



Research Article

Characterization and evaluation of native *Pseudomonas* spp. of south Gujarat against leaf blast in finger millet

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ABSTRACT: Seven *Pseudomonas* isolates were isolated from different locations of south Gujarat. Based on the morphological, biochemical and physiological characterization of these isolates, four bacterial isolates were identified as *Pseudomonas aeruginosa* (PaWP, PaWS, PaRS and PaNS) and three as *P. fluorescens* (PfWN, PfRB and PfNC). All the isolates were able to grow in 2 to 10% NaCl but, failed to grow at 12% concentration and recorded growth from 10 to 42°C temperature. All the isolates grew in 6 to 8 pH range but failed to grow at 4, 5 and 10 pH. Bio-efficacy of the seven native strains of *Pseudomonas* spp. was compared along with local commercial available biopesticides i.e. *P. fluorescens* (Aumgene, Pramukh and Pantnagar) and Hinosan. Three times spraying of *P. aeruginosa* Rambhas Strain at 0.6% (2×10^9 cfu/ml) at 15 days interval, starting at 21 days of transplanting was found significantly effective in the management of the leaf blast of finger millet (*Magnaporthe grisea* (Cke) Sacc.) by lowering disease intensity and increasing grain yield as well as fodder.

KEY WORDS: *Pseudomonas* spp., isolates, bioefficacy, finger millet blast

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INTRODUCTION

The injudicious application of chemicals has resulted in the development of resistance in pathogens and health hazards to the users. The search for effective alternative approaches to chemical control with no ecological hazards will contribute to sustainable agriculture (Jain *et al.*, 2009). 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. Their diversity, colonizing ability and mechanisms of action, formulation and application facilitate 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 was carried out to isolate, identify, characterize and evaluate the native isolates of *Pseudomonas* from south Gujarat.

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. Information pertaining to the locality, crop history, stage of the crop, etc. was noted along with the samples. Soil samples were analyzed on the day of collection. One gram of soil was mixed thoroughly in 10 ml sterile water and processed by serial dilution agar plate technique and was spread on King's B (KB) medium to isolate *Pseudomonas* spp. The plates were incubated at room temperature ($28 \pm 2^\circ\text{C}$) day and ($20 \pm 2^\circ\text{C}$) night for 24 h. 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 KB agar Petri plates separately for colony development. The individual colonies were examined for shape, size, structure of colonies and pigmentation after 48 h.

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

Sl. No.	Location	Habitat	Crop	Geographical status	Code
1	Hill Millet Research Station, NAU, Waghai.	Rhizosphere	Paddy	20.77' N73.50' E	PaWP
2		Rhizosphere	Finger millet	20.77' N73.50' E	PfWN
3		Soil	Finger millet	20.77' N73.50' E	PaWS
4	Hill Millet Research Station, NAU, Rambhas.	Rhizosphere	Banana	20.80' N73.62' E	PfRB
5		Ambika River	—	20.80' N73.62' 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

Biochemical tests

The KB002 biochemical test kit (Hi Media) was used for the characterization and detection of the Gram –ve bacteria. This contains citrate, lysine and ornithine utilization, urease, phenylalanine deamination, nitrate reduction, H₂S production, utilization of carbohydrates i.e. glucose, adenitol, lactose, arabinose and sorbitol. The result of identification was recorded as per interpretation chart provided along with kit.

The identification was further confirmed by biochemical characterization i.e. gelatin liquefaction, lipid, starch, arginine and casein hydrolysis, oxidase, catalase and KOH solubility test H₂S, acid and gas production, nitrate reduction, VP test and gram staining (Cappuccino and Sherman, 1992).

Physiological characterization

Growth at 4 and 41°C

Twenty four hours old cultures of the test organisms in nutrient broth were spotted on the trypticase soy agar (TSA) plates and incubated for 24 to 48 h at 4 and 41°C. The observations on growth were recorded at the end of incubation period.

Temperature

Test tubes containing nutrient agar were equally inoculated with fresh culture in triplicates and incubated at 4, 10, 15, 25, 30, 37, 42 and 60°C. After 24 h, the bacterial growth was recorded on nutrient agar media.

pH study

Test tubes of nutrient agar media with different pH (4, 5, 6, 7, 8, 10 and 12) were prepared in triplicates and after inoculation they were incubated at optimum temperature (24±2°C). The pH of media was maintained with the help of 0.1N HCl and 0.1% NaOH. The bacterial growth was recorded after 24 h.

Salt (NaCl) concentration

Test tubes of media at different NaCl concentrations (2, 4, 6, 8 and 10%) were prepared in triplicates and after inoculation they were incubated at optimum temperature. After 24 h, growth was recorded.

Bioefficacy of *Pseudomonas* spp. against leaf blast of finger millet (*Magnaporthe grisea* (Cke) Sacc.)

The field experiment was conducted for two years during Kharif- 2009-10 and 2010-11 at Hill Millet Research Station, Navsari Agricultural University, Waghai, Gujarat with the seven *Pseudomonas* spp. isolated from different locations and rhizosphere of various crops as well as local available biopesticides *P. fluorescens* (Aumgene, Pramukh and Pantnagar) and Hinosan (Table 1) with. The GN-4 (Red finger millet) cultivar was selected to evaluate efficacy of strains as it is mostly cultivated cultivar in south Gujarat region. The experiment was conducted in randomized block design with 12 treatments, replicated thrice with plot size of 2.25 x 1.5m. Three sprays of *Pseudomonas* spp. (0.6% of 2 × 10⁹ cfu/ml) and hinosan (0.1%) were given at 15 days interval, 1st spray was done at 21 days after transplanting. The leaf infection was recorded after 7 days of each spray using a numerical scale from 0-5 by examining 20 selected plants to know accurate intensity of blast in each treatment. Occurrence of leaf blast was recorded by visual observation following 0-5 scale as: 0 = No symptoms on the leaves, 1 = Small brown specks of pin head size spot to slightly elongated, necrotic gray spots with brown margin less than 1% leaf area affected, 2 = A typical blast lesion, elliptical 5-10 mm long, 1-5% of leaf area affected, 3 = A typical blast lesion, elliptical 1-2 cm long, 5-25% of leaf area affected, 4 = 25-50% of leaf area affected and 5 = More than 50% leaf area affected with coalescence of the lesions at the time of vegetative growth and per cent disease intensity (PDI) was calculated as suggested by Nagaraja *et al.*, (2007) and grain and fodder yield (kg/ha) was recorded after harvest.

RESULTS AND DISCUSSION

Morphological characterization

All the seven bacterial isolates showed differences in their morphological characters. The colony colour of PfWN, PfRB and PfNC isolates were yellowish, dull yellowish and yellowish white with high, medium and weak fluorescens, respectively. The isolates produced round, non-spreading colony and the cells were rod shaped.

PaWP and PaRS produced light green and dirty green colour while colonies were round and non-spreading with rod shaped cells. The fluorescence was high in PaRS and medium in PaWP isolate.

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.

The colony colour and fluorescens produced by the seven bacterial isolates on KB medium resembled the characteristics described for *Pseudomonas* spp. identification (Brenner *et al.*, 2005; Anonymous, 2010).

Biochemical characterization

All the bacterial isolates were negative to lysine, adenitol, lactose, arabinose and sorbitol utilization while positive to citrate utilization, urease test, H₂S production and glucose utilization. All the isolates were +ve in ornithine utilization except PfNC. Isolates PfRB, PfWN and PfNC were +ve to phenylalanine deamination while PaWP, PaWS, PaRS and PaNS were –ve. PaWP, PaWS, PaRS and PaNS isolates were positive to nitrate reduction and PfRB, PfWN and PfNC were –ve (Table 2).

All the isolates were positive to gelatin liquefaction, lipid, starch and casein hydrolysis, oxidase and catalase tests, arginine hydrolysis, KOH solubility, H₂S production, Voges- Proskauer test and motility tests. Gram's staining as well as acid and gas production tests were –ve in all the isolates. PfWN, PfRB and PfNC showed –ve while PaWP, PaWS, PaRS and PaNS were positive to nitrate reduction test. All the seven isolates were +ve to lipid hydrolysis, gelatin liquefaction, catalase test, H₂S production, arginine hydrolysis and urease test (Table 2).

PfWN, PfRB and PfNC were identified as *Pseudomonas fluorescens* while PaRS, PaNS, PaWP and PaWS were identified as *Pseudomonas aeruginosa* as per the keys mentioned by Stolp and Gadkari (1970).

Physiological characterization

All the isolates grew at 41°C temperature while only PfWN, PfRB and PfNC grew at 4°C temperature on TSA medium. PaWP, PaWS, PaRS and PaNS isolates did not grow at 4°C temperature. PaWP, PaWS, PaRS and PaNS isolates failed to grow while PfWN, PfRB and PfNC were able to grow at 4°C temperature indicating physiological properties of *P. aeruginosa* and *P. fluorescens*, respectively (Table 2). The present results are in conformity with the results obtained by Cakmaki *et al.* (2007), Kaur *et al.* (2010). Meera and Balabaskar (2012) identified thirty five isolates of *P. fluorescens* based on morphological and biochemical characterization. Twenty one strains of *P. fluorescens* isolated from different parts of Tamil Nadu showed +ve response for pigment production, oxidase test, KOH test, arginine dihydrogenase and –ve to starch hydrolysis (Kalaivani, 2005).

Temperature

All the isolates showed normal growth from 10 to 42°C temperature range. PfWN, PfRB and PfNC isolates were able to grow at 4°C temperature but PaWP, PaWS, PaRS and PaNS isolates were failed to grow. All the bacterial antagonists failed to grow at 60°C temperature (Table 3).

Salt concentration (NaCl)

All the isolates were able to grow in 2 to 10 per cent NaCl but failed to grow at 12 per cent (Table 3).

pH

The growth of all the isolates was +ve in 6 to 8 pH range while it was –ve at 4, 5 and 10 pH. The growth was observed only at 9 pH in case of PaRS while the rest of the isolates failed to grow (Table 3). The results corroborate with the findings of Stolp and Gadkari (1970), who indicated that optimum temperature and pH for *Pseudomonas* spp. were 30°C and 7–8.5, respectively. *Pseudomonas aeruginosa* grew upto 5 per cent salt concentration as reported by Keskin and Ekmekci (2008).

Evaluation against Leaf blast

There was significant reduction in the disease intensity of finger millet leaf blast in all the treatments as compared to the control. Significantly lower leaf blast intensity was recorded in edifenphos (12.00%) which was statistically at par with PaRS (17.67%), PaNS (18.00%) and PaWS (18.67%)—during 2009-10 (Table 4). In the year 2010-11, the lowest disease intensity was recorded in Edifenphos (10.67%) which was statistically at par with PaRS (13.33%) and PaNS (15.33%) (Table 4). The pooled results of application of *Pseudomonas* spp. against

Table 2. Characterization of native isolates of *Pseudomonas* spp.

Test	PaWP	PfWN	PaWS	PfRB	PaRS	PaNS	PfNC
Biochemical characterization							
Hi Media Kit (KB002)							
Citrate utilization	+	+	+	+	+	+	+
Lysine utilization	–	–	–	–	–	–	–
Ornithine utilization	+	+	+	+	+	+	–
Urease test	+	+	+	+	+	+	+
Phenylalanine deamination	–	+	–	+	–	–	+
Nitrate reduction	+	–	+	–	+	+	–
H ₂ S production	+	+	+	+	+	+	+
Glucose	+	+	+	+	+	+	+
Adanitol	–	–	–	–	–	–	–
Lactose	–	–	–	–	–	–	–
Arabinose	–	–	–	–	–	–	–
Sorbitol	–	–	–	–	–	–	–
Standard test protocol							
Gelatin liquefaction	+	+	+	+	+	+	+
Lipid hydrolysis	+	+	+	+	+	+	+
Starch hydrolysis	+	+	+	+	+	+	+
Casein hydrolysis	+	+	+	+	+	+	+
Oxidase test	+	+	+	+	+	+	+
Catalase test	+	+	+	+	+	+	+
Acid and gas production	–	–	–	–	–	–	–
Arginine hydrolysis	+	+	+	+	+	+	+
KOH solubility test	+	+	+	+	+	+	+
Nitrate reduction	+	–	+	–	+	+	–
H ₂ S production	+	+	+	+	+	+	+
Voges-Proskauer test	+	+	+	+	+	+	+
Gram staining	–ve	–ve	–ve	–ve	–ve	–ve	–ve
Motility	+	+	+	+	+	+	+
Physiological Characterization							
Growth at 4°C	–	+	–	+	–	–	+
Growth at 41°C	+	+	+	+	+	+	+

leaf blast of finger millet showed that significantly lower disease intensity was recorded in edifenphos (11.33%) which was statistically at par with PaRS (15.50%). Maximum leaf blast was recorded in untreated control (39.17%) (Table 4).

Two sprays of *P. fluorescence* @ 0.3 per cent i.e. first at 50 per cent flowering followed by second spray 10 days later were recommended for control of neck and

finger blast (Ramappa *et al.*, 2002). Kumar and Kumar (2011) revealed that seed treatment and two spray of *P. fluorescence* Pf-2 @0.6 per cent recorded lowest neck and finger blast with maximum yield of 2312.34 kg/ha. Patro *et al.* (2008) found that *P. fluorescence* (0.6%) as a seed treatment with two foliar sprays at 10 days interval was best for the management of finger 27.8%, leaf 1% and neck 18.3% blast with higher yield (3288 kg/ha) as compared to other treatments. Sitter *et al.* (1996) emphasized

Table 3. Physiological studies of native isolates of *Pseudomonas* spp.

Sl. No.		PaWP	PfWN	PaWS	PfRB	PaRS	PaNS	PfNC
1	Temperature							
	4°C	–	+	–	+	–	–	+
	10°C	+	+	+	+	+	+	+
	15°C	+	+	+	+	+	+	+
	25°C	+	+	+	+	+	+	+
	30°C	+	+	+	+	+	+	+
	37°C	+	+	+	+	+	+	+
	42°C	+	+	+	+	+	+	+
	60°C	–	–	–	–	–	–	–
2	Salt concentration (NaCl)							
	2%	+	+	+	+	+	+	+
	4%	+	+	+	+	+	+	+
	6%	+	+	+	+	+	+	+
	8%	+	+	+	+	+	+	+
	10%	+	+	+	+	+	+	+
	12%	–	–	–	–	–	–	–
3	pH							
	4	–	–	–	–	–	–	–
	5	–	–	–	–	–	–	–
	6	+	+	+	+	+	+	+
	7	+	+	+	+	+	+	+
	8	+	+	+	+	+	+	+
	9	–	–	–	–	+	–	–
	10	–	–	–	–	–	–	–

+: growth present -: No growth

that six strains of *P. fluorescence* and *P. putida* showed fungal inhibition in dual plate assay in the laboratory and reduced *E. coracana* blast severity in the field.

Grain Yield (kg/ha)

During 2009-10, significantly higher yield was recorded in edifenphos (3883 kg/ha) over control but was statistically at par with PaRS (3863 kg/ha), PfWN (3608 kg/ha), PaWS (3439 kg/ha) and PfRB (3413 kg/ha) (Table 4) while in 2010-11 significantly higher grain yield was recorded in the edifenphos (3927 kg/ha) but was statistically at par with the PaRS (3630 kg/ha) and PaNS (3422 kg/ha). The pooled results indicated that maximum yield was recorded in edifenphos (3895 kg/ha) which was at par with PaRS (3757 kg/ha). The lowest grain yield was recorded in untreated control (1403 kg/ha) (Table 4).

Fodder Yield (kg/ha)

Edifenphos recorded higher fodder yield (7743 kg/ha) over all the treatments but it was statistically at par with PaRS (6983 kg/ha) during 2009-10 (Table 4). The pooled results of application of *Pseudomonas* spp. showed that all the treatments were significantly more effective to increase fodder yield as compared to the control. Maximum yield was recorded in edifenphos (7547 kg/ha) and was statistically at par with PaRS (7480 kg/ha). The lowest fodder yield was recorded in the untreated control (5214 kg/ha) (Table 4).

Based on our studies, it is concluded that PaRS was found to be a better potent native isolate for controlling finger millet blast than rest of the isolates. PaRS may be inhibiting *Pyricularia grisea* by producing antibiotics, siderophores and plant growth stimulating substances. The reduction of disease by *Pseudomonas* may be due

Table 4. Effect of native isolates of *Pseudomonas* spp. on finger millet leaf blast, grain yield and fodder yield

Sl. No.	Treatment	PDI		Grain yield (kg/ha)				Fodder yield (kg/ha)					
		2009-10	2010-11	Pooled	PDC	2009-10	2010-11	Pooled	% Increase	2009-10	2010-11	Pooled	% increase
1	PaWP (0.6%)	31.84* (28.00)	29.14 (24.00)	30.49 (26.00)	33.62	2655	3283	2969	111.46	5744	5931	5838	11.96
2	PfWN (0.6%)	29.67 (24.67)	30.18 (25.33)	29.93 (25.00)	33.62	2655	3283	2969	111.46	5744	5931	5838	11.96
3	PaWS (0.6%)	25.54 (18.67)	25.99 (19.33)	25.77 (19.00)	36.17	3608	3234	3421	143.66	5883	6560	6222	19.33
4	PaWB (0.6%)	25.54 (18.67)	25.99 (19.33)	25.77 (19.00)	36.17	3608	3234	3421	143.66	5883	6560	6222	19.33
5	PaRS (0.6%)	24.77 (17.67)	21.26 (13.33)	23.02 (15.50)	51.49	3111	3304	3207	128.41	5689	5490	5589	7.19
6	PaNS (0.6%)	24.86 (18.00)	22.92 (15.33)	23.89 (16.67)	19.14	3413	2977	3195	127.56	6296	6109	6203	18.96
7	PfNC (0.6%)	29.75 (24.67)	26.92 (20.67)	28.34 (22.67)	60.42	3863	3630	3757	167.59	6983	7518	7480	44.74
8	Pf-1 (0.6%)	34.42 (32.00)	31.05 (26.67)	32.74 (29.33)	57.44	3439	3422	3431	144.37	6332	6578	6455	23.80
9	Pf-2 (0.6%)	34.62 (32.33)	29.72 (24.67)	32.18 (28.50)	42.12	3096	3224	3166	125.49	5956	5885	5921	13.55
10	Pf-3 (0.6%)	35.82 (34.33)	30.63 (26.00)	33.23 (30.17)	25.12	3274	3136	3205	128.27	6021	6167	6094	16.87
11	Edifenphos (0.1%)	20.02 (12.00)	18.93 (10.67)	19.48 (11.33)	27.24	3136	2975	3055	117.59	6129	6200	6164	18.22
12	Control	39.36 (40.33)	37.97 (38.00)	38.67 (39.17)	22.97	3363	3242	3303	135.25	5543	5480	5511	5.69
	S.Em ± (T)	1.99	1.94	1.31	71.07	3883	3927	3895	177.42	7743	7811	7547	46.45
	(T x Y)	—	—	1.96		1415	1392	1404		5537	4891	5214	
	P = 0.05 5% (T)	5.83	5.69	3.72		168.00	174.00	174.00	126.00	349.00	349.00	166.56	241.00
	(T x Y)	—	—	NS		—	—	—	171.00	—	—	—	341.00
	CV%	11.25	11.98	11.60		492.00	511.00	511.00	356.00	1022.00	1022.00	977.00	684.00
						—	—	—	NS	—	—	—	NS
						9.12	10.00	10.00	9.36	9.81	9.81	9.28	9.54

*Figures inside the parenthesis are the original values while those outside are arc sine transformed values. PDC: Per cent disease control over controls

to (i) activation of enzymes of phenylpropanoid metabolism and accumulation of PR-proteins in finger millet leaves; (ii) application of *Pseudomonas* isolates strengthen host cell wall structures resulting in restriction of pathogen invasion in plant tissue; (iii) increased amount of silicic acid in the leaves; (iv) activate induced systemic resistance; (v) induction of defense proteins *viz.* chitinase, α -1,3 glucanase, peroxidase (PO) and polyphenol oxidase (PPO) and (v) expression of defense gene against finger millet blast (Radjacommar *et al.*, 2004^b).

Thus, minimum finger millet blast disease intensity was recorded in treatments with PaRS isolate, it might be due to strongly synthesis and accumulation of chitinase, PO and PPO, expression of defense gene, production of silicic acid strengthen cell wall and activate phenylpropanoid metabolism and PR-proteins activity in PaRS. PaRS isolate shows potential for use as a promising bioagent in the management of finger millet blast.

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