

Original Research Article

<https://doi.org/10.20546/ijcmas.2017.608.096>

Isolation and Screening of Bacterial and Fungal Isolates for Plant Growth Promoting Properties from Tomato (*Lycopersicon esculentum* Mill.)

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ABSTRACT

Keywords

Pythium debaryanum,
Rhizoctonia solani, *Sclerotium rolfsii*,
Trichoderma harzianum,
Trichoderma viride,
Pseudomonas fluorescence, Soil borne pathogens.

Article Info

Accepted:
14 June 2017
Available Online:
10 August 2017

In the present study About 24 isolates of fungal bio-control agent particularly *Trichoderma* spp and 12 bacterial isolates were isolated from the rhizosphere of tomato which was identified based on morphological and cultural characteristics. Among 24 isolates of *Trichoderma* spp ten and twelve bacterial isolates were identified with antagonistic ability and tested against the test soil borne pathogens *P. debaryanum*, *R. solani* and *S. rolfsii*. The *Trichoderma harzianum*-7 and *Trichoderma harzianum*-1 were found effective in inhibition of mycelium (80.03), (62.53 and 95.1) against *P. debaryanum*, *R. solani* and *S. rolfsii* respectively under *in vitro* conditions. The efficient isolates further screened for functional plant growth promoting properties. Among twelve bacterial isolates, *Pseudomonas fluorescence*-3 recorded highest solubilization zone followed by *Pseudomonas fluorescence*-2. All the bio-agents showed positive results to ammonia production except *Trichoderma harzianum*-6. Similarly all the bio-gents exhibited positive results for HCN production. Highest HCN production was observed with the *Trichoderma viride*-2, 9, 10 and *Trichoderma harzianum*-4, 7, 8, 13 and bacterial isolates of *Bacillus subtilis*-2, 4 and *Pseudomonas fluorescence*-10. IAA production was observed with *Trichoderma* but the highest IAA production was noticed with the bacterial isolates of *Bacillus subtilis*-5, *Pseudomonas fluorescence*-2 and 12. All the bacterial isolates showed siderophore production in the plate assay of which the bacterial isolates of *Bacillus subtilis*-4, *Pseudomonas fluorescence*-1 showed prominent orange colour zone. The *Trichoderma viride* 9 and *Trichoderma harzianum*-2 showed lower production of siderophores.

Introduction

Tomato (*Lycopersicon esculentum* Mill.) is an important vegetable crop which belongs to family Solanaceae. It is grown widely throughout the world under various agro-climatic conditions and attained the status of one of the world's most popular vegetables after potato, due to its high nutritive and medicinal value, in addition to processing and export potential. Tomato (*Solanum lycopersicum* L.) is a major contributor to the

fruit vegetable diet of humans. It is cultivated in essentially all countries either in fields or in protected culture. Its many varieties are now widely grown, sometimes in greenhouses in cooler climates. Controlling such diseases mainly depend on fungicides treatments (Rauf, 2000). Tomato is prone to attack various fungal, bacterial, viral and nematode diseases at different stages of crop growth.

The soil borne fungal pathogens viz., *Pythium* spp., *Sclerotium rolfsii*, *Rhizoctonia solani* and *Fusarium oxysporum* f. sp. *lycopersici* are known to cause damping-off, root rot, basal stem rot and wilt disease respectively in tomato. The tomato crop is also highly susceptible to damping of disease at seedling stage in nursery beds and the fungus, *Pythium* spp., (Edson) Fitz is common both in summer and winter grown tomato crops causing losses to the extent of 50 per cent (Bisht *et al.*, 1997). Root rot caused by *Rhizoctonia solani* takes heavy toll in each season of its cultivation. It has been considered as the most devastating disease in Haryana destroyed the crop from early sowing until maturity, the disease may appear at any stage of growth in nursery beds as well as in field after transplanting. However, the damage is more marked at the early stage of the crop growth.

Sclerotium rolfsii, fungus is a soil borne facultative parasite having very wide host range which causes pre-emergence rot, damping off, collar rot, stem rot of tomato. This disease is also called by other names like southern blight, southern root rot, *Sclerotium* rot or white mold. The pathogen is polyphagous and non-target fungus. Biological control promises an environmental friendly disease management strategy for soil borne plant pathogens. Antagonistic fungi especially *Trichoderma* spp. and bacteria fluorescent *Pseudomonads*, *Bacillus* have been widely used against soil borne pathogens to reduce the population of *Sclerotium* spp., *Rhizoctonia* spp., *Phytophthora* spp. and *Pythium* spp. The biological control is the best alternative especially against soil borne pathogens. Biological control of pathogens, i.e., the total or partial destruction of pathogen populations by other organisms, occurs routinely in nature. Among the various antagonists used for the management of plant diseases, *Trichoderma* spp. plays a vital role. Recently, it was suggested that, *Trichoderma*

affects induced systemic resistance mechanism in plants against pathogens (Haggag and Amin, 2001; Prasad *et al.*, 2002; Hibar *et al.*, 2007; Jayalakshmi *et al.*, 2009). Among the various isolates of *Trichoderma*, *T. Asperellum*, *T. harzianum*, *T. virens*, *T. viride*, and *T. hamatum* are used against the management of various diseases of crop plants especially with soil borne pathogens. These filamentous fungi are very common in nature, with high population densities in soil and plant litters. Many studies have proved the potential of *Trichoderma* spp. as biological agents antagonistic to several plant pathogens (Tondje *et al.*, 2007; Houssien, *et al.*, 2010).

Materials and Methods

Isolation of the antagonistic microflora from the rhizosphere of tomato plants

Diseased and healthy plant samples with rhizosphere soil of tomato growing Mandals of Ranga Reddy districts on soil-borne fungal diseases during *kharif* 2013-14. Plants was collected in polythene bags from each survey plots and isolated immediately for antagonistic organisms by following serial dilution method.

Isolated fungal and bacterial antagonists from rhizosphere soil is transferred to culture tubes containing appropriate media for further examination. Identification was done up to genus level based on their cultural and morphological characterization (Barnett and Hunter, 1998).

Identification of *Trichoderma* spp. and antagonistic bacteria

Trichoderma spp. was identified based on keys as described by Rifai (1969), Rhizosphere bacteria were identified based on gram reaction of bacterial isolates was

determined by following Gram staining and Biochemical Characterization by comparison with Bergey's Manual of Determinative Bacteriology. *Trichoderma* spp. and antagonistic bacteria were maintained by periodical transfer onto Potato Dextrose Agar and Nutrient Agar, respectively.

Screening of pure isolates for plant growth promoting properties

Pure isolates were isolated by streaking isolates on respective media plates and screened for following Plant growth promoting properties.

Phosphate solubilization

Phosphate solubilizing ability of the isolate was checked on Pikovskaya (PVK) medium (Pikovskaya, 1948). Incorporated with tricalcium phosphate (TCP) [Ca₃ (PO₄)₅]. Phosphate solubilization index was evaluated according to the ratio of the total diameter (colony diameter + halo zone) and the colony diameter.

Indole Acetic Acid Production

Indole acetic acid production was tested according to Gorden and Weber (1951). Development of pink colour considered as positive for IAA production.

Siderophore Production

Siderophore production was estimated qualitatively on Chrome Azurol S (CAS) Agar medium (Schwyn and Neilands, 1987). Formation of yellow to orange coloured zone around the well indicates siderophore production.

Hydrogen Cyanide Production (HCN)

Pure isolate will be tested by inoculating on succinate agar using alkaline picric acid as a

reagent (Castric and Castric, 1983), brown colour of the filter paper positive for HCN production.

Ammonia production

Ammonia production determined by the method (Dye 1962) after growing the different cultures in peptone water broth.

The tubes were incubated at 30°C for four days, after which 1 ml of Nessler's reagent was added in each tube and observations were recorded in terms of a faint yellow colour, deep yellow to brownish colour. Three replicates per treatment were maintained.

Results and Discussion

About 24 isolates of fungal bio-control agent particularly *Trichoderma* spp and 12 bacterial isolates were isolated from the rhizosphere of tomato which was identified based on morphological and cultural characteristics. *Trichoderma* spp. and antagonistic bacteria were maintained by periodical transfer onto Potato Dextrose Agar and Nutrient Agar, respectively.

Among 24 isolates of *Trichoderma* spp ten and ten bacterial isolates were identified with antagonistic ability and tested against the test pathogens *P. debaryanum*, *R. solani* and *S. rolfsii*.

The *Trichoderma harzianum*-7 and *Trichoderma harzianum*-1 were found effective in inhibition of mycelium (80.03), (62.53 and 95.1) against *P. debaryanum*, *R. solani* and *S. rolfsii* respectively under *in vitro* conditions. All bacterial isolates morphologically and culturally characterized. These twelve isolates were gave gram negative and gram positive reaction. Further these isolates were characterized biochemical reactions using different sources.

Plate.1 Phosphate solubilization on pikovskayas agar medium



Plate.3 Siderophore production by *Trichoderma* and Bacterial isolates

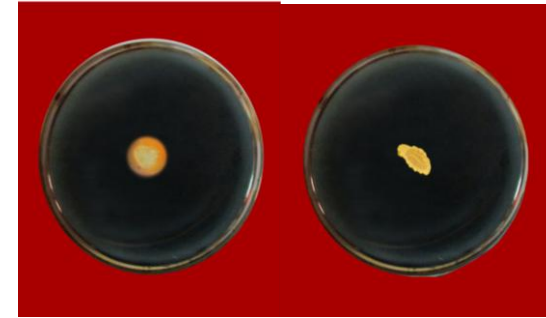


Plate.2 IAA production by *Trichoderma* and bacterial isolates



Plate.4 HCN production by *Trichoderma* and bacterial isolates



Table.1 Carbohydrate utilization of bio-chemical properties of rhizosphere bacteria isolated from tomato crop

S.No	Test	Bac-1	Bac-2	Bac-3	Bac-4	Bac-5	Bac-6	P.f-1	P.f-2	P.f-3	P.f-4	P.f-5	P.f-6
1	ONPG	-	-	-	-	-	-	-	-	-	-	-	-
2	lysine	-	+	-	+	-	-	-	+	+	-	+	-
3	ornithine	+	+	-	+	-	-	-	+	+	-	+	-
4	Urease	-	-	-	-	-	-	-	-	-	-	-	+
5	TDA	-			-	-	-	-			+	-	+
6	Nitrate	-	+	+	+	+	+	-	+	+	-	-	-
7	H2S	-	-	-	-	-	-		-	-		-	-
8	Citrate utilization	-	-	+	+	-	+	+	-	-	+	-	+
9	Vogesproskauer's	-	-	-	-	-	-	-	+	-	-	+	+
10	Methyl red	-	-	+	-	-	-	-	-	-	-	-	+
11	Indole	-	-	-	-	-	-	-	-	-	-	-	-
12	Malonate		-	-	-	+	+	-	-	-	-	--	-
13	Esculin hydrolysis	+	-	-	-	+	+	+	--	-	+	+	+
14	Arabinose	-	-	-	-	-	-	-	-	-	+	+	+
15	Xylose		-	-	-	-	-		-	-	+	+	+
16	Adonitol	-	-	-	-	-	-	-		-	+	-	-
17	Rhamnose	+	-		-	+	-	-	+	-	-	-	-
18	Cellobiose	+	-	+	-	+	-	-	-	-	-	+	-
19	Melibiose	+	-	-	-	-	-	-	+	+	+	+	+
20	Saccharose	+	+	-	-	+	-	+	-	-	+	+	+
21	Raffinose	+		-	-	+	-	-	-	-	+	+	-
22	Trehalose	+	+	-	-	+	-	-	+	+	+	+	+
23	Glucose	+	+	-	-	+	-	-	+	+	+	+	+
24	lactose	+	-	-	-	-	-	-	-	-	-	+	-

Bac - means *Bacillus subtilis* 1-6, *P.f* means- *Pseudomonas fluorescense* 1-6.

Table.2 Screening for plant growth promoting properties

Isolates of Bio-agents	Phosphate Solubilization			Ammonia production	IAA production	Siderophere production	HCN Production
	Zone diameter (mm)		Solubilization efficiency (%)				
	Solubilization zone	culture diameter					
<i>Trichoderma viride</i> -2	-	-	-	+++	-	-	+++
<i>Trichoderma viride</i> -6	-	-	-	++	-	-	++
<i>Trichoderma viride</i> -9	15.66	15	115.0	+++	+	+	+++
<i>Trichoderma viride</i> -10	-	-	-	+++	-	-	+++
<i>Trichoderma viride</i> -11	-	-	-	+	-	-	++
<i>Trichoderma harzianum</i> -1	-	-	-	+++	+	+	++
<i>Trichoderma harzianum</i> -2	-	-	-	++	-	+	+++
<i>Trichoderma harzianum</i> -5	-	-	-	+++	-	+	+++
<i>Trichoderma harzianum</i> -6	-	-	-	-	-	-	+++
<i>Trichoderma harzianum</i> -11	-	-	-	++	-	-	+++
<i>Bacillus subtilis</i> -1	-	-	-	+	+	+	
<i>Bacillus subtilis</i> -2	0.8	9	85	++	++	++	+++
<i>Bacillus subtilis</i> -3	-	-	-	++	+	+	-
<i>Bacillus subtilis</i> -4	18	7	117.1	+++	+++	+++	+++
<i>Bacillus subtilis</i> - 5	16	15	105.0	++	++	++	-
<i>Bacillus subtilis</i> -6	20	15	135	++	++	+	++
<i>Pseudomonas fluorescense</i> -1	18	6	138.4	+++	+++	+++	-
<i>Pseudomonas fluorescense</i> -2	2.6	5	140	+++	+	++	+++
<i>Pseudomonas fluorescense</i> -3	16	13	123.2	++	++	++	++
<i>Pseudomonas fluorescense</i> -4	-	-	-	+	++	++	++
<i>Pseudomonas fluorescense</i> -5	12	8	125	++	+	+	++
<i>Pseudomonas fluorescense</i> -6	22	11	130	++	+++	++	++

Biochemical characterization of bacterial isolates

Carbohydrate utilization patterns of the bacterial isolates were studied using biochemical kits supplied by Himedia and the reactions are presented in table 1 and Plate-1. The bacterial isolates tested could not utilize O-Nitrophenyl β -D galacto pyranoside (ONPG). The *Bacillus subtilis* isolates 2, 4 and *Pseudomonas fluorescens*-2, 3 and 5 could utilize decarboxylate lysine and ornithine and the *Pseudomonas fluorescens*-6 utilized urea as a nitrogen source. Out of twelve isolates, seven isolates (*Bacillus subtilis* isolates -2, 3, 4, 5, 6, *Pseudomonas fluorescens* 2 and 3) preferred nitrite as the nitrogen source whereas isolate *Pseudomonas fluorescens*-4 and *Pseudomonas fluorescens* isolate-6 utilized phenylalanine.

Among the carbon sources tested, most of the bacterial isolates (*Bacillus subtilis* isolates-1, 2, 5 and *Pseudomonas fluorescens* isolate-2, 3, 4, 5, 6) utilized glucose and trehalose as preferred carbon sources followed by saccharose (*Bacillus subtilis* isolates 1, 2, 5 and *Pseudomonas fluorescens* 1, 4, 5, 6) and melibiose (*Bacillus subtilis* isolate-1, *Pseudomonas fluorescens* 1, 2, 4, 5, 6). Raffinose as carbon source was utilized by the bacterial isolates *Bacillus subtilis*-1, 5 and *Pseudomonas fluorescens*-4 and 5 and Cellobiose by *Bacillus subtilis*-1, 3, 4 and *Pseudomonas fluorescens*-5. Arabinose and xylose was utilized by the bacterial isolates of *Pseudomonas fluorescens*-4, 5 and 6. Lactose and adonitol were found be the least preferred carbon source by the bacterial isolates. The ability of the bacteria to utilize different substrates containing carbon, hydrogen and oxygen varies with the battery of enzymes present in the bacteria (Cowan, 1974). For example, none of the isolates tested were found to possess β -galactosidase activity as they couldn't utilize ONPG.

Screening for plant growth promoting properties

In the present study, all the fungal and bacterial isolates were used to study the phosphate solubilization. Among twelve bacterial isolates, eleven bacterial isolates showed solubilization zone except *Pseudomonas fluorescens*-4. The *Pseudomonas fluorescens*-2 recorded highest solubilization zone followed by *Pseudomonas fluorescens*-1. All *Trichoderma* isolates did not show any phosphate solubilization zone (Table 2 and Plate 1).

All the bio-agents showed positive results to ammonia production except *Trichoderma harzianum*-6. The dark colour was recovered in *Trichoderma viride*-2, 9, 10, *Trichoderma harzianum*-2 and 5 and *Bacillus subtilis* isolates-4, *Pseudomonas fluorescens*-1 except bacterial isolate *Bacillus subtilis*-5. Similarly all the bio-agents exhibited positive results for HCN production. Highest HCN production was observed with the *Trichoderma viride* isolates-2, 9, 10, *Trichoderma harzianum*-2, 5, 6, 11 and bacterial isolates of *Bacillus subtilis*-2, *Pseudomonas fluorescens*-2 and 3 (Plate 4). IAA production was observed with *Trichoderma* but the highest IAA production was noticed with the bacterial isolates *Bacillus subtilis*-5, *Pseudomonas fluorescens*-2 and 6 (Plate 2). All the bacterial isolates showed siderophore production in the plate assay of which the bacterial isolates of *Bacillus subtilis*- 4, *Pseudomonas fluorescens*-1 showed prominent orange colour zone. The *Trichoderma viride*-9 and *harzianum*-2 showed lower production of siderophores (Plate 3).

The present study was undertaken in order to isolate and identify tomato-associated *Trichoderma* and rhizobacteria from various

tomato-producing sites and growing systems and to perform morphological, biochemical characterization. The current investigation provided strong evidence that tomato rhizosphere was populated by a numerous and a diverse array of rhizobacteria exhibiting a great morphological and biochemical diversity. Screening of their ability to synthesize plant growth-promoting and antifungal compounds revealed that the majority of isolates were able to produce siderophore, to solubilize phosphate and to synthesize IAA and that interestingly, some isolates cumulated the three plant growth promoting traits.. Thus, this study clearly demonstrated that tomato rhizospheric soils harbor a diversity of beneficial bacteria which may be promising biocontrol agents due to their interesting metabolic activity and their interesting antifungal potential displayed toward both target fungi. Moreover, their ability to produce plant growth-promoting compounds indicated that they may exhibit a bio-fertilizing action.

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How to cite this article:

Rajendra Prasad, M., B. Vidya Sagar, G. Uma Devi, S. Triveni, S.R. Koteswar Rao and Damodara Chari, K. 2017. Isolation and Screening of Bacterial and Fungal Isolates for Plant Growth Promoting Properties from Tomato (*Lycopersicon esculentum* Mill.). *Int.J.Curr.Microbiol.App.Sci*. 6(8): 753-761. doi: <https://doi.org/10.20546/ijcmas.2017.608.096>