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Impact of *Pseudomonas* spp. on Plant Growth, Lytic Enzymes and Secondary Metabolites Production

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Seven strains of *Pseudomonas* spp. were isolated from the south Gujarat region of India. Antifungal and bacterial activities of bacterial strains were evaluated against important plant pathogens *in vitro*, among them, PaRS was found most effective. The indole acetic acid production was recorded in all isolated *Pseudomonas* spp. Seed treatment with PaRS at 6 g/kg was significantly superior over other treatments for plant growth promotion (germination, shoot and root length, shoot and root weight, vigor index, and both shoot and root colonization) under standard roll towel method and pot conditions. The maximum siderophore production was observed in PaRS and medium production in PfRB, PaNS, and PfNC. PaNS and PaRS strains recorded strong HCN production but moderate production recorded in PaWP, PaWS, and PfNC. The maximum phosphate solubalization zone (22 mm) was found in PaRS. PaRS recorded maximum chitinase, ß-1,3-glucanase activity, hydrogen cyanide, and salicylic acid production as compared to other strains.

Keywords: *Pseudomonas*, plant growth, secondary metabolites, enzymatic activity, colonization

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INTRODUCTION

Various agrochemicals are available in the market for the management of pests and diseases, and a few of them cause harmful effects on the environment. Recently, consumers are demanding organic products in the world market which are safer for consumption (Kumar, 2009; Waghunde et al., 2016, 2021; Morales-Cedeño et al., 2021). The higher input cost, economical losses from pests and disease, climate change, as well as the risk of invasive pests resulted in a search for alternate low cost techniques and management practices (Waghunde et al., 2021). Biological control is an effective, eco-friendly, and economical practice, and the best alternative to agrochemicals for pest management (Iftikhar et al., 2020). The microorganism is one of the smartest living organisms on the earth because of its survivability under extreme conditions, andpresently many beneficial microorganisms are utilized for plant disease management (Shelake et al., 2019; Waghunde et al., 2021). The different agriculturally important microbes like *Trichoderma*, *Bacillus*, *Serratia*, *Pseudomonas* spp. were used to manage plant pathogens i.e., *Ganoderma boninense*, *Fusarium oxysporum* f. sp. *cubense*, *Burkholderia glumae*, *Xanthomonas oxyzae pv. Oryzae*, *Pseudomonas syringae*, *Pectobacterium carotovorum*, *Ralstonia solanacearum*, *Fusarium oxysporum* f. sp. *cucumerinum*, *Sclerotium rolfsii*, and *Pyricularia oryzae,* and also increase plant growth (Kishore et al., 2005a; Durairaj et al., 2017; Islam et al., 2018; Muniroha et al., 2019; Wong et al., 2021). The plant-growth-promoting bacteria comprising a different group has a beneficial effect on the host through the direct and indirect mechanism which not only helps plant growth but also

reduces plant disease (Durairaj et al., 2017; Islam et al., 2018; Wong et al., 2021). The maximum emphasis should be given to identification, characterization, and mass multiplication of native bioagents for the promotion of sustainable agriculture throughout the world (Waghunde et al., 2016; Verma et al., 2019, 2020). The successful use of native strains of *Pseudomona*s as bioinoculants for applications on crops requires these strains to compete satisfactorily with the indigenous microflora of soil, to survive, and to persist in adequate viable-cell numbers in the rhizosphere (Fischer et al., 2010). Some bioagents like *Pseudomonas* have the potential to manage a diverse group of pathogens by releasing volatile and non-volatile compounds (Kishore et al., 2005b).

The objectives of the present study were to isolate *Pseudomonas* spp. and *in vitro* evaluation against different important plant pathogens. The secondary metabolites production with the enzymatic activity of bacterial strains was also studied during the investigation.

MATERIALS AND METHODS

Isolation and *in vitro* Screening

The *Pseudomonas* strains were isolated during 2011–2012 from the Waghai region of south Gujarat, India which is especially known for organic farming. It is situated at a cross-section of latitude of 20.77′N and longitude of 73.50′E under the agro-climatic zone of the south Gujarat heavy rainfall zone, agroecological situation-I. The location, habitat, crop, and code of bacterial strains are mentioned in **Table 1**.

The bacterial strains were subjected to identification and based on the morphological, biochemical, and physiological characterization, among them four bacterial strains were identified as *Pseudomonas aeruginosa* (PaWP, PaWS, PaRS, and PaNS) and three as *P. fluorescens* (PfWN, PfRB, and PfNC).

The bacterial strains were evaluated against important fungal pathogens i.e., *Pyricularia oryzae* Cavara, *Colletotrichum falcatum* Went, *Fusarium moniliformae* Sheldon, *Macrophomina phaseolina* (Tassi) Goud, *Sclerotium rolfsii* Sacc., *Pythium aphanidermatum* (Eds.) Fitz., *Pestalotiopsis anacardia,* and *Lasiodiplodia theobromae* Pat.; and bacterial pathogens i.e., *Xanthomonas axonopodis* pv. *oryzae*, *Xanthomonas axonopodis* pv. *Malvacearum,* and *Xanthomonas axonopodis* pv. *citri in vitro* by dual culture (Dennis and Webster, 1971) and paper disc method (Thornberry, 1950), respectively. Seven days old mycelia disc (5 mm) of pathogen were placed on the opposite side of bacterial strain streaked (Vidhyasekaran et al., 1997). The plates were incubated at room temperature (25 ± 2°C) for 5 days and the radial growth of pathogens in treated and control plates were recorded. Each treatment was repeated three times. Two days old culture of bacterial pathogens was spread with the help of a sterile spreader on sterilized nutrient agar Petri plates. Three sterile 5 mm filter paper discs were dipped in the cell suspension (10^8 cells/ml) of *Pseudomonas* spp. and placed on a Petri plate spread with the pathogen. Three Petri plates were kept for each pathogen. Filter paper discs without dipping in *Pseudomonas* spp. considered as control. The Petri plates were incubated at room temperature (25 ± 2°C) for 2 days and inhibition was recorded.

Plant Growth Promoting Activity of PaRS

Roll Towel Method

An experiment was conducted to assess the efficacy of PaRS on seed germination and plant growth promotion ability by the standard roll towel method (ISTA, 1985) in the growth chamber.

The seeds of finger millet variety GN-4 were sterilized with 1% sodium hypochlorite (NaOCl) solution for 2–3 min then washed 2–3 times and air-dried for 15 mins. A talc and vermicompost-based formulation of PaRS applied to finger millet seed at 4, 6, and 8 g/kg seeds (10^7 cfu/g) and without PaRS was considered as control. Five repetitions of each treatment were made. One sheet of germination paper was wetted by distilled water and 25 seeds of the respective treatment were placed on the first sheet evenly. The rolled papers were incubated in a seed germinator at 25 ± 2°C and 95 ± 3% RH. Sterile water was added to maintain paper towel moisture. The emergence of seedlings from the grain was considered as successful germination and recorded after 7 days. Three seedlings were taken at random from each replication and the length of root and shoot were measured along with fresh root and shoot weight after 7 days. Plant growth promotion of finger millet seedling was assessed using Vigour Index (VI).

VI = percent germination × mean total length of seedling (root length + shoot length) (Baki and Anderson, 1973).

The PaRS colonization was assessed as per the method suggested by Papavizas and Davey (1960). Three seedlings from each replication were selected, a root and shoot portion was cut and transferred to a test tube containing 10 ml sterile water. After thorough shaking, the population of PaRS in the suspension was estimated by the dilution plate method using a KB medium containing rifamycine (195 μg/ml), penicillium G (90 μg/ml), actinomycin D (100 μg/ml), and streptomycin D (30 μg/ml). Fluorescence of the colony was checked under UV light. Three Petri dishes for each dilution and five replications were maintained. The colony forming unit was enumerated at 10^4 dilutions. The root samples were weighed and the population of bacteria was expressed per gram of root samples.

Pot Study

The treatment details in the roll towel method were repeated in pot conditions to validate the efficacy in pots under greenhouse conditions. The experiment was conducted in the greenhouse of the Department of Plant Pathology, N.M. College of Agriculture, Navsari Agricultural University, Navsari, Gujarat, India during the year 2011–2012. Five repetitions of each treatment were made and without PaRS treated seeds, which served as control. Plastic pots (300 × 300 mm) were used and filled with sterilized soil. Finger millet (GN-4) seeds were treated with PaRS formulation (10^7 cfu/g) 4, 6, and 8 g/kg seeds, and eight seeds were sown in each pot. Five seedlings of 4, 6, and 8 g/kg PaRS formulation treated seeds were kept in each pot for the pot experiment. The germination was recorded initially and the number of leaves, plant height, and root colonization (cells/g) were recorded at 15 day intervals. The root portion was cut and washed gently in 100 ml of sterile water in an Erlenmeyer flask to remove soil. CFU was enumerated at 10^6 dilution as per the procedure given by Papavizas and Davey (1960).

TABLE 1 | Sampling site location, habitat and code of bacterial strains used during experiment.

Sr. No.	Sampling site	Habitat	Geographical status	Code
1	Hill Millet Research Station, Navsari Agricultural University, Waghai	Paddy (Near to root zone)	20.77′N 73.50′E	PaWP
2		Nagli (Near to root zone)	20.77′N 73.50′E	PfWN
3		Nagli (Soil only)	20.77′N 73.50′E	PaWS
4	Hill Millet Research Station, Navsari Agricultural University, Rambhas	Banana (Near to root zone)	20.80′N 73.62′E	PfRB
5		Ambika River	20.80′N 73.62′E	PaRS
6	Krishi Vigyan Kendra, Navsari Agricultural University, Navsari	Farm Pond	20.95°N 72.93°E	PaNS
7	Livestock Research Station, Navsari Agricultural University, Navsari	Castor (Near to root zone)	20.95°N 72.93°E	PfNC

Qualitative and Quantitative Estimation of Siderophore

All the glassware used in the siderophore assays and in the preparation of the Chrome Azurol S blue agar medium (CAS) were soaked in a 2N HCl solution for 24 h. After removing from the acid solution, the glassware was invariably washed with double distilled water. The rest procedure followed as per Schwyn and Neilands (1987).

Preparation of CAS for the Detection of Siderophore

For one liter of blue agar CAS medium, 60.5 mg Dehydrated Chromo Azurol S (Hi Media) was dissolved in 50 ml water and mixed with 10 ml of iron solution (1 mM $FeCl_3 \cdot 6H_2O$ in 10 mM HCl). While stirring, a 40 ml aqueous solution containing 72.9 mg cetyl trimethyl ammonium bromide was slowly added, with continuous stirring, and the final solution was autoclaved. King's B agar medium was prepared with PIPES (30.2 g) and Difco agar (18.0 g). The pH of the medium was adjusted to 6.8 with the addition of 50 percent (w/w) sodium hydroxide (NaOH) solution and autoclaved it. Cooled CAS dye was added to the glass wall with gentle agitation to achieve mixing without the formation of foam. The 20 ml of CAS agar dye was added to each plate and stored in a refrigerator (4°C) for 24 h before use. The bacterial strains PaRS (10 μl) grown overnight were spotted on CAS plates and incubated at 25±2°C for 48 h. A Yellow to orange colored clear zone around the spotted colony was considered as a positive indication of siderophore production.

The CAS-shuttle assay method (Tank and Saraf, 2010) was used for the quantitative estimation of siderophore. The bacterial strains were grown in Fiss minimal medium at 25 ± 2°C for 48 h. The cell suspension was centrifuged at 2,700 rpm for 15 min, CAS assay solution was added to the culture supernatant. The solution was kept for 15 min and four repetitions of each strain were taken. The absorbance was done at 630 nm and measurement as per the below formula:

$$[(Ar - -As)/Ar]100 = \%siderophoreunits \quad (1)$$

where, Ar = absorbance of reference (minimal media + CAS assay solution), As = absorbance of sample (culture supernatant + CAS assay solution).

Qualitative and Quantitative Estimation of HCN

Whatman No. 1 filter paper was placed on the lid of the Petri plate to detect HCN production activity and the plates were sterilized. TSA medium amended with glycine (4.4 g/l) was sterilized and poured into the sterile plates as per the protocol of Wei et al. (1991). The seven native bacterial strains were streaked on the medium and filter paper placed in each plate was soaked with 2 ml sterile picric acid solution. The plates were sealed with parafilm in order to contain gaseous metabolites produced by the bacterial strains and allowed the chemical reaction with picric acid present in the filter padding. The color change of the filter paper was noted after 5 days at 30°C and the HCN production potential of bacterial strains was assessed as per Wei et al. (1991). On the basis of filter paper color change from yellow to light brown, brown, or reddish-brown was recorded as weak (+), moderate (++) or strong (+ + +) reaction, respectively.

For quantitatively estimation of HCN production, the bacterial strains were grown in the broth contained flask and the medium composition was the same except agar-agar. The filter paper strips (10 × 0.5 cm) were soaked in picrate solution and kept hanging position near to neck of the flask. It was incubated at 30°C in a rotary shaker and color change was observed after 4 days. The color was eluted by placing the changed filter paper in a test tube containing 10 ml of distilled water, and its absorbance was read by the spectrophotometer at 625 nm. Five repetitions of each treatment were made.

IAA Production

The native *Pseudomonas* bacterial strains were inoculated into the pre-sterilized SIM agar slants. The tubes were incubated for 48 h at room temperature (25 ± 2°C). After incubation, 10 drops of Kovac's reagent were added to each tube. The production of red color was considered as positive for indole production.

P-Solubilization

The bacterial strains were tested for their ability to solubilize insoluble inorganic phosphate on Pikovskaya's agar by adding the overnight grown culture. The plates were incubated for 48 hrs at room temperature. The diameter of the solubilization zone was measured and expressed in millimeters.

SA

The quantitative analysis of the SA was done using the method of Meyer et al. (1992). The purple iron–SA complex, developed in the aqueous phase was measured at 527 nm using a spectrophotometer. A standard curve was prepared with SA dissolved in the succinate medium. The quantity of SA in the culture filtrate was expressed as mg/ml (Meyer et al., 1992). Five repetitions of each treatment were made.

Enzymes

Pseudomonas cell suspension was grown at 28°C for 96 h and mixed in a rotary shaker containing 50 ml of chitin–peptone medium as per Lim et al. (1991). The centrifuged cell suspension supernatant was used as an enzyme source. The reaction mixture was incubated at 50°C for 4 h in a water bath. Chitinase activity was measured by the method Nelson (1944). A unit of chitinase was considered as 1 nmol of GlcNAc released per minute per mg of protein. The primary procedure followed in chitinase enzyme estimation, the same procedure repeated up to supernatant preparation except growing medium as peptone medium containing laminarin as per Lim et al. (1991). The reaction mixture was incubated at 40°C for 2 h in a water bath and ß-1,3-glucanase activity was measured as 1 nmol of glucose released per minute per mg of protein. The protein content for both the enzymes was determined as per Bradford (1976). Five repetitions of each treatment were made.

RESULTS

In vitro Evaluation

Antifungal and antibacterial properties of bacterial strains were evaluated by dual culture and paper disc methods under *in vitro* conditions. The PaRS strain recorded minimum mycelial growth (3.37, 3.53, 3.47, 3.40, 2.80, 2.47, 2.63, and 2.47 cm) against fungal pathogens and the maximum growth inhibition zone (20.6, 23.67, and 16.67) in the case of bacterial pathogens, as shown in **Table 2**. The PaNS and PfNC were also effective to manage mycelia growth after PaRS in the case of bacterial pathogens. PaNS and PaWP were effective against *P. grisea*, *C. falcatum*, *P. dermatum,* and *M. phaseolina* while PaNS and PaWS in the rest of the fungal pathogens as mentioned in **Table 2**.

PGPR and Colonization Activity of PaRS

Roll Towel Paper Method

All the doses of seed treatments (ST) with PaRS proved significantly superior over the control for seed germination. Among these, ST @ 6 g/kg seed was significantly superior over the rest and gave maximum germination (79.78%). The next best dose was @ 4 g/kg (62.89%). The lowest germination was recorded in untreated seeds i.e., the control (41.56%) (**Table 3**). Shoot and root length were significantly more in all the doses of ST tested as compared to control. The significantly larger shoot (7.94 cm) and root (2.59 cm) length were recorded in ST @ 6 g/kg than the other treatments. The next best treatment was ST @ 4 g/kg. The lowest shoot and root lengths were recorded in the untreated control (**Table 3**). All the doses of seed treatment of PaRS produced a significantly higher shoot and root weight as compared to control. Among these, significantly higher shoot (4.22 mg) and root (1.72 mg) weights were recorded in ST @ 6 g/kg than the rest of the doses. The next best dose was ST @ 4 g/kg. The minimum shoot (1.81 g) and root (0.49 g) weight was recorded in the control (**Table 3**). The vigor index was significantly higher in all the doses of seed treatments with PaRS as compared to control. Among these, seed treated @ 6 g/kg was significantly (838.97) superior as compared to other doses. The next best dose was 4 g/kg (458.89) followed by 2 g/kg (210.72). The lowest vigor index was recorded in the control (125.85) (**Table 3**). PaRS colonization was maximum ST at 6 g/kg in shoot (6.72×10^4 cfu/g) and root (4.83×10^4 cfu/g). The next best dose was 4 g/kg. The least PaRS colonization was recorded in 2 g/kg seed treatment. There was no bacterial colonization in the control (**Table 3**).

Thus, seed bacterization with *P. aeruginosa* @ 6 g/kg seed proved better for germination and plant height. Further, field confirmations of the results are required for practical utility and feasibility. This can be suggested to the farmers for cost-effective and eco-friendly management of finger millet blast.

Pot Study

An experiment was conducted to evaluate plant growth-promoting activity of PaRS and results are mentioned in **Table 4**. The percent germination was observed in ST @ 2, 4, and 6 g/kg and at 70% in control. A significantly higher plant height was recorded in ST at 6 g/kg at 15–60 days after transplanting (52.07–72.36 cm) as compared to other treatments. The next best treatment was ST @ 4 g/kg (47.47–65.20 cm) (**Table 4**). The minimum plant height was recorded in the untreated control (29.01–45.60 cm). Seed treatment @ 6 g/kg was significantly superior in producing a greater number of leaves (8.68–20.27) than the rest of the doses at 15–60 days after transplanting. The next best treatment was ST @ 4 g/kg (7.87–17.07). The least number of leaves was recorded in the control (4.63–11.20) (**Table 4**).

Colonization

The ability of PaRS for colonization was studied during the pot study and results are mentioned in **Table 4**. The bacterial colonization in the rhizosphere was significantly higher in all the treatments as compared to the control. Among these, the seed treatment @ 6 g/kg seeds proved superior at 15 (5.06×10^6 cfu/g), 30 (6.67×10^6 cfu/g), 45 (8.00×10^6 cfu/g) and 60 (7.06×10^6 cfu/g) days after transplanting having significantly higher bacterial counts as compared to the rest (**Table 4**). The next best in order of merit was seed treatment @ 4 g/kg while 2 g/kg was comparatively less effective. This showed that seed treatment @ PaRS 6 g/kg was better for increasing bacterial rhizosphere colonization. The seed treatment of PaRS recorded higher plant height, the greatest number of leaves, and largest colonization up to 60 days (**Table 4**).

Biochemical Characterization

The biochemical properties i.e., indole acetic acid, hydrogen cyanide, siderophore, and P-solubilizing of seven *Pseudomonas* spp. were studied and results were interpreted in **Table 5**.

TABLE 2 | Fungal mycelial growth and bacterial inhibition zone by *Pseudomonas* isolates.

Sr. No.	*Pseudomonas* isolates	*P. grisea*	*C. falcatum*	*P. aphanidermatum*	*P. anacardii*	*L. theobromae*	*M. phaseolina*	*F moniliformae*	*S. rolfsii*	*XCM*	XOO	XAC
1	PaWP	3.73^{de}	3.57^{d}	3.73^{c}	4.30^{bc}	3.69^{bc}	2.7^{cde}	4.07^{b}	2.7^{de}	15^{d}	21^{c}	14.67^{c}
2	PfWN	4.37^{bc}	4.7^{b}	3.80^{c}	4.57^{bc}	4.00^{b}	3.6^{b}	3.53^{bc}	3.73^{b}	11^{e}	17^{d}	9.33^{e}
3	PaWS	4.07^{bcd}	4.2^{bc}	4.40^{b}	4.00^{c}	3.07^{de}	2.83^{cd}	2.5^{e}	2.63^{e}	10.67^{e}	11.00^{f}	10.67^{d}
4	PfRB	4.53^{b}	4.53^{bc}	3.90^{bc}	4.80^{b}	3.63^{bcd}	3.1^{bc}	3.1^{cd}	3.1^{cd}	10.33^{e}	15.00^{e}	9.00^{e}
5	PaRS	2.7^{f}	2.8^{e}	3.03^{d}	3.13^{d}	2.37^{f}	2.2^{e}	2.3^{e}	2.1^{f}	23.33^{a}	26.00^{a}	18.67^{a}
6	PaNS	3.37^{e}	3.53^{d}	3.47^{cd}	3.40^{d}	2.80^{ef}	2.47^{de}	2.63^{de}	2.47^{ef}	20.67^{b}	23.67^{b}	16.67^{b}
7	PfNC	3.87^{cde}	3.97^{cd}	4.00^{bc}	4.07^{c}	3.38^{cd}	3.17^{bc}	3.17^{cd}	3.17^{c}	17^{c}	23.00^{b}	17.33^{b}
8	Control	8.93^{a}	8.77^{a}	8.63^{a}	8.70^{a}	9.00^{a}	9.00^{a}	9.00^{a}	9.00^{a}	0^{f}	0^{g}	0^{f}

Data are mean of three replications.
Means followed by the same letter in a column are not significantly different (P = 0.05) by DMRT.

TABLE 3 | Effect of seed bacterization by *P. aeruginosa* on plant growth promoting activity of Finger millet (Roll Towel Paper Method).

Sr. no.	Seed treatment	Germination (%)	Shoot length (cm)	Root length (cm)	Shoot weight (g)	Root weight (g)	Vigour index	PaRS colonization	
								Shoot ($\times 10^4$cfu/g)	Root ($\times 10^4$cfu/g)
1	2 g/kg	50.66^{c}	3.01^{c}	1.15^{c}	2.82^{c}	0.9^{c}	210.72^{c}	3.56^{c}	2.28^{c}
2	4 g/kg	62.89^{b}	5.49^{b}	1.74^{b}	3.43^{b}	1.44^{b}	455.59^{b}	5.44^{b}	3.94^{b}
3	6 g/kg	79.78^{a}	7.94^{a}	2.59^{a}	4.22^{a}	1.72^{a}	838.97^{a}	6.72^{a}	4.83^{a}
4	Control	41.56^{d}	2.11^{d}	0.92^{c}	1.81^{d}	0.49^{d}	125.85^{d}	0^{d}	0^{d}

Data are mean of five replications.
Means followed by the same letter in a column are not significantly different (P = 0.05) by DMRT.

PaRS showed high siderophore fluorescence while PfRB, PaNS, and PfNC recorded medium siderophore fluorescence (**Table 5**). The weak siderophore fluorescence was observed in PaWP, PfWN, and PaWS strain (**Table 5**). The maximum siderophore production was recorded in PaRS (12.20±0.08) followed by PaNS and PfNC (11.86 ± 0.16 and 10.06 ± 0.07) as mentioned in **Table 6**. The minimum siderophore production was recorded in PaWN (5.38 ± 0.06).

Among the 7 strains of *Pseudomonas* spp. tested for the HCN production, the highest production of HCN was recorded in isolate PaRS (0.084 ± 0.003) followed by PaNS (0.066 ± 0.003). The next best isolate for HCN production was PaWN, PaNC, PaWP, and PfRB. The lowest production was found in PaWS (**Table 6**).

All the seven native strain of *Pseudomonas* were positive to IAA production. PaRS showed high siderophore fluorescence while PfRB, PaNS, and PfNC recorded medium siderophore fluorescence (**Table 5**). The weak siderophore fluorescence was observed in PaWP, PfWN, and PaWS strain (**Table 5**). Strong HCN production was observed in PaNS and PaRS while moderate in PaWP, PaWS, and PfNC. The highest P-solubilizing zone (22 mm) was recorded in PaRS followed by PaNS (20 mm) as mentioned in **Table 4**. The P-solubilizing zone in PfWN, PaWS, PaWP, and PfRB was 18, 17, 15, and 14 mm, respectively. The lowest inhibition was recorded in the PfNC (10 mm) as mentioned in **Table 5**.

The SA production was found to be the maximum in PaRS (12.68 ± 0.36) followed by the PaNS (10.84 ± 0.39) and PfNC (10.24 ± 0.24) isolate (**Table 6**). The PaWP recorded the lowest (1.68 ± 0.07) SA production.

The lytic enzyme production supported antagonism, which is the ideal character of an effective bioagent for plant diseases management. The PaRS (76.24 ± 0.35 and 132.36 ± 0.31) recorded the highest activity of chitinase and b-1,3-glucanase enzymes followed by PaNS (54.38 ± 0.29 and 116.20 ± 0.26), respectively. The lowest chitinase activity was found in PaWN (12.36 ± 0.21), while PaWP (28.18 ± 0.06) isolate in b-1, 3-glucanase (**Table 6**).

DISCUSSION

In vitro Evaluation

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 *P. anacardii* (Patil, 2012) earlier. The native strains became most potent for the reduction of pathogen growth confirmed as per the results of Manjula et al. (2004), Sen et al. (2006), Razeena and Rasheed (2007), Sivakumar (2007),

TABLE 4 | Effect of seed bacterization by PaRS on plant growth promoting activity of Finger millet.

Sr. No.	Seed treatment	Germination (%)	Plant height (Cm)			No. of leaves/plant			Colonization (Rhizosphere)		
			15 days	30 days	45 days	60 days	15 days	30 days	45 days	60 days	15 days ($\times 10^6$cfu/g)	30 days ($\times 10^6$cfu/g)	45 days ($\times 10^6$cfu/g)	60 days ($\times 10^6$cfu/g)
1	2 g/kg	100	40.72^{c}	43.13^{c}	50.36^{c}	53.13^{c}	7.1^{c}	10.73^{c}	12.99^{c}	15.07^{c}	2.67^{c}	3.06^{c}	4.89^{c}	3.28^{c}
2	4 g/kg	100	47.47^{b}	49.27^{b}	58.65^{b}	65.2^{b}	7.87^{b}	11.67^{b}	14.91^{b}	17.07^{b}	3.72^{b}	4.5^{b}	6.44^{b}	4.89^{b}
3	6 g/kg	100	52.07^{a}	54.73^{a}	66.53^{a}	72.36^{a}	8.68^{a}	12.6^{a}	17.4^{a}	20.27^{a}	5.06^{a}	6.67^{a}	8^{a}	7.06^{a}
4	Control	70	29.01^{d}	34.27^{d}	42.47^{d}	45.6^{d}	4.63^{d}	8^{d}	9.13^{d}	11.2^{d}	1.11^{d}	1.28^{d}	1.44^{d}	0.83^{d}

Data are mean of five replications.
Means followed by the same letter in a column are not significantly different (P = 0:05) by DMRT.

and Vishwanathan and Samiyappan (2007). Reported that 14 bacterial isolates inhibit the mycelia growth of *M. phaseolina* due to HCN, chitinase, b-1,3-glucanase, and siderophore production. An antagonist exhibits direct and indirect mechanisms to inhibit pathogen growth. The production of volatile and non-volatile compounds along with lytic enzymes reduced the vegetative growth of pathogens. The present results are more or less similar to Arora and Verma (2017), who observed that fluorescent *Pseudomonas* spp. Inhibited the mycelia growth of *P. capsici* and *R. solani*. Yasmin et al. (2017) evaluated *P. aeruginosa* against BLB rice pathogen strains under *in vitro* conditions because of lytic enzymes and secondary metabolites production. In the present investigation, PaRS proved as a potential bioagent against important fungal as well as bacterial pathogens.

PGPR and Colonization

The results of the experiments are quite confirmative with the results of Ramanathan et al. (2002) who revealed that all the strains of *P. fluorescens* promoted plant growth. Ramamoorthy and Samiyappan (2002) supported that seed bacterization with *P. fluorescens* isolate *Pf*-1 effectively increased plant vigor and produced the maximum amount of IAA in the culture medium. The increase in plant biomass in respect to germination, plant height, root and shoot weight, and vigor index may be due to higher water and nutrient uptake, phytohormone production i.e., gibberellic acid, indole acetic acid, and siderophore production by PaRS. The antagonist inoculum dose and its delivery system determine and significantly influence their population in the crop rhizosphere (Fischer et al., 2010). PaRS might have also contained the enzyme 1-aminocyclopropane-1-carboxylate (ACC) deaminase, a hydrolase of ACC (the precursor of ethylene in the plant), and thus, in this way reduce the level of ethylene during plant development. PaRS could also increase K, Zn, and Mn uptake by the above plant parts. It might also be produced organic acids involved in phosphorus solubilization or the fixation of atmospheric N_2 (Smolin and Shabaev, 1992).

The results corroborate with, Bergsma-Vlami et al. (2005) who recorded the significant effect of plant species on the population densities of fluorescent *Pseudomonas* spp. The dynamics of bacteria population are a complex phenotype affected by many different traits and environmental factors. These are motility, synthesis of the O-antigen of lipopolysaccharide and cellulose, thiamine production, amino acid synthesis biotin production, and an isoflavanoid-inducible efflux pump.

Pseudomonas spp. synthesizes osmolytes to protect themselves against fluctuations in osmotic conditions and accumulate to higher levels to alleviate stress effects. The accumulated osmolytes enhance the stability of proteins and membranes under water-limiting environments. PaRS might have produced proline, free amino acid, sugars, and extracellular polysaccharides (EPS) under stress conditions than under non-stressed conditions, indicating the role of these metabolites in stress tolerance to maintain PaRS population. However, the concentration of protein should be reduced significantly under stress indicating the degeneracy under stress conditions. Under stress, proteins are used for polysaccharide production. PaRS might have EPS under stress conditions, EPS produced by bacterial cells forms as an

TABLE 5 | Biochemical properties of native isolates of *Pseudomonas* spp.

Sr. no.	*Pseudomonas* isolates	Biochemical properties			
		IAA	Siderophore	HCN	P-solubilizing zone (mm) after 48 h
1	PaWP	+	+	++	15
2	PfWN	+	+	+	18
3	PaWS	+	+	++	17
4	PfRB	+	++	+	14
5	PaRS	+	+++	+++	22
6	PaNS	+	++	+++	20
7	PfNC	+	++	++	10

IAA: *+, Presence; –, Absent.* ***Siderophore fluorescence:*** *+++, High; ++, Medium; +, Weak.* ***HCN production:*** *–, No HCN; +, Weak; ++, Moderate; +++, Strong.*

TABLE 6 | Secondary metabolites and enzymes productions by native isolates of *Pseudomonas* spp.

Sr. no.	*Pseudomonas* isolates	SA	Siderophore	HCN	Chitinase	b-1,3-glucanase
1	PaWP	1.68 ± 0.07	10.06 ± 0.07	0.034 ± 0.002	28.18 ± 0.06	42.08 ± 0.05
2	PfWN	2.16 ± 0.07	5.38 ± 0.06	0.0523 ± 0.001	12.36 ± 0.21	54.45 ± 0.51
3	PaWS	7.12 ± 1.09	8.10 ± 0.09	0.026 ± 0.001	42.12 ± 0.18	72.17 ± 0.32
4	PfRB	4.18 ± 7.95	7.12 ± 0.06	0.032 ± 0.001	34.56 ± 0.08	84.21 ± 0.21
5	PaRS	12.68 ± 0.36	12.20 ± 0.08	0.084 ± 0.003	76.24 ± 0.35	132.36 ± 0.31
6	PaNS	10.84 ± 0.39	11.86 ± 0.16	0.066 ± 0.003	54.38 ± 0.29	116.20 ± 0.26
7	PfNC	10.24 ± 0.24	10.06 ± 0.07	0.047 ± 0.006	50.06 ± 0.14	96.37 ± 0.21

Data are represented by the mean of five replicates ± standard deviation.

organo-mineral sheath around the colonies that creates a micro-environment that decreases the water potential decline. The level of glutamate in PaRS might have increased in response to osmotic stress conditions. The desiccation-tolerant cells also accumulate high levels of disaccharides such as trehalose, sorbitol, fructose, glucose, and sucrose, which protect cellular enzymes by replacing water around macromolecules and also stabilize cell membranes during desiccation (Sandhya et al., 2010).

PaRS population increased after 15 and 30 days, it might be due to that above-discussed characteristics that may be possessed in PaRS during rhizospheric osmotic stress conditions. The P-solubilisation and IAA production capacity of PaRS is better than in the others, hence helping to promote growth. The higher HCN production might be reducing the pathogen infection which helps to maintain the plant health as compared to rest. The higher siderophore production in PaRS probably helps the survivability and colonization. The decreased colonization after 45 and 60 days might be due to the optimum availability of protein in the PaRS and hence there is no conversion of protein in EPS.

Biochemical Characterization

The quantitatively HCN production results are similar to the result of Goswami et al. (2013), Reetha et al. (2014), Rijavec and Lapanje (2016) and Abd El-Rahman et al. (2019) under *in vitro* conditions.

The six isolates of bacteria (*Pseudomonas japonica* strain NBRC 103040, *Bacillus megaterium* strain CtST3.5, *Pseudomonas* sp. strain Gamma-81, *P. tolaasii* strain ATCC 33618, *P. chlororaphis* strain Lzh-T5, and *P. mosselii* strain CV25) inhibit the growth of *Agrobacterium tumefaciens* as reported by Abd El-Rahman et al. (2019). Reetha et al. (2014) found a similar result; they isolated *Pseudomonas* and *Bacillus* from the sunflower rhizosphere. The inhibition of *Macrophomina phaseolina was* found under *in vitro* conditions due to HCN production. Goswami et al. (2013) isolated *Pseudomonas* spp. from marine water and recorded IAA, HCN, phosphate solubilization, and siderophore production which increased chickpea and green gram growth. Rijavec and Lapanje (2016) found that *Fusarium moniliforme* EXF1, *F.graminearum* EXF2, *Pseudomonas syringae* pv. *syringae* z1, *P.syringae* pv. *coronafaciens* z1238, *Erwinia carotovora* pv. *carotovora* z87, and *Xanthomonas campestris* pv. *campestris* recorded growth inhibition in the presence of HCN.

The quantitative siderophore production results are similar to the results of Goswami et al. (2013), Arora and Verma (2017), and Nithyapriya et al. (2021).

The siderophore results also respond to the finding by Arora and Verma (2017) that 23 bacteria including *Pseudomonas*, *Rhizobium*, *Enterobacter*, *Chronobacter*, *Kosakonia*, *Beijerinck*ia, and *Pantoea* spp. genus produced the siderophore at different levels. The *Bacillus* sp. LSB2 produced siderophore which helped to increase plant growth and other biochemical parameters of sesame as recorded by Nithyapriya et al. (2021).

The results are less or more similar to Megha et al. (2007) who studied the quantitative estimation of 52

fluorescent *Pseudomonas* for GA, IAA, protease, and phosphate solubilization similarly to the present study. Meera and Balabaskar (2012) studied Gram staining, gelatin, liquefaction, catalase and oxidase test, starch hydrolysis, siderophore, and HCN production. Umamaheswari et al. (2008) detected activity of IAA, SA, HCN, and siderophore from *P. fluorescens* (PfCIAH-196) with the same results as obtained in the present study.

Statistical Analysis

The biochemical experiments were performed in five repetitions and the data is represented as mean_standard deviation (SD). The data were analyzed by One Way Analysis of Variance (ANOVA) and DMRT tests. The significant differences in the means were analyzed based on the DMRT test ($p < 0.05$).

DATA AVAILABILITY STATEMENT

The original contributions presented in the study are included in the article/supplementary material, further inquiries can be directed to the corresponding author.

AUTHOR CONTRIBUTIONS

AS and RW contributed equally with experiment conduction. All authors contributed to the article and approved the submitted version.

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