International Journal of Bio-resource and Stress Management https://ojs.pphouse.org/index.php/IJBSM

Crossref

IJBSM May 2024, 15(5): 01-13 **Article** AR5215

Research Article *Stress Management*

DOI: HTTPS://DOI.ORG/10.23910/1.2024.5215

Characterization and Field Evaluation of Potential Fluorescent *Pseudomonas* Isolates on Growth and Yield Attributing Characters in Chickpea

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ABSTRACT

The present investigation was conducted at the research and instructional farm, College of Agriculture, Indira Gandhi Krishi Vishwavidyalaya, Raipur, Chhattisgarh, India during *rabi* (October–December, 2019) to evaluate the influence of fluorescent Pseudomonas isolates on chickpeas through characterization and field evaluation. Eighty-eight rhizosphere soil samples were derived from different locations in Bastar and Bilaspur districts of Chhattisgarh, India. Potential isolates were required for fundamental research and practical application of plant growth-promoting rhizobacterial strains on different crops. Among eighty-eight isolates, ten fluorescent Pseudomonas (visible under UV light at 360 nm) emitting strong fluorescence were selected for the course of the investigation and characterized for the production of siderophores, phosphate solubilization, indole acetic acid, 1-aminocyclopropane-1-carboxylic acid deaminase, and analysis of antagonistic potential. Of the 10 Pseudomonas isolates, 9704 was identified as the highest phosphate solubilizer, BSP-23 was identified as the highest indole acetic acid producer, and BSP-19 was the highest siderophore producer. Fluorescent Pseudomonas isolates BS-1, BS-4, 9809, BSP-14, and BSP-19 were identified to possess 1-aminocyclopropane-1-carboxylic acid deaminase-producing ability following qualitative analysis. Our present investigation indicates that beneficial effects of fluorescent Pseudomonas could be achieved through simple and cost-effective seed treatment. Three fluorescent Pseudomonas strains, 9704, BS-14 and 9829, consistently increased seed germination and improved plant growth and yield (BS-37.9% and 9704-35.7%) attributes in chickpeas. Besides, confrontation assays performed against *R. bataticola* expressed varying levels of inhibition. The phylogenetic affinities of 16S rRNA gene sequencing resolved the species identities of selected isolates.

KEYWORDS: Chickpea, confrontation assay, PGPR, rhizosphere, *Rhizoctonia bataticola*, *Pseudomonas*

***Citation* (VANCOUVER):** Parashuram et al., Characterization and Field Evaluation of Potential Fluorescent *Pseudomonas* Isolates on Growth and Yield Attributing Characters in Chickpea. *International Journal of Bio-resource and Stress Management,* 2024; 15(5), 01-13. HTTPS://DOI.ORG/10.23910/1.2024.5215.

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Data Availability Statement: Legal restrictions are imposed on the public sharing of raw data. However, authors have full right to transfer or share the data in raw form upon request subject to either meeting the conditions of the original consents and the original research study. Further, access of data needs to meet whether the user complies with the ethical and legal obligations as data controllers to allow for secondary use of the data outside of the original study.

Conflict of interests: The authors have declared that no conflict of interest exists.

RECEIVED on 12th February 2024 RECEIVED in revised form on 28th April 2024 ACCEPTED in final form on 10th May 2024 PUBLISHED on 20th May 2024

1. INTRODUCTION

Chickpeas are an important food legume commodity and have a diverse use with specific consumer preferences in the global market. Chickpeas in India peaked at an all-time high of 11.23 tonnes during 2017–18 (Anonymous, 2019). Chickpea has a high protein content, low fibre content, low fat content, and is rich in folate and vitamins. It is also a good source of trace minerals (molybdenum and potassium) (Sandhu et al., 2023). Despite its potential as a crop, chickpea productivity has shown little improvement in recent decades due to its susceptibility to insect pests like *Helicoverpa armigera* Hübner, as well as diseases such as wilt caused by *Fusarium oxysporum* Schlechtend: Fr. f. sp. *ciceri* (Padwick), dry root rot from *Macrophomina phaseolina* (Tassi) Goidanich, and blight caused by *Ascochyta rabiei* (Passerini) Labrousse. Dry root rot, caused by the soil-borne fungus *M. phaseolina*, is a prevalent disease in both temperate and tropical regions globally. Specifically in India, where chickpea cultivation is widespread, this disease has become increasingly severe and results in a significant crop loss of 10–25% (Manjunatha et al., 2013). Previous studies have shown that bio-control agents, such as *Pseudomonas fluorescens*, have been successful in colonizing and decreasing the germination of sclerotia associated with *M. phaseolina*. This has led to the notion that they may serve as a promising bio-control solution for combating root rot (Srivastava et al., 2001).

Plant growth-promoting rhizobacteria (PGPR) facilitate plant growth through direct/indirect mechanisms *viz*., bio- fertilization, stimulates root growth, rhizo-remediation, reduces plant stress against dieases, antibiosis, induction of systemic resistance, and competes for nutrients and niches (Lugtenberg and Kamilova, 2009; Glick, 2014). The release of various organic compounds by plant roots, including organic acids, phytosiderophores, sugars, vitamins, and amino acids, serves as signaling molecules to increase microbial populations. This interaction between the root system and its rhizosphere demonstrates their connection with the biotic environment. (Bais et al., 2006; Pothier et al., 2007; Badri et al., 2009; Shukla et al., 2011; Drogue et al., 2013). Understanding the impact of microbial population's PGPR activity on both the rhizosphere and the entire plant remains a challenge. *In vitro* studies on bipartite interaction between roots and PGPR indicate various effects. PGPR may decrease the growth of primary roots (Dobbelaere et al., 1999), while increasing their number and length (Combes-Meynet et al., 2011; Chamam et al., 2013). Additionally, PGPR can stimulate root hair elongation under *in vitro* (Dobbelaere et al., 1999; Contesto et al., 2008). Furthermore, plants inoculated with PGPR exhibit increased root and shoot biomass (El Zemrany et al., 2006; Minorsky, 2008; Veresoglou and Menexes, 2010; Walker et al., 2012). Consequently, PGPR activity stimulates mineral and water uptake, promoting overall plant growth. Seed bacterization or inoculation with PGPR bacteria aids in their establishment in the soil and rhizosphere (Ma et al., 2016). However, the poor survival ability of bioinoculants hampers commercial production of biofertilizers (Ma et al., 2011). Improving the formulation of microbial inoculum to survive, establish, and develop in the soil is crucial for providing nutrients to plants. Treating seeds with PGP microbes offers comprehensive protection against various environmental stresses, promoting cost-effectiveness and eco-friendliness, thereby advancing precision agriculture (Hazra and Patanjali, 2016). Coating seeds with microbes efficiently facilitates inoculation and population development in the soil, ensuring higher safety and efficiency standards (Ma et al., 2019; Rocha et al., 2019). To significantly impact global sustainable agriculture, implementing plant-beneficial microorganisms requires seamless transition from laboratory to field applications. This study aims to assess the impact of seed treatment with potential fluorescent Pseudomonas isolates on the growth and yield characteristics of chickpea through characterization and evaluation.

2. MATERIALS AND METHODS

The present investigation was carried out in the Department of Plant Pathology and Department of Plant Molecular Biology and Biotechnology, College of Agriculture I.G.K.V., Raipur, Chhattisgarh, India. Field and pot experiments were carried out at research cum instructional farm, College of Agriculture, I.G.K.V., Raipur during *rabi* (October–December, 2019).

2.1. Collection and isolation of fluorescent Pseudomonas

Rhizospheric soil samples (88) collected from various locations in the Bastar and Bilaspur districts of Chhattisgarh were utilized to isolate fluorescent *Pseudomonas*. Isolation was performed using the standard serial dilution method on Kings's B agar plates (Reynolds Jackie, 2005). These plates were then incubated at 28°C for 48 hours. Single bacterial colonies of fluorescent *Pseudomonas* were identified under UV light (360 nm) and subsequently purified by streaking onto Kings's B media plates. Pure culture colonies of these isolates were subcultured and maintained on King's B media slants.

2.2. Phenotypic characterization of fluorescent pseudomonas isolates

Fluorescent Pseudomonas isolates underwent phenotypic characterization based on biochemical criteria using King's B medium. The test parameters included gelatin liquefaction, starch hydrolysis, nitrate reductase activity, and growth at 4°C and 42°C. Rapid antibiotic sensitivity

studies were conducted using the streak plate method outlined by Bauer et al. (1966). Sensitivity to kanamycin and carbenicillin was determined for all Pseudomonas isolates by adding 1 mg ml^{-1} of kanamycin and carbenicillin, respectively, to King's B medium.

2.3. Phosphate solubilization activity

Quantitative screening for phosphate solubilization in Pikovskya's broth followed the procedure outlined by Murphy and Riley (1962). Fresh cultures of potential Pseudomonas isolates in active growth phase were inoculated into Pikovskya's broth to assess their ability to solubilize supplemented phosphate. After seven days of incubation, the quantity of inorganic phosphate (Pi) released into the broth was measured. Phosphate solubilization activity was quantified in terms of tricalcium phosphate solubilization, with the measurement expressed as µg ml^{-1} of available orthophosphate, calibrated using the standard curve of potassium dihydrogen phosphate.

2.4. Indole acetic acid (IAA) production

For the production of indolic compound, an actively growing fresh culture of fluorescent Pseudomonas was inoculated into 20 ml of DF salts minimal media (Dworkin and Foster, 1958) in a 100 ml conical flask and incubated for 4 days at 28±2°C. The medium was supplemented with L-Tryptophan at a concentration of 1.02 g l^{-1} from a 5 mM filter-sterilized stock prepared in warm distilled water. After 72 hours of incubation, bacterial cells were removed from the culture medium by centrifugation at 5000 rpm for 5 minutes, and the pH of the medium of all isolates was recorded. Followed by, 1 ml of supernatant aliquot was vigorously mixed with 4 ml of Salkowski's reagent (Gordon and Weber, 1951), with a blank containing uninoculated DF salts minimal media. The mixture was allowed to stand at room temperature for 20 minutes before measuring absorbance at 535 nm in a colorimeter. Then, 4 ml of Salkowski's reagent was added to each tube, mixed vigorously, and incubated at room temperature for another 20 minutes. Absorbance at 535 nm was measured again in the colorimeter. The concentration of indole-3-acetic acid (IAA) in each culture was determined by comparing it with the standard curve.

2.5. ACC deaminase assay

For qualitative estimation of ACC deaminase activity, *Pseudomonas* isolates were cultured in 5 ml of Tryptic Soy Broth (TSB) medium and incubated at 28°C with shaking at 120 rpm for 24 hours. The cells were then harvested by centrifugation at 3000 rpm for 5 minutes, washed twice with sterile 0.1 M Tris-HCl (pH 7.5), and resuspended in 1 ml of 0.1 M Tris-HCl (pH 7.5). Followed by, the cells were spot inoculated on Petri plates containing modified DF (Dworkin and Foster) salt minimal medium supplemented with 2 mM ACC (1-aminocyclopropane-1-carboxylic acid) as the sole nitrogen source. Plates with only DF salt minimal medium without ACC served as the negative control, while plates with $(NH_4)_2SO_4$ (0.2% w/v) served as the positive control. The inoculated plates were then incubated at 28°C for 27 hours. Isolates exhibiting growth on ACC-supplemented plates were compared to the negative and positive controls. Selection was based on the ability of isolates to utilize ACC as a nitrogen source.

2.6. Siderophore production

For quantitative estimation of siderophore production, the chrome azurol sulfonate (CAS) assay (Schwyn and Neilands, 1987) was employed. Actively growing cultures of potential fluorescent Pseudomonas were inoculated into 20 ml of King's B broth in a 100 ml capacity flask and incubated for 72 hours at 28±2°C. Centrifugation was conducted at 3000 rpm for 5 minutes to pellet the bacterial cells. Following centrifugation, 0.5 ml of supernatant was pipetted out and mixed with 0.5 ml of CAS solution. Then, 10 µl of a shuttling solution (sulfosalicylic acid) was added. After incubating for 20 minutes, the color formed was determined using a spectrophotometer at 630 nm. King's B broth served as the blank, and a reference solution was prepared by adding CAS dye and shuttling solution to King's B broth. The values of siderophore released in King's B medium were expressed as a percentage.

2.7. Analysis of the antagonistic potential of isolated bacteria

Fluorescent Pseudomonas isolates were evaluated for their potential as a biocontrol agent against *R. bataticola*, the causal agent of root rot in chickpeas. A bipartite interaction assay was conducted following a confrontation method developed by Kotasthane et al. (2017). In this assay, the edges of a glass funnel were used to deposit the bio-agent inoculum surrounding the pre-inoculated fungal pathogen R. bataticola on a mixture of potato dextrose agar (PDA) and King's B media in equal volumes. Inoculation was performed by gently touching the edge of the funnel containing the Pseudomonas inoculum, encircling the pre-inoculated plant pathogenic fungi on agar at equidistant points. After 72 hours of incubation at 28±2°C, the inhibition zone was measured. The percent inhibition of the pathogen by fluorescent Pseudomonas compared to the control was calculated using the formula outlined by Vincent (1947)

Percent inhibition=(C-T)/T×100

Where, C=Growth of the pathogen in control; T=Growth of the pathogen in the presence of *Pseudomonas* isolates

2.8. Molecular identification of potential fluorescent pseudomonas isolates

The bacterial isolates were identified through partial 16S rRNA gene sequence analysis at the Molecular Biology

and Biotechnology Laboratory, College of Agriculture, I.G.K.V., Raipur. The bacterial 16S rRNA gene sequence was amplified via PCR using the eubacterial primer pair 27F (5'-AGAGTTTGATCMTGGCTCAG-3') and 1492R (5'-CTACGRRTACCTTGTTACGAC-3'). PCR products were purified using a MoBIO kit according to the manufacturer's instructions to eliminate PCR impurities. The purified PCR products were then sent to the Eurofins laboratory, Bangalore for Sanger sequencing. The resulting FASTA sequences were subjected to BLASTN analysis using the NCBI public domain database to identify the species of fluorescent Pseudomonas.

2.9. Assessment of growth and yield attributing characters of potential fluorescent pseudomonas isolates in soybean and chickpea

Field experiments were conducted at the Research cum Instructional Farm, College of Agriculture, I.G.K.V, Raipur, during *rabi* (October–December, 2019). to assess the efficacy of potential fluorescent *Pseudomonas* isolates on growth and yield attributes in chickpea (variety: INDIRA CHANA-1). Seed treatment involved spraying the potential *Pseudomonas* isolates onto seeds, followed by sprinkling talcum powder to ensure complete coating of the seeds with the bacterial culture. The treated seeds were then shade-dried and sown the following day.

Each treatment was replicated three times in a randomized block design, with an untreated control maintained for comparison. Observations such as plant height, root length, root volume, number of branches, grain yield, straw yield, biological yield, etc., were recorded.

2.10. Data analysis and statistical analysis

All pot and field experiments were carried out in rainout shelters and college farm fields arranged in completely randomized design and randomized block design respectively with three replications in each treatment. The data (biochemical and yield) were subjected to statistical analysis using WASP (Web Agri Stat Package) (http://icargoa.resin/wasp/idex.php). The critical difference at 0.01 and 0.05% level of significance was calculated for the observed values along with average and standard error

3. RESULTS AND DISCUSSION

3.1. Collection and isolation of fluorescent pseudomonas from soil

Rhizospheric soil of different crop plants derived from different locations of Bastar and Bilaspur districts of Chhattisgarh were used for isolation of fluorescent *Pseudomonas*. Soil samples were passed through a sieve to remove debris. Isolation of bacteria was done by standard serial dilution technique on King's B agar media and plates were incubated at 28°C for 48 hr. Single bacterial colonies of fluorescent *Pseudomonas* were detected under UV light (366 nm) and further purified by streaking onto the Kings'B media plates. Minute fluorescent bacterial colonies generated from a few bacterial cells were derived and were subcultured and maintained on King's B agar slants for future use. Ten isolates out of 88 emitting very strong fluorescence and producing 1×10^8 CFU g^{-1} on Kings B media were selected for the present study (Table 1).

Table 1: Potential Pseudomonas isolates used in the present study

Sl. No.	Isolates	Origin/location
1	BS-1	Brassica species rhizosphere
2.	BS-2	Brassica species rhizosphere
3.	BS-3	Brassica species rhizosphere
4	BS-4	Brassica species rhizosphere
5	9704	Jagdalpur
6	9829	Jagdalpur
7	9809	Jagdalpur
8	BSP-14	Bilaspur
9	BSP-19	Bilaspur
10	BSP-23	Bilaspur

3.2. Phenotypic characterization of fluorescent pseudomonas isolates

Pseudomonas isolates were characterized based on phenotypic characterization and antibiotic sensitivity tests (Table 2). Among 10 isolates eight isolates BS-1, BS-2, BS-3, BS-4, 9809, 9829, 9704, and BSP-14 hydrolyzed starch by formation of the yellow zone around the colony after flooding with Lugol's iodine solution, rapid gelatin hydrolysis was observed and also showed positive growth when incubated at 42°C on Kings B media. However, two isolates BSP-19 and BSP-23 failed to hydrolyse starch and formed semi-solidified gelatin agar medium after incubation at 4°C in a refrigerator. Sensitivity against two antibiotics, carbenicillin, and kanamycin was tested for selected potential *Pseudomonas* isolates. Among 10 isolates eight isolates BS-1, BS-2, BS-3, BS-4, 9809, 9829, 9704, and BSP-14 were able to grow in kanamycin supplemented medium where as two isolates BSP-19 and BSP-23 showed resistant to carbenicillin.

The present study aims to isolate a promising group of rhizobacteria that possess many characteristics that make them well-suited as a plant growth promoter and biocontrol agent (Weller, 1988; Lemanceau, 1992; Weller et al., 2002; Weller, 2007). Ten *Pseudomonas* isolates were characterized based on biochemical tests for differentiation of *P. fluorescence, P. aeruginosa, and P. putida* and most of

Table 2: Phenotypic and biochemical characterization of potential *Pseudomonas* isolates

Isolates	SH	GL	NR	Growth at 42°C	Growth at 4°C	CA	KA	PS	IAA P	SP	ACC Q	IRB
BS-1	Y	Y	Y	Y	N	S	R	141.5^{G}± 10.5	6.19^{DE}± 0.058	54.82^{CD}± 0.15	+	60.00
BS-2	Y	Y	Y	Y	N	S	R	162^{F}± 7.0	2.34^{F}± 0.022	46.78^{BC}± 0.028	-	47.20
BS-3	Y	Y	Y	Y	N	S	R	186.5^{E}± 1.5	24.29^{B}± 0.062	50.3^{CD}± 0.04	-	50.00
BS-4	Y	Y	Y	Y	N	S	R	191.5^{DE}± 5.5	10.37^{BCD}± 0.054	56.3^{B}± 0.005	+	54.44
9704	Y	Y	Y	Y	N	S	R	357.5^{A}± 1.5	3.29^{E}± 0.02	48.75^{BCD}± 0.023	-	61.10
9829	Y	Y	Y	Y	N	S	R	316^{B}± 5.0	7.63^{CDE}± 0.09	50.25^{CD}± 0.04	-	59.40
9809	Y	Y	Y	Y	N	S	R	194.5^{CDE}± 0.5	23.69^{AB}± 0.149	40.25^{D}± 0.082	+	61.10
BSP-14	Y	Y	Y	Y	N	S	R	198.5^{CDE}± 7.5	13.75^{ABC}± 0.041	50.66^{CD}± 0.15	+	60.50
BSP-19	N	N	N	N	Y	R	S	210C± 7.0	8.32^{BCD}± 0.068	79.71^{A}± 0.03	+	60.00
BSP-23	N	N	N	N	Y	R	S	206C± 4	28.08^{A} ± 0.085	22.1^{E}± 0.01	-	60.50
CD								18.156	0.236	0.253		
SEm±								5.764	0.074	0.079		
SEd±								8.152	0.105	0.112		
C.V.								3.947	23.611	7.623		

SH: Starch Hyrolysis; GL: Gelatine liquefaction; NR: Nitrate reductase; CA: Carbenicillin; KA: Kanamycin; PS: Phosphate solubilisation μg ml^{-1}; IAA P: IAA production μg ml^{-1}; SP: Siderophore production (%); ACC Q: ACC qualitative; IRB: % inhibition *Rhizoctonia* bataticola; S: Susceptible; R: Resistant; Y: (YES) growth; N: No growth; Positive (+ve), Negative (-ve); All the treatment values are average of three replications; All the treatments found significant at 5% probability level; Means followed by a common letter are not significantly different at the 5% level

the biochemical tests showed positive for *P. aeruginosa* except BSP-14 and BSP-23 which showed positive to *P. fluorescence*. Similar results were also reported by Blazevic et al. (1973) in diagnostic tests for the differentiation of *P. fluorescens* and *P. putida* isolates.

3.3. Phosphate solubilization activity (Liquid assay)

Phosphate solubilization activity revealed variations among all ten isolates in their utilization of supplemented tricalcium in the medium. Phosphate solubilization concentrations were expressed as μg ml^{-1}. In this investigation, phosphate solubilization varied from 108 μg ml^{-1} to 357.5 μg ml^{-1} (Table 2). The highest phosphate solubilization was observed in Pseudomonas isolate 9704 (357.5±1.5 μg ml^{-1}), leading to a 69.7% increase over the control, followed by 9829 (65.8%), BSP-19 (48.5%), BSP-23 (47.5%), BSP-14 (45.4%), 9809 (44.4%), BS-4 (43.6%), BS-3 (42%), BS-2 (33.3%), and BS-1 (23.6%).

Pseudomonas isolate 9704, isolated from the Jagdalpur region of the Bastar district and identified as P. aeruginosa through various biochemical tests, exhibited notable phosphate solubilizing ability among the ten isolates in vitro. Phosphorus plays a vital role in plant growth and development, involving several key functions such as photosynthesis and the transformation of sugars and starches in plant systems (Sultenfuss and Doyle, 1999). Paul and Sinha (2017) found that *Pseudomonas* strain KUPSB12 demonstrated an effective phosphate solubilization index of 2.85 on Pikovskya agar plates, along with a very high production of soluble phosphate (219±0.33 μg ml^{-1}) in a liquid medium. Similarly, Sharma et al. (2017) reported

that potential phosphate solubilizing *Pseudomonas aeruginosa* strain An-Mg, isolated from the rhizosphere soil of apple trees, could serve as both a biofertilizer and a potential biocontrol agent. Kloepper et al. (1988) highlighted phosphorus solubilization as the most readily available form for plant growth.

3.4. IAA (Indole acetic acid) production

The concentrations of IAA produced by 10 *Pseudomonas* isolates were evaluated by comparing it with a standard curve that was prepared by serial dilution (0–100 µg ml^{-1}) using the commercial available IAA. Quantity of IAA produced by ten *Pseudomonas* isolates ranged from 28.08±0.085 µg ml^{-1} to 2.34±0.022 µg ml^{-1}. Among ten *Pseudomonas* isolates highest IAA was produced by isolate BSP-23 (28.08±0.085 µg ml^{-1}) (Table 2).

Pseudomonas isolate BSP-23 was isolated from Bilaspur district soil samples and identified as *P. fluorescence by* performing different biochemical tests. IAA is responsible for increasing root surface area and root length and thereby provides the plants greater access to soil nutrients. Further PGP effects of isolate BSP-23 were evaluated in the field. *Pseudomonas* Isolates producing IAA have a stimulatory effect on plant growth and increase N, P, K, Ca, and Mg uptake (Farzana and Radizah, 2005). Sivasakthivelan and Saranraj (2013) analyzed that biocontrol strains *P. fluorescence* for IAA biosynthesis and studied the effect of its consequent manipulation on its PGP potential. Oberhänsli et al. (1991) reported the production of IAA by *P. fluorescens* isolated from tobacco rhizosphere

3.5. Siderophore production

3.5.1 Quantitative estimation (CAS assay)

Quantitative estimation of siderophore production by *Pseudomonas* isolates was done by CAS assay (chrome asurol assay). Using CAS assay percent siderophore unit was determined by spectrophotometer. *Pseudomonas* isolate BSP-19 (79.71%) produced the highest amount of siderophores followed by BS-4 (56.3%), BS-1 (54.82%), BSP-14 (50.66%), BS-3 (50.3%), 9829 (50.25%), 9704 (48.75%), BS-2 (46.78%), 9809 (40.25%) and BSP-23 (22.1%) (Table 2). The presence of siderophore in fluorescent *Pseudomonas* has been reported by several authors (Bakker and Schippers 1987; kloepper et al., 1980; Bezbaruah 1994).

3.6. ACC deaminase assay

All *Pseudomonas* isolates showed positive, no growth on negative control, and among 10 isolates five isolates showed growth in plates that are supplemented with ACC. *Pseudomonas* isolates BS-1, BS-4, 9809. BSP-14 and BSP-19 utilized ACC deaminase and degraded the ACC (Table 2).

Pseudomonas isolates BS-1, BS-4, 9809, BSP-14, and BSP-19 found to produce IAA, phosphate solubilization, and also tend to produce ACC deaminase enzyme which decreases the level of ethylene in the plant by utilizing ACC which is the precursor for ethylene and reduces stress in plants. Many reports say that IAA and ACC could work together and promote plant growth, especially root elongation (Glick et al., 1998; Glick, 2014). The major effect of seed inoculation with ACC producing rhizobacteria is plant root elongation, enhancement of rhizobial nodulation, and N, P, and K uptake (Nadeem et al., 2007). Magnucka and Pietr 2015 showed that the highest activity of the ACC enzyme exhibited by *Pseudomonas* isolates PO366 (wheat rhizosphere), PO283 (wheat rhizosphere), and RZ310 (rape rhizosphere) were 3.70, 4.32, and 16.50 mmol α-KB g^{-1} protein h^{-1} respectively in DF minimal media.

3.7. In-vitro antagonistic activity by fluorescent pseudomonas against macrophomina phaseolina

The efficacy of bio-control activity of ten *Pseudomonas* isolates was studied against the fungal pathogen *R. bataticola* following the confrontation assay technique (Kotasthane et al., 2017). There were differences in antagonistic abilities of ten *Pseudomonas* isolates against *R. bataticola* (Table: 2). All ten *Pseudomonas* isolates showed different degrees of growth inhibition of *R. bataticola* ranging from 47.2% to 61.1%. Confrontation assays revealed *Pseudomonas* isolate 9704 and 9809 as potential antagonists against *R. bataticola* with 61.1% inhibition (Figure 1) followed by BSP-14 and BSP-23 with 60.5% inhibition, BS-1 (60% inhibition), 9829 (59.4% inhibition), BSP-19 (60% inhibition), BS-4 (54.4% inhibition), BS-3 (50% inhibition) and BS-2 (47.2% inhibition).

Pseudomonas spp. produce antibiotic DAPG (2, 4 diacetylphloglucinol) is a major determinant of biological control of soil born plant pathogens (Thomashow and Weller 1996). Similar results were also obtained by Kotasthane et al. (2017) reported fluorescent *Pseudomonas* isolates P66, P141, P144, P166, and P174 were antagonists against *Rhizoctonia solani* and *Sclerotium rolfsii* following confrontation assays.

3.8. Molecular characterization of fluorescent pseudomonas isolates using genus and species-specific loci

Genomic DNA of ten *Pseudomonas* isolates was extracted as per the manufacturer's instructions using a HiPurA™ genomic DNA purification kit (Cat. no. K3100-02) and used for amplification using primer pairs. In the present investigation PCR amplification of 16S rRNA bacterial gene (1500 bp) using a 27F+1492R primer set resulted in a specific distinguishing amplification product. All ten *Pseudomonas* isolates resulted in positive reactions with 27F+1492R PCR primer sets and got an amplicon size of ~1150 bp (Figure 2).

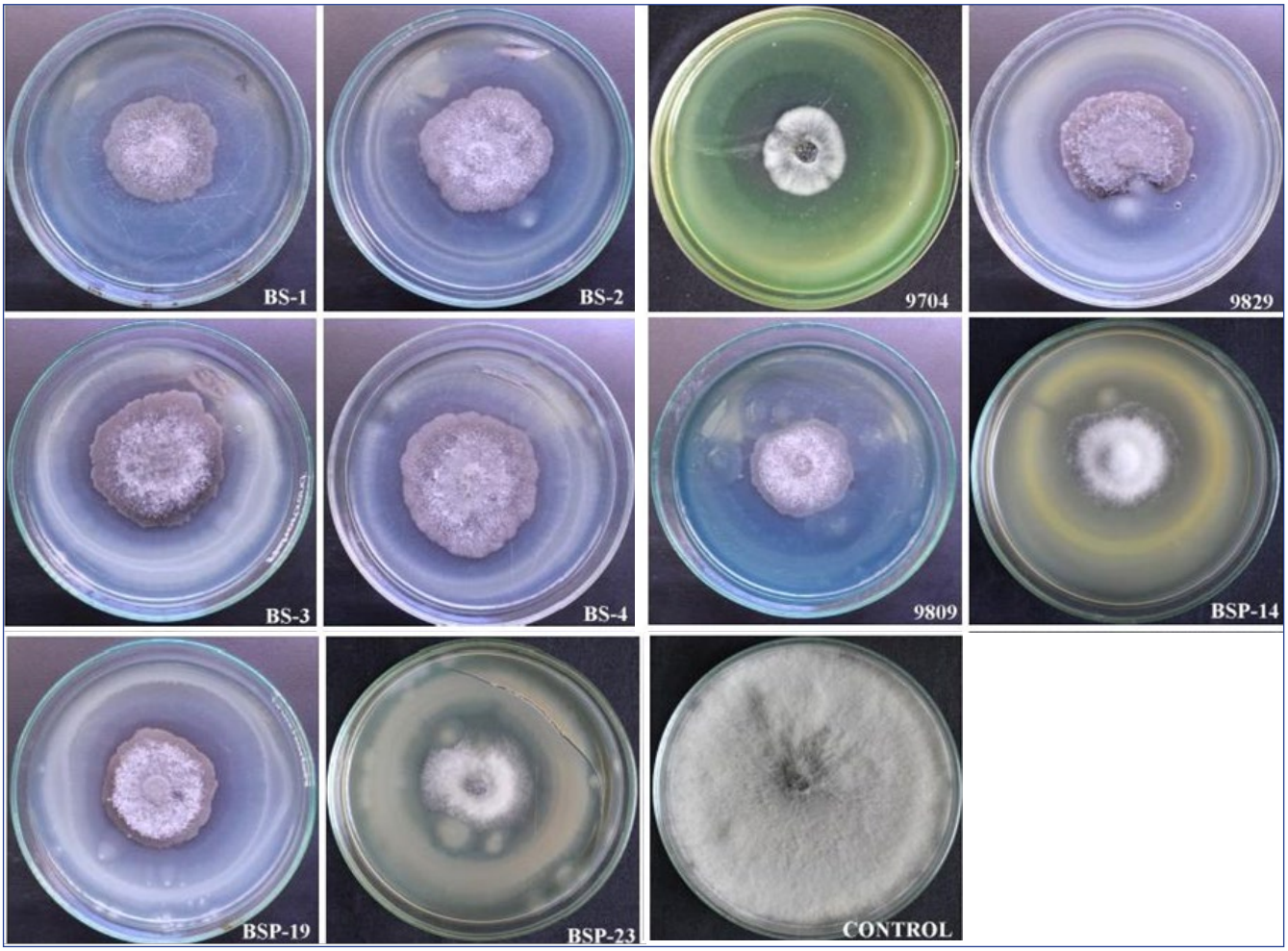

Figure 1: *In-vitro* antagonistic activity by *Pseudomonas* fluorescence against *Rhizoctonia bataticola* using confrontation assay

FASTA sequences so obtained were subjected to BLASTN analysis in NCBI public domain (https://blast.ncbi.nlm.nih.gov/Blast.cgi) (Table 3).

Sequencing analysis of ten *Pseudomonas* isolates was detected to be *P. aeruginosa* except BS-3 (*Pseudomonas chlororaphis* subsp. *chlororaphis* NZ_CP027712.1), BSP-14 (*Pseudomonas mesoacidophila* NZ_CP020738) and BSP-23 (*Pseudomonas mesoacidophila* NZ_CP020738) which were expected to be biotypes of *Pseudomonas fluorescence* (Bossis et al., 2000). Similar results were also obtained by Trivedi et al. (2015)"type":"article-journal"},"uris":["http://www.mendeley.com/documents/?uuid=29fb35e8-bcd5-46b3-990a-1a489e33b08d"]}],"mendeley":{"formattedCitation":"(Trivedi et al., 2015 based on nucleotides homology and phylogenetic analysis using 16S rDNA gene sequencing, treated sample was detected to be *Pseudomonas entomophila* (GenBank Accession No. AY907566) with 96% identity of gene sequencing data, which was nearest homolog species to *P. fluorescens* (Accession No. EF672049).

3.9. Plant growth-promoting response in chickpea (Indira chana-1) following seed bacterization with fluorescent Pseudomonas isolates

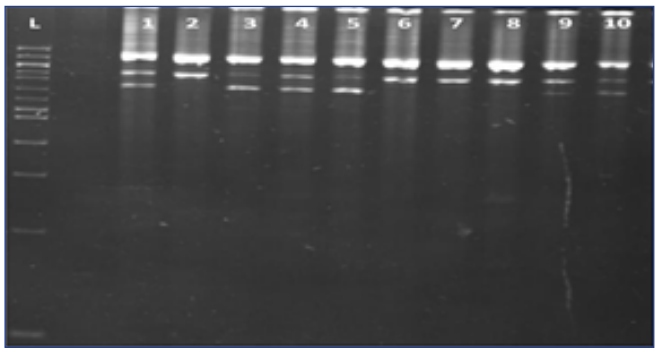

Figure 2: PCR amplification of 10 fluorescent *Pseudomonas* isolates generating 1150 bp bands through 27+1492 primer (L-100bp ladder)

3.9.1. Pot experiment

Pot experiments were carried out at the rainout shelter, College of Agriculture, I.G.K.V., Raipur during *rabi*-2019 to study the effect of *Pseudomonas* isolate on the growth and yield characteristics of chickpeas. The plant growth-

Table 3: 16S rRNA Gene Sequence analysis of ten *Pseudomonas* isolates using 27F+1492R primer set

Code of Query sequence	Species identity (Accession No.) of matching Sequence	% Identity	E-value
BS-1_16SF-GA 7542.ab1	*Pseudomonas aeruginosa* PAO1 (chromosome)(NC_002516)	98.696	0
BS-2_1492-R-GA-7542.ab1	*Pseudomonas aeruginosa* SP4527 (chromosome) (NZ_CP034409.1)	93.676	0
BS-3_16SF_GA_7542	*Pseudomonas chlororaphis* subsp. chlororaphis DSM 50083 (chromosome) (NZ_CP027712.1)	76.562	5.8E-09
BS-4-16S-F_GA-7542.ab1	*Pseudomonas aeruginosa* H25883 (chromosome) (NZ_CP033686.1)	99.259	0
9704_16SF_GA_7542	*Pseudomonas aeruginosa* BH9 (chromosome) (NZ_CP029713.1)	95.642	0
7542_9829-16SF	*Pseudomonas aeruginosa* isolate paerg004 (paerg004-contig000) (NZ_LR130531.1)	96.750	0
7542_9809_16SF_GA_7542	*Pseudomonas aeruginosa* PA34 (chromosome)(NZ_CP032552.1)	98.953	0
BSP-14_16SF_GA_7542	*Pseudomonas mesoacidophila* ATCC 31433 (chromosome 2) (NZ_CP020738)	86.580	0
BSP-19_16SF_GA_7542	*Pseudomonas aeruginosa* SP4527 (chromosome) (NZ_CP034409.1)	87.947	0
BSP-23_16SF_GA_7542	*Pseudomonas mesoacidophila* ATCC 31433 (chromosome 2) (NZ_CP020738)	86.254	0

Sequencing site: Eurofins laboratory Bangalore for Sanger's sequencing; Alphabets in parenthesis represent gene bank accession number; E-value represents number of hits

promoting ability of ten *Pseudomonas* isolates was evaluated in the chickpea cultivar (Indira chana-1) following seed treatment. Ten seeds of each treatment were sowed in each pot and three replications were maintained for each treatment. Observations were recorded after 90 days of sowing (Table 4). Most of the studied traits such as shoot length, root length, number of branches, and root volume were significantly different at the 5% probability level. Pot experiment revealed that shoot length does not differ much by seed inoculation with *Pseudomonas* isolates and varied from 46.33±5.36 cm to 33.33±0.88 cm. The highest shoot length (46.33±5.36 cm), was recorded in chickpea plants treated with *Pseudomonas* isolate BSP-14. *Pseudomonas* isolates BS-4 (57.33±5.92 cm) recorded the highest root length, which led to a 16.86 % increase over control (Figure 3). A distinct difference was seen in the root volume of chickpea plants treated with *Pseudomonas* isolates. The root volume of all the *Pseudomonas*-treated chickpea plants varied from 246.96 cm^3 to 94.99 cm^3. The highest root volume was recorded in chickpea plants treated with *Pseudomonas* isolate BS-2 (246.96 cm^3), which led to a 61.53% increase over control. Whereas, isolate BSP-19 recorded no difference in root volume in comparison to control.

Figure 3: PGP responses in chickpea (Indira chana-1) following seed bacterization with potential fluorescent *Pseudomonas* isolates under pot conditions

Figure 4: Efficacy of different fluorescent *Pseudomonas* isolates on plant growth promoting effects of chickpea (Indira chana-1) under field conditions; Variety: Indra chana-1; Plot size: 4×5 m^2; Seed rate: 200 grams $plot^{-1}$; Design: RBD

3.9.2. Field experiment

Field experiments were carried out at research cum instructional farm, College of Agriculture, I.G.K.V., Raipur during *rabi-2019* to study the effect of *Pseudomonas* isolate on the growth and yield of chickpea (Figure 4). Based on the results of variance analysis of chickpea fields data traits like mortality %, shoot length, the number of branches, filled pods, bundle weight, strawweight, and grain yield were significantly different at the 5% probability level (Table 4) the morality of chickpea plants was recorded in field plots 27 days after sowing. The highest mortality was recorded in check plots and the lowest mortality was recorded in chickpea plots treated with *Pseudomonas* isolate

Table 4: PGPR response in chickpea (Indira chana-1) following seed bacterization with fluorescent *Pseudomonas* isolates

Isolates	Plant growth-promoting response in chickpea (Indira chana-1) following seed bacterization with fluorescent *Pseudomonas* isolates (pot experiment)			
	Root (cm)	Root volume (cm^3)	Shoot (cm)	Branches
BS-1	44 ± 3.78^{BC}	104.48^{CD}	34.66 ± 0.66^{B}	4.66 ± 0.33^{CD}
BS-2	49.66 ± 5.23^{AB}	246.96^{A}	35 ± 1.73^{B}	5.33 ± 0.66^{ABCD}
BS-3	40.66 ± 4.97^{BCD}	104.48^{CD}	38 ± 2.64^{B}	5 ± 0.57^{BCD}
BS-4	57.33 ± 5.92^{A}	227.96^{AB}	35.33 ± 1.20^{B}	4.33 ± 0.33^{DE}
9704	47.66 ± 4.33^{AB}	227.96^{AB}	34.66 ± 0.33^{B}	5.33 ± 0.33^{ABCD}
9829	33.33 ± 1.66^{D}	132.98^{C}	37.66 ± 1.45^{B}	6 ± 0.57^{AB}
9809	44.66 ± 1.76^{B}	208.97^{B}	39 ± 1.52^{B}	5.66 ± 0.33^{ABC}
BSP-14	48.66 ± 0.88^{AB}	227.96^{AB}	46.33 ± 5.36^{A}	5 ± 0.57^{BCD}
BSP-19	33.66 ± 2.96^{CD}	94.99^{E}	35.66 ± 1.85^{B}	4.33 ± 0.33^{DE}
BSP-23	48.66 ± 0.88^{AB}	208.97^{B}	36.66 ± 2.18^{B}	6.33 ± 0.33^{A}
Control	47.66 ± 1.20^{AB}	94.99^{E}	33.33 ± 0.88^{B}	3.33 ± 0.33^{E}
CD (p=0.05)	10.393		6.5	1.32
CV	13.611		10.41	15.4

Table 4: Continue...

Isolates	Plant growth-promoting response in chickpea (Indira chana-1) following seed bacterization with fluorescent *Pseudomonas* isolates (field experiment)							
	Mortality (%)	Shoot (cm)	Branches	Pods $plant^{-1}$	BW (kg ha^{-1})	SY (kg ha^{-1})	GY (kg ha^{-1})	HI (%)
BS-1	3.69	64.18 ± 1.49^{D}	4.7 ± 0.26^{DEF}	41.5 ± 2.74^{BCD}	4000.00^{BCD}	2937.00^{BC}	1063.00^{AB}	26.58
BS-2	3.78	65.28 ± 1.37^{CD}	4.8 ± 0.24^{CDEF}	43.1 ± 1.14^{BC}	4333.00^{AB}	3352.16^{A}	981.16^{BC}	22.64
BS-3	4.26	63.74 ± 1.25^{DE}	4.7 ± 0.26^{DEF}	44.9 ± 2.03^{B}	4166.66^{ABC}	3105.83^{AB}	1061.83^{AB}	25.48
BS-4	4.1	65.03 ± 2.48^{CDE}	5.7 ± 0.21^{AB}	54.6 ± 2.60^{A}	4416.66^{A}	3263.33^{AB}	1153.33^{A}	26.11
9704	2.44	61.99 ± 0.96^{DEF}	5.5 ± 0.26^{ABC}	45.5 ± 2.22^{B}	4000.00^{BCD}	2885.83^{BCD}	1114.16^{A}	27.58
9829	3.83	67.86 ± 1.33^{BC}	5.7 ± 0.30^{AB}	54.2 ± 1.19^{A}	4000.00^{BCD}	2999.66^{ABC}	1000.33^{BC}	25
9809	3.26	71.34 ± 1.69^{A}	5.2 ± 0.24^{BCDE}	43.4 ± 2.45^{BC}	3666.66^{DE}	3160.16^{BCD}	773.10^{EF}	21.08
BSP-14	3.17	68.453 ± 1.18^{B}	5.3 ± 0.39^{ABCD}	45.4 ± 1.60^{B}	3583.33^{EF}	2677.16^{CD}	906.00^{CD}	25.28
BSP-19	3.53	67.65 ± 1.66^{BC}	6.0 ± 0.25^{A}	46.4 ± 2.63^{B}	3833.33^{CDE}	3154.00^{ABC}	846.00^{DE}	22.07
BSP-23	3.12	65.20 ± 2.57^{CD}	4.5 ± 0.22^{EF}	43.3 ± 2.23^{BC}	3500.00^{EF}	2650.00^{CD}	850.00^{DE}	24.28
Control	4.27	62.14 ± 1.52^{F}	4.3 ± 0.15^{F}	38.8 ± 1.34^{C}	3250.00^{F}	2534.66^{D}	715.50^{G}	22.01
CD (p=0.05)		4.694	0.741	6.082	0.79	0.784	0.209	
CV		9.882	16.24	15.003	5.97	7.784	6.417	

All the treatment values are average of three replications (Pot experiment); Values of Plant height, branches, and pods are an average of three replicates (10 plants per replication); Values of BW: Bundle weight; SY: Straw yield, GY: Grain yield; are average of three replicates; All treatments are found significant at a (p=0.05) probability level; Means followed by a common letter are not significantly different at (p=0.05) probability level

9704 (2.44%) in comparison to control plots which recorded 4.27% of mortal chickpea plants. In a field experiment, shoot length, the number of pods, bundle weight, straw weight, and grain yield parameters in chickpeas are influenced by ten *Pseudomonas* isolate treatments presented in Table 4. A significant increase in shoot length was observed in all

treatments over control. The maximum shoot length was observed in chickpea plants treated with *Pseudomonas* isolate 9809 (71.34±1.69 cm), which led to a 12.89% increase in shoot length over control. Whereas, isolate 9704 recorded a decreasing percentage of shoot length over control. Seed treatment with *Pseudomonas* isolate significantly affected filled pods. A significant increase in filled pods was recorded in chickpea plants treated with *Pseudomonas* isolates over untreated control. The highest number of filled pods was seen in chickpea plants treated with isolate BS-4 (54.6±2.60), which led to a 28.9% increase in filled pods over control.

Bundle weight, straw yield, and grain yield in chickpea plants treated with *Pseudomonas* isolates were markedly increased in comparison to untreated or controlled. The highest bundle weight (4416.66 kg ha^{-1}) was recorded in chickpea plants treated with *Pseudomonas* isolate BS-4 led to a 26.41% increase over control. The highest straw yield (3352.16 kg ha^{-1}) was recorded in chickpea plants treated with *Pseudomonas* isolate BS-2 led to a 24.38% increase over control. Grain yield in chickpeas varied from 1153.33 kg ha^{-1} to 715.5 kg ha^{-1} (Table 4). Highest grain yield was recorded in chickpea plants treated with *Pseudomonas* isolate BS-4 (1153.33 kg ha^{-1}) which led to 37.9% increase in grain yield. According to the result of the variance analysis harvest index was affected by all *Pseudomonas*-treated chickpea plants. The maximum harvest index was recorded in chickpea plants treated with *Pseudomonas* isolate 9704 (27.58%) and this response was probably due to higher grain yield and biomass.

Florescent *Pseudomonas* has been shown to produce an arsenal of metabolites that maintain plant health and increase plant growth and root development. However, most of the findings are limited to pot experiments (Bagnasco et al., 1998; O'Sullivan and O'Gara 1992; Dowling and O'Gara 1994). Ten *Pseudomonas* isolates tested in field trials produced an arsenal of metabolites and induced plant growth and yield. In field experiments, seed bacterization with *Pseudomonas* isolates has enhanced the growth of chickpeas (Kumar and Dube, 1992; Chaurasia et al., 2015; Shweta et al., 2008) nitrogen uptake, yield and its attributes of rice (*Oryza sativa* L. nitrogen uptake, yield and its attributes of rice (*Oryza sativa* L. These findings may be due to the increased synthesis of hormones by *Pseudomonas* isolate 9704. Gibberellins trigger the activity of specific enzymes such as amylase, which increase the availability of starch that promotes early germination (Gholami et al., 2009). Malleswari and Bagyanarayana (2013) studied that seed inoculation initiates the physiological processes of germination and helps in the proliferation of bioagents in the atmosphere. Therefore, using *Pseudomonas* isolates as seed inoculants is useful in enhancing germination in chickpeas. Shoot and root characters were significantly improved in chickpea cultivars treated with *Pseudomonas* isolates. *Pseudomonas* isolated from *Brassica*-specific (BS-2) rhizosphere showed enhanced root growth in chickpeas. Misko and Germida (2002) studied that *Pseudomonas* isolated from *B. napus* rhizosphere was shown to possess antifungal properties and to secrete the plant growth hormone indole acetic acid that can directly promote root growth. A low concentration of IAA can stimulate primary root elongation, whereas, high IAA levels stimulate the formation of lateral roots, decrease primary root length, and increase root hair formation (Patten and Glick, 2002; Dobbelaere et al., 1999).

4. CONCLUSION

Seed treatment was a simple and effective method to enhance the beneficial effects of fluorescent *Pseudomonas* for the delivery of bio-inoculants. Bacterial isolates (9704, BS-4, and 9829) consistently, improved seed germination, reduced seedling mortality, improved plant growth, and yield in chickpea. Phylogenetic affinities of the 16S rRNA gene sequences resolved BS-3 as *P. chlororaphis* sub sp *chlororaphis* and BSP-14 and BSP-23 as *P. mesoacidophilla* and other isolates BS-1, BS-2, BS-4, 9704, 9829, 9809, and BSP-19 as *Pseudomonas aeruginosa*.

5. ACKNOWLEDGEMENT

Authors acknowledge financial support provided by Department of Plant Pathology, Indira Gandhi Krishi Vishwavidyalaya, Raipur for carrying out this work.

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