



Original Research Article

Interstrain difference of phosphobacterial isolates of Bhendi rhizosphere for their growth promoting traits under in vitro conditions

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ABSTRACT

Keywords

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The phosphobacterial isolates viz., *Bacillus megaterium*, *B. cerus*, *B. polymyxa*, *B. subtilis*, *Pseudomonas fluorescens*, *P. putida* and *P. striata* were previously isolated and characterized from Bhendi rhizosphere soil samples from 15 different locations of Cuddalore district were screened for their efficiency in terms of plant growth promoting traits (phosphate solubilisation, IAA production, GA₃ production and siderophore content). The results revealed that the *Bacillus megaterium* isolate BRB - 2 obtained from Sakkangudi location recorded maximum production of IAA (60.15 µg 25 ml⁻¹ of broth), GA (5.58 µg 25 ml⁻¹ of broth), Siderophore such as Catechol type (7.39 µg ml⁻¹) and Salicylate type (7.65 µg ml⁻¹). With regard to *Pseudomonas* isolates, BRP - 7 obtained from Orathur location soil samples recorded maximum production of IAA (65.56 µg 25 ml⁻¹ of broth), GA (7.62 µg 25 ml⁻¹ of broth) and Siderophore such as Catechol type and Salicylate type of 8.45 µg ml⁻¹ and 8.82 µg ml⁻¹ respectively.

Introduction

Phosphorus is the second most important macronutrient required for the growth and development of many crop plants (Vance, 2001). Only 0.04–0.10% of phosphorus is present in the soil, of which 1.00–2.50% of phosphorus is easily available to plants, remaining exists in the unavailable form to plants (Lin, 1990). The amount of phosphorus in soil becomes the limiting factor, which play a vital role for the growth and development of plants (Saneoka *et al.*, 1989; Borch *et al.*, 1999; Kanako *et al.*, 2004).

Due to the low availability and insoluble form of phosphorus, it acts as a limiting factor. Throughout the world 30 million tones of soluble phosphorus fertilizer is applied directly in soil to increase the phosphorus content for the growth and development of plant due to its low availability. Furthermore, it is evidenced that 80% of aspired phosphorus becomes unavailable to the plants due to the formation of various inorganic polyphosphates (Holford, 1997; Lopez-Bucio *et al.*, 2000; Alam and Ladha, 2004).

The microorganisms found in the rhizosphere soil solubilize the phosphorus affectively from insoluble inorganic or organic phosphorus compounds, which was uptake by the plants easily because of the soluble or available form (Rodriguez and Fraga, 1999). They also produce some organic acids, phosphatases and chelating compounds, mineral acids and siderophores (Gaskins *et al.*, 1985; Podile and Dube, 1988; Schippers and Chanway, 1987; Singh *et al.*, 1989; Vinithadali and Muthuselvam, 2013). The phosphate solubilizing bacteria are eco-friendly and reduces the use of chemical fertilizers which causes environmental pollution and more expensive (Son *et al.*, 2006; Ayyadurai *et al.*, 2006).

Hariprasad and Niranjana (2009) isolated 43 PSB strains from 37 rhizosphere soil samples collected from tomato growing regions of Karnataka. Numerous reports have been published on the PSB in many crops plants, such as maize, wheat, rice and others (Chabot *et al.*, 1996; de Freitas *et al.*, 1997; Zaidi *et al.*, 2003). Wang *et al.*, (2009) screened 14 phosphate solubilizing bacteria strains in the rhizospheres of *Heveabrasiliensis*, *Eucalyptus* sp., *Acacia* sp., *Rhizophora apiculata* Blume, etc., from the Hainan ecosystem in China. In the present study the effective Bhendi phosphobacterial isolates were screened *in vitro* condition by using certain plant growth promoting traits.

Materials and Methods

The Bhendi Phosphobacterial isolates obtained previously from fifteen different locations of Cuddalore district were designated as BRB - 1 to BRB - 15 (*Bacillus* isolates) and BRP - 1 to BRP - 15 (*Pseudomonas* isolates) were utilized for the present study. The Interstrain different of *Bacillus* and *Pseudomonas* isolates for their growth promoting traits by various growth

promoting traits by using the standard protocols as described below.

Phosphate solubilization efficiency	:	Pikovskaya (1948); Morton (1955)
Indole acetic acid production	:	Gorden and Paleg (1957)
Gibberellic acid Production	:	Borrow <i>et al.</i> , (1955)
Siderophore production	:	Reeves <i>et al.</i> , (1983)

The *in vitro* experiments were conducted as triplicates. Statistical analysis of all the parameters was carried out through analysis of variance (ANOVA) (Gomez and Gomez, 1984).

Results and Discussion

Screening of *Bacillus* isolates for phosphate solubilisation efficiency

All the fifteen *Bacillus* isolates obtained from Bhendi rhizosphere soil samples from Cuddalore district were showed characteristically variable clear zones around their growth on Sperber's hydroxy apatite medium based on their solubilising activity. Further, the isolates were screened for release of phosphorus from tricalciumphosphate in Pikovskaya's broth, acid phosphatase activity, titrable acidity of the culture medium and change in the pH of the medium (Table 1).

The *Bacillus* isolates (BRB - 1 to BRB-15) were solubilizing tricalcium phosphates and the amount of phosphorus released from 100 mg of TCP in Pikovskaya's broth varied considerably. The isolate BRB-2 from Sakkangudi recorded higher phosphorus solubilisation of 31.32 mg followed by the BRB-7 from Orathur recorded 30.56 mg and the reference strain *Bacillus megaterium* MTCC-8755 solubilized about 29.62 mg of phosphorus from 100 mg of TCP. However,

these isolates and MTCC strain were showed statistically on par results.

The acid phosphatase activities of the fifteen isolates were ranged from 39.95 to 80.76 n moles of P-nitro phenol released $\text{min}^{-1} \text{mg}^{-1}$ of cell protein. The maximum amount of phosphatase activity was recorded by BRB-2 (80.76 n moles of P-nitro phenol released $\text{min}^{-1} \text{mg}^{-1}$ of cell protein) followed by the BRB-7 (79.43 n moles of P-nitro phenol released $\text{min}^{-1} \text{mg}^{-1}$ of cell protein) and reference strain *Bacillus megaterium* MTCC - 8755 with 77.89 n moles of P-nitro phenol $\text{min}^{-1} \text{mg}^{-1}$ of cell protein. Whereas, BRB-4 isolate recorded the minimum amount of phosphatase activity of 39.95 n moles of P-nitro phenol $\text{min}^{-1} \text{mg}^{-1}$ of cell protein.

The titrable acidity of *Bacillus* isolates (BRB-1 to BRB-15) were ranged from 2.35 to 6.58 g/l in the culture medium after 7 days growth. The reference strains *Bacillus megaterium* MTCC - 8755 recorded titrable acidity of 6.29 g/l, whereas, the isolate BRB-2 recorded titrable acidity of 6.58 g/l as the highest value. The change in pH of the culture medium after 7 days of growth varied from 3.02 to 6.43. The BRB-4 isolates from Keerapalaiyam showed significant reduction in a pH of 3.02.

Phosphate solubilizing bacteria strains are frequently screened by a plate assay method, in which insoluble phosphates such as tricalcium phosphates are used as sole sources of phosphorus. The strains that can produce halo/clear zone around the colony are selected (Pikovskaya, 1948; Katznelson *et al.*, 1962) did not show any visible clear/halo zones on agar plates could solubilize insoluble inorganic phosphates in liquid medium (Gupta *et al.*, 1994).

The phosphate solubilizing bacterial isolates were screened based on the ability to release

soluble phosphorous from apatite in the culture medium (Banik and Dey, 1982; Whitelaw *et al.*, 1999). Perez *et al.*, (2007) isolated a total of 130 heterotrophic bacterial isolates showing different degrees of mineral tricalcium phosphate solubilizing activities from NBRIP plates, of which 10 efficient solubilizers were selected for farther study.

In fact, the major source of phosphatase in soil is considered to be of microbial origin (Rodriguez and Fraga, 1999). The role of microorganisms in solubilizing insoluble phosphate in natural soil and making it to easily available form to plants is well known (Kundu and Gaur, 1984). Several reports indicate that phosphate solubilizing microorganisms include several genera of bacteria viz., *Bacillus*, *Pseudomonas*, *Rhizobium*, *Burkholderia*, *Achromobacter*, *Agrobacterium*, *Micrococcus*, *Aerobacter*, *Serratia*, *Flavobacterium*, *Enterobacter*, *Citrobacter*, *Klebsiella*, *Erwinia* etc., possess the ability of solubilizing and mineralizing phosphate (Nakas *et al.*, 1987; Strom and Lory, 1987; Rodriguez and Fraga, 1999; Wani *et al.*, 2007). Among bacteria, most efficient phosphate solubilizing bacteria belonged to the genera *Bacillus* and *Pseudomonas* (Dave and Patel, 1999). In relation to phosphate solubilizing bacteria characterized as *Pseudomonas*, members of these genera have been isolated from the rhizosphere, and their capacity to solubilize phosphorus has been assessed (Chung *et al.*, 2005; Herter *et al.*, 2006).

Vessey, (2003) reported that application of bacterial inoculants as biofertilizer to improve plant growth and increase yields of plants. However, Chung *et al.*, (2005) found that there is no direct correlation among *in-vitro* P solubilization and/or mineralization, plant P accumulation and soil available phosphorus. Plants secrete many organic

substances such as soluble sugars, amino acids and other compounds by roots, which could provide a constant source of nutrition and thus ensure a continued growth of the bacteria (Nardi *et al.*, 2000; Sunantha Posuk, 2000; Bais *et al.*, 2001).

A positive correlation between the degree of P solubilization and pH reduction was observed. The pH of the culture filtrate of phosphate solubilizing *Bacillus* varied from 4.0 to 6.5 and the amount of phosphate solubilization is directly correlated with decrease in pH of the culture medium (Arora and Gaur, 1978, 1979). Phosphate solubilizing bacteria reduced the pH of the medium consequent of the release of organic acids. This acid solubilization mechanism of phosphates was clearly reported by (Dave and Patel, 1999). Iron is an essential nutrient of plants, but it is relatively insoluble in soil solutions. Plant roots prefer to absorb iron as the more reduced ferrous (Fe^{2+}) ion, but the ferric (Fe^{3+}) ion is more common in well aerated soil although it is easily precipitated in iron-oxide forms (Salisbury and Ross, 1992).

Screening of *Bacillus* isolates for indole acetic acid and gibberellic acid production

The Indole acetic acid (IAA) and Gibberellic acid (GA_3) producing potential of *Bacillus* isolates obtained from the rhizosphere of Bhendiwere studied and the results were given in Fig. 1. The study clearly revealed that all the fifteen *Bacillus* isolates were able to produce phytohormones with varying amounts among them. The IAA production ranged from 17.73 to 60.15 $\mu\text{g } 25 \text{ ml}^{-1}$ of broth and GA_3 production ranged from 1.52 to 5.58 $\mu\text{g } 25 \text{ ml}^{-1}$ of broth levels. The BRB-2 isolate from Sakkangudi recorded higher levels of 60.15 and 5.58 $\mu\text{g } 25 \text{ ml}^{-1}$ of broth of IAA and GA_3 respectively. It was

observed that the reference strain MTCC-8755 was produced lesser quantities of 56.98 and 5.30 $\mu\text{g } 25 \text{ ml}^{-1}$ of broth of IAA and GA_3 respectively.

Screening of *Bacillus* isolates for siderophore production

Similar to phytohormones production, the siderophore contents were higher in the same isolate BRB-2 as 7.39 and 7.65 $\mu\text{g } 25 \text{ ml}^{-1}$ of broth of catechol type and salicylate type siderophores respectively. The reference strain MTCC-8755 recorded 7.08 and 7.33 $\mu\text{g } 25 \text{ ml}^{-1}$ of broth as the respective values (Fig. 2).

It was concluded that the *Bacillus* isolate from Sakkangudi was found to be efficient in terms of phosphate solubilisation potential, phytohormones production and siderophore production.

Screening of *Pseudomonas* isolates for indole acetic acid and gibberellic acid production

The results of the present study revealed that all the fifteen *Pseudomonas* isolates (BRP-1 to BRP-15) were able to produce substantial quantities of phytohormones namely gibberellic acid and indole acetic acid with variation in the quantity between them (Fig. 3). The higher amount of indole acetic acid was recorded in the Orathur isolate (BRP-7) with 65.56 $\mu\text{g } 25 \text{ ml}^{-1}$ of broth. It was followed by the BRP-2 isolated from Sakkangudi with 63.75 $\mu\text{g } 25 \text{ ml}^{-1}$ of broth and reference strain MTCC-9768 with 62.14 $\mu\text{g } 25 \text{ ml}^{-1}$ of broth. With regard to Gibberellic acid production, the BRP-7 isolate from Orathur recorded 7.62 $\mu\text{g } 25 \text{ ml}^{-1}$ of broth as higher value followed by BRP-2 isolate from Sakkangudi with 7.48 $\mu\text{g } 25 \text{ ml}^{-1}$ of broth and reference strain MTCC-9768 with 7.29 $\mu\text{g } 25 \text{ ml}^{-1}$ of broth.

Table.1 Screening of *Bacillus* isolates for phosphate solubilisation efficiency

Name of the Isolate	Phosphorous solubilized*	Acid phosphatase activity**	Titration Acidity (g/l)	pH of the culture filtrate
BRB-1	21.00	63.08	4.75	4.97
BRB-2	31.32	80.76	6.58	6.43
BRB-3	26.25	71.87	5.72	5.69
BRB-4	7.34	39.95	2.35	3.02
BRB-5	12.10	48.20	3.04	3.56
BRB-6	15.67	54.14	3.73	4.15
BRB-7	30.56	79.43	6.45	6.33
BRB-8	24.64	69.05	5.47	5.52
BRB-9	19.23	60.12	4.42	4.70
BRB-10	22.78	66.07	5.10	5.26
BRB-11	27.82	74.86	5.95	5.92
BRB-12	10.53	45.68	2.86	3.47
BRB-13	8.76	42.71	2.50	3.20
BRB-14	17.45	57.13	4.06	4.42
BRB-15	13.88	51.18	3.38	3.84
MTCC-8755	29.62	77.89	6.29	6.20
S.E.	0.87	1.46	0.15	0.12
C.D.(P = 0.05)	1.76	2.95	0.32	0.25

* mg of P released from 100 mg of tricalcium phosphate.

** n moles of p-nitro phenol released min⁻¹ mg⁻¹ of cell protein

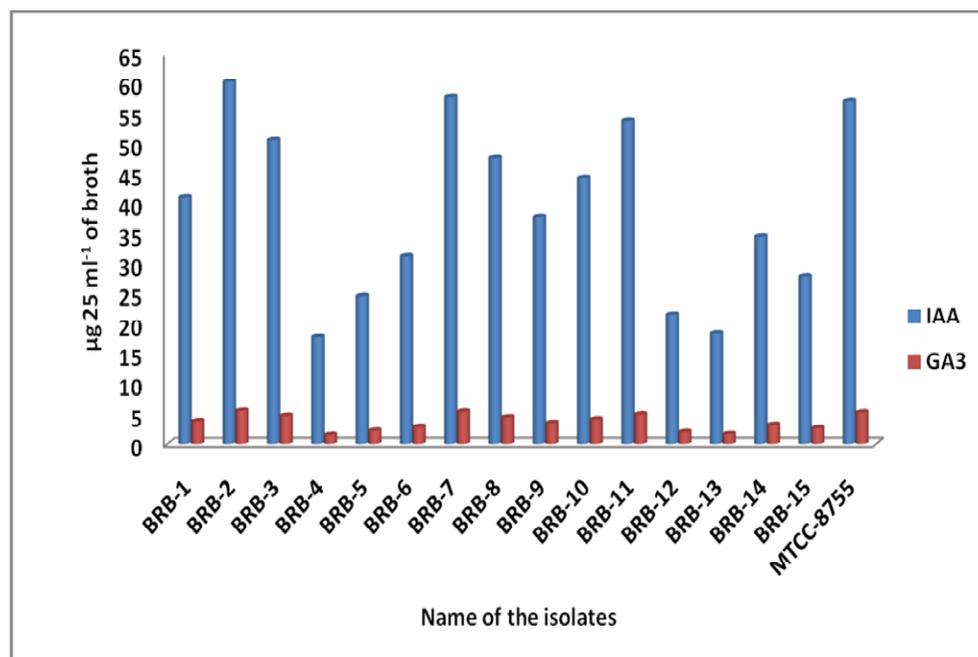


Fig.1 Screening of *Bacillus* isolates for indole acetic acid and gibberellic acid production

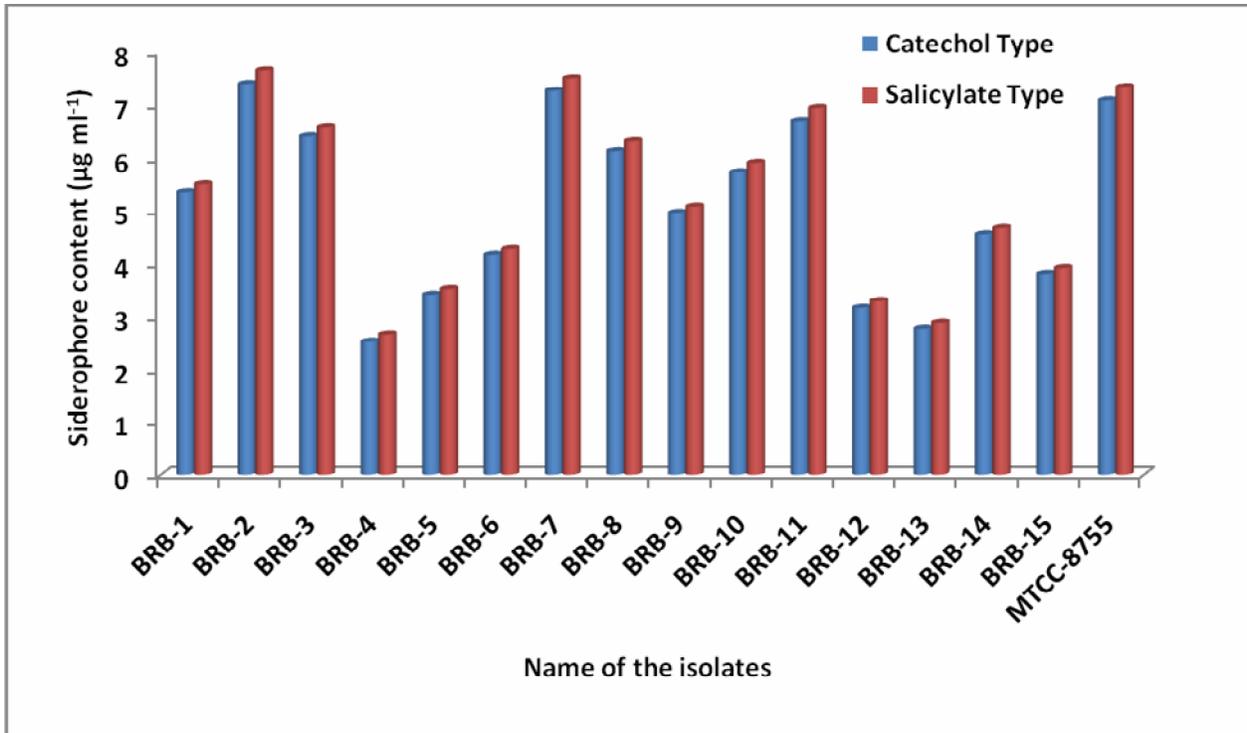


Fig.2 Screening of *Bacillus* isolates for siderophores production

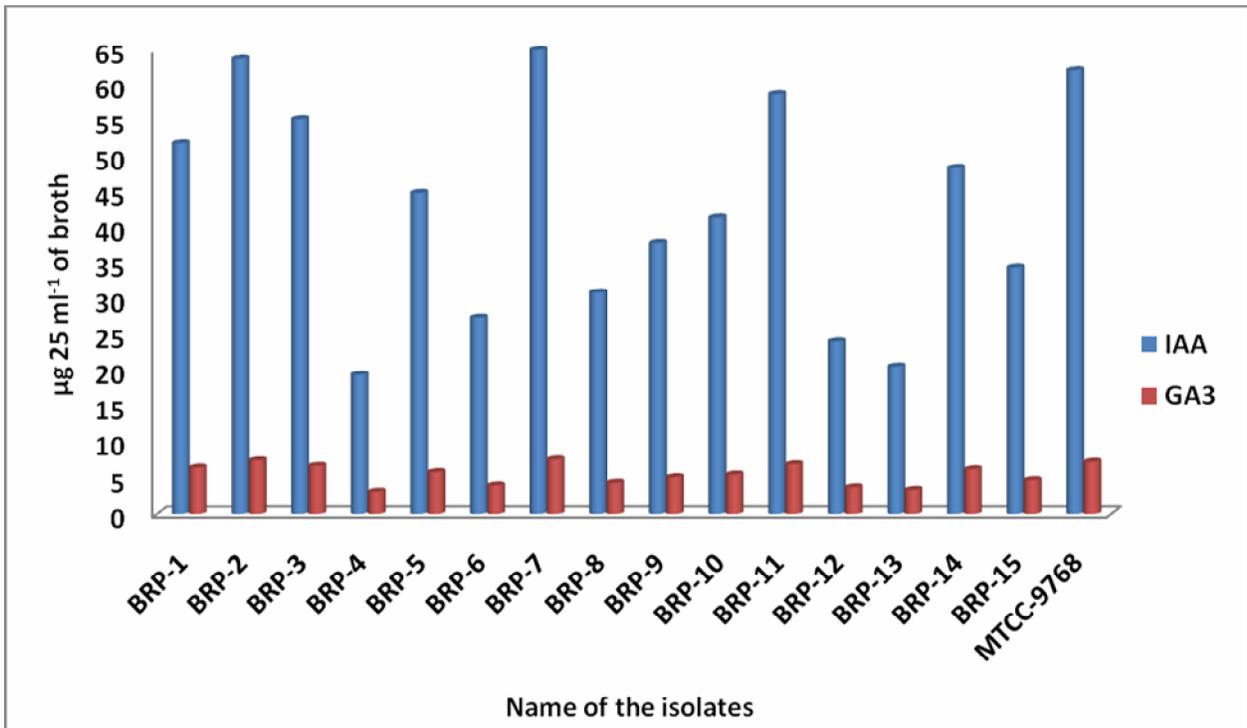


Fig.3 Screening of *Pseudomonas* isolates for indole acetic acid and gibberellic acid production

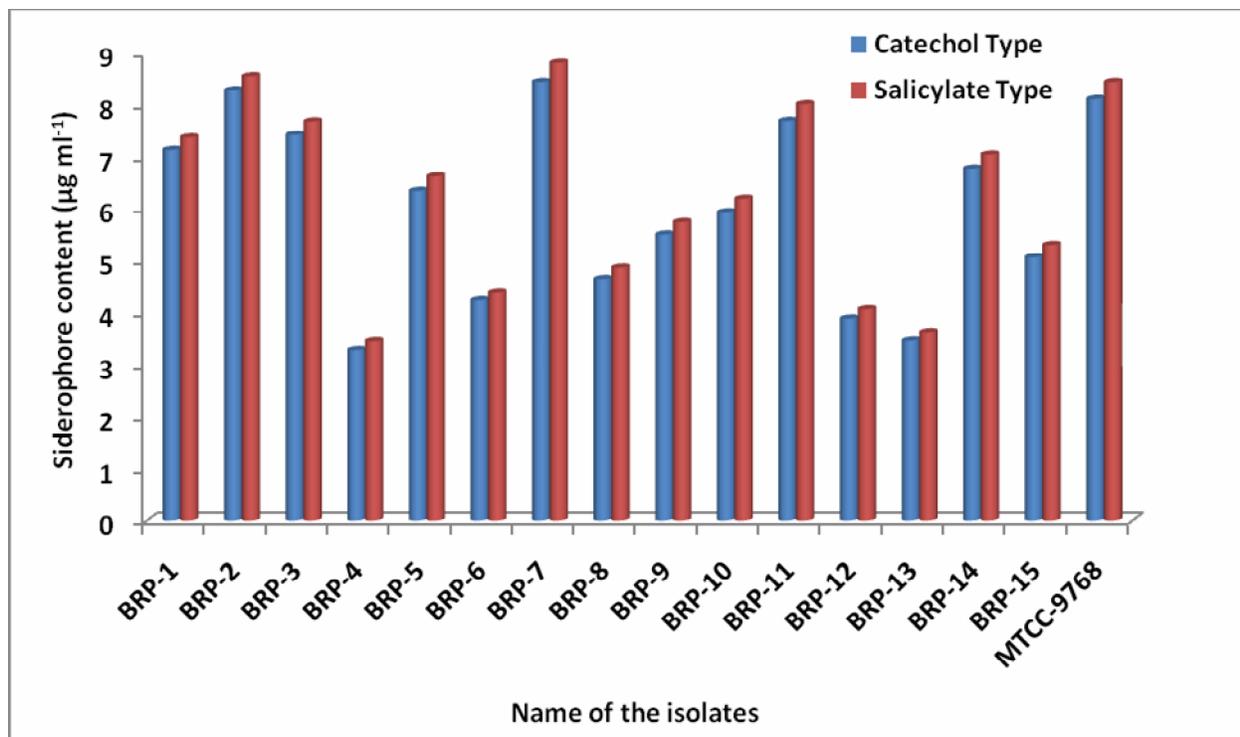


Fig.4 Screening of *Pseudomonas* isolates for siderophores production

However, these isolates BRP-7, BRP-2 and MTCC strain were showed statistically on par results.

Screening of *Pseudomonas* isolates for siderophore production

Similar to phytohormones production all the isolates were produced siderophores in varying quantities between them. The *P. fluorescens* isolate from Orathur location (BRP-7) recorded higher amounts of siderophore as 8.45 and 8.82 µg ml⁻¹ of catechol and salicylate types respectively. And the reference strain MTCC-9768 recorded 8.12 and 8.45 µg ml⁻¹ as corresponding values (Fig. 4). From the above screening studies on phytohormones and siderophore production, the isolate *P. fluorescens* BRP-7 from Orathur was selected for further research.

Ahamad *et al.*, (2005) reported the production of IAA by 11 isolates of *Pseudomonas* from different crop plants in

the range of 5.34 to 22.4 mg/ml. Similarly Karnwal (2009) also reported the varying amounts of IAA production by fluorescent *Pseudomonas*. Anandaraj and Sarma (2003) reported that growth-promoting strains of fluorescent *Pseudomonas* were found to synthesize phytohormones viz., IAA and Gibberellic Acid (GA). Bacterial siderophore (Pseudobactin and ferrioxamine B) were inefficient as iron sources for plants and the rhizosphere siderophore producing bacteria can be in competition with the plant for iron.

In fact, the vast majority of research on microbial siderophore in the rhizosphere is associated with their bio control activities due to their competitive effects with plant pathogens (Hefte *et al.*, 1994). Production of siderophore by agriculturally beneficial isolates and its role in Fe mobilization was reported by several workers (Saxena *et al.*, 1986; Kumar and Dube, 1991; Sharma and Johri, 2003).

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