Indian Journal of Agricultural Sciences **84** (5): 560–4, May 2014/Article

Phosphate solubilising *Streptomyces* spp obtained from the rhizosphere of *Ceriops decandra* of Corangi mangroves

D V SUBHASHINI[1] and ANIL KUMAR V[2]

Central Tobacco Research Institute, Rajahmundry, Andhra Pradesh 533 105

Received: 22 August 2012; Revised accepted: 4 March 2014

ABSTRACT

Phosphate-solubilizing potential of *Streptomyces* spp isolated from the rhizosphere soil of *Ceriops decandra* (Griffth) Ding Hou mangrove plant was studied using culture media supplemented with insoluble tricalcium phosphate which became transparent after few days of incubation. Fifteen phosphate-solubilizing strains of *Streptomyces* St-1 to St-15 were isolated from the rhizosphere of mangroves during 2010-11. Phosphate-solubilizing activity of these isolates was evaluated by the formation of halos (clear zones) around the colonies growing on solid medium containing tribasic calcium phosphate as a sole phosphorus source. St-3 was the most active phosphate solubilizing strain among the isolates followed by St-11. Studies on the biomass production of St-3 and St-11 showed an increase in growth at pH 7.2 after 7, 15 and 21 days of incubation at temperature 30°C and 37°C. Culture filtrate of St-3 alone showed the production of IAA. Present study reveals that the production of organic acids by the mangrove rhizosphere microorganisms may be a possible mechanism involved in the solubilization of insoluble calcium phosphate.

Key words: Indole-3-acetic acid, Mangrove, Phosphate-solubilizing microorganisms, Rhizosphere, *Streptomyces*

Corangi mangroves of Andhra Pradesh are one of the biggest wetland forests in India and are rich in biodiversity. Different genera of mangrove plants, viz. *Avicennia marina*, *Excoecaria agallocha, Brugiera gymnorrhiza* etc were available in this region. *Ceriops decandra* (Griffth) Ding Hou is one of the largest mangrove plant species widely distributed in Corangi and Konaseema deltaic zones. It is an evergreen tree in the inner mangrove forests in Andhra Pradesh and occurs in harder and higher muddy soil of the polyhaline zone, and forms pure stands on better drained soils and shows stunted growth in exposed and highly saline sites (Selvam and Karunagaran 2004). Several microflora having potential for bioactive compounds are reported from the rhizosphere soils of mangrove plants (Sinha 2012). *Streptomyces* are major contributors to biological buffering of soils and have role in organic matter decomposition conducive to crop production (Berdy 2005). Phosphate solubilizing ability of *Streptomyces* isolated from *Heritiera fomes* may be due to morphological, physiological and metabolic differences at species level (Kundu *et al*. 2002). Phosphate solubilising microbes play an important role in soil fertility and plant growth (Subhashini and Padmaja 2011). Very few reports are available on phosphate solubilization by *Streptomyces* in mangroves. (Vazquez *et. al*. 2000). In mangrove ecosystem phosphorus is present in insoluble inorganic forms such as tricalcium phosphate, iron and aluminium phosphates. However, mangrove plants do not exhibit phosphorus deficiency due to the presence of phosphate solubilizers in rhizosphere (Khamna *et al*. 2010).

Matsukawa *et al.* (2007) have reported that various *Streptomyces* spp including *S. violaceus*, *S. scabies*, *S. griseus*, *S. exfoliatus*, *S. coelicolor* and *S. lividans*, secrete indole-3-acetic acid (IAA) when fed with tryptophan. *S. albidoflavus* strain produced an amount of 34 µg/ml of IAA at optimal culture conditions. Secondary metabolites produced by this strain were found to have an inhibitory effect on plant pathogenic fungi (Narayana *et al.* 2007).

Aim of the present investigation is to demonstrate the presence of insoluble phosphate solubilising *Streptomyces* spp in the rhizosphere of semi-arid mangrove species *Ceriops decandra,* to isolate and identify the *Streptomyces* species, to measure their phosphate solubilising potential *in vitro*, and to identify the indole-3 –acetic acid produced by these *Streptomyces*.

MATERIALS AND METHODS

Soil samples were collected during 2010-11 from mangrove rhizosphere soils of *Ceriops decandra* (*Rhizophoraceae*) from Kandikuppa mangrove forests of Konaseema delta 16"36' 30.62" N, 82"12' 28.49" E located at east coast of East Godavari district, Andhra Pradesh.

[1]Principal Scientist (Agricultural Microbiology) (e-mail: dv_subhashini@rediffmail.com); [2]Research Scholar, Department of Biotechnology, Acharya Nagarjuna University (e mail: anilchemical@gmail.com)

This delta was created by the river Godavari & Bay of Bengal. The soil samples were collected around 5 cm depth from the surface of the earth using sterile spatchula and collected in sterile polythene bags and immediately brought to the laboratory for further analysis. Soil samples were pretreated with $CaCO_3$ (10:1 w/w) and incubated at 37^0C for 4 days in order to reduce the incidence of bacteria and molds. Soil dilution plate technique was employed to isolate the *Streptomyces* strains on enriched medium Starch Casein Agar (SCA) containing caseinpowder – 1.0g, starch- 10g, sea water 37.0 ml, agar – 15g; final pH (at 25°C) – 7.2±0.2.

Mangrove rhizosphere soil *Streptomyces* were isolated by dilution plate method using different media such as SCA medium, and ISP-5 (HiMedia) meant for specific isolation (Subhashini 2010). Weighed about 2 g of soil sample in a 500 ml conical flask containing 200 ml sterile distilled water. Kept the conical flask on orbitary shaker for 30 min at 200 rpm. Prepared about 250ml SCA medium and sterilized at 121°C, 15 lbs pressure. After cooling down poured it into Petri plates and left for some time for solidification. The soil solution (100 µl) from the conical flask was spread over the Petri plates containing SCA medium. Incubated the plates at 37°C for 24-72 hr. The colonies obtained were purified by quadrant streaking method. The cultural properties and growth characteristics of the isolates were studied on defined culture media such as glycerol asparagine agar ISP 5 (L-aspargine 1.0 g, dipotassium phosphate 1.0 g, trace salts solution (ml) 1.0 ml, agar 20.0 g, distilled water 1000 ml). The plates were incubated at 30°C and observations were recorded on the 7th, 15th and 21st days.

The *Streptomyces* colonies grown on SCA were screened for phosphate solubilization on Pikovskaya (PVK) medium containing yeast extract – 0.5 g, dextrose – 10.0 g, calcium phosphate – 5.0g, ammonium phosphate – 0.5g, potassium chloride – 0.2g, magnesium sulphate – 0.1g, manganese sulphate – 0.0001g, ferrous sulphate 0.0001g at pH 7.2 (Srivastav *et al.* 2004). The pre-isolated *Streptomyces* were inoculated on solidified plates and incubated. The colony forming clear zones was considered as positive and selected for the production of biomass on PVK broth at different pH (4.0, 5.0, 6.0, 7.2, 8.0 and 9.0) and temperature (30°and 37°C) with 7, 15 and 21 days of incubation period.

Screening of isolates for phosphate solubilization was done according to Dave and Patel (2003). These organisms were grown in PVK broth medium of pH 4.0, 5.0, 6.0, 7.2, 8.0 and 9.0. Incubation was carried out for seven days at 30°C and 37°C. Halo zone formation around colony was measured and considered as positive culture for phosphate solubilization.

The selected organisms were grown in liquid PVK medium at selected pH and temperature for the analysis of released phosphate content in the culture filtrate (Bhargava and Raghupathi 1993). Three experimental sets were prepared (i) PK media to which 25 ml of 0.5% TCP was added (ii) media plus 0.5 g TCP and 0.2% NaCl and, (iii) media plus 0.5 g TCP and 3% NaCl and inoculated with fresh culture of selected organisms in triplicate (Gupta *et al.* 2010). These sets were incubated for 7, 15 and 30 days. The total phosphate content available in 25 ml of culture filtrate was measured by using UV spectrophotometer at 420 nm and expressed in µg/ml. In each experimental setup, the final pH of culture filtrate was also measured at different interval of incubation period.

The production of IAA by phosphate solubilising positive *Streptomyces* isolates (St-3 and St-11) was determined according to the method of Bano and Musarrat (2003). Phosphate solubilizing positive isolates were grown on yeast malt extract (YM) agar and incubated at 30°C for one week, and were transferred to 100 mL YM broth containing 2 mg/mL L-tryptophan. These cultures were incubated at 30°C with shaking at 125 rpm for 5-10 days and then harvested by centrifugation at 10000 rpm for 20 min. Supernatant (filtrate) was collected in a fresh flask (Khamna *et al*. 2010). One milliliter of the supernatant was mixed with 2 mL of Salkowski reagent, the appearance of a pink color indicated IAA production.

St-3 strain was grown in 200 mL of YM broth containing 2 mg/mL L-tryptophan at a pH of 7.0. IAA was extracted from the supernatants with ethyl acetate according to the method described by Ahmad *et al.* (2005). Ethyl acetate fractions (10-20 mL) were applied to TLC plates (0.25 mm, Merck.) and developed in butanone-ethyl acetate-ethanol-water (3:5:1:1). Spots with Rf values identical to authentic IAA were identified under UV light (254 nm) by spraying the plates with Ehmann's reagent.

RESULTS AND DISCUSSION

Rich biodiversity of *Streptomyces* isolates was observed in soils obtained from mangrove rhizosphere of *Ceriops decandra*-Rhizophoraceae from Kandikuppa mangrove forests of Konaseema delta, East Godavari district of Andhra

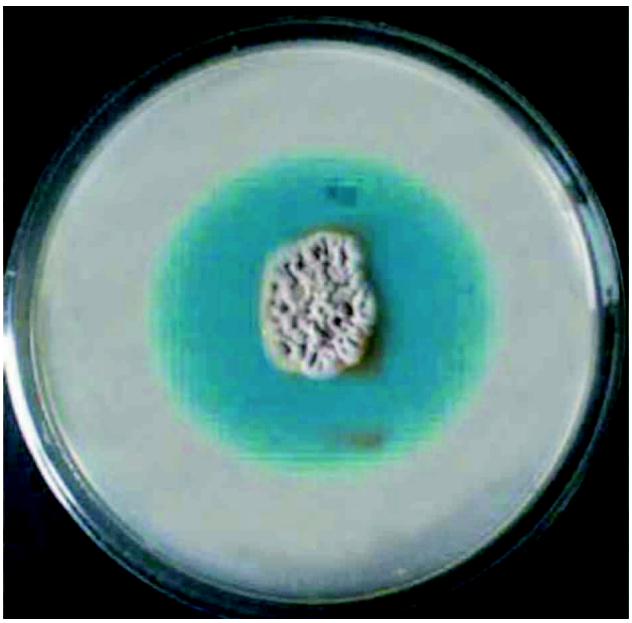

Fig 1 *Streptomyces* strain (St-3) showing phosphate solubilization (halo zone of 25mm) on PVK medium.

Table1 Growth and phosphate solubilsation efficiency of *Streptomyces* strains in different media and temperatures

Strain	Color of the mycelium on ISP	Color of the mycelium in ISP-5	Phosphate solubilization on PVK agar	Growth on PVK agar at 30°C	Growth on PVK agar at 37°C
St 1	Light grey	White	-	*	*
St 2	Brown	Brown	-	*	*
St 3	Light grey	Light grey	+	**	***
St 4	White	White	-	*	*
St 5	Green	Green	-	*	*
St 6	Brown	Brown	-	*	*
St 7	Light brown	Light brown	-	*	*
St 8	White	White	-	*	*
St 9	White	White	-	*	*
St 10	Brown	Brown	-	*	*
St 11	White	White	+	**	***
St 12	White	White	-	*	*
St 13	White	White	-	*	*
St 14	White	White	-	*	*
St 15	White	White	-	*	*

(+) solubilization; (–) no solubilization; *** excellent; ** good; * moderate

Pradesh. Isolates of pure culture grew well on the differential agar media forming isolated colonies. After soil inoculation, the Petri plates were incubated for a period of 7 to 10 days. All the 15 isolates were designated with St (*Streptomyces*). Morphological and cultural characteristics were observed on 4 day cultures of isolates grown on ISP 5 medium. Cultural characteristics of S*treptomyces sps* were tabulated in Table 1. In the present study two potential strains showed positive phosphate solubilizing activity among total 15 isolates. These strains showed larger zones of solubilization indicating an efficient solubilization of phosphate than the other remaining strains. St-3 strain showed 28 mm clear zone as it indicates phosphate solubilization, and the other St-11 strain also showed 15mm clear zone indicating phosphate solubilization on PVK medium (Fig 1).

Studies on the biomass production of St-3 and St-11 showed an increase in growth at pH 7.2 after 7,15 and 21 days of incubation at temperature 30°C and 37°C (Fig 2 and 3). Both the potent strains (St-3 and St-11) were evaluated for their activity in different cultural conditions (pH and temperature). Both strains St-3 and St-11 showed halo zones at pH 6.0, 7.2 and 8.0 and temperatures 30°C and 37°C, whereas no phosphate solubilization was noticed at pH 4.0, 5.0 and 9.0. *Streptomyces* St-3 exhibited solubilization zone at both temperatures and at all three pH ranges 6.0, 7.2 and 8.0 without the addition of NaCl. The two isolates of *Streptomyces* St-3 and St-11 could solubilize TCP on PVK agar plate at pH 7.2. There was variation in the phosphate solubilization potential of 2 isolates of *Streptomyces*. Very poor phosphate solubilization by St-11 was observed at

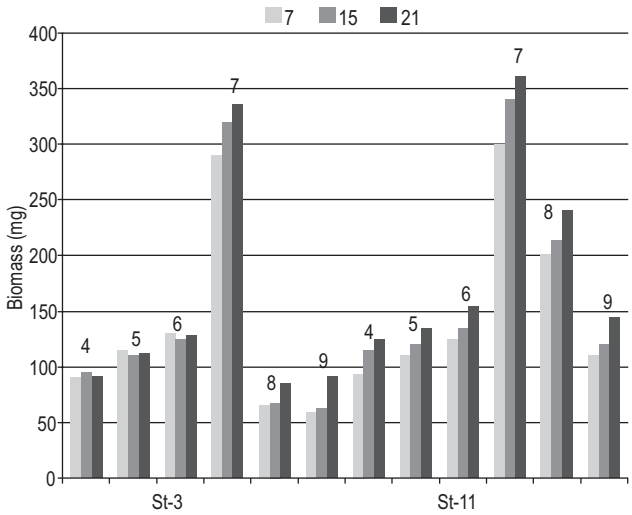

Fig 2 Effect of pH on the growth of *Streptomyces* spp (St-3 and St-11) on PVK broth (7, 15 and 21 are days of incubation); labels above bar indicate pH.

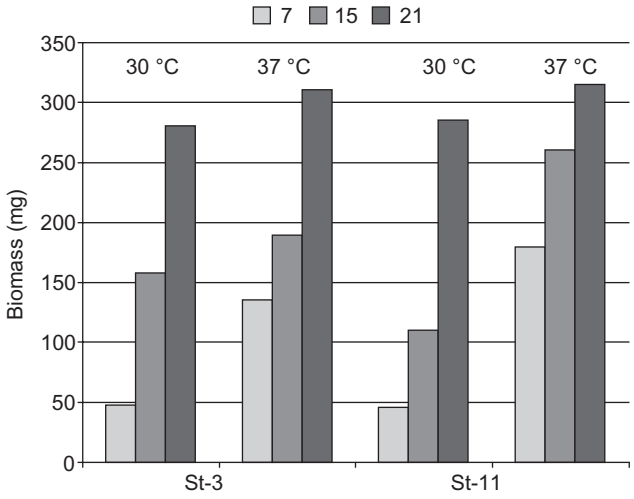

Fig 3 Effect of temperature on the growth of *Streptomyces* spp (St-3 and St-11) on PVK broth (7, 15 and 21 are days of incubation)

Table 2 Phosphate solubilization by St-3 in broth culture at different temperature and pH of the medium

Experimental condition			P content (ug/ml)		
NaCl (%)	Temperature (ºC)	pH	7 days	15 days	21 days
0	30	4.0	0.00	0.00	0.00
		5.0	0.00	0.00	0.00
		6.0	24.1 ± 1.12	2.1 ± 0.70	0.00
		7.2	40.93 ± 2.86	24.23 ± 0.84	28.37 ± 0.84
		8.0	12.80 ± 1.95	5.3 ± 2.63	3.43 ± 2.68
		9.0	0.00	0.00	0.00
	37	4.0	0.00	0.00	0.00
		5.0	0.00	0.00	0.00
		6.0	13.033 ± 1.59	0.00	0.00
		7.2	46.14 ± 2.07	31.04± 3.46	41.54 ± 0.30
		8.0	10.26 ± 0.03	2.2 ± 0.55	08.00
		9.0	0.00	0.00	0.00
0.2	30	4.0	0.00	0.00	0.00
		5.0	0.00	0.00	0.00
		6.0	31.86 ± 0.55	20.80 ± 3.70	24.23 ± 0.84
		7.2	46.27 ± 0.06	42.5 ± 3.21	44.02 ± 1.10
		8.0	25.6 ±1.17	0.00	0.00
		9.0	0.00	0.00	0.00
	37	4.0	0.00	0.00	0.00
		5.0	0.00	0.00	0.00
		6.0	26.53 ± 0.93	0.00	0.00
		7.2	36.00 ± 0.69	17.13 ± 2.31	27.06 ± 0.45
		8.0	11.09±0.02	3.98±0.32	9.78±2.40
		9.0	0.00	0.00	0.00
3.0	30	4.0	0.00	0.00	0.00
		5.0	0.00	0.00	0.00
		6.0	0.00	0.00	0.00
		7.2	48.28±0.19	20.21±1.21	36.02±3.00
		8.0	0.00	0.00	0.00
		9.0	0.00	0.00	0.00
	37	4.0	0.00	0.00	0.00
		5.0	0.00	0.00	0.00
		6.0	0.00	0.00	0.00
		7.2	31.7 ± 2.45	15.3± 2.45	21.45± 0.61
		8.0	12.80 ± 1.95	17.13 ± 2.31	25.08 ± 1.66
		9.0	0.00	0.00	0.00

Table 3 Phosphate solubilization by St-11 in broth culture at different temperature and pH of the medium

Experimental condition			P content (ug/ml)		
NaCl (%)	Temperature (ºC)	pH	7 days	15 days	21 days
0	30	4.0	0.00	0.00	0.00
		5.0	0.00	0.00	0.00
		6.0	10.09±0.01	0.00	0.29±0.09
		7.2	22.09±1.41	0.00	3.03 ±1 .40
		8.0	0.48±0.19	0.00	0.00
		9.0	0.00	0.00	0.00
	37	4.0	0.00	0.00	0.00
		5.0	0.00	0.00	0.00
		6.0	0.00	0.00	0.00
		7.2	43.61±2.13	26.51±0.42	39.01±2.00
		8.0	08.71±2.11	0.23±0.01	2.04±0.01
		9.0	0.00	0.00	0.00
0.2	30	4.0	0.00	0.00	0.00
		5.0	0.00	0.00	0.00
		6.0	0.00	0.00	0.00
		7.2	22.49±3.12	9.25±0.03	14.92±0.01
		8.0	0.00	0.00	0.00
		9.0	0.00	0.00	0.00
	37	4.0	0.00	0.00	0.00
		5.0	0.00	0.00	0.00
		6.0	0.00	0.00	0.00
		7.2	32.00±3.01	12.61±2.19	29.83±0.02
		8.0	0.00	0.00	0.00
		9.0	0.00	0.00	0.00
3.0	30	4.0	0.00	0.00	0.00
		5.0	0.00	0.00	0.00
		6.0	0.00	0.00	0.00
		7.2	9.61±1.41	0.00	1.49±0.41
		8.0	0.00	0.00	0.00
		9.0	0.00	0.00	0.00
	37	4.0	0.00	0.00	0.00
		5.0	0.00	0.00	0.00
		6.0	0.00	0.00	0.00
		7.2	0.00	0.00	0.00
		8.0	0.00	0.00	0.00
		9.0	0.00	0.00	0.00

0.2% of NaCl, at all the pH ranges 4.0 to 9.0 at 30°C and 37° C, and at 3.0% of NaCl, St-11 showed no phosphate solubilization at all pH and temperature ranges (Table 2 and 3).

The present study confirms that phosphate solubilizing microorganisms solubilize insoluble phosphates mainly by secreting acids into the medium (Dave and Patel 2003). It was also suggested that the production of organic acids by mangrove rhizosphere microorganisms is a possible mechanisms involved in the solubilization of insoluble calcium phosphate (Vazquez *et al.* 2000). The phosphate solubilizers St-3 and St-11 were screened for the production of IAA and only St-3 culture filtrate showed formation of pink colour when Salkowski reagent was added indicating the production of IAA. The results obtained are in agreement with the findings of Khamna *et al.* (2010). IAA was extracted from the supernatants with ethyl acetate, purified and spots generated on TLC plates were identified and correlated with original IAA. The present investigation clearly indicates the potential of *Streptomyces* strains obtained from mangrove rhizosphere soil that can be exploited as promising biofertilizers.

REFERENCES

Ahmad F, Ahmad I and Khan M S. 2005. Indole acetic acid

production by the indigenous isolates of *Azotobacter* and fluorescent *Pseudomonas* in the presence and absence of tryptophan. *Turkish Journal of Biology* **29**: 29–34.

Bhargava B S and Raghupathi H B. 1993. Analysis of plant materials for macro and micronutrients. (*In*) *Methods of Analysis of Soil, Plants, Waters and Fertilizers*, pp 49–82. Tandon HLST (Ed). Fertilizer Development and Consultation Organization, New Delhi.

Bano N and Musarrat J. 2003. Characterization of a new *Pseudomonas aeruginosa* strain NJ-15 as a potential biocontrol agent. *Current Microbiology* **46**: 324–8.

Berdy J. 2005. Bioactive microbial metabolites. A personal view. *Journal of Antibiotics* **58**: 1–26.

Dave A and Patel H H. 2003. Impact of different carbon and nitrogen sources on phosphate solubilization by *Pseudomonas fluorescens*. *Indian Journal of Microbiology* **43**: 33–6.

Gupta N, Sahoo Dolly and Basak Uday Chand. 2010. Evaluation of *in vitro* solubilization potential of phosphate solubilising *Streptomyces* isolated from phyllosphere of *Heritiera fomes* (mangrove). *African Journal of Microbiology Research* **4**(3) : 136–42.

Khamna S, Akira Yokota, John F Peberdy and Sisamorn Lumyong. 2010. Indole-3-acetic acid production by Streptomyces spp isolated from some Thai medicinal plant rhizosphere soils. *European Asian Journal of Biosciences* **4** : 23–32.

Kundu B S, Gera R, Sharma N, Bhatia A and Sharma R. 2002. Host specificity of phosphate solubilising bacteria. *Indian Journal of Microbiology* **42** : 19–21.

Matsukawa E, Nakagawa Y, Limura Y and Hayakawa M. 2007. Stimulatory effect of indole-3-acetic acid on aerial mycelium formation and antibiotic production in *Streptomyces* spp. *Actinomycetologica* **21** : 32–9.

Narayana K J P, Prabhakar P, Vijayalakshmi M, Venkateswarlu Y and Krishna P S J. 2007. Biological activity of phenylpropionic acid from a terrestrial Streptomycetes. *Polish Journal of Microbiology* **56** : 191–7.

Selvam V, Karunagaran V M. 2004. Coastal wetlands: Mangrove conservation and management. (*In*) Orientation guide 1. *Ecology and Biology of Mangroves*. M S Swaminathan Research Foundation, Chennai.

Sinha B. 2012. Global biopesticide research trends: a bibliometric assessment. *Indian Journal of Agricultural Sciences* **82**(2) : 95–101.

Srivastav S, Yadav K S and Kundu B S. 2004. Prospects of using phosphate solubilizing *Pseudomonas* as biofungicide. *Indian Journal of Microbiology* **44**: 91–4.

Subhashini D V. 2010. Biological control of damping-off (*Pythium aphanidermatum*) using *Streptomyces lavendulae* isolate 21 on tobacco seedbeds. *Journal of Biological Control* **24**(4): 338–42.

Subhashini D V and Padmaja K. 2011. Population dynamics and screening of phosphate-solubilizing bacteria isolated from tobacco *(Nicotiana tabacum)* – based cropping systems. *Indian Journal of Agricultural Sciences* **81**(8): 740–3.

Vazquez P G, Holguin M E, Puente A, Cortes L and Bashan Y. 2000. Phosphate-solubilising microorganisms associated with the rhizosphere of mangroves in a semiarid coastal lagoon. *Biology and Fertility of Soils* **30**: 460–8.