

## Original Research Article

<https://doi.org/10.20546/ijcmas.2017.606.333>

## Biochemical Differentiation of *Pseudomonas fluorescens* of Assam Soil and their Utility in Management of Bacterial Wilt of Solanaceous Crops

Thalhun L. Kipgen\* and L.C. Bora

Department of Plant Pathology, Assam Agricultural University, Jorhat-785013, Assam, India

\*Corresponding author

### ABSTRACT

Potential of antagonistic rhizobacteria *Pseudomonas fluorescens* in the management of bacterial wilt of different solanaceous crop, viz., tomato, brinjal and chilli caused by *Ralstonia solanacearum* was evaluated under in vitro and in vivo conditions. A total of twenty five (25) *P. fluorescens* isolates were collected from different rhizospheric soils of Assam and their characterization was done. Five (5) isolates produced fluorescent yellow green or bluish green diffusible pigment of variable intensities on King's B medium under UV light (365 nm). These five isolates were identified to be classified under Biovar I (Pf-A1, Pf - A4, Pf - A7), Biovar II (Pf-A6) and Biovar III (PfA8) respectively, according to their biochemical differentiations. Dual culture tests using paper disc assay revealed that the strains Pf-A8 could cause highest inhibition in growth of *R. solanacearum* (tomato) by 76.0 per cent, 63.0 per cent (Brinjal) and 72.2 per cent (Chilli), respectively. Application of Pf-A8 based bio formulation as seed treatment, root treatment and soil application exhibited lowest wilt incidence (1.0%) in tomato plants, 2.0 per cent in brinjal plants and 4.0 per cent in chilli plants, respectively. The population dynamics of the pathogen and antagonist in crop rhizosphere soils showed significantly increase in all treatments as compared to controls. Corresponding to the enhancement of *P. fluorescens* population in the treated rhizosphere soil there was decline of *R. solanacearum* population. The correlation studies established a negative correlation between PWI and population density of *P. fluorescens* as well as between population densities of *R. solanacearum* and *P. fluorescens*. The finding furthers supports the biocontrol properties of the antagonistic strain *P. fluorescens*. The isolate Pf-A8 strains could serve as promising bioagent although needs further in situ investigations.

#### Keywords

*Ralstonia solanacearum*,  
*Pseudomonas fluorescens*,  
Isolate,  
Bioagent,  
Wilt,  
Population dynamics.

#### Article Info

Accepted:  
26 May 2017  
Available Online:  
10 June 2017

### Introduction

The bacterial wilt solanaceous crops caused by *Ralstonia solanacearum* is an important soil borne bacterial plant pathogen with worldwide distribution and a wide host range of more than 200 species in 50 families (Hayward, 1991). Some of its economically important plant hosts include tomato, potato, eggplant, pepper, tobacco, banana, chilli, and peanut (French and Sequeira, 1970). Tomato

(*Lycopersicon esculentum* Mill), Brinjal (*Solanum nigrum*) and chilli (*Capsicum annum* L) are some popular and widely grown vegetable crops in the world and are major source of income for the small and marginal farmers. The major constraint in their production is the bacterial wilt disease as it causes severe plant mortality and yield loss up to an extent of 90 to 100 per cent (Kishun,

1989). *R. solanacearum* a ubiquitous soil borne bacterial pathogen, and its management by single method of control is very difficult. Environmental damage, cost and high labour inputs are some of the drawbacks of conventional chemointensive methods of disease management. Use of biological control by antagonistic microorganisms could be important alternative management technique that can be integrated with other practices for effective disease management at the field level. Primarily *Pseudomonas fluorescens* has been identified as an important microorganism with ability for plant growth promotion, increase yield and reduce severity of many plant diseases (Wei *et al.*, 1996). The present study was made to isolate new and aggressive native strains of *P. fluorescens* from Assam soil, characterize them and evaluated their potential biocontrol activity for suppressing bacterial wilt of solanaceous crops.

### **Materials and Methods**

The experiments were carried out in the laboratory and Green House of the Department of Plant Pathology, Assam Agricultural University, Jorhat. Cultures of *R. solanacearum* were isolated from infected tomato, brinjal and chilli plants using Triphenyl Tetrazolium Chloride (TTC) agar medium. The virulent colonies characterized by dull white colour, fluidal, irregularly round with light pink centres were further streaked on TTC medium to get pure colonies of the bacterium. Morphological, cultural and biochemical characterization were done inside the laboratory of Department of Plant Pathology, AAU Jorhat. Inoculum was prepared by using 24 hr old bacterial suspension, adjusted to optical density (O.D) 0.5 in Spectrophotometer (Spectronic 20) using blue filter (425nm) to obtain a bacterial population of  $1 \times 10^8$ cfu/ml.

Seeds of tomato (cv. Pusa Ruby), brinjal (cv. Pusa Kranti) and chilli (cv. Bor Bhut) were sown on earthen pots (26cm x 22cm x 32cm) simulating nursery beds. The pots were filled with sand and potting medium in the ratio 1:3 respectively. For the pathogenicity test, a set of three 30 days old brinjal plants were injected with suspension of *R. solanacearum* (@  $1 \times 10^8$ cfu/ml) following root inoculation technique (Winstead and Kelman, 1952). Another set of three seedlings were inoculated with sterile distilled water to serve as control.

### **Isolation and characterization of *P. fluorescens***

Isolates of *P. fluorescens* were collected from the rhizospheric soils sampled from different districts of Assam. One gram of soil from each rhizospheric sample was mixed with 10 ml of sterile water and vortex for 10 min to obtain standard soil suspension. Isolation of *P. fluorescens* was made by following serial dilutions (10<sup>-1</sup> to 10<sup>-8</sup>) using King's B medium. A well separated individual colonies with fluorescent yellowish-green and fluorescent yellow pigments were marked and detected by viewing under UV light (366 nm) after 24 hr. The individual colony was picked up and streaked on to fresh KMB slants. The slants were covered with mineral oil and preserved at 4°C for further use.

For morphological, cultural, physiological and biochemical characterization of *P. fluorescens*, pure cultures of each isolate were streaked on fresh King's B agar Petri plates separately for colony development and gram staining, examined for shape, colony elevation, colony edge and pigment production.

Biochemical properties like were studied following the guidelines described in Bergey's Manual of Determinative Bacteriology 9th edition (Holt *et al.*, 2000;

Maki *et al.*, 2011). Production of HCN and siderophore test by the bacterial antagonists were also determined (Payne, 1994).

Aggressive strains of *P. fluorescens* based on their ability to produce fluorescent yellowish-green and fluorescent yellow pigments, HCN production, siderophore production, etc., were identified and selected to test their antagonistic property against *R. solanacearum* in vitro and use as bio formulation for management of bacterial wilt disease in tomato, brinjal and chilli in vivo.

### **In vitro evaluation of *P. fluorescens* against *R. solanacearum***

In vitro tests for evaluation of *P. fluorescens* against *R. solanacearum* was conducted following dual culture method (Anuratha *et al.*, 1990). *R. solanacearum* was grown in conical flask containing 40 ml of nutrient broth, incubated at 25°C±1°C for 48 hrs. One ml of bacterial suspension was mixed with 15 ml of molten KMB and poured onto a sterile Petri plate to get bacterial lawn. Sterile filter paper disc (0.8 cm) was laid on the agar surface at the centre of the Petri plate and 25µl of 48h old broth cultures of selected *P. fluorescens* were applied to each disc. Plates were then incubated at 25°C±1°C for 120 hrs. The diameter of the inhibition zones around the disc was measured using antibiotic zone scale.

The efficacy of *P. fluorescens* based bio formulation in controlling bacterial wilt of tomato, brinjal and chilli were evaluated in the greenhouse using susceptible variety viz., tomato (var. Pusa Ruby), brinjal (var. Pusa Kranti) and chilli (var. Bor Bhoot), following complete randomized design (CRD). For preparation of substrate based bio formulation of aggressive *P. fluorescens*, finely sieved talcum powder was filled in 1 kg capacity polypropylene (PP) bags and sterilized at

121°C. Pure cultures of *P. fluorescens* grown in KB slants were washed with sterile distilled water and 15 ml of this suspension was added aseptically to 1 lit of Nutrient broth contained in conical flask. The flask after thorough stirring was incubated at 28±1 °C for 72 h to obtain a bacterial concentration of 1×10<sup>7</sup>cfu/ml. Then 15 ml of the bacterial cells were inoculated in to the PP bags containing talcum powder. To facilitate greater adherence property of the substrate, 10 ml of sticker, carboxy-methyl cellulose (CMC @1%) was added aseptically. Similarly, 10 ml of an osmoticant (mannitol @ 1%) was added to impart the substrate a higher moisture retaining property. The polypropylene bags after thorough mixing were incubated at 28±1 °C for 7 days.

### **Evaluation of different *P. fluorescens* based bio formulation against bacterial wilt of solanaceous crops**

Different *P. fluorescens* based bio formulation was applied as seed treatment, root treatment and soil application to evaluate the efficacy of the antagonists in controlling the bacterial wilt in tomato, brinjal and chilli crops. For seed treatment, paste slurry of each substrate based formulation was prepared by mixing 100 g of the formulation in 200 ml of water. To the paste slurry, tomato, brinjal and chilli seeds were dipped @ 1000 seed/100 ml of paste solution for 1 hr to coat the seed with the formulations. The coated seeds were then removed from the slurry and spread over a paper in cool and dry place (under shade for overnight) for drying. Treated seeds are then sown in the nursery.

For root treatment, each of the substrate based formulations was mixed with water @ 20 g in 1000 ml to prepare 2 per cent bio formulation solution. At the time of transplanting uprooted plants from nursery were root dipped (@1000seedlings/1000 ml) in the solution for

1 h. treated seedlings were dried under shade for 1 h before transplanting in to earthen pots. For soil application, each of the substrate based formulations was mixed with soil contained in earthen pots @ 5% solution. The mixture was applied @ 100 ml/pot (containing 20 kg of garden soil) after 15 days of sowing, at the base of the plants. After application of formulation, it was kept under net house to protect from direct exposure to sunlight. Five replications for each treatment with 5 plants per replication was maintained. The tomato, brinjal and chilli plants challenged with *R. solanacearum* cell suspension @ 10<sup>8</sup>cfu/mL by following root inoculation technique (Blair *et al.*, 1971). The controls included only the pathogen inoculated treatment (inoculated control). The number of wilted plants in each treatment was continuously recorded up to 90 days after inoculation with pathogen. The number of completely wilted plants was tabulated for each formulation and the percent (%) wilt incidence was calculated.

For quantitative determination of pathogen and antagonist population in the rhizosphere soil, the population of the antagonist and the pathogen in the pot soil was estimated 90 days after transplanting following the serial dilution plate technique. The correlation studies between percent wilt incidence (PWI) and population of *P. fluorescens* and also between *P. fluorescens* and the pathogen *R. solanacearum* were also carried out.

## Results and Discussion

Different strains of wilt pathogen *R. solanacearum* isolated from bacterial wilt infected tomato, brinjal and chilli plant from Assam were found to be basically similar in relation to their morphological and biochemical properties (Table 3). In TTC medium, the colony showed virulent creamy white /whitish fluidal irregular round colonies

with light pink centre; which are the characters of virulent *R. solanacearum*. The pathogenicity tests established the isolated bacteria from wilted tomato, brinjal and chilli plants were different strains of *R. solanacearum*, the causative agent of bacterial wilt disease.

Altogether, 25 isolates of *P. fluorescens* associated rhizosphere soils of Assam were collected, out of which, five (5) isolates were found to produce fluorescent yellow-green or bluish green diffusible pigment of variable intensities on KMB medium under UV light (Table 1). Earlier studies suggest that fluorescent Pseudomonads are most active and dominant bacteria inhabiting the rhizosphere of diverse crop plants that could produce fluorescent yellow green diffusible pigment on specific medium like KMB (Reddy and Rao, 2009).

Morphologically, *P. fluorescens* are gram's negative short rods with a variation of size from 0.6 to 0.8µm × 1.7 to 1.9µm. Colonies were circular convex with smooth and glistening surface, shiny water soluble and fluorescently pigmented appearance under UV light (Table 3). Biochemically, all native isolates of *P. fluorescens* showed similar results with regard to KOH test (+), catalase (+), arginine dehydrolase (+), oxidase (+), gelatin liquefaction (+), dextrose utilization (+), citrate utilization (+), fluorescent pigment production (+), growth at 4°C (+), growth at 27°C (+) and starch hydrolysis (-). However other biochemical tests like levan formation, denitrification, and H<sub>2</sub>S gas production tests showed variation in reaction. Earlier, Kuarabachew *et al.*, (2007) characterized fluorescent Pseudomonads on the basis of biochemical tests such as fluorescent production, levan formation, certain carbohydrate utilizations and morphological features of the isolates.

Results of biochemical tests recorded were compared with the similar tests outlined for beneficial bacteria (Holt *et al.*, 2000) and they were tentatively placed in biovar I, II and III (Table 5). On the basis of the results of levan, formation, denitrification and H<sub>2</sub>S gas production tests, *P. fluorescens* isolates were placed under three biovars. The isolates were also found HCN and siderophore positive for all five isolates (Table 3). Pf-A8 produced highest (80.86%) siderophore followed by Pf-A2 (76.39 %). Siderophore play an important role in the plant growth because of their ability to supply iron (Ramos-Solano *et al.*, 2010). On the other hand, Ramette *et al.*, (2003) reported that HCN was a broad spectrum antimicrobial compound involved in biological control of root diseases by many plant associated fluorescent pseudomonads.

The result of inhibitions produced (Table 2) by different isolates of *P. fluorescens* against Tomato *R. solanacearum* revealed that the highest inhibition was produced by isolate Pf-

A8 with suppression of 76.0 per cent growth, followed by Pf-A6 with 67.0 per cent growth suppression. In case of Brinjal *R. solanacearum*, the highest inhibition was produced by isolate Pf-A8 with suppression of 63.0 per cent growth followed by isolate Pf-A1 with suppression of 60.0 per cent growth. Similarly, in case of Chilli *R. solanacearum*, isolate Pf-A8 produced highest inhibition followed by isolate Pf-A1 with suppression of 72.2 per cent and 48.9 per cent growth, respectively. The antagonistic ability of Pf isolates might be due to the production of secondary metabolites like siderophore, hydrogen cyanide and due to the production of antibiotics like 2-4-diacetylphloroglucinol (DAPG), pyrrolnitrin (PRN), pyoluteorin (PLT), etc. (Hass and Keel (2003). Earlier, Nath *et al.*, (2015) screened the antagonistic potential of bioactive microorganisms like *P. fluorescens* under in vitro condition and observed highest inhibition of 57.70 per cent against *R. solanacearum*.

**Table.1** Sources of *R. solanacearum* isolates associated with rhizospheric soils of Assam

Isolated code	Species	Origin/District	Rhizospheric Soil of Associated Plant
PfA1	<i>P. fluorescens</i>	Jorhat	Rice
PfA4	<i>P. fluorescens</i>	Bokakhat	Teak
PfA6	<i>P. fluorescens</i>	Karbianglong	Wild Banana
PfA7	<i>P. fluorescens</i>	Karbianglong	Mango
PfA8	<i>P. fluorescens</i>	Karbianglong	Pineapple

**Table.4** Suppression of *R. solanacearum* of tomato, brinjal and chilli (cm diam.) by different *P. fluorescens* after 120 h of incubation *in vitro*

<i>P. fluorescens</i> Strains	<i>R. solanacearum</i> (Tomato)		<i>R. solanacearum</i> (Brinjal)		<i>R. solanacearum</i> (Chilli)	
	Colony diam.(cm) after 120h	Growth suppression (%)	Colony diam.(cm) after 120h	Growth suppression (%)	Colony diam.(cm) after 120h	Growth suppression (%)
Pf-A1	4.1	46	5.4	60	4.4	48.9
Pf-A4	4.1	46	4.2	38	4.0	44.4
Pf-A6	6.0	67	5.7	47	4.1	45.6
Pf-A7	3.8	42	3.6	40	4.3	47.8
Pf-A8	6.8	76	3.4	63	6.5	72.2
S.Ed	0.7		0.6		0.5	
CD	1.2		1.0		0.9	



**Table.2** Morphological and biochemical characters of different *Pseudomonas fluorescens* (Pf) and *Ralstonia solanacearum* (Rs)

Test/ Isolates	PfA 1	PfA 4	PfA 6	PfA 7	PfA 8	Rs (Tomato)	Rs (Brinjal)	Rs (Chilli)
Colony elevation	C	C	C	C	C	C	C	C
Colony edge	O	O	O	O	O	O	O	O
Surface & water soluble	S+	S+	S+	S+	S+	S+	S+	S+
Gram stain	-	-	-	-	-	-	-	-
Shape of cell	R	R	R	R	R	R	R	R
Spore formation	-	-	-	-	-	-	-	-
Motility	+	+	+	+	+	+	+	+
7% NaCl	+	+	+	+	+	-	-	-
Fl. pigment	+	+	+	+	+	-	-	-
KOH	+	+	+	+	+	+	+	+
Catalase	+	+	+	+	+	+	+	+
Oxidase	+	+	+	+	+	+	+	+
Gas prod	-	-	+	-	-	+	+	+
Starch hydrolyse	-	-	-	-	-	-	-	-
Gelatin hydrolyse	+	+	+	+	+	-	-	-
Dextrose utilize	+	+	+	+	+	+	+	+
Growth at 4°C	+	+	+	+	+	+	+	+
Growth at 41°C	-	-	-	-	-	-	-	-
Growth at 27°C	+	+	+	+	+	+	+	+
Levan prod	+	+	+	+	-	-	-	-
Arginine hydrolyse	+	+	+	+	+	-	-	-
Citrate utilize	+	+	+	+	+	+	+	+
Denitrification	-	-	+	-	+	+	+	+
HCN	+	+	+	+	+	-	-	-
Siderophore (%)	75	76	70	75	81	-	-	-

C=convex, O=round, S+= smooth, glistening and water soluble, R= rod shaped, -=negative and +=positive

**Table.5** Effect of different *P. fluorescens* based bioformulation on bacterial wilt incidence (%), population dynamics of *R. solanacearum* and *P. fluorescens* ( $1 \times 10^9$  cfu/g) in tomato rhizosphere after 90 days of transplanting (DAT)

Treatment	Wilt incidence (%)*	Population density of <i>P. fl</i> **	Population density of <i>R. sol</i> **
T1: Pf-A1	2.0 (8.13)	14.68 (1.17)	0.56 (-0.25)
T2: Pf-A4	32.0 (34.45)	8.54 (0.93)	4.74 (0.67)
T3: Pf-A6	1.0 (5.74)	15.3 (1.18)	1.2 (0.08)
T4: Pf-A7	18.5 (25.47)	9.7 (0.97)	2.8 (0.45)
T5: Pf-A8	1.0 (5.74)	15.92 (1.20)	0.24 (-0.62)
T6: Inoculated control	76.0 (60.60)	0.02 (-1.7)	19.66 (1.29)
S.Ed	0.05	0.05	0.12
CD	0.08	0.08	0.21

\*=angular transformed value, \*\*=logarithm transformed value

**Table.3** Differentiation of *P. fluorescens* based on phenotypic characterizations

Type of Biovar	Strains of <i>P. fluorescens</i>
Biovar I	<i>Pf</i> -A1, <i>Pf</i> -A4 and <i>Pf</i> -A7 (3)
Biovar II	<i>Pf</i> -A6 (1)
Biovar III	<i>Pf</i> -A8 (1)

**Table.6** Effect of different *P. fluorescens* based bioformulation on bacterial wilt incidence (%), population dynamics of *R. solanacearum* and *P. fluorescens* ( $1 \times 10^9$  cfu/g) In brinjal rhizosphere after 90 DAT

Treatment	Wilt incidence (%)*	Population density of <i>P. fl</i> **	Population density of <i>R. sol</i> **
T1: <i>Pf</i> -A1	2 (8.13)	15.8 (1.20)	0.33 (-0.49)
T2: <i>Pf</i> -A4	12.0 (20.27)	10.78 (1.03)	3.58 (0.55)
T3: <i>Pf</i> -A6	8.0 (16.43)	13.48 (1.13)	1.70 (0.23)
T4: <i>Pf</i> -A7	11.0 (19.37)	10.3 (1.01)	2.87 (0.46)
T5: <i>Pf</i> -A8	4.0 (11.54)	14.28 (1.15)	1.88 (0.27)
T6: Inoculated control	68.0 (55.55)	0.03 (-1.52)	19.02 (1.28)
S.Ed	0.07	0.07	0.19
CD	0.12	0.12	0.33

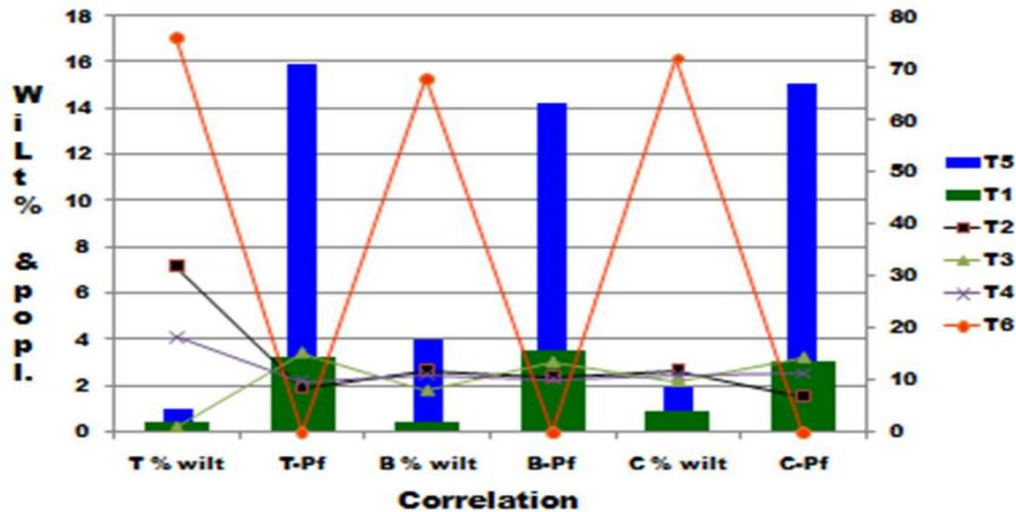
\*=angular transformed value, \*\*=logarithm transformed value

**Table.7** Effect of different *P. fluorescens* based bioformulation on bacterial wilt incidence (%), population dynamics of *R. solanacearum* and *P. fluorescens* ( $1 \times 10^9$  cfu/g) In chilli rhizosphere after 90 DAT

Treatment	Wilt incidence (%)*	Population density of <i>P. fl</i> **	Population density of <i>R. sol</i> **
T1: <i>Pf</i> -A1	4.0 (11.54)	13.92 (1.14)	1.74 (0.24)
T2: <i>Pf</i> -A4	12.0 (20.27)	6.94 (0.84)	6.00 (0.78)
T3: <i>Pf</i> -A6	9.8 (18.24)	14.4 (1.16)	1 (0.00)
T4: <i>Pf</i> -A7	11.0 (19.37)	11.26 (1.05)	3.4 (0.53)
T5: <i>Pf</i> -A8	2.0 (8.13)	15.12 (1.18)	1.36 (0.13)
T6: Inoculated control	72.0 (58.05)	0.02 (-1.7)	18.44 (1.26)
S.Ed	0.07	0.07	0.16
CD	0.12	0.12	0.28

\*=angular transformed value, \*\*=logarithm transformed value

**Fig.1** Correlation between the population density of *P. fluorescens* and PWI in tomato, brinjal and chilli rhizosphere soil



The result of the effects of *P. fluorescens* based bio formulation in vivo revealed that the wilt incidence in tomato, brinjal and chilli decreased significantly by different bio formulations compared to inoculated control. The lowest wilt incidence in tomato (1.0%) was recorded in the treatment with Pf-A8 based bio formulation and Pf-A6 based bio formulation applied as seed treatment, root treatment and soil application followed by Pf-A1 based bio formulation (2.0%) (Table 4). Tomato plants treated with only *R. solanacearum* (inoculated control) showed highest disease incidence (76.0%). In brinjal plants, the lowest disease incidence was exhibited by the bio formulation Pf-A1 based bio formulation (2.0%) followed by Pf-A8 based bio formulation (4.0%) and Pf-A6 based bio formulation (8.0%) (Table 6). Brinjal plants treated with only *R. solanacearum* (inoculated control) showed highest disease incidence (68.0%). Similarly, the lowest disease incidence in chilli treated plants was exhibited by the bio formulation Pf-A8 (2%) followed by Pf-A1 based bio formulation (4%) and Pf-A6 based bio formulation (9.8%) (Table 7). Chilli plants treated with only *R. solanacearum* showed highest disease incidence (72.0%). The results

are found in agreement with Bora and Deka (2007) who found that application of *P. fluorescens* based biopesticide (Biofor-Pf) as combination of seed treatment, root application and soil application at transplanting showed minimum wilt incidence.

The reduced in incidence of *R. solanacearum* up to 50 per cent in banana, 49 per cent in brinjal and 36 per cent in tomato due to *P. fluorescens* treatment was also recorded by Anuratha and Gnanamanickan (1990). Srivastava *et al.*, (2010), Nath *et al.*, (2015) also obtained significant reduction in bacterial wilt incidence in solanaceous crop by application of *P. fluorescens* alone or in consortia. For the effective management of any soil borne disease, the introduced antagonist should colonize root (Weller, 1984). Root zone application of *P. fluorescens* increased rhizosphere population of the bacteria since some of these strains have the ability to colonize the roots (Vidhyasekaran and Muthamilan, (1995).

Results of the population dynamics of *R. solanacearum* and *P. fluorescens* in the rhizosphere soil of tomato, brinjal and chilli



assayed 90 days after transplanting (DAT) are presented in tables 4, 6 and 7, respectively. Population dynamics of *P. fluorescens* in rhizosphere soil showed significant increase in all treatments as compared to inoculated controls. Corresponding to the enhancement of *P. fluorescens* population in the treated rhizosphere soil there was decline of *R. solanacearum* population. Similar phenomenon was observed by Bustamante *et al.*, (1989), when they recorded reduction of *R. solanacearum* population with corresponding increase in *P. fluorescens* population in the rhizosphere.

The correlation studies established a negative correlation between the size of *P. fluorescens* population and per cent wilt incidence in tomato, brinjal and chilli (Fig. 1). Although the correlation values were found statistically non-significant, negative correlation indicated that with increase in the size of antagonists population there was corresponding decrease in the PWI in the plants that maybe due to the fact that higher population of antagonists and their activities resulted either in suppression of the pathogen population or exclusion of pathogens from soil rhizosphere. Bora and Deka (2007); Chakravarty and Kalita (2012) observed similar phenomenon in tomato and brinjal, when they recorded negative correlation between *P. fluorescens* and PWI as well as between population densities of *R. solanacearum* and *P. fluorescens*. Nath *et al.*, (2016) observed negative correlation between bacterial wilt incidence and yield of tomato. Earlier, Weller and Cook (1983) also correlated the influence of antagonist population with suppression of disease incidence, and they suggested that *P. fluorescens* inhibit pathogens by competing with them for nutrients or by producing siderophore, antibiotics or HCN and May also produce substances that stimulates plant growth. The finding furthers supports the biocontrol properties of the antagonistic strain

*P. fluorescens*. In addition to the environmental factors, the PWI is chiefly dependent on the population of *R. solanacearum* in the soil.

### Acknowledgements

The authors are highly grateful to AAU, Jorhat for providing facilities and Maulana Azad National Fellowship for Minority Students, UGC for financial assistance to carry out the study.

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**How to cite this article:**

Thalhun L. Kipgen and Bora, L.C. 2017. Biochemical Differentiation of *Pseudomonas fluorescens* of Assam Soil and their Utility in Management of Bacterial Wilt of Solanaceous Crops. *Int.J.Curr.Microbiol.App.Sci.* 6(6): 2796-2806.  
doi: <https://doi.org/10.20546/ijcmas.2017.606.333>