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Morphological Characterization and Antagonstic Effect of Native *Pseudomonas* Spp. of South Gujarat

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An experiment was conducted in Department of Plant Pathology, N. M. College of Agriculture, N.A.U., Navsari to isolate, characterize and evaluate antagonistic efficacy of *Pseudomonas* spp. The six *Pseudomonas* spp. were isolate from different locations of south Gujarat and evaluated antagonistic efficacy *in vitro* against *Pyricularia oryzae* Cavara (Finger millet blast), *Colletotrichum falcatum* Went (Sugarcane red rot), *Fusarium moniliformae* Sheldon (Sugarcane wilt), *Macrophomina phaseolina* (Tassi) Goud (Chickpea root rot), *Sclerotium rolfsii* Sacc. (Groundnut stem rot), *Pythium aphanidermatum* (Eds.) Fitz. (Tobacco damping off), *Pestalotiopsis anacardii* (Pestolatia blight of mango), *Lasiodiplodia theobromae* Pat. (Stem end rot of mango), *Xanthomonas axonopodis* pv. *oryzae* (Bacterial leaf blight of rice), *Xanthomonas axonopodis* pv. *malvacearum* (Bacterial leaf blight of cotton) and *Xanthomonas axonopodis* pv. *citri* (Citrus canker) pathogens. Among the six isolates, PaRS (*Pseudomonas aeruginosa* Rambhas soil) showed maximum growth inhibition and minimum growth of pathogens. PaRS has dirty green colour and colonies were round and non-spreading with rod shaped cells.

Key words: *Pseudomonas*, Morphology and Antagonism

The novel technologies in all areas of agriculture have improved agricultural production, but it affecting the environment. The recent challenge faced by modern agriculture is to achieve satisfactory control of plant disease in environment friendly manner (Chettri *et al.*, 2010). The search for effective alternative approaches to chemical control which have minimal deleterious effects, more ecofriendly and will contribute to the goal of sustainability in agriculture is needed (Jain *et al.*, 2009). Biological control is a potential alternative to chemically based disease control. Because of this increasing awareness of the problems associated with the chemically based disease control, biological control has received considerable attention during the last 40 years as a potential alternative (Cook and Baker, 1983). Plant growth promoting bacteria (PGPR) are indigenous to soil as well as the plant rhizosphere and play a major role in the biocontrol of plant pathogens. Recent progress in their diversity, colonizing ability and mechanisms of action, formulation and application should facilitate their development as reliable biocontrol agents against plant pathogens. *Pseudomonas fluorescens*, *P. putida* and *P. aeruginosa* strains are known to be beneficial to plants. Some strains have been recognized for a long time as biocontrol agents (Mercado-Blanco *et al.*, 2007). Present study is done to isolate,

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characterize and evaluate the native isolates of *Pseudomonas* under south Gujarat condition.

MATERIALS AND METHODS

Isolation and identification of *Pseudomonas*

The soil samples were collected from the paddy and finger millet fields of Waghai, banana field and Ambika river of Rambhas, farm pond of K.V.K. and castor rhizosphere from LRS farm, NAU, Navsari (Table 1). The soil samples of rice, banana, finger millet and castor rhizosphere were collected from ten plants of each field randomly. Each sample was taken separately in polythene bags, tied with a rubber band and labeled. Soil samples were analyzed on the day of collection. One gram of soil was mixed thoroughly in 10 mL sterile water and processed to follow serial dilution plate technique and was spread on King's B (KB) medium to isolate *Pseudomonas* spp.. The plates were incubated at room temperature (28±2°C) for 24 hrs. Colonies that developed on KB plates were observed under UV light on a transilluminator. The colonies fluorescing under UV light were picked up, purified and preserved in nutrient broth. The *Pseudomonas spp.* isolated from different rhizosphere was coded in Table 1.

Morphological study

Pure cultures of the selected isolates were streaked on King's B (KB) agar Petri plates separately for colony development. The individual colonies were examined for shape, size, structure of colonies and pigmentation after 48 hrs.

Antagonistic evaluation

All the isolates of *Pseudomonas spp. viz*., *Pseudomonas aeruginosa* Waghai-1 (PaWP), *P. fluorescens* Waghai-2 (PfWN), *P. aeruginosa* Waghai-3 (PaWS), *P. fluorescens* Rambhas-1 (PfRB), *P. aeruginosa* Rambhas-2 (PaRS), *P. aeruginosa* Navsari-1 (PaNS), *P. fluorescens* Navsari-2 (PfNC) were used for the study of antagonism against important fungal and bacterial pathogens. The fungal and bacterial pathogens used were: *Pyricularia oryzae* Cavara (Finger millet blast), *Colletotrichum falcatum* Went (Sugarcane red rot), *Fusarium moniliformae* Sheldon (Sugarcane wilt), *Macrophomina phaseolina* (Tassi) Goud (Chickpea root rot), *Sclerotium rolfsii* Sacc. (Groundnut stem rot), *Pythium aphanidermatum* (Eds.) Fitz. (Tobacco damping off), *Pestalotiopsis anacardii* (Pestolatia blight of mango), *Lasiodiplodia theobromae* Pat. (Stem end rot of mango), *Xanthomonas axonopodis* pv. *oryzae* (Bacterial leaf blight of rice), *Xanthomonas axonopodis* pv. *malvacearum* (Bacterial leaf blight of cotton) and *Xanthomonas axonopodis* pv. *citri* (Citrus canker). Dual culture technique (Dennis and Webster, 1971) was used to test the fungal pathogens and paper disc method (Thornberry, 1950) for bacterial pathogens.

Dual Culture Technique

Sterilized Potato Dextrose Agar (PDA) i.e. 20 ml was poured aseptically in sterilized Petri plates of 90 mm diameter. Mycelial discs (5 mm) of 7 days old actively growing culture of the test pathogen was cut separately with the help of sterilized cork borer and placed on solidified PDA. The 48 hrs old bacterial culture was streaked opposite to pathogen. The experiment was repeated thrice along with their controls where test pathogen was alone placed for growth comparison. All the inoculated plates were incubated at room temperature (25 + 2^0C).

The radial growth of the test pathogens in treated and control plates were recorded after 4 days of incubation and the per cent inhibition of mycelial growth of the pathogens was calculated by using formula suggested by Vincent (1927).

$$PGI = \frac{100 \quad (DC \quad - \quad DT)}{DC}$$

Where,

PGI = Per cent growth inhibition

DC = Average diameter of mycelial colony of control

DT = Average diameter of mycelial colony of pathogens in treatments

Paper disc method

In paper disc method, 48hrs old culture of bacterial pathogens was spread with the help of sterile spreader on sterilized Nutrient Agar (NA) Petri plates. Three sterile 5 mm filter paper discs were dipped in the cell suspension (10^8 cells/ml) of *Pseudomonas spp.* for five min. and placed on the pathogens spreaded Petri plate. Three Petri plates were kept for each pathogen. Filter paper disc without dipping in *Pseudomonas spp.* and placed in NA Petri plate served as control. The Petri plates were incubated at room temperature (25+2^0C) for 2

days and surface area of inhibition was measured and compared with the control.

RESULTS AND DISCUSSION

Morphological characterization

The colony colour of PfWN, PfRB and PfNC isolates were yellowish, dull yellowish and yellowish white with high, medium and weak fluorescens, respectively. These isolates were round, non- spreading colony and rod shaped cells (Table 2). PaWP and PaRS produced light green and dirty green colour while colonies were round and non-spreading with rod shaped cells. The fluorescens was high in PaRS and medium in PaWP isolate (Table 2).

The colony of PaWS and PaNS were green and bluish green in colour. The colony shape was irregular and spreading in nature having rod shaped cells with medium fluorescens in both the isolates (Table 2).

Antagonistic effect of *Pseudomonas spp.* against different pathogens under *in vitro* condition

Pyricularia grisea

The minimum mycelial growth of the pathogen (2.70 cm) was recorded in PaRS which was significantly superior over the rest. The next best isolate for growth inhibition was PaNS (3.37 cm). The highest mycelial growth was recorded in PfRB (4.53 cm). Maximum growth inhibition was recorded in isolate PaRS (69.77%) followed by PaNS (62.31%), PaWP (58.22%), PfNC (56.69%), PaWS (54.46%), PfWN (51.11%) and PfRB (49.24%) (Table-3).

Colletotrichum falcatum

Among all the native isolates of

Table 1. Location, habitat, crop and code of native strains of *Pseudomonas spp*

S. No.	Location	Habitat	Crop	Geographical status	Code
1	Hill Millet Research Station, NAU, Waghai.	Rhizosphere	Paddy	20.77' N 73.50' E	PaWP
2		Rhizosphere	Nagli	20.77' N 73.50' E	PfWN
3		Soil	Nagli	20.77' N 73.50' E	PaWS
4	Hill Millet Research Station, NAU, Rambhas.	Rhizosphere	Banana	20.80' N 73.62' E	PfRB
5		Ambika River	-	20.80' N 73.62' E	PaRS
6	Krishi Vigyan Kendra, NAU, Navsari	Farm Pond	-	20.95° N 72.93° E	PaNS
7	Livestock Research Station, NAU, Navsari.	Rhizosphere	Castor	20.95° N 72.93° E	PfNC

Table 2. Morphological characteristics of native isolates of *Pseudomonas spp*

S. No.	Isolates	Colony Morphology			Cell shape	Fluorescence
		Colour	Shape	Nature		
1	PaWP	Light Green	Round	Non-Spreading	Rod	++
2	PfWN	Yellowish	Round	Non-Spreading	Rod	+++
3	PaWS	Green	Irregular	Spreading	Rod	++
4	PfRB	Dull Yellowish	Round	Non-Spreading	Rod	++
5	PaRS	Dirty Green	Round	Non-Spreading	Rod	+++
6	PaNS	Bluish Green	Irregular	Spreading	Rod	++
7	PfNC	Yellowish white	Round	Non-Spreading	Rod	+

+++ = High fluorescence, ++ = Medium fluorescence, + = Weak fluorescence

Table 3. Antagonistic effect of native *Pseudomonas* isolates against fungal pathogens

S. No.	Isolates	*P. grisea*		*C. falcatum*		*P. aphanidermatum*		*M. phaseolina*	
		Mycelial growth (cm)	Mycelial growth Inhibition (%)	Mycelial growth (cm)	Mycelial growth Inhibition (%)	Mycelial growth (cm)	Mycelial growth Inhibition (%)	Mycelial growth (cm)	Mycelial growth Inhibition (%)
1	PaWP	3.73	58.22	3.57	59.35	3.73	56.78	2.70	70.00
2	PfWN	4.37	51.11	4.70	46.34	3.80	56.00	3.60	60.00
3	PaWS	4.07	54.46	4.20	52.04	4.40	49.03	2.83	68.52
4	PfRB	4.53	49.24	4.53	48.30	3.90	54.83	3.10	65.56
5	PaRS	2.70	69.77	2.80	68.09	3.03	64.85	2.20	75.56
6	PaNS	3.37	62.31	3.53	59.69	3.47	59.87	2.47	72.59
7	PfNC	3.87	56.69	3.97	54.73	4.00	53.66	3.17	64.81
8	Control	8.93	-	8.77	-	8.63	-	9.00	-
	S.Em.±	0.08	-	0.10	-	0.10	-	0.08	-
	C.D. at 5%	0.24	-	0.31	-	0.30	-	0.24	-
	C.V.%	3.11	-	3.97	-	3.99	-	3.77	-

Table 4. Antagonistic effect of native Pseudomonas isolates against fungal pathogens

S. No.	Isolates	*F. moniliformae*		*S. rolfsii*		*P. anacardii*		*L. theobromae*	
		Mycelial growth (cm)	Mycelial growth Inhibition (%)	Mycelial growth (cm)	Mycelial growth Inhibition (%)	Mycelial growth (cm)	Mycelial growth Inhibition (%)	Mycelial growth (cm)	Mycelial growth Inhibition (%)
1	PaWP	4.07	54.81	2.70	70.00	4.30	50.55	3.69	59.07
2	PfWN	3.53	60.74	3.73	58.52	4.57	47.46	4.00	55.56
3	PaWS	2.50	72.22	2.63	70.74	4.00	54.04	3.07	65.93
4	PfRB	3.10	65.56	3.10	65.56	4.80	44.76	3.63	59.63
5	PaRS	2.30	74.44	2.10	76.67	3.13	63.99	2.37	73.70
6	PaNS	2.63	70.74	2.47	72.59	3.40	60.87	2.80	68.89
7	PfNC	3.17	64.81	3.17	64.81	4.07	53.25	3.38	62.41
8	Control	9.00	-	9.00	-	8.70	-	9.00	-
	S.Em.±	0.10	-	0.06	-	0.10	-	0.10	-
	C.D. at 5%	0.31	-	0.19	-	0.29	-	0.31	-
	C.V.%	4.79	-	2.99	-	3.67	-	4.44	-

Pseudomonas, PaRS (2.80 cm) was found significantly superior than the other isolates in its efficacy against the pathogen. The next best treatment in order of merit was PaNS (3.53 cm) followed by PaWP (3.57 cm). Isolates PfNC (3.97 cm), PaWS (4.20 cm) and PfRB (4.53cm) showed comparatively higher growth while it was highest in PfWN (4.70cm). Maximum growth inhibition was recorded in PaRS (68.09%) followed by PaNS (59.69%), PaWP (59.35%), PfNC (54.73%), PaWS (52.04%), PfRB (48.36%) and PfWN (46.34%) (Table-3).

Pythium aphanidermatum

As per the results interpreted in the Table-3 the significantly highest mycelium growth inhibition with lower mycelial growth (3.03 cm) was recorded in PaRS as compared to rest. The next best treatment in order of merit was PaNS (3.47 cm) followed by PaWP (3.73 cm). Isolates PfWN (3.80 cm), PfRB (3.90 cm) and PfNC (4.00 cm) were medium in their efficiency. Isolate PaWS recorded the highest mycelium growth (4.40 cm). Maximum growth inhibition was recorded in PaRS (64.85%) followed by PaNS (59.87%), PaWP (56.78%), PfWN (56.00%), PfRB (54.83%), PfNC (53.66%) and PaWS (49.03%).

Macrophomina phaseolina

Minimum mycelium growth (2.20 cm) was recorded in PaRS which was significantly superior over the rest. The next best treatment in order of merit was PaNS (2.47 cm) followed by PaWP (2.70 cm). Isolate PaWS (2.83 cm), PfRB (3.10 cm) and PfNC (3.17 cm) were also potent antagonists for inhibiting the mycelial growth. Isolate PfWN recorded maximum mycelial growth (3.60 cm) and found inferior in its efficacy. Maximum growth inhibition was recorded in PaRS (75.56%) followed by PaNS (72.59%), PaWP (70.00%), PaWS (68.52%), PfRB (65.56%), PfNC (64.81%) and PfWN (60.00%) (Table-3).

Fusarium moniliformae

Isolate PaRS (2.30 cm) was significantly superior as compared to the rest except PaWS (2.50 cm). The next best in order of merit was PaNS (2.63 cm). PfRB (3.10 cm), PfNC (3.17 cm) and PfWN (3.53 cm) were comparatively less effective against the pathogen. The highest mycelial growth was recorded in PaWP (4.07 cm). Maximum growth inhibition was recorded in PaRS (74.44%) followed by PaWS (72.22%), PaNS (70.74%), PfRB (65.50%), PfNC (64.81%), PfWN (60.74%) and PaWP (54.81%) (Table-4).

Sclerotium rolfsii

PaRS isolate (2.10 cm) was significantly more effective than the other isolates tested as it produced minimum mycelial growth of the pathogen. The next best isolate was PaNS (2.47 cm) followed by PaWS (2.63 cm) in their efficacy. Isolates PaWP (2.70 cm), PfRB (3.10 cm) and PfNC (3.17 cm) were also quite effective in inhibiting the mycelial growth. Maximum mycelial growth was observed in PfWN (3.73 cm). Maximum growth inhibition was recorded in PaRS (76.67%) followed by PaNS (72.59%), PaWS (70.74%), PaWP (70.00%),

Table 5. Antagonistic effect of native *Pseudomonas* isolates against bacterial pathogens

S. No.	Isolates	Average Inhibition Zone (mm)		
		Xanthomonas axonopodis pv. malvacearum	*Xanthomonas axonopodis* pv. *oryzae*	*Xanthomonas axonopodis* pv. *citri*
1	PaWP	15.00	21.00	14.67
2	PfWN	11.00	17.00	9.33
3	PaWS	10.67	11.00	10.67
4	PfRB	10.33	15.00	9.00
5	PaRS	23.33	26.00	18.67
6	PaNS	20.67	23.67	16.67
7	PfNC	17.00	23.00	17.33
8	Control	0.00	0.00	0.00
	S.Em.±	0.42	0.51	0.41
	C.D. at 5%	1.27	1.54	1.22
	C.V.%	5.45	5.21	5.87

PfRB (65.56%), PfNC (64.81%) and PfWN (58.52%) (Table-4).

Pestalotiopsis anacardii

Significantly lowest mycelial growth of the pathogen (3.13 cm) was observed in PaRS as compared to the rest and which was statistically at par with PaNS (3.40 cm). The next best isolate was PaNS (4.00 cm) followed by PfNC (4.07 cm). PaWP (4.30 cm) and PfWN (4.57 cm) were moderately effective. The lowest effective native isolate was PfRB (4.80 cm). Maximum growth inhibition was noticed in PaRS (63.99%) followed by PaNS (60.87%), PaWS (54.00%), PfNC (53.25%), PaWP (50.55%), PfWN (47.56%) and PfRS (44.76%) (Table-4).

Lasiodiplodia theobromae

Among the different native isolates of the south Gujarat, PaRS (2.37 cm) recorded significantly lowest mycelial growth of the pathogen as compared to rest. The next best isolate was PaNS (2.80 cm). Comparatively higher growth of the pathogen was recorded in PaWS (3.07 cm) followed by PfNC (3.38 cm), PfRB (3.63 cm) and PaWP (3.69 cm). The highest growth of the pathogen was recorded in PfWN (4.00 cm). Maximum growth inhibition was recorded in PaRS (73.70%) followed by PaNS (68.89%), PaWS (65.93%), PfNC (68.89%), PfRB (59.63%), PaWP (59.20%) and PfWN (55.56%) (Table-4).

Xanthomonas axonopodis* pv. *malvacerarum

PaRS Isoalte (23.33 mm) proved significantly superior showing maximum growth inhibition zone than the other native isolates tested. The next best isolate in order of merit was PaNS (20.67 mm) followed by PfNC (17.00 mm). Isolate PaWP (15.00 mm), PfWN (11.00 mm) and PfRB (10.67 mm) were moderately effective and the lowest zone of inhibition was recorded in PaWS (10.33 mm) (Table-5).

Xanthomonas axonopodis* pv. *oryzae

As per the results interpreted in the Table-5 maximum zone of inhibition (26.00 mm) was recorded in PaRS and was significantly superior over the rest. The next best isolate in order of merit was PaNS (23.67 mm) followed by PfNC (23.00 mm). Isolates PaWP (21.00 mm), PfWN (17.00 mm) and PfRB (15.00 mm) were moderately effective and PaWS (11.00 mm) was least effective.

Xanthomonas axonopodis pv. citri

The isolate PaRS was significantly superior over all the isolates tested. Maximum inhibition zone was recorded in PaRS (18.67 mm). The next best isolate was PfNC (17.33 mm) followed by PaNS (16.67 mm). The isolate PaWS (14.67 mm), PaWS (10.67 mm) and PaWP (9.33 mm) were comparatively less effective. Isolate PfRB (9.00 mm) recorded the least inhibition zone (Table-5).

As per the results obtained in *in vitro* evaluation of native isolates, overall, all the isolates proved potential biocontrol agents for all the fungal and bacterial pathogens tested. Among these, the native and beneficial isolate PaRS proved the most effective against all the pathogens tested. Hence, PaRS isolate was selected for further study.

The colony colour and fluorescens produced by the seven bacterial isolates on KB medium resembled to the characteristics described for *Pseudomonas* spp. identification in (Brenner *et al.*, 2005 and Anonymous, 2010). The efficiency of different strains of *P. fluorescens* and *P. aeruginosa* were also reported significant against *P. oryzae* (Gnanamanickam and Mew, 1992), *S. rolfsii* and *Xanthomonas campestris* pv. *malvacearum* (Bhowmik *et al.*, 2002), *F. moniliformae* (Sharma *et al.*, 2007), *C. falcatum* (Sangeetha *et al.*, 2009), *P. aphanidermatum* (Muthukumar *et al.*, 2010), *R. solani* and *M. grisea* (Reddy *et al.*, 2010), *L. theobromae* (Nath, 2010) and *Pestalotiopsis anacardii* (Patil, 2012) earlier. The present results are more or less similar with these results. The present investigation clearly shows that native isolate PaRS is proved potential bioagent for the important fungal as well as bacterial pathogens causing seed and soil borne diseases.

The inhibition of the pathogens by PaRS may be directly or indirectly by producing secondary metabolites such as antibiotics, hydrogen cyanide that inhibit soil pathogens or by synthesizing cell-wall-degrading enzymes or competing for colonization sites, nutrients, etc. In the secondary metabolites, 2,4-diacetylphloroglucinol, phenazine-1-carboxylic acid, pyrrolnitrin and hydrogen cyanide are a key compound in the suppression of plant pathogens produced by *Pseudomonas* spp (Mercado-Blanco *et al*., 2007). Minimum mycelial growth of the pathogen by PaRS may be resulted by direct antagonistic effect, competition for nutrients, microelements (predominantly Fe^{3+}) and antibiosis properties present in PaRS.

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