Short Scientific Report *Journal of Plantation Crops, 2012, 40 (1): 61-64*

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

(Manuscript Received: 05-09-11, Revised: 13-12-11, Accepted: 08-02-12)

Keywords: Coconut rhizosphere, phosphate solubilizing microorganisms, plant growth promoting attributes

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 [$Ca_3(PO_4)_2$] on solid medium. Phosphate solubilization efficiency (%) on agar medium was calculated as Z–C/C × 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 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 tri-calcium phosphate (Vazquez *et al.*, 2000). The results also revealed that there is no direct relationship between the solubilization efficiency (%)

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

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)
Pseudomonas sp. HSF 126	32	333	173.47 (±2.43)
Pseudomonas sp. HSF 132	40	300	200.07 (±0.88)
Pseudomonas sp. KnSF 227	42	283	244.17 (±3.99)
Unidentified isolate RNF 267	30	250	217.53 (±4.56)
Bacillus sp. RSB 17	34	140	162.55 (±2.50)
Bacillus sp. RSB 22	30	100	176.16 (±1.08)

Values in parentheses represent standard error of mean

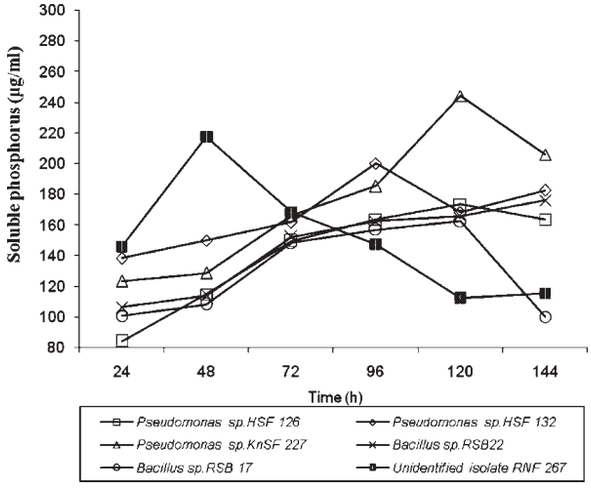

Fig. 1. Quantitative phosphate solubilization by efficient phosphate solubilizers

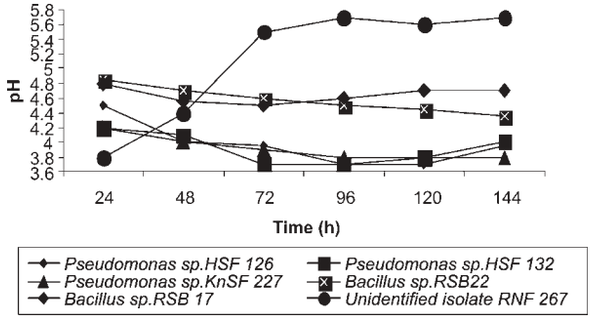

Fig. 2. Changes in pH of the culture fluid during phosphate solubilization by efficient phosphate solubilizers

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	*Enterobacter cloacae*	*Kluyvera ascorbata*	0.325	4.78
Pseudomonas sp. KnSF 227	*Pseudomonas plecoglossicida*	*Pseudomonas putida* Biotype B	0.433	3.187
Pseudomonas sp. HSF 132	*Pseudomonas putida* Biovar B	*Pseudomonas putida* 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.

Acknowledgements

We thank Indian Council of Agricultural Research, New Delhi, India for funding this network project on 'Application of Microorganisms in Agriculture and Allied Sectors'. Priya George is grateful for the award of Senior Research Fellowship.

References

Alikhani, H.A., Saleh-Rastin, N. and Antoun, H. 2006. Phosphate solubilization activity of rhizobia native to Iranian soils. *Plant Soil* **287**:35-41.

Brick, J.M., Bostock, R.M. and Silverstone, S.E. 1991. Rapid *in situ* assay for indole acetic acid production by bacteria immobilized on a nitrocellulose membrane. *Appl. Environ. Microbiol* **21**: 613-618.

Chakraborty, B.N., Chakraborty, U., Saha, A., Sunar, K. and Dey, P.L. 2010. Evaluation of Phosphate Solubilizers from Soils of North Bengal and Their Diversity Analysis. *World J. Agric. Sci* **6**(2): 195-200.

Dworkin, M., and Foster, J.W. 1958. Experiments with some microorganisms which utilize ethane and hydrogen. *Microbiology* **75**: 502-603.

Gyaneshwar, P., Kumar, G.N., Parekh, L.J. and Poole P. S. 2002. Role of soil microorganisms in improving P nutrition of plants. *Plant Soil* **245**: 83-93.

Jackson, M.L. 1967. Soil chemical analysis. Prentice hall of India Private Limited, New Delhi. pp. 183-214.

Nair, S.K. and Subba Rao, N.S. 1977. Distribution and activity of phosphate-solubilizing microorganisms in the rhizosphere of coconut and cocao under mixed cropping. *J.Plant.Crops* **5**:67-70.

Nishimori, E., Kita-Tsukamoto, K. and Wakabayashi, H. 2000. *Pseudomonas plecoglossicida* sp. nov., the causative agent of bacterial haemorrhagic ascites of ayu, *Plecoglossus altivelis. Int. J. Syst. Bacteriol* **50**: 83-89.

Palaniswami, C., Thomas, G.V., Dhanapal, R., Subramannian, P. and Maheswarappa, H.P. 2005. Biomass waste from coconut as nutrient source. In: Proceedings of ICAR short course, 'Utilization of wastes from plantation crops for improving soil health and fertility, crop production, post-harvest output and socio-economic status of farmers'. pp. 19-27.

Ponmurugan, P. and Gopi, C. 2006. Distribution pattern and screening of phosphate solubilizing bacteria isolated from different food and forage groups. *J. Agronomy* **5**(4): 600-604.

Rosas, S.B., Andres, J.A., Rovera, M. and Correa, N. 2006. Phosphate-solubilizing *Pseudomonas putida* can influence the rhizobia-legume symbiosis. *Soil Biol. Biochem* **38**: 3502-3505.

Schwyn, B. and Nielands, J.B. 1987. Universal chemical assay for the detection and determination of siderophores. *Anal. Biochem.* **160**: 47-56.

Sharma, K., Dak, G., Agrawal, A., Bhatnagar, M. and Sharma, R. 2007. Effect of phosphate solubilizing bacteria on the germination of *Cicer arietinum* seeds and seedling growth. *J. Herb. Med. Toxicol.* **1**: 61-63.

Srivastava, S., Yadav, K.S. and Kundu, B.S. 2004. Prospects of using phosphate solubilizing *Pseudomonas* as biofungicide. *Indian J Microbiol* **44**: 91-94.

Sundara, B., Natarajan, V. and Hari, K. 2002. Influence of phosphorus solubilizing bacteria on the changes in soil available phosphorus and sugarcane yields. *Field Crops Res.* **77**: 43-49.

Thomas, G.V., Iyer, R. and Bopaiah, B. M. 1991. Beneficial microbes in the nutrition of coconut. *J. Plant. Crops* **19**(2): 127-138.

Vazquez, P., Holguin, G., Puente, M.E., Lopez-Cortes, A. and Bashan, Y. 2000. Phosphate-solubilizing microorganisms associated with the rhizosphere of mangroves in a semi-arid coastal lagoon. *Biol. Fertil. Soils* **30**: 460-468.

Yazdani, M., Bahmanyar, M. A., Pirdashti, H. and Esmaili, M. A. 2009. Effect of Phosphate solubilization microorganisms (PSM) and plant growth promoting rhizobacteria (PGPR) on yield and yield components of Corn (*Zea mays L.). Proc. World Acad. Science, Eng. Technol.* **37**: 90-92.

Zaidi, A., Khan M. S., Ahemad M. and Oves M. 2009. Plant growth promotion by phosphate solubilizing bacteria. *Acta Microbiol. Immunol. Hung* **56**: 263-284.

Central Plantation Crops Research Institute
Kudlu P.O., Kasaragod - 671 124, Kerala, India

Priya George
Alka Gupta*
Murali Gopal
Litty Thomas
George V. Thomas

Corresponding Author: agcpcri@yahoo.co.in