



Screening and *in vitro* evaluation of phosphate solubilizing bacteria from rhizosphere and roots of coconut palms (*Cocos nucifera* L.) growing in different states of India

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Coconut (*Cocos nucifera* L.), a traditional plantation crop, is grown in 93 countries across the world and supports the livelihood of more than 80 million small and marginal farmers. Phosphorus deficiency is a major constraint to most of the crop production system. The results of the permanent manurial experiment at Central Plantation Crops Research Institute (CPCRI), Kasaragod, Kerala, India revealed that in long run P is becoming the most limiting nutrient for coconut production (Palaniswami *et al.*, 2005). Zaidi *et al.*, (2009) reported that among the heterogeneous and naturally abundant microbes inhabiting the rhizosphere, the phosphate solubilizing microorganisms (PSM) including bacteria have provided an alternative biotechnological solution in sustainable agriculture to meet the P demands of plants. Phosphate solubilizing microorganisms were found to be widely distributed in coconut growing soils (Thomas *et al.*, 1991) and the predominant bacteria solubilizing phosphate in coconut soils were *Pseudomonas* spp. and *Bacillus* spp. (Nair and Subba Rao, 1977). Presence of phosphate solubilizers were also reported in other plantation crops like tea, rubber and mandarin (Chakraborty *et al.*, 2010).

In an attempt to develop an effective phosphate solubilizing bacterial inoculum for improving phosphorus uptake by coconut crop, heterotrophic bacteria were isolated from the rhizosphere and roots of coconut palm growing in different states of India viz., Kerala, Karnataka, Tamil Nadu, Andhra Pradesh and Maharashtra. All

the isolates were evaluated for their abilities to solubilize phosphate [$\text{Ca}_3(\text{PO}_4)_2$] on solid medium. Phosphate solubilization efficiency (%) on agar medium was calculated as $Z-C/C \times 100$, by measuring the diameter of solubilization zone (Z) and colony diameter (C), (Srivastava *et al.*, 2004). Bacterial isolates with highest phosphate solubilization efficiency (%) selected from each group of isolates were further tested for quantitative phosphate solubilization in Pikovskaya's liquid medium containing insoluble tri-calcium phosphate (0.5 %). Water soluble phosphorus in the culture supernatant was estimated by the chlorostannous reduced molybdophosphoric acid blue method as described by Jackson (1967). The pH of the culture supernatant was measured by pH meter (Eutech, Singapore). Efficient isolates were identified by conventional identification methods including morphological, physiological and biochemical tests according to Bergey's Manual of Determinative Bacteriology- 9th edition and Nishimori *et al.* (2000). The results were validated by Biolog® GEN III microplate identification system (Biolog, CA, USA), which provided 94 phenotypic tests (71 carbon source utilization assays and 23 chemical sensitivity assays). Other potential plant growth promoting attributes such as production of IAA (Brick *et al.*, 1991), ACC deaminase (Dworkin and Foster, 1958) and siderophores (Schwyn and Neilands, 1987) were determined.

A total of 512 bacterial isolates were isolated from rhizosphere soil and roots of coconut palms growing in different states. Of these, 156 isolates

were fluorescent *Pseudomonas* spp., and 327 were endospore forming *Bacillus* spp. (Table 1). Twenty nine isolates were neither *Bacillus* spp. nor fluorescent *Pseudomonas* spp. and were grouped as unidentified bacteria. A total of 284 isolates (56%) were identified as phosphate solubilizing microorganisms (PSM) as evidenced by the dissolution halos on Pikovskaya's agar. This included 45% endophytic *Bacillus* spp., 33% rhizospheric *Bacillus* spp. and 94% of rhizospheric fluorescent *Pseudomonas* spp. Fifty seven per cent of the unidentified isolates were phosphate solubilizers. Among diverse group of phosphate solubilizers, fluorescent pseudomonads were more widespread than others. Phosphate solubilization efficiency (%) of the isolates ranged from 13 to 333%. Fluorescent pseudomonads exhibited the maximum solubilization efficiency (333%) and 14 of them showed >100% efficiency. The solubilization efficiency (%) of *Bacillus* spp. varied from 16 to 140% and the phosphate solubilizing efficiency of unidentified isolates varied from 33 to 250%. It is reported that high proportion of PSM is concentrated in the rhizosphere and they are metabolically more active than from other sources (Vazquez *et al.*, 2000).

Six isolates, *Pseudomonas* sp. HSF 126, *Pseudomonas* sp. HSF 132, *Pseudomonas* sp. KnSF

Table 1. Details of rhizospheric and endophytic isolates obtained from the coconut palms growing in different states

State (s)	Name of place (s)	Number of isolates				
		RSF	RSB	EB	RUI	EUI
Kerala	HDMSCS, CPCRI	15	9	18	5	6
	Chengannur	7	13	5	0	0
	Kunnamkai	16	30	6	1	2
	Vadakkenchery	10	13	4	1	1
	Thopumpady	12	15	4	0	0
	Total	60	80	37	7	9
Karnataka	Tumkur	27	21	22	0	0
	Kidu	33	34	33	2	0
	Total	60	55	55	2	0
Tamil Nadu	Coimbatore	2	10	8	2	0
	Pollachi	9	10	5	0	2
	Total	11	20	13	2	2
Maharashtra	Ratnagiri	16	22	5	6	1
Andhra Pradesh	Ambajipetta	9	29	11	0	0
	Net Total	156	206	121	17	12
				512		

RSF - fluorescent pseudomonads from rhizosphere, RSB - *Bacillus* spp. from rhizosphere, EB - *Bacillus* spp. from roots, RUI - Unidentified isolates from rhizosphere, EUI - Unidentified isolates from roots

227, unidentified isolate RNF 267, *Bacillus* sp. RSB 17 and *Bacillus* sp. RSB 22 were selected as potent phosphate solubilizers as they possessed solubilization efficiency ranging from 100 to 333% (Table 2). All six bacterial isolates were capable of dissolving insoluble phosphate in liquid medium, albeit to a different extent. The solubilization of tricalcium phosphate in the liquid medium ranged between 84.18 µg/ml and 244.17 µg/ml with variations among different isolates at different time intervals. It was observed that most of the isolates increased the soluble phosphorus content in the culture broth on longer incubation with some exemptions (Figure 1 and 2). *Pseudomonas* sp. 227 recorded the maximum phosphate solubilization (244.17 µg/ml) at 120 h. Whereas in unidentified bacterium RNF 267, the peak soluble phosphorus content (217.525 µg/ml) was recorded at 48 h and phosphate solubilization reduced after 48 h. *Pseudomonas* sp. 132 recorded maximum soluble phosphorus (200.065 µg/ml) at 96 h incubation. The solubilization of tricalcium phosphate in the liquid medium by different isolates was accompanied by a drop in pH (up to 3.7) from an initial pH of 5.9 (Fig. 1 and 2). Maximum drop in the pH was observed with the maximum P solubilization by each isolate. Alikhani *et al.*, (2006) reported that the release of soluble P was significantly correlated with a drop in the pH of the culture filtrates indicating the importance of acid production in the mobilization process. These results corroborate with similar observations made with rhizobia (Alikhani *et al.*, 2006), and other bacteria mobilizing P from tricalcium phosphate (Vazquez *et al.*, 2000). The results also revealed that there is no direct relationship between the solubilization efficiency (%)

Table 2. Phosphate solubilizing potential of six selected isolates

Phosphate solubilizers	P-solubilization zone (mm) on Pikovskaya's agar	Solubilization efficiency (%) on Pikovskaya's agar	Maximum P-solubilization in Pikovskaya's broth (µg/ml)
<i>Pseudomonas</i> sp. HSF 126	32	333	173.47 (±2.43)
<i>Pseudomonas</i> sp. HSF 132	40	300	200.07 (±0.88)
<i>Pseudomonas</i> sp. KnSF 227	42	283	244.17 (±3.99)
Unidentified isolate RNF 267	30	250	217.53 (±4.56)
<i>Bacillus</i> sp. RSB 17	34	140	162.55 (±2.50)
<i>Bacillus</i> sp. RSB 22	30	100	176.16 (±1.08)

Values in parentheses represent standard error of mean

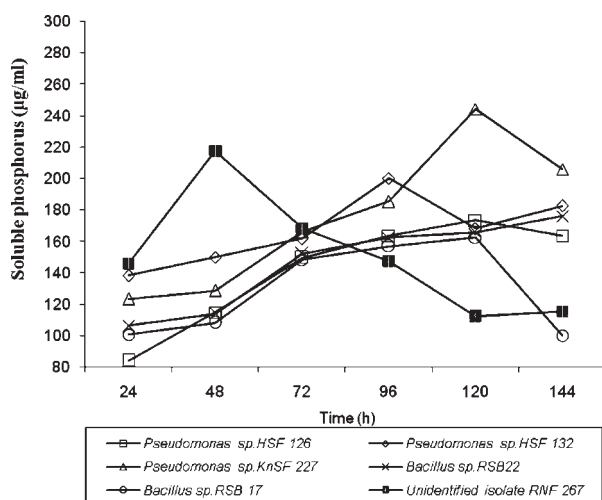


Fig. 1. Quantitative phosphate solubilization by efficient phosphate solubilizers

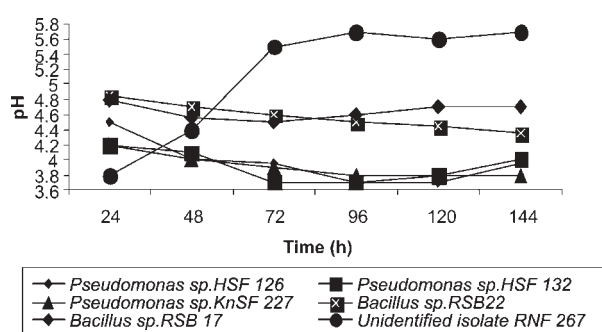


Fig. 2. Changes in pH of the culture fluid during phosphate solubilization by efficient phosphate solubilizers

and quantification results (Table 2). Although *Pseudomonas* sp. HSF 126 was more efficient on solid medium, *Pseudomonas* sp. KnSF 227 solubilized maximum phosphorus in liquid medium. Similar observations were reported by Srivastava *et al.*, (2004) and Ponmurugan and Gopi (2006). Comparatively lower level of soluble phosphorus was detected in *Bacillus* spp. (93.7 – 176.6 µg/ml) inoculated broths (Fig.1 and 2).

The efficient phosphate solubilizers were identified as *P. putida* biovar B HSF 132, *Enterobacter cloacae* RNF 267 (Bergey's Manual of Determinative Bacteriology 9th ed.) and *Pseudomonas plecoglossicida* KnSF 227 (Nishimori *et al.*, 2000) based on the results of the morphological, physiological and biochemical tests. Biolog provided the identification of HSF 132 as *P. putida* Biotype B with similarity index 0.617 and

distance 5.087, validating the conventional identification. Biolog assay of RNF 267 and KnSF 227 isolates gives similarity index lower than the acceptable value (Table 3), hence, the identity of these isolates were retained as *Enterobacter cloacae* RNF 267 and *P. plecoglossicida* KnSF 227.

Table 3. Conventional and Biolog identity of efficient phosphate solubilizers

Isolate No.	Conventional identification	Biolog® GEN III microbial ID system	Similarity index value	Distance value
Unidentified isolate RNF 267	<i>Enterobacter cloacae</i>	<i>Kluyvera ascorbata</i>	0.325	4.78
<i>Pseudomonas</i> sp. KnSF 227	<i>Pseudomonas plecoglossicida</i>	<i>Pseudomonas putida</i> Biotype B	0.433	3.187
<i>Pseudomonas</i> sp. HSF 132	<i>Pseudomonas putida</i> Biovar B	<i>Pseudomonas putida</i> Biotype B	0.617	5.087

Production of plant growth-promoting hormone (IAA), ACC deaminase was observed in all the three efficient phosphate solubilizing isolates and the production of siderophore was observed in *P. plecoglossicida* KnSF 227 and *P. putida* Biovar B HSF 132. These mechanisms in addition to the phosphate solubilization activity may contribute to enhanced plant growth. It has been reported that indirect growth promotion by phosphate solubilizing microorganism (PSM) is achieved by reducing pathogen infection via the antibiotic or siderophores which are synthesized and supplied by the bacteria (Rosas *et al.*, 2006). And many reports are there about the enhancement of plant growth and yield by phosphate solubilizing bacteria in corn (Yazdani *et al.*, 2009), *Cicer arietinum* (Sharma *et al.*, 2007) and sugarcane (Sundara *et al.*, 2002). Phosphate solubilizing potential and their ability to produce IAA, ACC-deaminase and siderophores makes *E. cloacae* 267, *P. putida* Biotype B HSF 132 and *P. plecoglossicida* KnSF 227 prospective candidates for use as bio-inoculants for achieving sustainable organic farming of coconut.

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