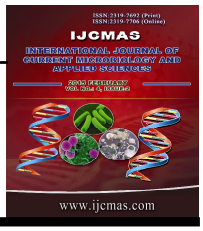
Int.J.Curr.Microbiol.App.Sci (2015) 4(2): 720-730

International Journal of Current Microbiology and Applied Sciences
ISSN: 2319-7706 **Volume 4 Number 2 (2015) pp. 720-730**
http://www.ijcmas.com



Original Research Article

Influence on growth and fruit yield of tomato (*Lycopersicon esculentum Mill.*) plants by inoculation with P*seudomonas fluorescence* (SS5): Possible role of plant growth promotion

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K e y w o r d s

Plant growth promoting rhizobia, *Pseudomonas fluorescence*, Indole acetic acid, Phosphate-solubilization, Siderophores

A B S T R A C T

A total of 28 *Pseudomonas fluorescence* isolates were obtained from tomato plant rhizospheres and were characterized on the basis of biochemical tests and plant growth-promoting activities. *Pseudomonas fluorescens* SS5 showed the best plant growth-promoting activity as well as enhanced productivity in tomato crop. In this experiment we were studied to examine its role as a plant growth promoting rhizobia (PGPR) in enhancing the growth and yield of the tomato plant. *Pseudomonas fluorescence*, isolate was known to several plant growth- promoting activities, including production of phytohormone and antibiotic substance. *Pseudomonas fluorescence* was shown to produce IAA. The production of IAA by the *P. fluorescence* was stimulated in vitro by the addition of tryptophan (500µg ml^{-1}). Besides, the strain also exhibited significant production of both the siderophore and hydrogen cynide (HCN) on chrome azurol S and King's B media, respectively. In this investigation were studied the physiological basis of yield improvement with the application of SS5. To quantify the effect of *P. fluorescence* on yield by the tomato plants and to ascertain the mode of action of *P. fluorescence* in plants**.** In pot culture, and field trials *P. fluorescence* (SS5) enhanced the growth of tomato plants. Significant increase in root and shoot weight, length, fruit yield per plant, and total fruit yield was recorded. The strain SS5 was significantly rhizopheric competent and stabilized in the rhizosphere, without disturbing the normal indigenous bacterial population.

Introduction

Over the past two decades the agricultural policy in India has undergone a major change to meet the increased demand of food in the global competitive scenario through diversification and emphasis on sustainable production systems. The role of plant growth promoting bacteria (PGPB) have been extensively studied as biofertilizers to increase the yield of agronomically important crops such as wheat (Khalid et al 2004), corn (Mehnaz and Lazarovits 2006) Beneficial effects of the

introduction of specific microorganisms on plant growth have been reported for numerous crops, including tomato (*Lycopersicon esculentum* Mill.) grown under green house in organic media (Guo et al., 2004) or field conditions (Kokalis-Burelle et al., 2002). Plant growth promoting rhizobacteria (PGPR) are defined as root colonizing bacteria (rhizobacteria) that exert beneficial effects on plant growth and development. PGPR are known to survive both in rhizosphere and phyllosphere (Krishnamurthy et al, 1998). Fluorescent pseudomonads are among the most effective rhizosphere bacteria because in addition to disease control, (Dubeikovsky et al., 1993.)

Such beneficial microorganism referred as PGPR (plant growth promoting bacteria) or PGPF (plant growth promoting fungi) enhance plant growth through numerous mechanisms including the protection of roots against infection by minor and major pathogens (Whipps, 1997, 2001), Plant growth-promoting rhizobacteria (PGPR) are free-living, soil-borne bacteria, which enhance the growth of the plant either directly or indirectly (Kloepper et al., 1980; Glick, 1995). The direct mechanisms involve nitrogen fixation, phosphorus solubilization, HCN production, production of phytohormones such as auxins, cytokinins and gibberellins, and lowering of ethylene concentration (Kloepper et al., 1989).

Among plants growth regulator s indole-3-acetic acid (IAA) is the most common natural auxin found in plants and its positive effect on root growth and morphology is believed to increase the access to more nutrient in the soil (Vessey, 2003). IAA affects plant cell division, extension, and differentiation; stimulates seed and tuber germination; increases the rate of xylem and root development; controls processes of vegetative growth; initiates lateral and adventitious root formation; mediates responses to light, gravity and florescence; affects photosynthesis, pigment formation, biosynthesis of various metabolites, and resistance to stressful conditions (Spaepen and Vanderleyden, 2011). In recent years, considerable interest has been paid to rhizobacteria, which are aggressive root colonizer s and produce siderophores . Siderophores provide an advantage in survival of both plants and bacteria.(Leong J, 1986) . Previously reported *Pseudomonas aeruginosa* GRC2 isolated from potato rhizosphere, which has the ability to produce antifungal metabolites ,phosphate solublization, HCN (Hydrocyanic Acid), IAA (indole-3- acetic acid) and also inhibits the growth of fungal pathogens.(Pandy et al., 2007).

Phosphorus (P), the second important plant growth-limiting nutrient after nitrogen, is abundantly available in soils in both organic and inorganic forms (Khan et al., 2009). Despite of large reservoir of P, the amount of available forms to plants is generally low. Plants are unable to utilized phosphate because 95-99% phosphate present in the insoluble, immobilized, and precipitated form (Pandey et al., 2007).

Pseudomonas sp. is ubiquitous bacteria in agricultural soils and has many traits that make them well suited as PGPR. Considerable research is underway globally to exploit the potential of one group of bacteria that belong to fluorescent *pseudomonas*. Recently Pandey et al. (2013) reported that *Pseudomonas* strains were plant growth promoting endorhizospheric bacteria inhabiting sunflower (*Helianthus annus*).

Root colonization comprises the ability of bacteria to establish on or in the plant root,

to propagate, survive and disperse along the growing root in presence of the indigenous microflora Representatives of many different bacterial genera have been introduced into soils, onto seeds, roots, tubers or other planting materials to improve crop growth. Despite the fact that iron is the fourth most abundant element on earth, in aerobic soils, iron is not readily assimilated by either bacteria or plants because ferric ion or Fe+3, which is the predominant form in nature, is only sparingly soluble so that the amount of iron available for assimilation by living organisms is extremely low (Ma, 2005). Both microorganisms and plants require a high level of iron, and obtaining sufficient iron is even more problematic in the rhizosphere where plant, bacteria and fungi compete for iron (Guerinot and Ying, 1994).

The direct benefits of bacterial siderophores on the growth of plants have been demonstrated by using radiolabeled ferric-siderophores as a sole source of iron showed that plants are able to take up the labeled iron (Robin et al., 2008), by inoculation with the siderophore producing *Pseudomonas* strain and grown under iron limiting conditions in mung bean plant, showed reduced chlorotic symptoms and an enhanced chlorophyll level compared to un inoculated plants (Sharma et al., 2003).

Schippers et al. (1987) documented an increase in fresh weight of root and shoot of tomato, cucumber, lettuce and potato as a result of bacterization with *Pseudomonas* strains. Kloepper et al. (1989) reported 57 per cent yield increase and enhanced plant growth due to seed application of beneficial rhizobacteria in canola.

Seed and soil application of PGPR strains showed significant plant growth promotion with increased runner length and increased leaf number per plant in chickpea (Singh et al., 2014). In nature, beneficial rhizosphere and development in microbe-rich and nutrient-poor environments. The growth substrates used in micro propagation are usually devoid of beneficial microorganisms. By introducing such micro-organisms to the substrates, it would be possible to lower fertilizer and pesticide inputs and grow the plants in a more sustainable way. In the present work, we studied the plant growth promoting characteristics and root colonization ability of *P. fluorescence* SS5. Its effect on plant growth, physiology, and fruit yield of tomato in pot culture, and field study in tomato plants.

Materials and Methods

Microorganism

Twenty eight bacterial isolates were use in this screen including the *Pseudomonas fluorescence* SS5. These organisms were identified based on the cultural, morphology and biochemical characteristics and results of SS5 (*Pseudomonas fluorescence*) which was isolated from tomato rhizosphere from a farmer's field at Shahganj, District- Sehore, Madhya Pradesh. One set of isolates was collected from 1 month old plants, and another set was collected from 2 month old plants. The plants roots were carefully shaken to remove excess soil. Isolation was performed by serial dilution plating of soil suspension on modified King's B (KB) agar which contained; glycerol, 8.0ml/L; MgSO4,0.4g/L; proteose peptone,20.0g/L; K2HPO4,4.0g/L; agar,18.0g/L; pH to 7 ± 0.2 prior to autoclaving.

Plates were incubated at 28˚C. The isolates strain were maintained on modified King's B agar slants at 4 C and also as 30% glycerol stocks at -80C. Biochemical

characterization of the bacterial strain was carried out according to Bergey's Manual of Determinative Bacteriology (Holt JG, et al, 1994).

Indole Acetic Acid Production

The production of Indole Acetic Acid (IAA) was determined according to Bric et al., (1991). Log phase culture of fluorescent Pseudomonad strains were inoculated in 15 ml KB broth amended with tryptophan (500µg/ml) and incubated at 28^{o}C for 48h. Cells were removed by centrifugation at 10,000 rpm for 15min. 1ml supernatant was transferred to a fresh tube to which 50 µl of 10 mm orthophosphoric acid and 2 ml of salkowski's reagent (1ml of 0.5M FeCl3 in 50ml of 35% HClO4) were added.

The mixture was incubated at room temperature for 25 min and absorbance measured at 530nm. The level of IAA produced was estimated by a standard curve of IAA (Merck, India). Each test was replicated three times. Pure IAA was used for preparing the standards of 0, 5, 10, 15, 20, 25, 30, 35, 40, and 45 mg L-1.

Detection of siderophore production

The production of siderophore was determined by (Schwyn and Neilands, 1987). The chrome – azurol S (CAS) agar plates were spot inoculated of CAS amino acid with log phase culture of *Pseudomonas fluorescence* strains and observed for colour change from blue to orange. Each test was replicated three times.

Phosphate solubilization activity

Phosphate solubilization ability of *pseudomonas fluorescence* strains were checked by spot inoculation (Pandey et al., 2002) on the Pikovskaya agar medium (Himedia, Mumbai), and incubated at 28^{o} C and 72^{o} C. the inoculated plates were observe for zone formation around the bacterial growth. Each test was replicated three times.

Detection of HCN Production

Production of HCN was observed according to Schippers and Baker and (1987). The log phase culture of fluorescent pseudomonad strains were spread on KB medium containing glycin (4.5g/l) a sterilized filter paper saturated with 1% solution of picric acid and 2 % sodium carbonate was placed in upper lid of Petri dishes. The Petri dishes was then sealed with paraffin and incubated at 28^{o} C for 4 days. A change in colour of the filter paper from yellow to reddish brown was recorded as an index of cyanogenic activity.

Seed bacterization

Tomato seed were surface sterilized by soaking 70% ethanol for 4 min and subsequently in 2% hypochloric acid for 1 min. the seeds were rinsed thoroughly 4 times with sterilized distilled water. Seed were inoculated by soaking for 1 h in a suspension of *P. fluorescence* SS5 (5X 10^{6} Bacteria ml^{-1}). Control consists of uninoculated seeds.

The soil for pot experiment was collected from in 0 to 15 cm depth from the farmer field. The soil was analyzed for various physiochemical characteristics. The general characteristics of the soil were: pH 7.7; EC 2.2 dS m^{-1}; available nitrogen 72.8 mg kg^{-1}, phosphorus 8.23 mg kg^{-1}, potassium 245 mg kg^{-1} and organic carbon 4.0 g kg^{-1}. The soil was clayey in texture and classified as Typic Haplusterts.

A pot culture and field experiment were conducted to studied the response of plants to *Pseudomonas fluorescence* SS5 and its

effect on tomato plant morphology and yield.

Pot culture study

The soil was transferred to 14 kg plastic pots. Bacterized and nonbacterized Seed were sown in two sets of treatments: (i) bacterized seed (ii) nonbacterized seed (control). The seed germination was noted on the 14th day of sowing. Hybrid Vipul tomato seeds was used in pot culture experiment. The pots were irrigated routinely. The experiment was conducted in a completely randomized block design and each treatment was replicated five times. Seedling growth, phenology, were recorded. Two plants from each pot were randomly selected for recording the data. The data were analyzed statically by using analysis of variance to find out the significance levels.

Field study

A field experiment was carried out at farmer's Farm, Shahganj during 2012-13 seasons. The bacterized and non bacterized treated tomato hybrid 'Vipul' seeds were sown in the field on winter; each of the following treatments had a plot size of 5 m^2. Two weeks after emergence, plants were thinned and single plant was maintained per hill.

Agronomical measurement of tomato plants

All observation (leaf area measurement, plant height, shoot and root length measurement, number of branches, nodes, flowers, fruits, yield and yield attributes characters) recorded were used agronomical standard methods.

Result and Discussion

In the present study, the production of secondary metabolites, viz., IAA, HCN, and siderophore, has been assessed to elucidate the agronomical significance of the soil isolate *Pseudomonas fluorescence* strain SS5. *Pseudomonas fluorescence* strains SS5 clearly indicate the production of substantial amounts of IAA during growth in nutrient broth. The presence of tryptophan in the medium substantially enhanced the IAA production. The strain SS5 also showed the production of fluorescence color on nutrient agar plates on the UV gel document (Fig 1).

The strain SS5 also show the production of siderophore on CAS agar plates (Fig 2) Appearance of a reddish brown zone surrounding the inoculums on CAS agar plates. The strain SS5 exhibited significant growth inhibitory activity against a fungi and the size of zone of inhibition produced with the strain SS5 was noticed (Fig 3). Further, screening of the strain SS5 for HCN production. A remarkable change in color from yellow to reddish brown with strain SS5 this color change indicates HCN production. The data revealed a decreased level of HCN production under low iron conditions (Fig 4.). Detection and characterization of plant growth activity produce by *pseudomonas fluorescence* strain SS5 show in (Table 1& Fig 6).

Effect of *Pseudomonas fluorescence* Strain SS5 on tomato plants-

Tomato plant tended to increased significantly the growth parameters (plant height, leaf number and area, fresh and dry weight) in the inoculated of *Pseudomonas fluorescence* SS5 compared to control tomato plant. The different agronomical parameters were observed in tomato plant leaves of appropriate stage of analysis and the values obtained are presented in (Tables 2, 5, & 6,).

Pot culture experiment

Basic studies on morphological traits of pot grown Tomato plants as given in (Table-2). Showed that *pseudomonas fluorescence* SS-5 resulted in significant increase as measured on 70 (DAS) day after sowing, in leaf area, leaf weight, number of buds, flowers, fruits / plant. Contribution to 19 % increase in yield (209 gm / plant) over control in yield (175 gm/plant). All produced yield on par with control only and demonstrates the significant effect of treated plants on yield and yield contributing parameters.

Field Experiments

In the present study, measured initial soil fertility status of the field experimental site data pertaining to the availability of major nutrients namely nitrogen, phosphorus and potassium are presented in (Table 3). The soils from agricultural area having pH are near to neutral (7.7). Available nitrogen (N) 72.8 mg/kg. Available phosphorus (P) 8.24 mg/kg. Available potassium is 253 mg/kg.

Measured post harvest soil fertility status of the control and bacterized field experimental site. pH EC and OC (organic carbon) and the availability of major nutrients namely N, K, P are presented in (Table 4)

The increased physiological yield and yield attributes characters effect on these parameters by P. fluorescence SS5 finally reflecting on the positive increased in total Biomass and yield (Table 6). The increase in yield with *P .fluorescence* treatment was higher than the increase in biomass suggesting greater partitioning of biomass towards yield. In field experiment Fruit yield (Fresh wt Kg/ha) increase in 57% and total biomass (dry wt kg/ha) increase in 28% over the control. Amongst the yield attributing characters *P. fluorescence* influenced the fruit number to a greater extent than fruit weight suggesting either increased production or higher retention of fruiting forms in *P. fluorescence* plants.

P. fluorescence (SS5) in tomato significantly increased the yield over control. The pot experiment increase in tomato yield was 19% and treated plants on yield and yield contributing parameters and physiological traits of pots grown tomato plants. In field experiment Fruit yield (Fresh wt Kg/ha) increase in 57% and total biomass (dry wt kg/ha) increase in 28% over the control. However, there is species variability in response to in *P. fluorescence* (SS5) indeterminate crops like tomato contributed more to fruit number, and fruit weight.

The greater yield and biomass production of *P. fluorescence* treated plants was found to be associated with increased rate of uptake of nutrients and thus they had higher nutrient uptake. Further *P. fluorescence* treated plants had higher growth and yield as compared to control, thus demonstrating the yield enhancing properties of *P. fluorescence* SS5 used as plant growth promoter bacteria. *P. fluorescence* treated tomato plants had significantly higher physiological efficiency indicating better ability to transfer acquired nutrient into economic yield.

Results of the current study showed the positive impacts of *P. fluorescence* on growth of tomato plant compared to control. So as a simple and safe method, the seeds of tomato plant before planting can be inoculated with *P. fluorescence* to improvement plant growth efficiency. It appears that can led to improve quantity and quality of tomato (*Lycopersicon esculentum*) plant by accumulated the organic and inorganic components. Generally, using

bacterization treatment of seeds could be a promising technique for agricultural improvements but extensive research is required on different crops. These results proved that plant growth regulators produced by *Pseudomonas* species could also play a critical role in plant growth promotion.

Table.1 Detection and characterization of plant growth promoting activity produced by *Pseudomonas fluorescence* Strain SS5

Test	***Pseudomonas fluorescencs* SS5**
Hydrogen cyanide production	+++
Indole acetate acid production	+++ (25mg/ml)
Siderophore production	+++
Phosphate solubilization	+++ (19mm)

+++ = High production, ++ = Moderate production.

Table.2 Effect of control and bacterized plants on morphological traits at 70 days after Sowing and yield and yield attributing characters (fruit number and weight) at harvest of pot grown tomato plants

Treatments	**Leaf area (cm^2)**	**Stem wt (g)**	**Root wt (g)**	**Leaf wt (g)**	**No. of buds/ plant**	**No. of flowers/ plant**	**Fruits/ plant**	**Fruit wt (g)**	**Yield (g/plant)**	**% Increase over control**
Control	537	3.96	1.49	5.23	7	7	14	12.45	175	19
Bacterized	886	7.36	2.34	8.61	20	14	22	9.96	209	

Table.3 Initial soil fertility status of the experimental site

Treatments	**Initial soil fertility (mg/kg soil)**					
	pH	EC	OC %	Av. N	Av. K	Av. P
Control	7.7	EC 2.2 dS m-1	0.54%	72.8	253	8.24

EC= Electrical Conductivity, OC= Organic Carbon, Av. N= Available Nitrogen, Av. K= Available Potassium, Av. P= Available Phosphorous

Table.4 Post harvest soil fertility status of the experimental site

Treatments	**Post harvest soil fertility (mg/kg soil)**					
	pH	EC	OC %	Av. N	Av. K	Av. P
Control	7.6	EC 2.2 dS m-1	0.48%	70.93	260.33	12.77
Bacterized	7.9	EC 2.3 dS m-1	0.58%	72.20	346.00	14.03

EC= Electrical Conductivity, OC= Organic Carbon, Av. N= Available Nitrogen, Av. K= Available Potassium, Av. P= Available Phosphorous

Table.5 Effect of control and bacterized plants on morphological traits at 70 days after Sowing and yield and yield attributing characters (fruit number and weight) at harvest of field grown tomato plants

Treatments	Leaf area (cm2)	Stem wt (g)	Root wt (g)	Leaf wt (g)	No. of buds/ plant	No. of flowers/ plant	Fruits/ plant	Fruit wt (g)	Yield (g/plant)
Control	622	4.17	1.98	6.93	8	10	18	13.85	201
Inoculated	967	8.72	2.89	9.71	26	19	28	11.50	289

Table.6 Effect of *P. fluorescence* SS-5 on yield and total biomass of field grown tomato plants

Treatment	Fruit yield (Fresh wt Kg/ha)			% increase over control	Total biomass (dry wt kg/ha)			% increase over control
Control	4211	4321	4266	57	1477	1673	1575	28
Inoculated	6682	6760	6721		1926	2082	2004	

Fig.1 Production of fluorescence by fluorescent pseudomonad strain SS5 on Nutrient agar plates

Fig.2 Production of siderophore by fluorescent pseudomonad strain SS5 on chrome azurol (CAS) agar plates

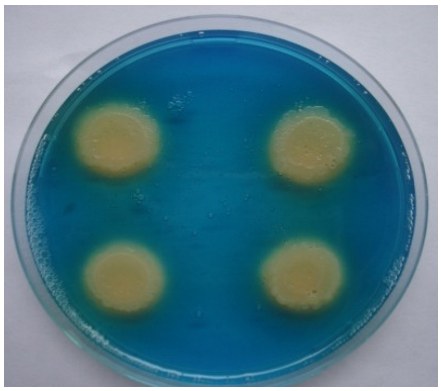


Fig.3 Production of Phosphate solublization by fluorescent pseudomonad strain SS5 on Pikovskaya agar medium plates (Himedia, Mumbai)

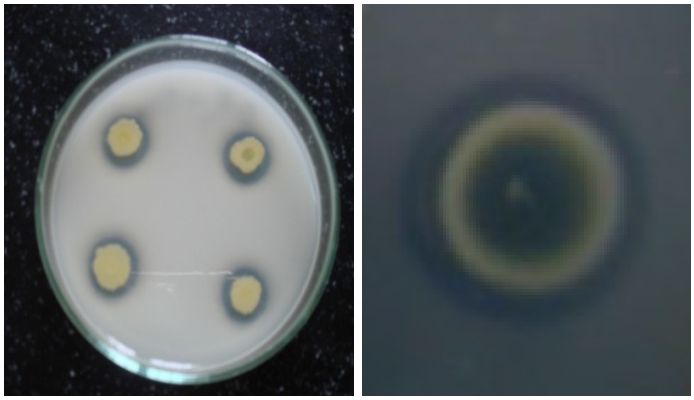
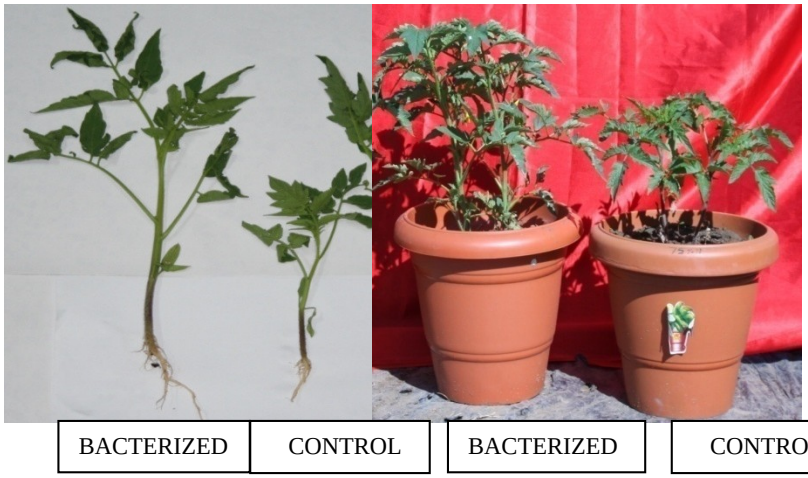


Fig.4 Production of HCN (hydrogen cynide) by fluorescent pseudomonad strain SS5 on King's B (KB) agar plates

Figure.5 Schematic figure showing field and pot study respectively



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