

## Isolation and Characterization of *Bacillus* sp with *In-vitro* Antagonistic Activity against *Fusarium oxysporum* from Rhizosphere of Tomato

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### ABSTRACT

The present investigation was carried out to isolate and characterize antagonistic bacteria against wilt causing fungal pathogen i.e. *Fusarium oxysporum*, from the rhizosphere of tomato. Fifty-six bacterial strains were isolated from the rhizosphere soil samples of healthy tomato fields, collected from different locations of Faridabad district, Haryana, India. Out of these, ten isolates were found to be antagonistic against the tested fungal pathogen i.e. *Fusarium oxysporum*, under *in-vitro* conditions. On the basis of percentage inhibition of radial growth of *Fusarium oxysporum*, isolate TNAM5 was found to be the most effective antagonistic rhizobacteria. Based on its morphological and biochemical properties along with 16s rRNA sequence analysis, it was identified as a *Bacillus* sp., with close nucleotide identity to *Bacillus subtilis* group. Average percentage inhibition given by this isolate was 47.77% and it was found to produce diffusible and volatile antifungal metabolites along with hydrogen cyanide and ammonia. Effect of physiological parameters on the growth and antagonistic behavior of the potential isolates was also examined. Optimal conditions for antagonistic activity were found to be 28°C and pH 6.5, while the maximum growth was observed at 35°C and pH 7.0. However, increase in salinity did not significantly affect ( $P > 0.05$ ) the antagonistic behavior or growth of the isolates and they were found to withstand NaCl concentration up to 8.0% (w/v). The present study, hence, provides a potential biocontrol agent for *Fusarium oxysporum*, however, field studies of this isolate as soil inoculant in tomato are required in order to establish its actual performance.

**Keywords:** Rhizobacteria, Biocontrol, Percentage inhibition, Tomato wilt.

### INTRODUCTION

Owing to their devastating effects on plant health and crop yield, plant pathogenic microbes impose a major threat to food production as well as ecosystem stability. So far, the use of chemical pesticides has remained the method of choice to control plant pathogens due to their proficient and consistent performance along with the ease of application. However, in the light of developing sustainable agricultural practices and increasing public awareness about the

ill-effects of agrochemicals, research directed towards the development of alternative and complementary pathogen control methodologies is highly required. Exploring the inherent inhibitory potential possessed by many microbes against the phytopathogens may prove to be the alternative and environment-friendly substitute of chemical pesticides. Biocontrol methods utilizing antagonistic microorganisms associated with the plant rhizosphere have great potential for control of soil borne plant pathogens. Huge volume

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of literature has been generated in the last few decades that reports the efficient use of rhizosphere microflora to control fungal pathogens in a variety of plants (Anjaiah *et al.*, 2006; Siddiqui and Akhtar, 2007; Sahu and Sindhu, 2011).

Tomato (*Solanum lycopersicum* L.) is one of the most important vegetable crops worldwide and constitutes 72% of the total fresh vegetables produced worldwide (Hanssen *et al.*, 2010). Fusarium wilt of tomato is an economically important and most widespread disease of tomato that is found worldwide (Ploetz, 2005). It is caused by a soil borne fungus, *Fusarium oxysporum* f. sp. *lycopersici* that affects the vascular system of plant and severely reduces the yield.

Currently used means of controlling *Fusarium oxysporum* include use of fungicidal chemicals and resistant cultivars, crop rotation with non-host varieties of the fungus, use of clean equipment, and raising beds to promote soil drainage and dry soil surface (Smith *et al.*, 1988). Tomato is the heaviest consumer of chemical fungicide like dithane M45 and dithiocarbamate in various African countries like Ghana (Ntow *et al.*, 2006); Tanzania (Ngowi *et al.*, 2007) and in Sub Saharan Africa (Matthews *et al.*, 2003).

The present study, therefore, aimed at isolation and characterization of antagonistic bacteria against wilt causing fungal pathogen *Fusarium oxysporum* from the rhizosphere of tomato, which can be further developed as biocontrol soil inoculum for tomato crop.

## MATERIALS AND METHODS

### Site Description

The study site is located at south-east of Haryana state in northern India and lies at 28° 25' 16" N latitude, 77° 18' 28" E longitude and an elevation of 198 meters. The soil used for this study was sandy loam.

### Sampling

Sampling was carried out during July-August 2011. Twenty-six samples of rhizosphere soil and the roots were randomly collected from tomato fields that had been cultivated with tomato for at least two consecutive years, at seven different villages of Faridabad district, Haryana, India. The selected plants were healthy and their age was less than thirty days. The samples were collected in plastic bags and stored at 4°C till further processing.

### Isolation of Rhizobacteria

Rhizobacteria were isolated from the collected soil samples according to the method of Bashan *et al.* (1993). Inoculation was carried out by spread plate method and the media used were King's B agar (selective media for *Pseudomonas*), glycerol-arginine agar (selective media for actinomycetes) and nutrient agar. Incubation was carried out at 30°C for 24 to 48 hours or more (one or more weeks) in case of glycerol-arginine agar plates. The isolated colonies of rhizobacterial strains were randomly selected and further purified by streaking. Pure isolates were maintained as glycerol stocks at -80°C for further use.

### Fungal Pathogen

The fungal pathogen used in this study i.e. *Fusarium oxysporum* (ITCC 4998) was obtained from ITCC, IARI, New Delhi, India.

### Screening of Rhizobacteria for Antagonism against *Fusarium oxysporum*

All the rhizobacterial isolates so obtained were evaluated for their antagonistic activity against mycelial growth of *Fusarium oxysporum* using dual culture technique (Gupta *et al.*, 2001). A 5 mm agar disc of a

five-day old culture of fungal pathogen was placed in the centre of potato dextrose agar (PDA) plates. Twenty four hour old culture of each isolate was streaked parallelly on either side of the fungal disc at a distance of 2 cm. The plates with only centrally placed fungal disc, but without bacterial streaks, served as the control. The inoculated plates were incubated at  $28\pm 2^{\circ}\text{C}$  for five days and inhibition of the radial growth of the pathogen was measured. Each treatment was replicated three times. Colony diameter of the fungal pathogen was measured and compared with the control. Percentage inhibition of the pathogen by the rhizobacterial strain over the control was calculated by using the formula given by Vincent (1947) as follows:

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

Where,  $I$  = Percent inhibition of mycelium;  
 $C$  = Growth of mycelium in the control,  $T$  = Growth of mycelium in the treatment.

#### Elucidation of Antagonistic Mechanism

Bacterial isolates showing antagonistic activities against tested pathogen i.e. *Fusarium oxysporum*, were further examined for elucidation of the possible mechanism underlying their antagonistic behaviour.

#### Hydrogen Cyanide (HCN) Production

HCN production by rhizobacterial isolates was tested according to the method described by Wei *et al.* (1991). Plates with Whatman No.1 filter paper pads inside their lids were poured with glycine supplemented ( $4.4 \text{ g l}^{-1}$ ) trypticase soy agar (TSA) medium and streak inoculated with twenty four hours old bacterial isolates. The filter paper padding was soaked with sterile picric acid solution and the lid was closed. Inoculated plates were sealed properly and were given an incubation of five days at  $30^{\circ}\text{C}$  and then observed for color change of the filter paper padding. Degree of HCN production was

evaluated according to the color change, ranging from yellow to dark brown.

#### Protease Production

Proteolytic activity was determined using skimmed milk agar (Kumar *et al.*, 2005). Overnight activated cultures were spot inoculated on skimmed milk agar plates and given an incubation of two days at  $30^{\circ}\text{C}$ . Afterwards, plates were observed for the formation of a clear zone around the bacterial growth, which indicated a positive proteolytic activity.

#### Production of Diffusible Antifungal Metabolites

The method described by Montealegre *et al.* (2003) was used to determine the production of diffusible antifungal metabolites by antagonistic rhizobacterial isolates. Overnight activated bacterial cultures were stab inoculated in the centre of PDA plates covered with a cellophane membrane and incubated at  $28^{\circ}\text{C}$  for 72 hours. Afterwards, the membrane with the bacterial growth was removed from the plate and it was inoculated with a 5 mm disk of the test fungus (pathogen) in the centre. Plates were further incubated at  $28^{\circ}\text{C}$  for 5 days and the growth of fungus was measured. Cellophane membrane covered PDA plates inoculated with sterile distilled water in place of bacteria and further inoculated with the test fungus served as the control. Colony diameter of the fungal pathogen was measured and compared with the control. Percentage inhibition of fungal growth was calculated and production of diffusible antifungal metabolites was recorded as nil, low, medium, and high.

#### Production of Volatile Antifungal Metabolites

Production of volatile metabolites having antagonistic activity against fungal pathogens was tested by paired plate



technique of Fiddaman and Rossall (1993) with some modifications. A petri plate containing nutrient agar medium was streak inoculated with a loopful of 48 hours old rhizobacterial isolate. A second petri plate containing PDA was inoculated with a 5 mm plug of the activated test fungus (pathogen) at the centre of the plate. Both half plates were sealed together and the paired plates were incubated at 28°C for five days. Control set of paired plates was designed with only the test fungus on PDA half plate inverted over unstreaked nutrient agar half plate. The experiment was conducted in triplicates. After incubation period the paired plates were observed for inhibition of fungal growth as compared to the control. Colony diameter of the fungus was measured and compared with the control set. Percentage inhibition of radial growth of the fungus was calculated as mentioned before and production of volatile antifungal metabolites was recorded as nil, low, medium, and high.

#### **Production of Ammonia**

Ammonia production was tested in peptone water. Freshly grown cultures were inoculated in 10 ml peptone water and incubated for 48-72 hours at 30 °C. Afterwards, 0.5 ml Nessler's reagent was added to each tube. Development of brown to yellow color was taken as a positive reaction for ammonia production (Cappuccino and Sherman, 2010).

#### **Identification of Antagonistic Rhizobacteria**

Isolated antagonistic strains were characterized on the basis of various morphological and biochemical features according to Bergey's manual of determinative bacteriology (Holt *et al.*, 1994) as per the standard procedures (Aneja, 2003; Cappuccino and Sherman, 2010). Further, the molecular characterization was

carried out for the best antagonistic isolate (in terms of percentage inhibition of mycelial growth) i.e. TNAM5 on the basis of the 16s rRNA sequencing by Macrogen Inc. Korea. The 16s rRNA region was sequenced using universal primers 518F and 800R and compared with sequences deposited at the National Center for Biotechnology Information (NCBI) using BLAST.

#### **Study of Physiological Parameters on Antagonistic Behavior of Rhizobacteria**

The antagonistic strains isolated against *Fusarium oxysporum* were further examined for determining the optimal physical conditions required for their growth and antagonistic activity. Three parameters were studied i.e. temperature, pH, and salinity. For temperature, three variants were used: 28, 35, and 42°C, five variants were tested for pH: 6.5, 7.0, 7.5, 8.0, and 8.5 and seven variants for salinity: sodium chloride (NaCl) concentration of the media at 0.5, 1.0, 1.5, 2.0, 4.0, 6.0 and 8.0% (w/v). Effect of these variables on the growth of antagonistic rhizobacterial isolates was determined in their respective isolation media (either of the two: King's B agar or nutrient agar). An aliquot of 500 µl of overnight activated antagonistic rhizobacterial cultures was inoculated in 10 ml of nutrient media maintained at different pH and NaCl concentrations and incubated for 48 hours at the above mentioned temperatures i.e. 28, 35, and 42°C, after which growth was estimated at 600nm and the corresponding cfu ml<sup>-1</sup>, after appropriate dilution of the culture.

Variation of antagonistic properties of the isolates with these physical parameters was examined using dual culture test in PDA plates as described earlier with similar variants of temperature, pH, and salinity as used for examination of the growth. Percentage inhibition of the radial growth of the fungus, at each variable, was recorded.

All the experiments were conducted in triplicates.

### Statistical Analysis

The data obtained in this study was subjected to analysis of variance (ANOVA) for a completely randomized design and the means were compared using post-hoc Tukey's HSD test with  $P < 0.05$  being accepted as significant.

## RESULTS

### Isolation and Selection of Antagonistic Rhizobacteria

A total of fifty six isolates were obtained from the rhizosphere of healthy tomato plants from different locations of Faridabad district, Haryana, India, out of which ten isolates showed *in-vitro* antagonistic potential against *Fusarium oxysporum* when tested on PDA plates using dual culture technique (Table 1). Average percentage

inhibition of the pathogen was observed as 29.54% and it varied significantly between the isolates ( $P < 0.05$ ). On the basis of percentage inhibition of the radial growth of the test pathogen, isolate TNAM5 was found to be the best antagonist (47.77% inhibition). Average percentage inhibition given by isolates of nutrient agar medium was almost 33%, while that of King's B agar medium was 26.19%.

### Identification of Antagonistic Rhizobacteria

Amongst the ten antagonistic strains, five were isolated on King's B agar while the other five, including the best antagonist TNAM5, were isolated on nutrient agar medium. All the strains isolated on nutrient agar medium were Gram-positive, spore forming, and motile rods. Further identification of the best isolate TNAM5 on the basis of 16s rRNA sequencing followed by BLAST showed that it had 100% nucleotide identity with seven strains of *Bacillus subtilis* i.e. KISR-1; CE1; SG05;

**Table 1.** *In-vitro* inhibition of *Fusarium oxysporum* by rhizobacterial isolates from tomato on the basis of dual culture technique <sup>a</sup>.

S. No.	Isolate	Radial growth (in mm)	Percentage inhibition of radial growth
1	TNAM5	47.00±0.45h	47.77±0.48a
2	TNAM27	55.03±0.45g	38.85±0.50b
3	TNAM4	57.07±0.11f	36.59±0.13c
4	TKB12	60.00±0.17e	33.33±0.19d
5	TKB6	65.03±0.42d	27.74±0.46e
6	TKB2	67.90±0.36c	24.56±0.40f
7	TNAM13	68.00±0.17c	24.44±0.19f
8	TKB9	69.13±0.32b	23.18±0.35g
9	TKB13	70.07±0.4b	22.15±0.44g
10	TNAM 7	74.93±0.11a	16.74±0.13h
	Mean percentage inhibition		29.54±9.40

<sup>a</sup> The values given are mean (n= 3) with standard deviation. Means in the same column followed by same letter are not significantly different at  $P < 0.05$  (Tukey's Honestly Significant Difference test).



318; K21; NBY44; TAT1-8, and *Bacillus* sp. EL31410, *Bacillus* sp. LS03 as well as *Bacillus tequilensis* KNUC9029. The next two best isolates i.e. TNAM27 and TNAM4 were also identified as belonging to *Bacillus* sp on the basis of their morphological and biochemical characteristics.

Five antagonistic strains, isolated on King's B agar were Gram-negative, rod shaped, motile, and oxidase positive and showed fluorescence when observed under UV light (366 nm). They were identified as *Pseudomonas* strains on the basis of morphological and biochemical characteristics.

### Elucidation of Antagonistic Mechanism

All the ten antagonistic isolates were found to produce more than one kind of antifungal compounds under *in-vitro* conditions (Table 2). All the ten antagonistic isolates produced ammonia, 80.0% of them produced HCN, while other diffusible metabolites and proteases were produced by 70.0% of the isolates. Production of volatile antifungal metabolites was observed for 60.0% of the isolates. Isolate TNAM5, TNAM27, and

TNAM4 were found to produce all the tested antifungal metabolites in medium to high range, except proteases. Isolate TNAM4 was observed as the strongest producer of HCN. None of the isolates demonstrated highly effective proteases against the tested fungal pathogen, however, weak to moderately effective proteases were produced by seven antagonistic isolates.

### Diffusible Antifungal Metabolites

As stated above, seven antagonistic isolates produced diffusible antifungal metabolites in PDA plates and the level of inhibition of the fungus under their effect varied significantly among the isolates ( $P < 0.05$ ). Isolate TNAM5 showed maximum inhibition of 47.78% due to diffusible antifungal metabolites, while isolate TKB12 showed the least inhibition of only 12.15%. Average percentage inhibition by all the seven isolates, against *Fusarium oxysporum*, was calculated as 26.37% (Table 3). Antagonistic isolates tested negative for the release of diffusible antifungal metabolites were TKB6, TKB13, and TNAM13.

**Table 2.** Production of various antifungal compounds by rhizobacterial isolates against *Fusarium oxysporum*<sup>a</sup>.

S. No.	Isolate	HCN production	NH <sub>3</sub> production	Protease production	Diffusible antifungal metabolites	Volatile antifungal metabolites
1	TKB2	+	+	+	+	-
2	TKB6	++	+	++	-	+
3	TKB9	+	+	++	+	+
4	TKB12	++	+	+	+	-
5	TKB13	-	+	+	-	+
6	TNAM4	+++	+	-	+	+
7	TNAM5	++	+	-	+	+
8	TNAM 7	-	+	+	+	-
9	TNAM13	++	+	+	-	-
10	TNAM27	++	+	-	+	+

<sup>a</sup> -: Nil; +: Low production; ++: Medium production, +++: High production.

**Table 3.** Inhibition of *Fusarium oxysporum* by diffusible antifungal metabolites produced by antagonistic rhizobacterial isolates<sup>a</sup>.

S. No.	Isolate	Radial growth (in mm)	Percentage inhibition of radial growth
1	TNAM5	47.00±0.20a	47.78±0.22a
2	TNAM27	55.37±0.32b	38.48±0.36b
3	TNAM4	60.23±0.25c	33.07±0.28c
4	TKB9	69.93±0.11d	22.30±0.13d
5	TNAM 7	74.43±0.4e	17.30±0.45e
6	TKB2	77.83±0.29f	13.52±0.32f
7	TKB12	79.07±0.11g	12.15±0.13g
Mean percentage inhibition			26.37±13.64

<sup>a</sup> The values given are mean (n= 3) with standard deviation. None of the means in the same column are similar at  $P < 0.05$  (Tukey's Honestly Significant Difference test). Isolate TKB6, TKB13 and TNAM13 were tested negative for production of diffusible antifungal metabolites.

### Volatile Antifungal Metabolites

Six antagonistic isolates produced volatile antifungal compounds against *Fusarium oxysporum* with significantly varied level of antagonistic potential ( $P < 0.05$ ). An average percentage inhibition of 31.21% was observed. Isolate TNAM5 gave maximum inhibition i.e. 48.59%, while isolate TKB9 showed the least percentage inhibition of 21.48% (Table 4). Isolate TKB2, TKB12, TNAM7, and TNAM13 were tested negative for production of volatile antifungal metabolites.

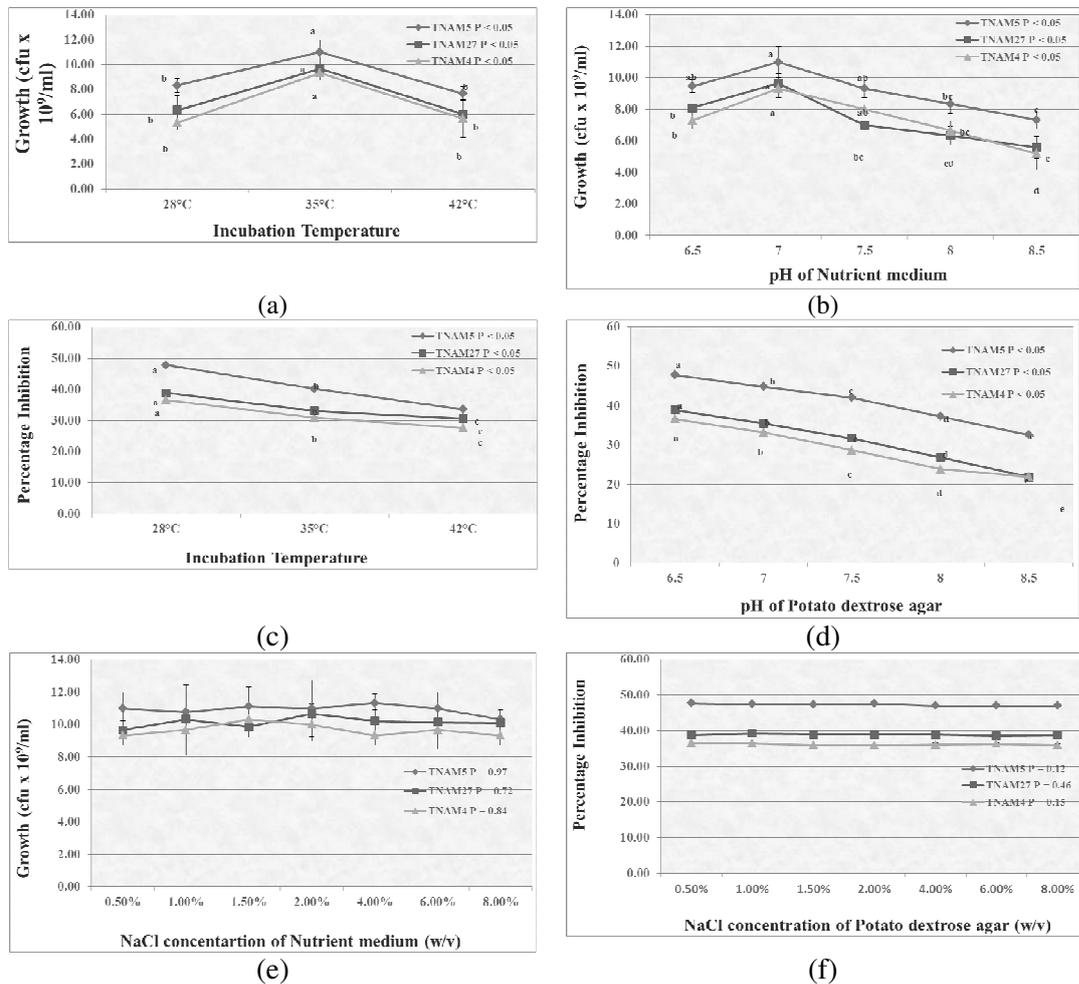
**Table 4.** Inhibition of *Fusarium oxysporum* by volatile antifungal metabolites produced by antagonistic rhizobacterial isolates<sup>a</sup>.

S. No.	Isolate	Radial growth (in mm)	Percentage inhibition of radial growth
1	TNAM5	46.27±0.40e	48.59±0.45a
2	TNAM27	56.50±0.20d	37.22±0.22b
3	TNAM4	62.27±0.25c	30.81±0.28c
4	TKB6	65.73±0.46b	26.96±0.51d
5	TKB13	70.10±0.53a	22.11±0.59e
6	TKB9	70.67±0.58a	21.48±0.64e
Mean percentage inhibition			31.21±10.33

<sup>a</sup> The values given are mean (n= 3) with standard deviation. Means with the same letter in the same column are not significantly different at  $P < 0.05$  (Tukey's Honestly Significant Difference test). Isolate TKB2, TKB12, TNAM7, TNAM13 were tested negative for production of volatile antifungal metabolites.

### Effect of Physical Parameters on Growth and Antagonistic Behavior of Rhizobacteria

Amongst the tested physiological parameters, temperature and pH were found to significantly affect the growth (Figures 1a and b) as well as antagonistic activity (Figures 1c and d) of the isolated antagonistic strains ( $P < 0.05$ ). All the antagonistic strains were found to give maximum inhibition to the tested fungal pathogen at 28°C and pH 6.5. Increase in incubation temperature



**Figure 1.** Effect of temperature on growth (a) pH on growth (b) temperature on antagonistic activity (c) pH on antagonistic activity (d) salinity on growth (e) salinity on antagonistic activity (f) of best antagonistic isolates from tomato rhizosphere. (Values given are the mean (n= 3) and error bars are  $\pm$ standard deviation. Means of the same series sharing a common letter are non-significant at  $P < 0.05$  (Tukey's Honestly Significant Difference test).

resulted in reduced antagonistic activity and minimum antagonism was observed at 42°C. Isolate TNAM5 showed 47.77% inhibition of radial growth of fungus at 28°C, which decreased to 33.59% at 42°C. Similarly, as pH was raised from 6.5 to 8.5, with an increment of 0.5, the percentage inhibition of the pathogen decreased significantly. At pH 6.5, 47.78% inhibition of radial growth of *Fusarium oxysporum* was shown by isolate TNAM5, which decreased to 32.56% at pH 8.5. Optimal conditions for the growth of the antagonists were observed as 35°C and pH 7.0. A shift in temperature below and above this

value resulted in considerable reduction in the growth. However, adequate level of growth was still observed at 28 and 42°C in all cases (Figure 1a). Likewise, optimal pH value for the growth of antagonistic isolates was identified as 7.0 and minimal growth was observed at pH 8.5 (Figure 1b).

In contrast to this, increase in salt (NaCl) concentration didn't significantly affect the growth (Figures 1-e) or antagonistic behavior (Figures 1-f) of the isolates ( $P > 0.05$ ). Similar level of percentage inhibition of fungal pathogen up to salinity level of 8.0% NaCl and

the growth of antagonistic isolates was also optimally observed at this salt concentration.

## DISCUSSION

Fusarium wilt of tomato is an economically important disease that is found worldwide. The inefficiency of currently practiced methods to control the disease along with the need to develop sustainable methods of disease management has started the hunt for a suitable alternative. Plant growth promoting rhizobacteria (PGPR) have emerged as most promising choice in this direction. Antagonistic activities of PGPR have been reported against several soil borne fungal pathogens of plants like *Phytophthora capsici* (Lee *et al.*, 2008), *Rhizoctonia solani* (Asaka and Shoda, 1996), *Pythium ultimum* (Lee *et al.*, 2000) and many others. Here, we report the antifungal properties of rhizobacterial isolates of tomato in semi-arid agroclimatic zone, against *Fusarium oxysporum*. Amongst the ten antifungal strains isolated in this investigation, *Bacillus* was observed to have the strongest antagonistic potential against the tested pathogen. Rhizobacteria belonging to *Bacillus* and *Pseudomonas* species have been previously reported to inhibit various wilt causing strains of *Fusarium oxysporum* in plants like chickpea (Landa *et al.*, 2004; Karimi *et al.*, 2012), eggplant (Yildiz *et al.*, 2012), lily (Chung *et al.*, 2011), lentil (Akhtar *et al.*, 2010) as well as tomato (Larkin and Fravel, 1998; Adebayo and Ekpo, 2004). However, this appears to be the first report of antagonistic activity of *Bacillus* isolate of semi-arid agroclimatic zone, against tomato wilt causing *Fusarium oxysporum*.

Further, on the basis of the results obtained, it may be observed that a significant difference existed among the different strains of the same genera isolated from the same ecological niche in terms of their antagonistic behavior against a particular pathogen. It may be noted that percentage inhibition of radial fungal growth

by different isolates of *Bacillus* and *Pseudomonas* varied widely in the present study ( $P < 0.05$ ). Divergence in their capacity to produce effective antifungal compounds may explain this disparity among the antagonistic isolates of the same genera.

According to the observations made in this study, production of diffusible and volatile antifungal molecules along with compounds like ammonia and HCN seems to be the primary source of inhibition of the tested fungal pathogens. Isolate TNAM5 belonging to *Bacillus sp.* was found as a strong producer of volatile and diffusible antifungal compounds, a character that has been previously well established for various strains of *Bacillus* (Asaka and Shoda, 1996; Wang *et al.*, 2007; Dunlap *et al.*, 2011). Efficiency of volatile and diffusible antifungal compounds produced by different *Bacillus* isolates also varied significantly ( $P < 0.05$ ) and so was the case with other antifungal compounds as well.

In order to exhibit their plant growth promotion and protection capabilities, the foremost requirement for the PGPR is to colonize the suitable sites in the rhizosphere so as to establish themselves in the soil. The soil tested in the present study was slightly alkaline and moderately saline (Kumar, 2007; Sargaonkar *et al.*, 2008). It is known that for bacteria to grow and sustain in arid saline-alkaline soils, it is very essential to withstand high salt concentration and high temperature conditions (Egamberdiyeva and Khandakar, 2008). Antagonistic strains isolated in this investigation have been found to grow reasonably well in a broad range of temperature i.e. between 28 to 42°C, while the maximum growth was observed at 35°C. Though the optimal temperature for the inhibitory action of the isolates against the tested fungus was recorded as 28°C, they were found to be effective up to 42°C. Average temperature found in the site of study for the present investigation reached up to 41°C in the month of May and June. This indicates that the isolated *Bacillus* strain possessing antagonistic activities against the tested



fungus pathogens may sustain well in this site.

Further, the average pH found in this zone is 6.5 and 8.7 (Kumar, 2007). We have found that isolate TNAM5 showed maximum antagonistic activity at pH 6.5, however, reasonably good level of antagonism was observed up to pH 7.5. Moreover, optimum pH for its growth was observed at 7.0 and it grew well up to pH 8.0. None of the isolated antifungal strains responded to variations in salinity as provided by different NaCl concentrations in the media i.e. from 0.5 to 8.0%. All the strains showed proficient growth and antagonistic activities up to 8.0% NaCl (w/v). Thus, it may be inferred that the isolate obtained in the present study will be adequately effective in establishing itself in the field conditions and inhibiting the fungal pathogen when applied as a biocontrol agent in this area.

The present study has, therefore, provided a potential bacterial isolate suitable for controlling tomato wilt causing fungus *Fusarium oxysporum* in Faridabad agroclimatic zone. However, it is suggested that a detailed investigation must be carried out to evaluate isolate TNAM5 for its field performance to control *Fusarium oxysporum* in tomato, before it can be established as a biocontrol soil inoculant.

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## جداسازی و تشخیص درون شیشه ای *Bacillus sp* با فعالیت آنتاگونیستی ضد *Fusarium oxysporum* فراریشه گوجه فرنگی

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### چکیده

هدف مطالعه حاضر جداسازی و تشخیص باکتری های آنتاگونیست قارچ بیمارگر (پاتوژن) و عامل پژمردگی یعنی *Fusarium oxysporum* فراریشه گوجه فرنگی بود. پنجاه و شش سویه باکتری از فراریشه و نمونه های خاک مزارع سالم گوجه فرنگی در نقاط مختلف بخش فریدآباد در هاریانای هند جمع آوری شد. از میان آنها، ده سویه در شرایط درون شیشه ای ضد عامل بیمارگر مورد آزمون یعنی *Fusarium oxysporum* بودند. بر مبنای درصد بازدارندگی رشد شعاعی *Fusarium oxysporum* جدایه TNAM5 موثرترین باکتری آنتاگونیستی فراریشه بود. شناسایی آن بر پایه خواص ریخت شناسی و بیوشیمیایی آن همراه با تحلیل توالی (ترادفی) 16s rRNA نشان داد که این جدایه یک *Bacillus sp* است که با گروه *Bacillus subtilis* هویت نوکلئوتیدی نزدیکی دارد. میانگین بازدارندگی این جدایه برابر ۴۷.۷۷٪ بود و آشکار شد که متابولیت های ضد قارچ نشأت کننده و فراری همراه با پروتئاز و آمونیا تولید می کند. اثر پارامترهای فیزیولوژیکی روی رشد و رفتار آنتاگونیستی جدایه های مستعد نیز بررسی شد. شرایط بهینه برای فعالیتهای آنتاگونیستی  $28^{\circ}\text{C}$  و اسیدیته ۶/۵ بود در حالیکه بیشترین رشد در  $35^{\circ}\text{C}$  و اسیدیته ۷ مشاهده شد. از سوی دیگر، افزایش شوری تاثیر معنی داری ( $P > 0.05$ ) روی رشد یا اثر آنتاگونیستی جدایه ها نداشت و آنها غلظت ۸.۰٪ نمک طعام بر حسب (w/v) را تحمل کردند. به این قرار، پژوهش حاضر عامل مستعدی برای کنترل زیستی *Fusarium oxysporum* شناسایی کرده است هر چند که برای اطمینان از کار کرد واقعی این جدایه به عنوان ماده تلقیح خاک آزمون های صحرائی د مزارع گوجه فرنگی ضروری است.