formed calculated using the extinction co-efficient of 9630/m. The enzyme activity was expressed as nmol transcinnamic acid/min/g of fresh plant weight (Dickerson *et al*. 1984).

RESULTS AND DISCUSSION

In vitro screening

All the nine strains of *Bacillus* spp. inhibited *R. solanacearum* and the inhibition zone ranged from 11 to 22mm. Among nine strains of *Bacillus* spp. three of them, viz. NBAII 63, 65 and 71 were found highly inhibitory against *R. solanacearum.* Highest inhibition zone (22 mm) was recorded in the *Bacillus* strain NBAII-63 which was collected from the soils of reserved forests of Western Ghats of Kerala, India followed by NBAII 71 (18 mm) and NBAII 65 (17 mm) (Table 1).

The *in vitro* tests on bacterial characterization revealed that all the strains of *Bacillus* were Gram-positive rod shaped aerobic endospore-forming bacteriam producing opaque colonies. Nine promising *Bacillus* isolates were characterized through 16S rDNA analysis and the accession numbers assigned (Table 1). Amplification of 16S rRNA gene by PCR resulted in a product approximately 1.5 kb in size for all the strains. Sequencing of the PCR product followed by BLAST searches revealed that the *Bacillus* spp. showed 99% similarity to the strains deposited in GenBank. The results of 16S rDNA analysis of *Bacillus* strains were consistent with the physiological characters of the isolates as reported in Bergey's Manual of Systematic Bacteriology (1986). The morphological, physiological characterization and BLAST searches revealed that all the bacterial isolates are *Bacillus* spp. All the isolates of *Bacillus* spp. produced indole acetic acid (IAA) at varying degrees from 130.6 to 174.2µg/ml. High amount of IAA (174.2 µg/ml) produced by *Bacillus* strain NBAII 63 (174.2 µg/ml) followed by NBAII 71 (169.5 µg/ml) and NBAII 65 (152.6 µg/ml). Siderophore an iron chelating agent was produced by all the nine strains of *Bacillus* spp. and highest producer was *Bacillus* strain 63 (1.32µg/ml) followed by the isolates NBAII 71(1.18 µg/ ml) and NBAII 65 (1.12 µg/ ml). High degree of variation in phosphorous solubilization ability is observed among the strains of *Bacillus* (variability data were provided in the table). The strain NBAII 63 solubilize the highest phosphorous (55.3 µg/ml) followed by NBAII 71 (52.1 µg/ml) and NBAII 63 (49.2 µg/ml). Variability data were provided in the Table 1.

All three *Bacillus* strains performed better in increasing the growth and reducing the wilt incidence under green house condition. Highest root length (8.2 cm), shoot length (51.2 cm), fresh shoot weight (42.4 g), fresh root weight (6.3 g), shoot dry weight (6.8 g) and root dry weight (2.9 g) and lowest wilt incidence (15.2%) were recorded when the seedlings were treated with the *Bacillus* strain NBAII 63 followed by the Strain 71 and 65 as compared to the pathogen *R. solanacearum* alone (Table 2). The highest reduction (74.75%) in wilt incidence was recorded in the strain 63 followed by 67.74% in the strain NBAII 71 and 61.46% in the strain NBAII 65. The highest wilt incidence 60.2% was recorded when the seedlings treated with pathogen *R. solanacearum* (Table 2).

Tomato plants showed increased activity of peroxidase (PO), polyphenol oxidase (PPO) and phenyl alanine ammonia lyase (PAL) up to 8 days of inoculation of *R. solanacearum*. The highest activity of peroxidise (1.97 changes in absorbance/min/g of tissue) was observed in tomato plants whose roots were treated with *Bacillus*

Table 1 *In vitro* evaluation of *Bacillus* sp. on plant growth promoting attributes of tomato.

Bacillus strains	Geographical location	Inhibition zone against *R. solanacearum* (mm)	Strain identity and gen bank accession		16S rRNA amplification	IAA production (µg/ ml)	Siderophore production (µg/ ml)	Phosphorus solubilization (µg/ ml)
NBAII 33	Coimbatore 11.0183° N, 76.9725° E	16±2.62	*Bacillus cereus*	HQ162491	+	140.9±2.95	1.12±0.82	45.6±3.19
NBAII 63	Devikulam 10.0626° N, 77.1040° E	22±2.64	*Bacillus megaterium*	HQ162492	+	174.2±3.32	1.32±0.62	55.3±2.85
NBAII 25	Varanasi 25.2800° N, 82.9600° E	13±2.64	*Bacillus subtilis*	HQ162493	+	139.1±3.85	0.91±0.41	40.2±3.95
NBAII 7	Guntur 16.3008° N, 80.4428° E	15±4.38	*Bacillus cereus*	HQ162494	+	135.7±2.93	1.11±0.71	44.4±4.09
NBAII 71	Bangalore 12.9667° N, 77.5667° E	18±3.80	*Bacillus cereus*	HQ162495	+	169.5±2.78	1.18±0.50	52.1±3.93
NBAII 65	Jorhat 26.7500° N, 94.2200° E	17±3.00	*Bacillus megaterium*	HQ162496	+	152.6±3.85	1.12±0.72	49.2±4.01
NBAII B3	Hyderabad 17.3700° N, 78.4800° E	12±2.17	*Bacillus flexus*	KF322121	+	135.1±2.57	0.89±0.26	27.5±3.98
NBAII B4	Dindigul 10.3500° N, 77.9500° E	13±1.90	*Bacillus cereus*	KF322122	+	137.1±3.00	0.90±0.40	39.2±4.47
NBAII B12	Ganganagar 13.0210° N, 77.5880° E	11±2.64	*Bacillus cereus*	KF322127	+	130.6±4.43	0.86±0.57	25.1±4.55
Control (*R. solanacearum* alone)		2±0.46						
SEM		0.21				0.99	0.05	0.59
CD (P=0.05)		0.95				2.11	0.19	1.99

Table 2 Evaluation of antagonistic *Bacillu*s spp. on growth and wilt incidence of tomato under glasshouse condition

Bacillus strains	Shoot length (cm)	Shoot fresh wt. (g)	Shoot dry wt. (g)	Root length (cm)	Root fresh wt. (g)	Root dry wt. (g)	Wilt incidence (%)	% wilt reduction over control
NBAII 71	45.2±3.46	37.2±3.05	5.2±2.52	7.5±2.02	5.9±1.59	2.2±1.81	19.4±3.68	67.74±4.91
NBAII 63	51.2±4.01	42.4±3.37	6.8±3.14	8.2±3.46	6.3±1.80	2.9±1.25	15.2±3.49	74.75±3.24
NBAII 65	42.3±3.27	35.3±3.89	4.1±2.30	6.2±2.79	5.2±1.86	1.9±1.00	23.2±2.48	61.46±2.72
Control (*R. solanacearum*)	35.2±1.30	39.1±2.30	3.1±0.92	4.7±0.93	4.1±0.74	1.2±0.33	60.2±1.48	
SEM	0.17	0.04	0.06	0.09	0.05	0.08	0.91	
CD (P=0.05)	0.51	0.12	0.19	0.24	0.16	0.17	2.11	

strain 63 at 8 days after inoculation as compared to strain NBAII 71 (1.62/min/g), NBAII 65 (1.53/min/g) and control (1.12/min/g) (Fig 1). There was a gradual decrease in the activity of all these three enzymes after 8 days of inoculation in all the strains including control. Tomato plants whose roots treated with *Bacillus* strain 63 showed the highest activity

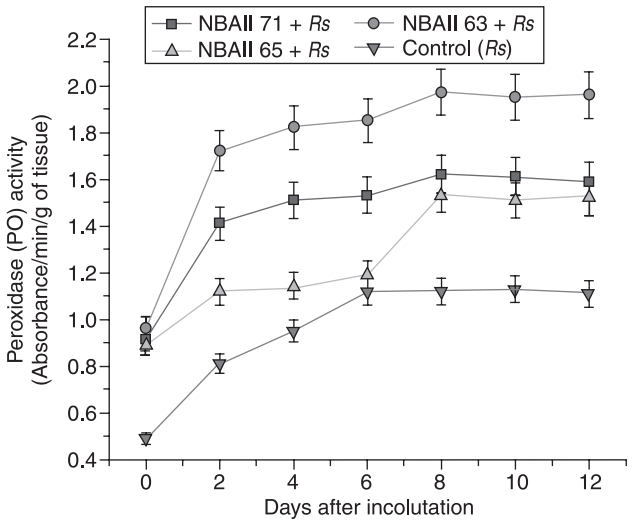

Fig 1 Peroxidase (PO) activity in tomato plants treated with *Bacillus* strains under glasshouse condition.

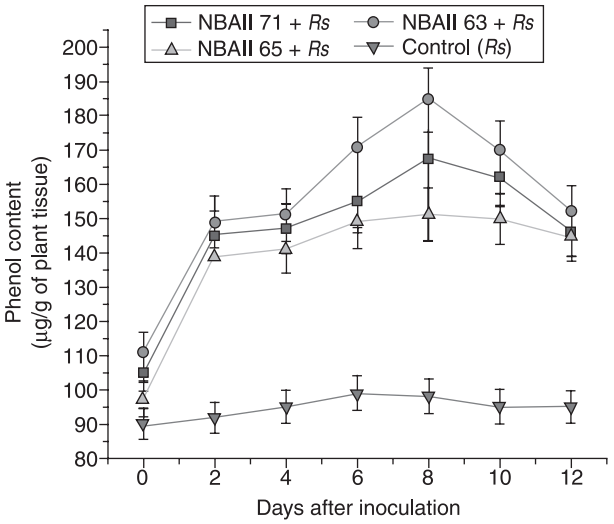

Fig 2 Phenol content in tomato plants treated with *Bacillus* spp. under glasshouse condition.

of PPO (0.94 changes in absorbance/min/g of tissue) at 8 days of inoculation as compared to strain NBAII 71 (0.72/ min/g), NBAII 65 (0.66/min/g) and control (0.19/ min/g). PAL activity was recorded as 103 nmol transcinnamic acid/ min/g of tissue at 0 day of inoculation in the case of NBAII 63 strain, and it was increased to 162 nmol/min/g at 8 days of inoculation in the same strain. In the case of strain 71 the activity was 149 at 8 days after inoculation as compared to strain 65 where it was 151 nmol/min/g and in the control the value was 99 nmol/min/g.

There was a significant increase in phenol content in the tomato plant roots dipped with strain 63 as compared to other strains and control (Fig 2). The maximum phenol content (185 µg/g of plant tissue was observed in the tomato plants whose roots were treated with *Bacillus* strain 63 at 8 days after inoculation as compared to strain NBAII 71 (167 µg/g), NBAII 65 (151 µg/g) and control (98 µg/g) (Fig 2).

Microorganisms that can grow and proliferate in the rhizosphere are ideal to use as biocontrol agents, since the rhizosphere provides the front-line defense against the attack by root pathogens. In this study, nine *Bacillus* spp. collected from the rhizosphere were evaluated for their plant growth promotion and disease suppression against *R. solanacearum in vitro*. The results revealed that all the *Bacillus* spp. inhibited the mycelial growth of *R. solanacearum* and the inhibition ranged from 11 to 22 mm. This inhibitory activity of *Bacillus* spp. was due to production of antifungal compounds or metabolites released into the growing medium (Dihazi *et al*. 2012). Iturin and surfactin are antimicrobial cyclic lipopeptides produced by antagonistic *Bacillus* spp. and the antimicrobial lipopeptide producing-*Bacillus* strain shows biological control activity against several kinds of plant diseases. Genetic diversity of iturin producing strains of *Bacillus* species antagonistic to *Ralstonia solanacerarum* causing bacterial wilt disease in tomato was studied in detail (Singh *et al.* 2013). In the present study, the rhizosphere bacteria belonging to genus *Bacillus* collected from various locations to find the potential strains suppressing the bacterial wilt and increasing the growth of plants. *Bacillus* spp. from the rhizosphere have been reported to be effective against a variety of soil borne pathogens and they are able to do this using diverse mechanisms (Choudhary and Johri 2009, Kloepper *et al.* 2004). The present study proved that three promising *Bacillus* strains, i.e NBAII 63 (*B. megaterium*), NBAII 71 (*B. cereus*), NBAII 65 (*B. megaterium*) were found effective in increasing the growth and reducing the wilt incidence to 15.2, 19.4 and 23.2% respectively from 60.2% (control) (Table 2). The reduction in wilt incidence for the three strains ranged from 61 to 75% (Table 2). All nine *Bacillus* strains have good phosphate solubilization ability and produced considerable quantity of siderophore and indole acetic acid which improved the growth of tomato plants helped to suppress the wilt incidence. These results are in accordance with the earlier studies reporting the antagonizing activity of *Bacillus* species against *R. solanacearum* (Li *et al*. 2008, Almoneafy *et al*. 2012).

Physiological changes in plants are induced and plant defense enzymes are triggered when they are invaded by pathogenic/antagonistic microorganisms. Induction of systemic resistance in plants by application of any antagonistic organism is thought to be the best alternative for protecting the plants from pathogens. This present study clearly proved that the *Bacillus* spp. induced the defense enzymes and phenols against the bacterial wilt pathogen *R. solanacearum*. Many studies have shown that members of bacterial genera can induce systemic resistance in different plants for control of soil-borne diseases (Nagorska *et al*. 2007). Some members of *Bacillus* genera are able to produce various lytic enzymes (e.g. chitinase and β-1, 3-glucanase) and antibiotics, along with induction of systemic resistance of plants, such as increasing the activities of plant defense related enzymes of peroxidase, polyphenol oxidase and phenylalanine ammonia lyase (Jayaraj *et al.* 2004). Oxidative

enzymes such as po and ppo, can catalyze the formation of lignin and other oxidative phenols, and contributes in formation of defense barriers by changing the cell structure and defense system get actuated against pathogens (Thilagavathi *et al*. 2007). These enzymes have been reported to correlate with the defense activities against pathogens in several plant species (Thilagavathi *et al*. 2007). Phenolics are toxic to the pathogens and they inhibit the growth of the pathogens. Increase in phenolics content has been found to be due to increase in synthesis of phenylalanine ammonia lyase in plants. This study proved that there was increase in the activity of peroxidise, polyphenol oxidase, phenylalanine ammonia lyase and phenol content in tomato plants whose roots dipped with the *Bacillus* spp. up to 8 days of inoculation. These results were in confirmation with the previous reports (Ramanujam *et al*. 2012, Nakkeeran *et al*. 2006) which revealed that the application of bacterial antagonists *P. fluorescens*, *Bacillus subtilis* increased the level of the defense enzymes peroxidase, polyphenol oxidase and phenylalanine ammonia lyase 3-4 days of inoculation of pathogen. Elicitation of induced systemic response by use of *Bacillus* strains has been documented on tomato and pepper against fungal and bacterial diseases (Akgul and Mirik 2008). In the present study it was seen that when roots of tomato seedlings were treated with *Bacillus* strains, an increased level of phenolic compounds was observed as compared to control. Present study indicated that *Bacillus* spp. increased the activity of phenolic enzymes and phenols in tomato plants against bacterial wilt pathogen. These bacterial strains could effectively be utilized for the management of wilt disease of tomato.

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