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Improvement of Oil yielding crops yield attributes using plant growth promoting rhizobacteria

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Abstract Siderophores are small molecules that can easily bind to ferric iron. As a chelating agent, they transport iron molecules inside the bacterial cell for various biochemical reaction. At present study, characterization of few siderophores producing bacteria from the soil samples collected from Salem district, Tamil Nadu. The siderophores production was assayed qualitatively and quantitatively through Chrome Azural S and the results showed positive for the strains PS01 and PS02 that grown in succinate medium. In pot culture studies Brassica nigra.L Sesamum indicum.L showed significantly increasing in the root length, shoot length, fresh weight, dry weight and total chlorophyll and carotenoids significantly increased in treated plant .The antagonist activity of the siderophore producing Pseudomonas spp against fungal pathogen Fusarium oxysporum. This result showed that Pseudomonas spp is a good producer of siderophore which can be beneficial for its antagonistic activity towards fungal pathogen and increasing the Oil yielding crops.

Keywords: *Brassica nigra* L., chlorophyll, carotenoids, *Pseudomonas* spp, siderophore, *Sesamum indicum* L.

Introduction

Plant growth promoting rhizobacteria (PGPR) directly associate with plant roots and can exist within root tissue (as endophytes), on the surface of plant roots (the rhizoplane), or within the zone of soil specifically influenced by the root system (the rhizosphere). Characterized by their plant-growth promoting properties, PGPR are a diverse group of bacteria that produce a wide range of enzymes and metabolites, influence nutrient acquisition, modulate hormone levels, and ameliorate the negative impacts of biotic and abiotic stressors (Ngumbi and Kloepper 2016). *Pseudomonas* are excellent candidates of PGPR and can produce hydrogen cyanide, siderophores, protease, antimicrobial compounds and possess phosphate solubilizing activity (Noori *et al.*, 2012). Microbes release siderophores to scavenge iron from these mineral

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phases by formation of soluble Fe^{3+} complexes that can be taken up by active transport mechanisms. Many siderophore are non-ribosomal peptides. (Hu *et al*., 2011). At present nearly 500 siderophores are reported from selected microorganisms. A great variation is seen in siderophores structures from one species to another (Alli and vidhale *et al*., 2013). Biological control has emerged as a very popular alternative because it offers a way of controlling pathogens that does not involve the use of chemicals .Siderophores (derived from the greek meaning 'iron carriers') are low molecular weight (below 1000Da), ferric –ion-specific chelating agents produced by bacteria and fungi to compact low iron stress (Ngamau *et al*., 2014).Sesame (*Sesamum indicum* L.) seed has rich source of fatty acids and antioxidants, so it is used as healthy and nutritive additive to prepare several foods. The yield of sesame has been affected by several abiotic and biotic factors. The seedlings emergence, plant growth and the yield of *sesame* are inhibited up to 40%, during the effect of destructive pathogens of *Fusarium oxysporum* and *Macrophomina phaseolina*, which cause wilt and charcoal rot disease (Kumar *et al.,* 2011). Siderophore producing PGPR play a vital role in Fe nutrition of plants and therefore in plant growth promotion leading to healthy plants, which are vital for increasing the crop and food yield. Siderophores of rhizobacteria provide iron nutrition to the plants and help in plant growth promotion.(Shaink *et al.,* 2014). Siderophore producing PGPR play a vital role in Fe nutrition of plants and therefore in plant growth promotion leading to healthy plants, which are vital for increasing the oil yielding crops *Brassica nigra.L* and *Sesamum indicum* L. Siderophores of rhizobacteria provide iron nutrition to the plants and help in plant-growth promotion. They prevent the plant pathogens from iron nutrition; thereby restricting its growth, and thus, help in biological control of phytopathogens.

Materials and methods

Isolation of soil microbes

Rhizosphere soils were collected from plant rhizosphere region Pagalpatti, Pannapatti. Poosaripatti (Omalur), Salem Tamilnadu. The collected soil sample was brought to the laboratory in sterile polythene bag under aseptic conditions the soil sample was air dried and subjected to the isolation of bacteria by spread plate technique.

Soil Analysis

The soil samples were air dried and stored in a refrigerator at 5℃ for analysis. The pH of the soil samples was measured with PH meter using 1:2.5

soil/wet system (Elico, India) and Electrical Conductivity (EC) was measured using an electrical conductivity bridge (Elico type CM82,India)and was expressed as dSm¯ ˙The available nitrogen content in the soil was estimated by the alkaline permanganate method as described by (Subbiah and Asija 1956).Soil phosphorus was estimated by the method by the given by (Olsen *et al.,* 1954). Potassium content of the soil was estimated by the method of (Jackson, 1973).

Biochemical Characterization of soil bacteria

Gram Staining: Gram staining was detected by method described by (Cappuccino and Sherman, 2002).

Indole test (Morello *et al*., 2002): Two ml of peptone water was inoculated with 5ml of bacterial culture and incubated for 48 hours. 0.5ml of Kovac's reagent was added shakes well and examined after 1 minute.A red colour in the reagent layer indicated Indole. In negative case there is no development of red colour

Methyl red (MR) and Voges Proskauer (VP) test (Morello, *et al*., 2002): MRVP broth was prepared and 5ml broth is transfer in culture tubes inoculated with bacterial strains and two test tubes were kept as control. The test tubes were incubated at 28℃ for 48h.Five drops of MR indicator were added to each test tube including the control and observed the change in colour similarly ten drops of VP-I reagent and 2-3 drops of VP-II reagent were added to other incubated test tubes and control also. Observed the test tubes for colour change and compared with control.

Citrate utilization test (Ruchhoft *et al*., 1931): The isolates were carried out by inoculating the test organism in test tube containing Simon's citrate medium and this was inoculated for 24 hours to 72 hours. The development of deep blue colour after incubation indicates a positive result.

Catalase activity (Taylor and Achanzar, 1972): The isolates were streaked on yeast extract mannitol agar slant and incubated for 3 days at 28^{o}C. The formation of effervescence upon addition of 1ml of 1% hydrogen peroxide was positive to catalase activity.

Oxidase production (Gaby and Hadley, 1957): The isolates were streaks on yeast extract mannitol agar plates and incubated for 3 days at 28^{o}C. After incubation, a loop full of isolates was placed over oxidase disc (N, N – Tetra methyl- Para-phenyl diamine dihydro chloride). Development of blue (or) purple colouration was positive to oxidase production.

Urease activity (Mac Faddin, 2000): The isolates were streaked on Christensen's urea agar slants and incubated for 3 days at 28^{o}C Observed the

slant for a colour change at 6 hours, 24 hours, and every day for up to 6 days. Urease production is indicated by a bright pink colour on the slant that may extend into the butt.

Antibiotic susceptibility test (Baure *et al*., 1996): The strains were cultured on slants of the solidified Nutrient agar medium and suspended directly in sterile saline without any change in the antibiotic resistance pattern. Antibiotic disks (3disks/plate) were applied on the plates with a dispenser and after 3 days incubation at 37^0C. The antibiotic disks used were the following Chloromphencol^{-10}, Chloromphencol^{-25}, and Chloromphenicol^{-50}.

IAA (Indole-3-acetic acid) production: IAA (Indole-3-acetic acid) was detected by method described by (Ahmad *et al*., 2005).

Phosphate solubilization test (Chen *et al*., 2006): Phosphate solubilization ability of isolates was detected by spotting them on Pikovskaya's agar plates. The plates were then incubated at 28±1℃ for 3 days and then observed for the appearance of clearing zones around the colonies (due to solubilization of inorganic phosphate by producing organic acid by bacteria).

Siderophores production: The *pseudomonas* isolates were inoculated in King's B broth and incubated for 15 days. After 15 days incubation period the culture was centrifuged at 10,000rpm to 15min cell free supernatant were used for the following tests.

Siderophore assay: Siderophore assay was carried out based on the CAS shuttle assay of (Payne 1994). The siderophore content in the aliquot was calculated by using following formula % of siderophore units= $\frac{(\mathrm{Ar}-\mathrm{As})\times 100}{\mathrm{Ar}}$ Where, Ar= absorbance of reference at 630 nm (CAS reagent) As= absorbance of sample at 630 nm

Ferric chloride test: To 0.5ml of culture filtrate 0.5ml of 2% aqueous ferric chloride solution was added. Appearance of reddish brown /orange colour was positive indication of siderophore production.

Arnow's test (Arnow, 1937): To 1ml of culture filtrate 1ml of 0.5N hydrochloric acid, 1ml of nitrite molybdate reagent and 1ml of 1N sodium hydroxide were added. The formation of red coloured solution was considered as indication of the presence of catechole type of siderophore.

Tetrazolium test: To a pinch of tetrazolium salt were added to 2drops of 2N sodium hydroxide and 0.1ml of the culture filtrate. Appearance of a deep red color was indication of hydroxamate type of siderophore (Snow, 1954).

Isolation of fungal pathogen *Fusarium* spp.: *Fusarium* species were isolated based on its colony and morphological characteristics (Singh *et al*., 1991).Isolated colonies were sub cultured onto potato dextrose agar (PDA) slants and stored at 4^0C. Six day old cultures were used throughout the study.

Antagonistic activity of *Pseudomonas* sp Bio control assay: Sterile Potato Dextrose Agar was prepared and solidified in petridishes. The well size 6mm diameter was made aseptically in the agar plate. Forty µ1 of partially purified siderophore was added to the well and allowed to diffuse radially for 1 hour at low temperature. The inoculums size of 6×10^3 spores of plant pathogenic fungi was swabbed over the surface of the PDA plates and incubated at room temperature for 48 hours. After incubation, the zone of inhibition of the mycelia growth was measure.

Pot culture: Seeds of *Brassica nigra, Sesamum indicum* were soaked in water overnight and surface sterilized with 0.1% mercury chloride for 5min and washed several time with sterilized distilled water. Garden soil and sand (2:1) w/w was stream sterilized for 3 days and then distributed in each parts and then seeds were sown. After germination the seedling were thinned out two, three. The pots were arranged over a slap in the green house. The plants were irrigated with nitrogen free sterile a tap water on alternate days, Plants were harvested at 45 DAI (Day after Inoculation) and separated into leaves, shoot and roots**.** The plant length (cm) was calculated in bio inoculants treated plants and non-inoculants plants. Three plants were taken each pot to measure the mean value for all the treated and control plants.

Estimation of photosynthetic pigments (mg/g): Chlorophyll a, b and carotenoids contents were extracted from respective dose of leaves and estimated according to the method of (Arnon 1949) and the carotenoids content was determined according to the method of (Krick and Allen 1965). Chlorophyll content was calculated using the formula of Arnon (1949).

Soil microbial population (cfu/g.soil): Microbial population in the soil samples was determined by the dilution plate's techniques. The results were expressed as colony forming units per gram soil. Dry weight basis, Nutrient Agar (NA), Rose Bengal agar medium (RBA), Potato dextrose agar medium (PDA).Each culture medium was also used in the liquid form without added for dilution preparation. In addition soils from sites of *in vitro* study area.

Results

Rhizosphere soils were collected from Pagalpatti, Pannapatti, Pusaripatti (Omalur) Salem district, Tamil Nadu. The soil sample was analyzed for pH, Electrical conductivity, and NPK analysis (Table 1). Bacterial isolates was isolated by serial dilution method. The isolates were followed by biochemical test these was *Pseudomonas aeruginosa* , *Pseudomonas fluorescence* and *Bacillus cereus* they were named as from PSA01, PSA02, BAC03. (Figure:1,2,3).

Table 1. Soil Characteristics

Location	pH	EC	N (mg/kg)	P (mg/kg)	K (mg/kg)
Pagalpatti	8.1	0.4	113	1.5	231
Pannapatti	7.9	0.3	125	4.5	185
Pusaripatti	7.3	0.2	98	3.5	85

EC = Electrical conductivity
Nitrogen = 0 to 113 mg/kg-low, 113 to 181 mg/kg-medium, 181and above mg/kg-high
Phosphorus = 0.0 to 4.5 mg/kg-low, 4.6 to 9.0 mg/kg-medium, 9.0 and above mg/kg-high
Potassium = 0 to 46 mg/kg-low, 47 to 113 mg/kg-medium, 113 and above mg/kg-high

Table 2. Biochemical characterization of bacterial isolation

S.No	Test	PSA01	PSA02	BAC03
1.	Gram staining	_	_	+
2.	Indole production	_	+	_
3.	Methyl Red	_	+	+
4.	Voges-proskaur	_	+	_
5.	Citrate utilization	+	+	+
6.	Phosphate solubilization	+	+	+
7.	Siderophore production	+	+	_
8.	Catalase activity	+	+	+
9.	Oxidase production	_	+	+
10.	Urea's activity	_	+	+

(+)- Positive, (-) Negative

Table 3. Antibiotic Sensitivity test of (PSA01, PSA02, and BAC03*)*

S.No.	Commercial antibiotic	Inhibition zone diameter (mm)		
		PSA01	PSA02	BAC03
1.	Streptomycin^{-25}	16 mm	18 mm	15mm
2.	Chloromphecol^{-25}	22 mm	24mm	19mm
3.	Chloromphecol^{-50}	24 mm	28mm	22mm

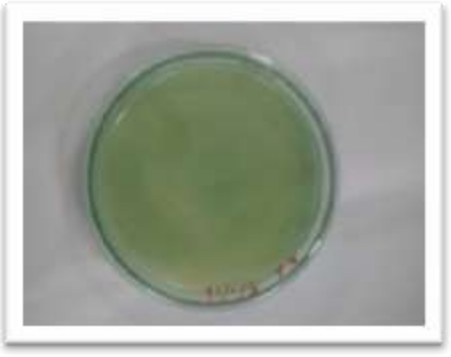

Figure 1. PSA01

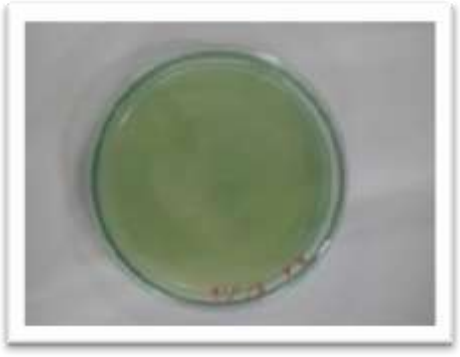

Figure 2. PSA 02

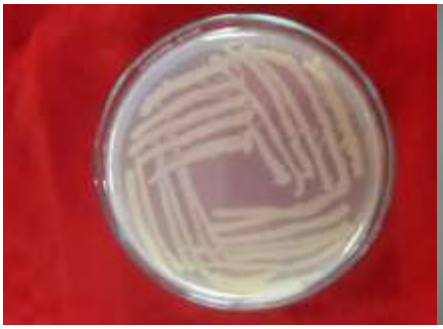

Figure. 3 BAC3

Figure. 4 Siderophore test

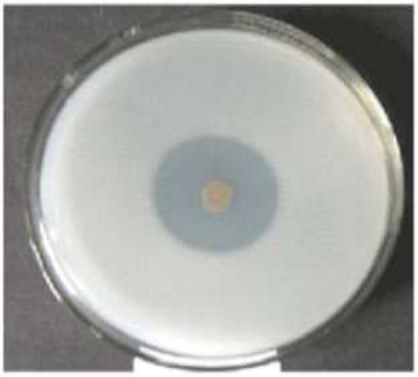

Figure 4. Phosphate solubilization

Figure 5. *Fusarium* spp.

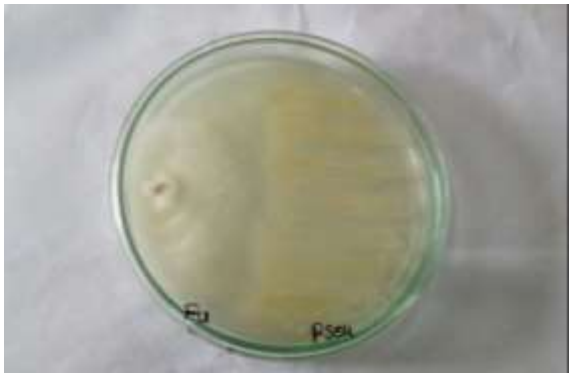

Figure 6. Antagonistic activity

Table 4. CAS assay based on the color change

S.No	Culture Marking	CAS Agar Plate Zone diameter in (mm)
1		PSAO2
2	PSAO1	7mm

Pot culture studies

After 45 days of inoculation (DAI), compared to isolates BAC03, PSA02&PSA01 significantly enhanced the plant shoot length, root length and leaf length. Inoculation of PSA02 increased *Brassica nigra.* L root length (15cm). Similarly PSA01 also increased *Sesasmum indicum.L* root length (11.5cm) when compared to that of control (10cm) & BAC03 (10.5). PSA02 increased shoot length (26cm), similarly PSA01 also increased shoot length (24.4cm) when compared to that of control (19.3cm) & BAC03 (19.9). PSA02 increased leaf length (4.1cm), similarly PSA01 also increased leaf length (4cm) when compared to that of control (3.1cm) & BAC03 (3.5). PS02 increased number of nuts (8), similarly also increased PSA01 number of nuts (5) when compared to that of control (2) & BAC03 (3). Fresh weight of *Sesasmum indicum.L* and *Brassica nigra.L* enhanced PSA02 (12.1g) PSA01 (10.2g) while comparing with control (1.8g) BAC03 (5.8g). Similarly dry weight also remarkably increased when compared with control (0.5g) & BAC03 (2.4g) PSA02 dry weight (6.4g) PSA01 dry weiigth (5.2g).

Figure 9. Pot culture studies on *Sesamum indicum* L.

Table 6. Growth parameter of (*Sesasmum indicum* .L) 45 DAI (Day After Inoculation)

Treatment	Leaf length (cm)Mean ±SD	Shoot length (cm)Mean ±SD	Root length (cm)Mean ±SD	Fresh weight(g)	Dry weight(g)
Control	0.3 ±0.1	0.3 ±0.1	1.5 ±0.5	0.9 ±0.75	0.3 ±0.41
PSA02	4 ±1.80	17.2 ±1.71	3.6 ±1.40	1.7 ±1.90	0.7 ±0.43
PSA01	2 ±1	4 ±1.80	1.3 ±0.44	1.1 ±0.90	0.07 ±0.33
BAC03	3.2 ±0.57	2 ±0.70	1.5 ±0.2	1.3 ±0.02	0.07 ±0.01

Table 7. Estimation of Total chlorophyll and carotenoids for (*Sesamum indicum* L.)

Treatment	Total Chlorophyll mg g-1	Carotenoids mg g-1
Control	0.056±0.056	0.26±0.04
PSAO2	3.981±0.012	0.91±0.19
PSAO1	3.678±0.229	0.04±0.02
BACO3	3.674±0.241	0.52±0.05

Figure 10. Pot culture studies on (*Brassica nigra* L.)

Table 8. Growth parameter of (*Brassica nigra* L.) 45 DAI (Day after Inoculation)

Treatment	Leaf length (cm)Mean±SD	Shoot length (cm)Mean±SD	Root length (cm)Mean±SD	Fresh weight(g)	Dry weight(g)
Control	0.9±0.50	3.8±0.76	1.5±0.5	1.0±0.20	0.01±0.04
PSA02	4±1	16±1.52	3.3±2.08	1.7±0.64	0.17±0.29
PSAO1	1.2±0.20	8±0.70	1.3±0.15	0.8±0.46	0.1±0.11
BAC03	4±1.52	9.2±0.3	3±15	0.3±0.23	0.06±0.09

Table 9. Estimation of Total chlorophyll and carotenoids for (*Brassica nigra* L.)

Treatment	Total Chlorophyll mg g-1	Carotenoids mg g-1
Control	2.43±0.388	0.21±0.03
PSA02	9.24±0.122	0.32±0.16
PSAO1	8.12±0.120	0.09±0.09
BACO3	8.284±0.061	0.22±0.06

Table 10. Impact of after inoculated soil Counting of Microbial population at 45 DAI (cfu/g soil)

S.no	Treatments	Total bacterial population in soil (cfu/g soil)
1	Control	1.3 X107
2	PSA01	4.1 X107
3	PSA02	6.5 X107
4	BCA03	3.2 X107

Cfu – Colony forming unit

Discussion

Pseudomonas gram negative, gram staining test it was confirmed that isolated bacteria belonged to genus *Pseudomonas*. In Gram's staining, the morphology of isolated *Pseudomonas* strains showed Gram-negative, pink colored, medium rod shaped appearance. These findings agreed with the findings reported by earlier researchers (Tripathi *et al*., 2011). In this study, the qualitative estimation of siderophores by *Pseudomonas fluorescens* and *Pseudomonas aeruginosa* isolates showed that they were powerful producer of siderophores under limited iron on King's B medium. The production of siderophores by *Pseudomonas fluorescens and Pseudomonas aeruginosa* isolates indicated that these bacteria isolates can be used biocontrol against soil borne phytopathogens. Similarly, reported that *Pseudomonas fluorescens* and *Pseudomonas aeruginosa* as siderophore producer on King's B medium. *Pseudomonas fluorescens* was able to give higher yields of siderophores under iron stress conditions (Sayyed *et al*., 2005). Application of growth parameter of *Pseudomonas aeruginosa* isolates on *Brassica nigra* L. and *Sesamum indicum* L. seed were brought significant increases on growth and yield of crop.

Summary

Rhizosphere soils were collected from Pagalpatti, Pannapatti, Pusaripatti (Omalur) Salem district, Tamil Nadu. The soil sample was analyzed for pH, Electrical conductivity, and NPK analysis (Table 1). Bacterial isolates was isolated by serial dilution method. The isolates were followed by biochemical test these was *Pseudomonas aeruginosa*, *Pseudomonas fluorescence* and *Bacillus cereus* they were named as from PSA01, PSA02, BAC03. The isolates were the amount of phosphate solubilization and siderophore production by compared to isolates (BAC03, PSA01& PSA02).In highest phosphate solubilization activity was PSA02 with a zone of clearance 0.5mm. In siderophore test maximum amount of hydroxamate production was identified by PSA02. In pot culture studies *Brassica nigra* L., *Sesamum indicum* L. showed significantly increase in the root length, shoot length, fresh weight, dry weight and total chlorophyll and carotenoids significantly increased in treated plant .The antagonist activity of the siderophore producing *Pseudomonas* spp. against fungal pathogen *Fusarium oxysporum.* This result showed that *Pseudomonas* spp. is a good producer of siderophore which can be beneficial for its antagonistic activity towards fungal pathogen and increasing the Oil yielding crops.

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