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REGULAR ARTICLE

STUDIES ON ISOLATION AND CHARACTERISATION AND ITS EFFECT OF SEED INOCULATION OF PGPR (*PSEUDOMONAS FLUORESCENS*) ON YIELD OF TOMATO

U. Sakthivel, S. Mahalakshmi, B. Karthikeyan*

Department of Microbiology, Annamalai University, Annamalainagar – 608 002, Tamilnadu, India

SUMMARY

Soil samples of five different locations of rhizosphere of tomato. The sample used for identification of *Pseudomonas fluorescens* and characterisation based on biochemical characteristics. The best isolate PF-5 used for seed treatment of with tomato variety PKM-1. The higher fruit yield was recorded in the triple combination of *Pseudomonas fluorescens* + *Azotobacterchroococcum* + *Azospirillum brasilense* @ 900g/plant-1 followed by *Pseudomonas* + *Azotobacter*.

Keywords: Tomato (*Lycopersicon esculantum*), PGPR, *Pseudomonas fluorescens*, *Azotobacter chroococcum*, *Azospirillum brasilense*.

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***Corresponding Author**, *Email*: **balakar@yahoo.com**

1. Introduction

Tomato (*Lycopersicon esculantum*, Miller) is an important solanaceous crop. India is the world second largest producer of vegetables next to china. The soil organisms that could improve the plant vigour by providing the growth and reducing soilborne diseases are potential biological agent to increase the tomato yield. Moreover, they are environmentally safe and cause no pollution and toxicity. Saprophytic fluorescent *Pseudomanas* are very common in soil and they are also formed in plant rhizosphere, where they seem to have a stimulating effect on plant growth.

Plant growth promoting rhizobacteria (PGPR) *Pseudomonas fluorescens* are free living bacteria that are able to colonize plant root and promote plant growth [9]. The improved plant growth and crop yield is attributed to disease suppression. Iron chelation, antibiotic production, enhanced nutrient uptake [13] and seedling emergence promotion and by plant phytohormone production [13,3].

Siderophores are iron chelating compounds secreted by bacteria on or around the roots that affect the growth of the plants rhizobacteria. The PGPR, being more potent chelators, starve the deleterious rhizobacteria of their iron

nutrient, thus protect the plants from the harmful effects of DRB, resulting in better growth and yield.

Tomato is one of the highest responsive crop for microbial inoculants owing to their root biomass production and more colonization of beneficial microorganisms which are interdependent with these considerations, the present research study was formulated with the ultimate aim of identifying a potential, most efficient strain of PGPR for inoculating tomato to increase the crop productivity.

2. Materials And Methods

Isolation of plant growth promoting rhizobacteria from the rhizosphere soil of tomato plants and purified in the medium B of King's et al. [8]. The seedling vigour index was computed from 5 to 14 days after sowing and the procedure was suggested by Abdul-Baki and Anderson [1].

Vigour index = Germination % x Plant height

Gram staining method is followed by the Crabree and Hindstill [4]. The starch hydrolysis for the utilization by the microorganisms [15]. Motility test carried out in semisolid agar medium. Starch hydrolysis and Gelatin hydrolysis is done by (Stolpe and Godkeri) [15]. Quantitative estimation of Indole Acetic Acid (IAA) by PGPR isolates [12]. Antibiotics evaluation in the presence and absence of iron. The best strain PF-5 (*Pseudomonas fluorescens*) was selected. Effect of different micronutrient on the growth of *Pseuomonas fluorescens* (PGPR) PF-5 in the Dowsen's medium [5].

Effect of PGPR (PF-5) on fruit yield of Tomato var PKM-1:

The experiment was conducted in Department of Agricultural Microbiology pot culture yard December-2008 to April-2009.The tomato variety PKM-1 seeds were treated and sown. The following treatments are given below.

Treatment details:

T0 - Control

T1 - *Pseudomonas*

T2 -*Azotobacter*

T3 - *Azopirillum*

T4 - *Pseudomonas* + *Azotobacter*

T5 - *Pseudomonas* + *Azospirillum*

T6 - *Pseudomonas* + *Azotobacter* + *Azospirillum*

3. Result

The Pant growth promoting rhizobacteria (PGPR) was enumerated in the rhizosphere soil of Tomato of five different locations .Among the five locations Orathur (PF-5) was found to be recorded the maximum population (22.33 X 106) followed by Sethiyathope PF-1 (20.66 X106), Keerapalaiyam PF-2 (18.33 X 106), Kiliyanur PF-3 (9.66 x 106) and Sakkanguidi PF-4 (12x106) Table-1. The Characterization of *Pseudomans fluorescens* based on the gram staining reactions, physiological and biochemical tests like Catalase activity,Starch hydrolysis,Gelatin liquefaction and culture identified as Pseudomonas fluorescens are presented in the Table-2. Screening of *Pseudomanas fluorescens* isolates obtained from the rhizosphere of tomato was tested for efficiency presented in Table -3. Among the five isolates, PF-5 recorded the highest germination percentage of 95, plant height. IAA production by PGPR isolates *Pseudomans fluorescens* was given in the (Table-4). The among of IAA production of these isolates was expressed in µg/ml) followed by PF-1 isolates (11.70 µg/ml). The minimum production of IAA was formed in PF-2 recorded about (10.54 µg/ml) PF-3 isolate recorded about (10.50 µg/ml). Accordingly, the best isolates of PGPR (PF-5) was designated as used for further study.

Table 1. Enumeration of PGPR (*Pseudomonas fluorescens*) in the rhizosphere soils of Tomato

S.No.	Rhizosphere soil	Microbial Population x 106
1.	Sethiyathope	20.66
2.	Keerapalaiyam (PF-2)	18.33
3.	Kiliyanur	9.66
4.	Sakkangudi	12.00
5.	Orathur	22.33

Table 2.Characterization of *Pseudomonas fluorescens*

S.No.	Characters studied	Reaction of PGPR isolates of Tomato				
		PF1	PF2	PF3	PF4	PF5
1.	Gram staining reaction	-ve	-ve	-ve	-ve	-ve
2.	Motility	+ve	+ve	+ve	+ve	+ve
3.	Catalase activity	+ve	+ve	+ve	+ve	+ve
4.	Starch hydrolysis	-ve	-ve	-ve	-ve	-ve
5.	Gelatin liquefaction	+ve	+ve	+ve	+ve	+ve
6.	Fluorescent pigment production	+ve	+ve	+ve	+ve	+ve
7.	Culture identification	*Pseudomonas fluorescens*				

The invitro antibiosis produced by *Pseudomans fluorescens* against plant pathogens in presence and absence of Iron (FeCl3), the zone of inhibition to pathogenic fungi, produced by the organism in two types of media tried, confirmed the ability of the organism to inhibit fungal pathogens (Table-5).

The zone of inhibition were wider in most cases in King's B Medium. However the inhibition zones were reduced in the presence of available iron in the medium. The highest inhibition zone of 10.00 mm was observed to both the pathogenic organism viz., *Rhizoctonia* and *Pythium* tested in the absence of iron.

The effect of different micronutrient viz., $FeSO_4$, $MnSO_4$, $CuSO_4$ at different concentrations on the growth of *P. fluorescens* was studied. In general 10 ppm concentration of the different micronutrient tested were found to be optimum for the growth of *P. fluorescens*. Whereas in the other concentration 25 and 50 ppm were proportionately reduced the growth of *Pseudomonas fluorescens*. Among the micronutrients studied $MnSO_4$ was found to be maximum growth followed by $FeSO_4$, $ZnSO_4$ presented inTable-6.

The effect of individual and combined inoculation of PGPR of (*Pseudomonas*, *Azotobacter* and *Asozpirillum*) on fruit yield of tomato was studied and the results are presented in Table-7.

Table 3.Screening of PGPR isolates for efficiency

S.No.	PGPR isolates	Germination percentage (%)	Plant height (cm)	Vigour index
1.	PF - 1	90	17.00	1598
2.	PF - 2	85	13.77	1170
3.	PF - 3	82	12.52	1027
4.	PF - 4	82	10.06	824
5.	PF - 5	94	18.16	1604

Table 4. Indole Acetic Acid production by PGPR isolates

S.No.	PGPR isolates	Amount of IAA produced (µg/ml)
1.	PF - 1	11.70
2.	PF - 2	10.54
3.	PF - 3	10.50
4.	PF - 4	11.50
5.	PF - 5	12.64

Table 5. Invitro Antibiosis produced by *Pseudomonas fluorescens* PF-5 against plant pathogens in presence and absense of iron (FeCl3) (100 µgL-1)

S.No.	Plant pathogens	Zone of Inhibition (mm)			
		PDA medium		KB medium	
		Without Iron	With Iron	Without Iron	With Iron
1.	*Phythium* sp.	4.0	4.0	10.0	6.2
2.	*Rhizoctonia* sp.	4.0	2.0	10.0	8.2

It was observed that the fruit yield of tomato was significantly increased by all the treatment when compared to control. The maximum fruit yield 900.00 (g plant-1) was recorded in combined of the treatment of *Pseudomonas* + *Azotobacter* + *Azospirillum* followed by the treatment (T1).

Among the individual treatments, the inoculation of *Pseudomonas* (T1) was found to be best and recorded 710g plant-1 of fruit yield followed by *Azotobacter* (646.00 g plant -1) and *Azospirillum* (620.00g plant-1).

4. DISCUSSION

Plant growth promoting rhizobacteria include a wide variety of soil bacteria when grown in association with a host plant result in stimulation of growth of their host. The members of the genera are *Azotobacter, Azospirillum, Bacillus, Pseudomonas, Arthrobacter, Rhizobium* are considered as plant growth promoting bacteria [7, 2].

Table 6. Effect of different micronutrients on the growth of *Pseudomonas fluorescens* PF-5

S.No.	Micronutrients	Concentration in ppm	OD values
1.	MnSO4	10	0.82
		25	0.53
		50	0.46
2.	Zn SO4	10	0.35
		25	0.20
		50	0.09
3.	CuSO4	10	0.31
		25	0.28
		50	0.07
4.	FeSO4	10	0.53
		25	0.22
		50	0.18

The PGPR stimulate plant growth by a variety of mechanisms including production of siderophore, synthesis of antibiotics, production of phytohromones, enhancement of phosphate uptake by the plant, nitrogen fixation and synthesis of enzymes that regulate plant ethylene levels [7].

Table 7.Effect of individual and combined inoculation of (PGPR) (*Pseudomonas*, *Azotobacter* and *Azospirillum*) on fruit yield in tomato variety PKM-1

Treatments	Fruit yield (g plant-1)
T0 – Control	410.00
T1 – Pseudomonas	710.00
T2 – Azotobacter	646.00
T3 – Azospirillum	620.00
T4 – Ps + Azot	820.00
T5 – Ps + Azs	700.00
T6 – Ps + Azot + Azs	900.00
SEd	50.42
CD 5%	10.810

The study revealed that the ubiquitous nature of plant growth promoting rhizobacteria with inconsistent population load as influenced by soil and environmental factors in the rhizosphere of tomato soils collected from five different locations of Chidambaram Taluk where it is grown as Monocrop. Among the five locations, Orathur has recorded the maximum population of *Pseudomonas fluorescens* (22.33 x 106) followed by Sethiyathope. Earlier reported by [3] isolated about 500 fluorescent strains of Pseudomonas from the rhizosphere of different plants namely Tomato, Potato, Corn and Vine.

Fig. 1. Culture of *Pseudomonas fluorescens* Colony in *Pseudomonas* slant

The five isolates of tomato rhizosphere soil were screened for the efficiency to promote plant growth. The isolate obtained tomato from Orathur was found to be the most efficient in increasing the growth and vigour index of tomato variety PKM-1. The most efficient tomato (PF-5) isolate was chosen for further study.

The efficiency of PGPR isolate on the improvement of plant growth and yield were studied by several research workers [11].

Fig.2. Overall view of Pot culture

The IAA producing capacity of the isolate was considerable and recorded 12.64 µg/ml of the culture filtrate. The IAA producing capacity of *fluorescens pseudomonas* was already reported by Kloepper et al. [11] and Tankandsaraf [16]. The role of IAA in the metabolism and growth promoting activity is very well established.

Fig.3.Control treatment (T0)

The effect of micronutrient on the growth of *Psedomonas flourescens* revealed that MnSO4 exerted most significant effect following by FeSO4, ZnSO4 and CuSO4. The role of MnSO4 especially as activator of decarboxylase, dehydrogenase, peroxidise and found to be important for the growth of the bacteria.

Fig.4. Combined treatment (T6) *Pseudomonas*, *Azotobacter*, *Azospirillum*

In the present study the plant growth promoting rhizobacteria *P. fluorescens* was studied for their effect on the growth and yield of Tomato variety PKM-1. The combined treatment T6 (*Pseudomonas* + *Azotobacter* + *Azospirillum*) was found exert the maximum influence on various growth parameters of PKM-1 tomato variety and recorded the highest values yield parameters studied. Siddique, [14]early reported that the inoculation of *Pseudomonas fluorescens* + *Azotobacter chroococcum* + *Asopirillum brasilense* in combination recorded higher plant growth and yield as well as control of the nematode.

Among the individual inoculation treatments the performance of *P. fluorescens* on the various growth parameters of tomato variety PKM-1 excelled than the other individual treatments of *Azotobacter* and *Azospirillum*. *P. fluorescens* inoculation alone significantly increased plant growth, dry matter production and yield of tomato crop [17].

The nutrients enrichment of rhizosphere soil inoculated with microbial inoculant was reported [6] and attributed to the increased soil microbiologist process that contribute towards fertility status of the soil [10].

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