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Potential plant growth-promoting properties of *Pseudomonas* species isolated from the Rhizosphere of the Soybean plant

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ABSTRACT

Pseudomonas species has gained major attention in the agricultural industry because of its widespread application in various biotechnological processes. It is one of the rhizobacterial groups that have an important role in plant growth promoter and plant health. In the present study, *Pseudomonas* species isolated from soybeans rhizosphere and identified based on biochemical reactions. Various tests were performed for the determination of the growth promoter were based on Indole Acetic acid, phosphate solubilization, and seed germination test, etc. Twenty-five isolates were identified as *Pseudomonas* species that produced Indole Acetic acid, phosphate solubilization, and promote enhancement of root length, shoot length, or the number of lateral roots. Among those 25 isolates, 9 isolates showed Indole Acetic acid production and phosphate solubilization properties. Based on excellent growth promoter, 4 isolates of *Pseudomonas* species were taken for final screening which was S-3, S-16, S-23, and S-24 as potential isolates of *Pseudomonas* species that could be applied as inoculants of the soybean plant. The study suggested that all *Pseudomonas* strains enhanced plant growth in the soybean plant.

Keywords: *Pseudomonas* species. Soybean and Plant Growth Promotion

INTRODUCTION

Bacteria that inhabit the rhizosphere may influence plant growth by contributing to a host plant's endogenous pool of bioactive compounds such as phytohormones, antibiotics, siderophores (Patten and Glick, 2002; Mubarik *et al.,* 2010). Those kinds of the bacterial group are well-known as Plant Growth Promoting Rhizobacteria (PGPR). PGPR is considered to promote plant growth directly or indirectly. Indirect effects are related to the production of metabolites, such as antibiotics, siderophores, or HCN, that decrease the growth of phytopathogens and other deleterious microorganisms. Direct effects are dependent on the production of plant growth regulators or improvements in plant nutrients uptake (Ahmad *et al.,* 2005; Bai *et al.,* 2003). Due to their bioactive properties, secondary metabolites have been traditionally mined from producing organisms for use in the pharmaceutical industry. Pharmacologically significant antibacterial secondary metabolites such as penicillin and vancomycin inhibit bacterial cell wall synthesis (Rai *et al.,* 2003; Allen and Nicas, 2003) while tetracycline and erythromycin inhibit bacterial protein synthesis (Metcalf *et al.,* 2002; Schlunzen *et al.,* 2003). *Pseudomonas* species has gained major attention in the agricultural industry because of its widespread application in various biotechnological processes. An important ubiquitous member of this group, *Pseudomonas aeruginosa* is an opportunistic pathogen of plants and humans (Walker *et al.,* 2004; de Bentzman and Plesiat, 2011). The conscious agricultural applications of *Pseudomonas aeruginosa* not only pose a threat to human health and environment but also raise relevant ecological issues such as the evolution of multi resistant bacteria and pathogenicity (Gerber, 1973).

The soybean-wheat cropping system is the predominant cropping system in central India followed by soybean chickpea. Soybean (*Glycine max* L. Merrill) contains 40% protein, and 20% oil, as well as bioactive molecules and isoflavones, whereas durum wheat (Triticum turgidum var. durum) provides approximately 2% more protein along with a higher content of beta-carotene than wheat (*Triticum aestivum*). Thus, the adoption of the soybean-wheat cropping system can help alleviate malnutrition and also improve the socioeconomic status of farmers of central India (Kathiresan *et al.,* 1999; Mandal *et al.,* 2002.).

Screening of effective *Pseudomonas* species based on their functional traits, soil enzyme activities, and plant nutrients acquisition has been a challenging task because of wide variations in their functionality. The objective of the present study was the isolation of *Pseudomonas* species from the agricultural soil and its characterization, production of plant growth-promoting compounds, and evaluation of its growth-inhibiting activities for sustainable agriculture.

MATERIALS AND METHODS

Sample collection

Soil samples were collected from Jalna District (Marathwada regions, India), for isolation of plant growth-promoting of *Pseudomonas* species. Soil samples (approx. 500 g) were collected by using clean, dry, and sterile polythene bags and stored in ice boxes and transported to the laboratory where they were kept in a refrigerator at 4°C until analysis (Marathe, *et al.,* 2015).

Isolation and Identification of bacteria

Rhizosphere soil samples were screened for *Pseudomonas* species using the dilution method with King's B Agar as a semi selective medium. *Pseudomonas* species. isolates were identified distinct morphological characteristics, including pigments, colony form, elevation and margin; texture; and opacity based on Bergeys' Manual of Systematic Bacteriology.

Estimation of Indole Acetic Acid (IAA)

IAA production was calculated quantitative analysis of IAA was performed using the method of Loper and Scroth (1986) at different concentrations of tryptophan (0, 50, 150, 300, 400, and 500 mg/ml). isolate *Pseudomonads* were grown for 48 h on kings B media. Fully grown bacterial cultures were centrifuged at 3600 rpm for 30 min. The supernatant (2 ml) was mixed with two drops of orthophosphoric acid and 4ml of the Salkowski reagent (50 ml, 35% of perchloric acid, 1ml 0.5M $FeCl_3$ solution). The development of the pink color indicates IAA production. Optical density was taken at 530nm with the help of a spectrophotometer with the help of a standard graph of IAA (Hi-media) obtained Concentration of IAA produced by cultures was measured in the range of 10–100 mg/ml.

Phosphate solubilization

The ability of the test isolates to solubilize insoluble inorganic phosphate was tested by spotting 10 µl overnight cultures on Pikovskaya's agar plates and incubating at 28-30^{0}C for 2-3 days. The isolates which showed a clear zone of solubilization of tricalcium phosphate (TCP) around the colony were noted as phosphate solubilizers. The diameter of the zone of TCP solubilization was measured (Pikovskaya, 1948).

Germination seed assay

The seedling bioassay was conducted based on the method described by (Dey *et al.,* 2004). For seedling bioassay, each *Pseudomonas* species isolate, was grown in King's B medium agar plates at room temperature for 24 h. The inoculants for treating seeds were prepared by suspending cells from agar plates in nutrient broth to gain approximately 1010 cells per mL. Germinating parameters were measured after 7 days of incubation including the length of the primary root, shoot, and numbers of lateral roots.

RESULTS AND DISCUSSION

Presently *Pseudomonas* specie one of the appealing candidates for the plant growth promoter of plant diseases (Weller, 1988). Among the *Pseudomonas* species, fluorescent pseudomonads make-up a dominant population in the rhizosphere and possess several properties that have made them biocontrol of choice (Johri *et al.,* 1997).

Isolation and screening of bacteria

After incubation growth was observed on all plates of nutrient agar. However, only the greenish colored colonies, which are peculiar characteristics of *Pseudomonas* species were selected as potential isolates. A total of 16 isolates were obtained from selected soil samples. These isolates were labeled as S1 to S16. In the present study total of 40 soil samples were collected from the Jalna districts of Marathwada. A total of 25 Pseudomonas species were isolated from rhizospheric soil of crops i.e. soybean from the Marathwada region of Maharashtra state and maintained in the pure culture were characterized, identified, and studied for their functional diversity. 25 isolates were selected as secondary metabolites producer, whereas among the 25 isolates 5 isolates were selected as an excellent secondary metabolite producer.

Identification of the isolate

After growth on nutrient agar isolate S3, S6, and S15 showed greenish yellow colored colonies by producing a diffusible pigment. The biochemical characters were performed by using standard methods described in Bergey's manual of determinative bacteriology. According to King E.O. *et al.,* (1954) *Pseudomonas aeruginosa* colonies appear green to bluish-green due to the production of pyocyanin pigments. The results obtained with morphological and biochemical characteristics (Table: 1) for S3, S6, and S15 were compared with the characters of reference Pseudomonas *aeruginosa* (Bergey's Manual of Determinative Bacteriology) and it was found that SS1 exhibits more similarity with the *Pseudomonas aeruginosa.*

Table 1: Morphological and Biochemical Characterization of isolate

Sr. No	Morphological Character	Results	Biochemical test	Results
1.	Gram staining	Gram-negative	Catalase test	+
2.	Motility	Motile	Oxidase test	+
3.	Cell shape	Rod	Sugar utilization test	-
4.	Greenish pigment	Present	Citrate utilization test	+
5.	Capsule	Absent	Casein hydrolysis test	-
6.	Spore	Absent		

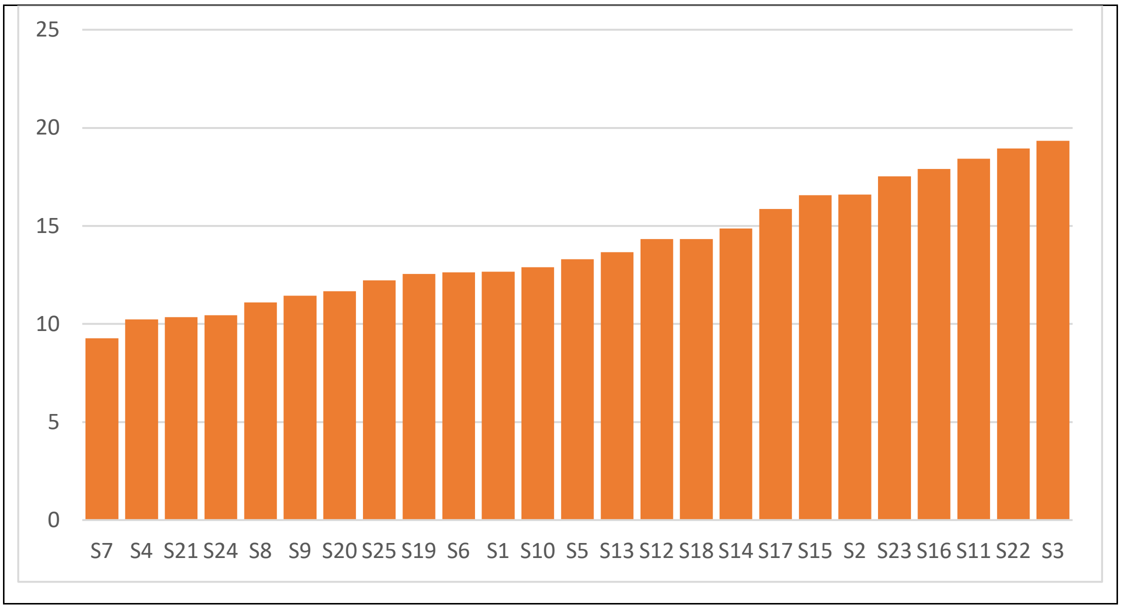
Three bacterial isolates efficient in bioactivity against selected human pathogens were identified as *Pseudomonas* species by using criteria given Bergey's Manuale of Systematic Bacteriology for identification. The identified *Pseudomonas* species showed Citrate, VP, Gelatinase, citrate utilization, Catalase, and Oxidase test positive.

Screening for production of Indole-3-acetic acid (IAA): Among the 25 isolates, only 7 isolates produced IAA, it was proved by both qualitative and quantitative methods (Table 2, Fig. 1). The presence of pink color indicates positive for IAA production qualitatively. In the quantitative analysis, maximum quantity of IAA was produced by the isolate S3 (19.34mg/ml), followed by S11 (18.43 mg/ml), S15 (16.56 mg/ml) S16 (17.90 mg/ml) S23 (18.95mg/ml) and S23 (17.53 mg/ml). Minimum production was observed in isolates S4, S7, S21 and S24 whose concentration were found to be (10.23 mg/ml), (9.27 mg/ml), (10.35 mg/ml) and (10.45 mg/ml) respectively. Isolates S8, S9, and S20 were statistically similar in IAA production, ranging from 10-11 mg/ml. The results also suggested that IAA may act on a common regulatory cascade leading to morphogenesis and secondary metabolism (Diemaite, 2004; Jeyanthi & Ganesh, 2013). The present study deals with monitoring the ability of *Pseudomonas aeruginosa* to produce plant growth promoters for stimulating plant growth along with the effect of inoculants on soil profile as well as the chlorophyll content of the leaves.

Table 2 : Qualitative and Quantitative analysis of IAA Production

Sr.No	Strain No	Screening for IAA production	Quantification of IAA (mg/ml)
1.	S1	+	12.67
2.	S2	++	16.60
3.	S3	+++	19.34
4.	S4	+	10.23
5.	S5	++	13.31
6.	S6	++	12.64
7.	S7	+	9.27
8.	S8	++	11.10
9.	S9	++	11.45
10.	S10	++	12.89
11.	S11	+++	18.43
12.	S12	++	14.33
13.	S13	++	13.67
14.	S14	++	14.87
15.	S15	+++	16.56
16.	S16	+++	17.90
17.	S17	+++	15.87
18.	S18	++	14.33
19.	S19	++	12.56
20.	S20	++	11.67
21.	S21	++	10.35
22.	S22	+++	18.95
23.	S23	+++	17.53
24.	S24	+	10.45
25.	S25	++	12.23

Figure 1: Quantitative analysis of IAA Production by *Pseudomonas* species



Screening of Phosphate Solubilizing *pseudomonas* species:

All 25 isolates were taken for the screening of Phosphate solubilization, among the 25 isolates 11 isolates were for potent phosphate solubilizing properties. In the total bacterial population percentage contribution of different pseudomonas species showed variations at different sites. In all the samples, the contribution of the genus *Pseudomonas aeruginosa was* more as they represented by a large number of species. The percentage contribution of pseudomonas species spelled considerable variations. Among the P-solubilizers, in the total bacterial population, *Pseudomonas aeruginosa* (S16) contributed a maximum of 17.5% and ranked first among all. The contribution of the S3 strain was significant with 15%, S23 contributed 7.5%, and S24 contributed 9.3%. These species together contribute 24.81% in the total bacterial population (Table 3).

Table 3: Phosphate Solubilization by *Pseudomonas* species

Sr.No	Code No	Name of Species	Zone of solubilization
1.	S3	*Pseudomonas aeruginosa*	22
2.	S11	*Pseudomonas aeruginosa*	20
3.	S16	*Pseudomonas aeruginosa*	24
4.	S23	*Pseudomonas aeruginosa*	20
5.	S24	*Pseudomonas aeruginosa*	22

Effect on Seed Germination, Root and Shoot Length

The influence of *Pseudomonas aeruginosa* S16 on seed germination and *Soybean (Glycine max)* plant growth promotion was studied by pot culture technique. It was found that the selected *Pseudomonas* strain has a notable positive effect on seed germination, seed size, and on the root and shoot length of the plant, as compared with the control P (uninoculated seeds). The result of the study showed a 25% increase in seed germination, in PP, whereas no effect was observed in PIAA. 36 %, 50 %, and 33% increase in root length was also observed in pot PP, and PIAA respectively. Surprisingly after 20 days, an 84% increase in shoot length was found in PP while a 90% and 73% increase in PIAA was also recorded. The 25% reduction in time of germination reduction in PP and PIAA when compared with control. A similar effect of different experiment on seed size was also found (Table 4)

A previous report of Mohite B. (2013), proposed that the IAA producing rhizosphere soil bacterial isolates were significantly augmented the plant height and root length of crop plants along with an increase in chlorophyll content when compared with control. IAA also induce proliferation of lateral roots and root hairs and thus increase nutrient absorbing surfaces; this may lead to greater rates of nutrient absorption. This in turn would be expected to significantly increase the shoot length of the plant (Shahab *et al.* 2009).

Table 4: Effect of *Pseudomonas aeruginosa* (S3) and IAA on seed germination, root length and shoot length of *Soyabean*

Parameter	PP	PIAA	P
Seed Germination (%)	100	87	72
Germination Time (days)	3	3	3
Root Length (cm) (after 20 days)	7.3 ± 0.43	8.7± 0.24	4 ± 0.12
Shoot length (cm) (after 20 days)	12.3 ± 0.10	10.8 ± 0.23	8.5 ± 0.34
P: Plant,: PP: Plant + *P. aeruginosa*, :PIAA: Plant + IAA			

PGPR affects plant growth in two different ways, indirectly or directly. The direct promotion of plant growth by PGPR entails either providing the plant with a compound that is synthesized by the bacterium, for example, phytohormones or facilitating the uptake of certain nutrients from the environment (Glick, 1995). The indirect promotion of plant growth occurs when PGPR lessen or prevent the deleterious effects of one or more phytopathogenic organisms. This can happen by producing antagonistic substances or by inducing resistance to pathogens (Glick, 1995). PGPR, as bio-control agents, can act through various mechanisms, regardless of their role in direct growth promotion, such as by known production of auxin phytohormone (Patten and Glick, 2002), a decrease of plant ethylene levels (Glick *et al.,* 2007) or nitrogen-fixing associated with roots (Döbereiner, 1992).

CONCLUSION

In conclusion, all the features lead to the conclusion that the secondary metabolites producing strains *of Pseudomonas* species under study having multiple plant growth-promoting characteristics play a vital role in iron nutrition and growth promotion of soybean crop. Further studies are required to prove the efficiency of the strains as biofertilizers and bio-pesticides at the field level.

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