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Isolation, characterization of potential phosphate solubilizing bacteria from sesame (*Sesamum indicum* L.,) rhizospheres' soil

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Abstract
Sesame is a most medicinal value rich oil seed crop. Plant growth promoting rhizobacteria was most predominant microbes present in the root region of the soil. Phosphate is the one of the most major nutrient for crop growth and development. In oil seeds phosphorus play an important role in the quality of the oil. Using of phosphate solubilizing bacteria can solubilize the unavailable form of phosphorus in to available form. In the present study focus on isolation, characterization and efficiency of the different phosphobacteria isolates from sesame crop. There are 26 different phosphate solubilizing bacteria can obtain and designated as Sesame Rhizosphere Phosphobacteria (SRP) from different locations of Cuddalore district Tamil Nadu, India. Among the 26 isolates based on the phosphate solubilizing index only five isolates SRP 4, SRP 6, SRP 11, SRP 16 and SRP 21were efficient phosphate solubilizers. So the five isolates was morphologically and biochemically characterized as two different genera *Pseudomonas* sp., and *Bacillus* sp., among the plant growth promoting activity of the different phosphobacteria isolates SRP 16 was recorded maximum production of IAA, GA3, cytokinin, EPS and ACC deaminase production. Also, the phosphobactera isolate SRP 16 only can able to grow under 25 to 45 °C temperature at 4 to 9 level of pH. Other isolates were found to grow only at the temperature of 35°C, at 4 to 9 tested pH.

Keywords: phosphobacteria, plant growth promoting rhizobacteria

Introduction
Plant Growth Promoting Rhizobacteria (PGPR) (Stephane Compant, 2005) [1] are beneficial microorganisms that are closely associated with plants and offer an excellent combination of traits useful for both disease control and increased nutrient availability, resulting in evident desirable changes in plant growth, seed germination, and metabolic activities. An essential property for a PGPR in enhancing plant yields is the ability to convert insoluble phosphates (both organic and inorganic) molecules into a form accessible to the plant (Igual *et al*. 2001: Rodrguez *et al*. 2006) [8].

Sesamum indicum L., a medicinal oil seed crops often known as sesame, has been farmed for edible oil and food from ancient times (about 2350 B.C.). In Asia, Africa, and South America, the plant is widely farmed (Anila kumar *et al* 2010, Haruna *et al* 2011). Sesame seed is in high demand for a variety of medicinal and nutritional purposes. Routine cultivation, on the other hand, frequently falls short of meeting demand (Mahrous *et al* 2015).

Organic and inorganic phosphates are two types of phosphates that may be found in soil. The type of phosphorus that plants require the most for growth and development is present in the inorganic form and is made available to them by PSB (phosphate solubilizing bacteria) converting it to soluble form, therefore inoculating plants with PSB directly enhances growth and yield. Bacillus, Rhizobium, and Pseudomonas are the bacteria with the most efficient phosphate solubilizing microorganisms. Two nodulating chickpea species, *Mesorhizobium ciceri* and *Mesorhizobium mediterraneum* are known to be effective phosphate solubilizers within Rhizobia (Rivas R *et al*., 2006) [2].

Materials and Methods
Isolation of phosphobacteria from sesame rhizospheric soils
Sesame cultivating area was selected around the ten different locations of Cuddalore district of Tamil Nadu, India (Table 1). The plants were slowly to pull out from the soil, the soil around the root region was carefully collected from different zip-lock plastic bags. To air-dry the different soil samples into the shed condition for 2 days. Then 5 grams of airdried soil samples were added into 100 ml conical flask containing 50 ml of sterile distilled water kept into shaker for 160 rpm for 20 min for the complete dissolving of the soil into the water. Followed by serially diluting the samples up to 10^{-6} to reduce the microbial populations in the samples. Take 0.5 ml of an aliquot from 10^{-5} and 10^{-6} dilution to well sterile Petri plates, and add 15ml of double-time sterilized pickovsky medium containing Ca_3Po_4 as a phosphate source, then mix the plates clockwise and anticlockwise directions for 3-4 times for even distribution of the sample. To incubate the Petri plates containing samples for one week at 280C. after the incubation to purify the colonies around the hallow zones. The different purified isolates were designated as (Sesame Rhizospheric Phosphobacteria) SRP 1to SRP 26. Among the different isolates SRP 4, SRP 6, SRP 11, SRP 16 and SRP 21isolates can produce a maximum of solubilization index compared to other isolates (Fig 1). The purified colonies were maintained in glycerol stocks for further studies.

Identification of the different phosphobacteria isolates from sesame rhizospheric soil

The five (SRP 4, SRP 6, SRP 11, SRP 16 and SRP 21) efficiently purified isolates were morphologically and biochemically characterized for the genus level identification. The morphological characteristics color, size and shape of the different bacterial isolate were identified. Each isolated different bacterial culture was examined for gram reaction, motility, spore, and catalase, IMVIC test, oxidase test, urease production, indole, Methyl red test, H_2S production was conducted by the readymade kit of micro express biochemical identify kit.

Efficiency of the different phosphate solubilizing bacteria

Qualitative determination of phosphate solubilizing activity on agar medium

The Phosphate solubilization index (PSI) of each isolate was recorded after seven days of incubation using the following equation.

$$PSI = (X+Y)/Y$$

Where: PSI = Phosphate Solubilization index, X = Halo zone diameter, Y = Colony diameter

Qualitative estimation of IAA production

The production of IAA was quantitatively estimated by isolates culture 24 hr broth was centrifuge at 5000 rpm for 10 min. addition of 0.5 mL of the culture supernatant to 4 mL of Salkowsky's reagent to 10 ml test tube and incubate the test tube under room temperature for 1 h under dark conditions. To observe pink colour after the incubation and the intensity of pink colour developed was measured using a UV spectrophotometer at 535 nm.

Quantitative estimation of IAA production

indole-3-acetic acid (IAA) production of phosphobacterial isolates was estimated by, HPLC method proposed by (Priyadharsini and Muthukumar 2017). Bacterial cultures were grown in LB medium, respectively with tryptophan (0.1 g L^{-1}) and incubated at 30 ^{0}C for 7 days. IAA was quantified with high performance liquid chromatography (HPLC: Agilent 1100, Waldbronn, Germany) equipped with a UV detector with absorbance at 280 nm and C-18 column (39 x 300 mm). Mobile phase: Methanol: water (80:20: v/v) was used at a flow rate of 1.5 mL min^{-1}. Sample volume (10 µL) was injected using a sample injector. Analyte peaks were compared with internal standards being added to the medium. Based on the peaks level to quantitively estimate IAA and measured as µg ml^{-1}.

Qualitative estimation of GA_3 and cytokinin production

GA_3 production was Quantitatively estimated (µg/ml) by the phosphobacterial isolates was done according to the starch agar halo test method described by Rajan (2010) [6]. Production of cytokinin (µg/ml) was estimated in the culture filtrate of the phosphobacterial isolate was done using cucumber cotyledon greening bioassay as described by Rajan (2010) [6].

Qualitative estimation of ACC deaminase production

The production of ACC deaminase of the different phosphobacteria isolates was screened using DF salts minima medium amended with 0.2% ammonium sulfate (w/v) (Jayakumar *et al*. 2019) [5].

Qualitative estimation of EPS production

The production of Exopolysaccharide was estimated by was observed according to the methods described by (Orsod *et al.* 2012)

Ph vs. temperature test

The efficiency of isolates to grow in pikovskya liquid medium adjusted to pH 4, 5, 6, 7, 8, and 9 in contrast to various temperatures of 20, 25, 30, 35, 40, 45, and 50°C was evaluated. (Zhu F. *et al.,* 2011) [4].

Result and Discussion

Among the different isolates was screened based on the phosphate solubilizing index (PSI). The maximum PSI was recorded on isolates SRP 4 (2.10 mm), SRP 6 (2.32 mm), SRP 11(2.90 mm), SRP 16 (2.64 mm) and SRP 21(2.14 mm) presented in Fig 1. Among the different isolates the five isolates were morphologically and biochemically characterized and two different genera *Pseudomonas* spp., and *Bacillus* sp., were found (Table 1). Soil bacteria that have been reported to mobilize poorly available phosphorus via solubilization and mineralization include *Pseudomonas* spp., *Agrobacterium* spp., and *Bacillus circulans* (Babalola and Glick, 2012) [10].

The selected five isolates were screened based on the different efficiency like phosphate solubilization, IAA production, GA_3 (Gibberellic acid) production, cytokinin (µg/ml) Production, ACC deaminase production (Table 2) and pH vs. temperature test was conducted.

Among the different phosphobaterial isolates SRP 16 can perform the highest production of IAA (38.46 µg/ml) followed by SRP 6, SRP 4, SRP 21(32.65, 28.41, 26.93 µg/ml) and the least production of IAA was recorded on SRP 11 (24.18 µg/ml), Most or studies from the earlier work showed that IAA producing organisms are Gram negative (Lindow *et al*., 1998: Datta and Basu, 2000). Few Gram-positive strains belong to Bacillus strain known to produce IAA (Wahyudi *et al*., 2011) [11].

The gibberellic acid production was recorded maximum on isolate SRP 16 GA_3 (1.94 µg/ml) followed by SRP 11, SRP 4, SRP 21 (1.58, 1.31, 1.18 µg/ml) and the least production of GA3 was recorded on SRP 6 isolate. Cytokinin production was highest on isolate SRP 16 (1.21µg/ml) followed by SRP 6, SRP 4, SRP 21 (0.83, 0.65, 0.52 µg/ml) and the minimum Cytokinin production recorded on SRP 11 isolate. ACC deaminase production was positive for all the different phosphobacterial isolates. (Table 3). The variation of IAA production among the PGPR was reported by Prakash and Karthikeyan, 2013 in which ten bacterial strains isolated from Acoruscalamus rhizospheric soil of Melaiyar and Nagapattinam districts in Tamil Nadu and were identified as *Azospirillum* spp., *Bacillus* spp., *Pseudomonas* spp., and *Azotobacter* spp. The isolate SRP 16 was recorded the maximum Exopolysaccharide production compare to other isolates. Followed by SRP 11, SRP 6 and SRP 4. The isolate SRP 21 was recorded negative production of EPS.

Among the different phosphobacterial isolates showed the different response of growth in combinations of medium with different pH (pH 4 to 9) and incubation temperature (20°C to 50°C) (Table 3). The phosphobactera isolate SRP 16 only can able to grow under 25 to 45 °C temperature at 4 to 9 levels of pH. Other isolates were found to grow only at

the temperature of 35°C, at 4 to 9 tested pH. The pH and temperature test detail were presented in Table 3. Poonam and Ghosh AK (2011) [12], reported none of the isolates able to grow at high temperature (52°C). Prescott HK (2002) [13], discussed that cardinal temperatures for a particular species are not rigidly fixed but often depend to some extent on other environmental factors such as pH and available nutrients and also it varies between microorganisms.

The pure culture of efficient phosphate solubilizing strain SRP 16 presented in Fig 2 and the fluorescence character was estimated by UV transilluminator Fig 3.

Conclusion

Aim of the study is focusing on isolation characterization and efficiency of the different phosphate solubilizing bacteria from different locations of cuddalore district. The study mainly focusing on the maximum efficient phosphobacteria having the EPS producing properties for the biofilm formation. Among the different isolates SRP 16 perform better efficiency (IAA, Phosphate solubilization, ACC, GA3, Cytokinin and EPS production) to other isolates, so we select the isolate for molecular level of characterization and the selected strain to be use for *Trichoderma* based biofilmed biofertilizer production.

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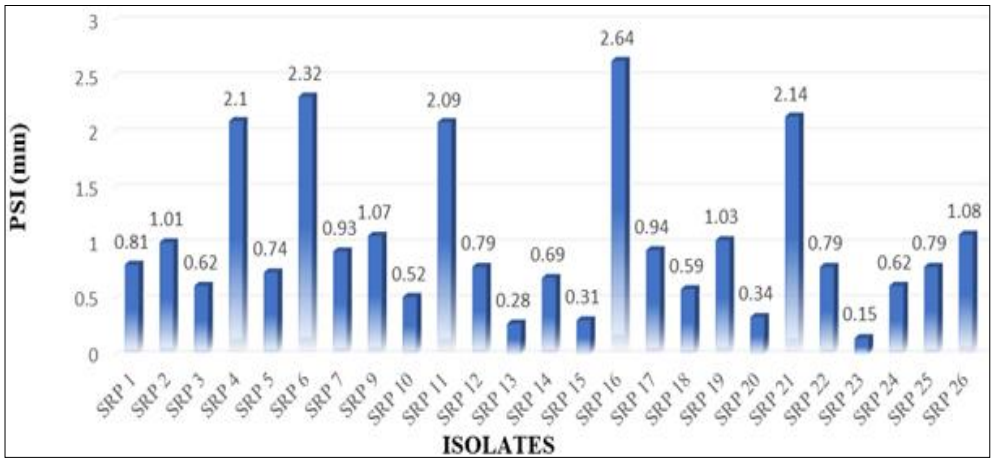

Fig 1: Phosphate Solubilization index

Table 1: Morphological and biochemical characterization of an efficient phosphobacterial isolates

Isolates	Shape	Spore	Gram reaction	Pigments	Catalase	Citrate	H_2S	Indole	MR	VP	Tentatively Identify as
SRP 4	Rod	-	-	-	+	+	-	-	-	-	*Pseudomonas* sp.
SRP 6	Rods	+	+	-	+	+		-	-	+	*Bacillus* sp.
SRP 11	Rods	+	+	-	+	+		-	-	+	*Bacillus* sp.
SRP 16	Rod	-	-	Fluorescence	+	+	-	-	-	-	*Pseudomonas* sp.
SRP 21	Rod	-	-	Yellow	+	+	-	-	-	-	*Pseudomonas* sp.

Table 2: Plant growth Efficiency of the different Phosphobacteria isolates

Isolates	Qualitative estimation of IAA production	Quantitative estimation of IAA production (µg/ml)	Qualitative estimation of ACC production	Qualitative estimation of GA_3 production (µg/ml)	Cytokinin production (µg/ml)	EPS production
SRP 4	+	28.41	++	1.31	0.65	+
SRP 6	++	32.65	+	0.93	0.83	+
SRP 11	+	24.18	++	1.58	0.421	++
SRP 16	+++	38.46	+++	1.94	1.21	+++
SRP 21	+	26.93	++	1.18	0.52	-

Table 3: Effect of pH and temperature on different phosphobacteria isolates

Isolates	pH						Temperature
	4	5	6	7	8	9	
SRP 4	-	+	+	+	+	-	20
	-	+	+	+	+	-	25
	-	+	+	+	+	+	30
	+	+	+	+	+	+	35
	-	+	+	+	+	-	40
	-	-	+	+	-	-	45
	-	-	-	-	-	-	50
SRP 6	-	-	-	+	-	-	20
	-	-	+	+	+	-	25
	-	+	+	+	+	+	30
	+	+	+	+	+	+	35
	-	-	+	+	-	-	40
	-	-	-	+	-	-	45

	-	-	-	-	-	-	50
SRP 11	-	-	-	-	-	-	20
	-	+	+	+	+	-	25
	+	+	+	+	+	-	30
	+	+	+	+	+	+	35
	-	-	+	+	-	-	40
	-	-	-	+	-	-	45
	-	-	-	-	-	-	50
SRP 16	-	+	+	+	+	-	20
	+	+	+	+	+	-	25
	+	+	+	+	+	+	30
	+	+	+	+	+	+	35
	-	-	+	+	+	+	40
	-	-	+	+	-	-	45
	-	-	-	-	-	-	50
SRP 21	-	-	-	-	-	-	20
	-	+	+	+	+	-	25
	+	+	+	+	+	-	30
	+	+	+	+	+	+	35
	+	-	+	+	-	-	40
	-	-	-	-	-	-	45
	-	-	-	-	-	-	50

Fig 2: Pure culture of SRP 16

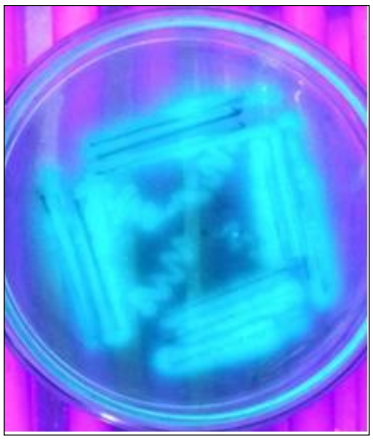

Fig 3: SRP 16 Fluorescence under UV Transilluminator

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