

# OCCURRENCE AND THE ACTIVITIES OF PHOSPHATE SOLUBILIZING MICROORGANISMS ASSOCIATED WITH COCONUT BASED CROPPING SYSTEMS

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## ABSTRACT

The phosphate solubilizing and competitive saprophytic ability of certain bacteria and fungi associated with coconut based cropping systems were studied. The population of phosphate solubilizing bacteria were higher in coconut based high density multispecies cropping, multistoreyed cropping and mixed farming systems as compared to that in coconut monocrop. *Bacillus*, *Pseudomonas* and *Micrococcus* and *Aspergillus*, *Fusarium* and *Penicillium* were the genera of P-solubilizing bacteria and fungi identified. No seasonal variation in the population of P-solubilizing bacteria and fungi was recorded. *Bacillus polymyxa* (45.2%), *Bacillus subtilis* (40.8%) and *Pseudomonas striata* (43.3%) were the bacteria which showed greater P-solubilizing ability under *in vitro* conditions. Among the fungi, *Aspergillus niger* (2 isolates) and *Penicillium citrinum* have recorded higher P-solubilizing ability. *Aspergillus niger* and *Penicillium citrinum* have shown greater competitive saprophytic ability, while *Bacillus subtilis*, *Bacillus polymyxa* and *Pseudomonas striata* showed better survival in sandy loam soil.

## INTRODUCTION

In most tropical soils, phosphorus is present predominantly in the form of inorganic compounds. The soil microorganisms play a very significant role in mobilizing P for the use of plants by bringing about changes in pH of the soil microenvironment and producing chelating substances which leads to the solubilization of native as well added insoluble phosphorus. Although bacteria have been used in the commercial preparation of inoculum to improve the P, uptake and the growth of the plant, fungi seem to be better agent in the dissolution of phosphate. The P availability can be increased by inoculating soil with phosphate solubilizing microorganisms along with application of low grade rock phosphate fertilizer. The occurrence of phosphate solubilizing microorganisms has been reported in coconut (Nair and Subba Rao, 1977; Thomas *et al.*, 1985; Thomas and Shantaram, 1986; Bopaiah, 1988). The present investigation, aims at studying occurrence, activities and competitive saprophytic ability of phosphate solubilizing microorganisms associated with coconut based cropping systems.

## MATERIALS AND METHODS

### Collections and enumeration of P-solubilizers from soil

Soil samples were collected from the coconut based high density multispecies cropping, multistoreyed cropping, mixed farming and coconut monocropping plots of Central Plantation Crops Research Institute, Kasaragod in October 1990. The collection of soil was done using soil auger from the root region of coconut, cacao, and napier grass at 0-25 cm depth (five samples each). In another experiment soil samples were collected in May, July, October and December from two depths (0-25 and 26-50 cm) of coconut monocropping plot to record the seasonal variation. The soil samples are stored in polythene bags at 4°C before the enumeration. The soils are sandy loam with a pH of 5.2 - 5.8 and are low in organic matter content.

The population P-solubilizing bacteria and fungi were enumerated by soil dilution and plate count method (Timonin, 1940) using Pikovskaya's medium containing tricalcium

phosphate (calcium phosphate tribasic-Sigma chemicals). Bacteria and fungi producing clear zone of solubilization around the colony were counted after 2 and 4 days of incubation respectively. The isolates of P-solubilizing bacteria (13 isolates) and fungi (7 isolates) were selected through preliminary screening and maintained for further studies.

### *In vitro* P-solubilizing ability

#### Bacteria

The P-solubilizing ability of thirteen bacterial isolated were tested using Pikovskaya's broth medium (Pikovskaya, 1948). The tricalcium phosphate was added to 50 ml medium in 100 ml Erlenmayer flasks to provide 25 mg 'P' and the flasks were sterilized. The flasks were inoculated with 0.5 ml of 48 hr old bacterial culture grown in Nutrient broth and incubated at  $30 \pm 2^\circ\text{C}$  for one week (three replicates each). The bacterial cells were separated by centrifugation at 15,000 rpm for 15 minutes. The available P in the supernatant was determined colorimetrically (Black, 1965). The pH of the culture broth was determined. The bacterial isolates were identified by following the morphological, cultural, physiological and biochemical tests (Buchanan and Gibbons, 1974).

#### Fungi

The pikovskaya's broth was taken in 50 ml lots in 100 ml Elenmayer flasks and tricalcium phosphate was added as insoluble 'P' at a concentration of 50 mg per flask. The flasks were autoclaved at 1.1 kg/sq. cm for 15 min. The inoculum of fungi (seven isolates) was prepared separately in petridishes containing Potato Dextrose Agar (PDA) by seeding. The flasks were inoculated with 9 mm diameter mycelial disc using a cork borer along the margin of the fungal colony (three replicates each). The flasks were incubated at  $30 \pm 2^\circ\text{C}$  for 21 days. After incubation the contents were filtered and centrifuged at 15,000 rpm for 15 min. The soluble P content in the filtrate were analysed colorimetrically (Black, 1965). The percentage 'P' solubilized by the fungal isolate was calculated after subtracting the amount of available 'P' in the control flask without fungal inoculation. The pH of the culture filtrate and mycellium dry weight (dried at  $70^\circ\text{C}$  to constant weight) were recorded.

### Survival of bacteria in soil

Ten g portion of sandy loam soils were taken in 25 ml test tubes and autoclaved at 1.1 kg/sq cm for 1 hr and sterilization was done consequently for 3 days. The moisture content of the soil was adjusted to 60 per cent water holding capacity. The tubes were inoculated with one ml of bacterial suspension containing  $10^8$  cell  $\text{ml}^{-1}$ . Soil was thoroughly mixed and incubated at  $30 \pm 2^\circ\text{C}$  for 30 days. The bacterial numbers surviving were determined by soil dilution and plate count method using nutrient agar. The percentage survival of bacteria was calculated based on the initial count.

### Competitive saprophytic ability of fungi

The competitive saprophytic ability (CSA) of fungi was determined by following the cambridge method with slight modification (Lucas, 1955). The fungal inoculum was prepared in rice bran fortified with 2% urea and 0.1% potassium phosphate (w/w). The full range of inoculum-soil (unsterilized garden soil) mixture contained the following percentage (w/w) of inoculum : 100 (pure culture), 20,10,2 and 0 (soil alone) in petri plates. The substrate, 1 cm long paddy straw bits (25 no) were implanted in the different soil: fungal inoculum mixture. The moisture content was adjusted 60% water holding capacity. After 15 days of incubation at  $35^\circ\text{C}$ , the straw bits were examined for the colonisation of fungi microscopically. Twenty five straw bits were examined at each inoculum dilution (3 replicates). The colonisation of maximum number of bits at a low inoculum potential was considered as criterion for better competitive saprophytic ability.

## RESULTS AND DISCUSSION

The population of phosphate solubilizing bacteria and fungi in different coconut based cropping and coconut monocropping systems are presented in Table I. The data indicate that, the counts of phosphate solubilizing bacteria were higher in different coconut based cropping systems as compared to coconut monocropping. Similarly, higher numbers of P-solubilizers were recorded in coconut -cocoa mixed cropping system as compared to coconut monocrop (Nair and Subba Rao, 1977). In the present study, the

population of fungi did not reveal much variation in all the cropping systems studied. There was no seasonal variation in the occurrence of P-solubilizing bacteria and fungi. The number of P-solubilizing bacteria and fungi were higher in 0-25 cm as compared to 26-50 cm soil depth. *Bacillus subtilis*, *Bacillus polymyxa*, *Bacillus cereus*, *Bacillus spp.*, *Micrococcus spp.*, *Pseudomonas striata* and *Pseudomonas spp.* are the P-solubilizers identified in different coconut based cropping systems.

The phosphate solubilizing ability of bacteria isolated from the root zone of different coconut based cropping systems *in vitro* has showed that the two isolates of *Bacillus subtilis*

**Table I. Phosphate solubilizing microorganisms in coconut based cropping systems (average of 5 replications).**

Cropping system and crop	Bacteria (x 10 <sup>4</sup> )	Fungi (x 10 <sup>3</sup> )
A. Coconut - HDMSC. Coconut	3.11	0.86
B. Coconut - multistoreyed Cropping. Coconut	4.84	1.30
Cacao	2.64	1.66
C. Coconut - mixed farming. Coconut	5.00	1.30
Napier grass	3.70	1.25
D. Coconut - monocropping. Coconut	1.84	1.25
C.D. at 5%	0.89	0.45

**Table II. *In vitro* phosphate solubilizing ability of bacteria isolated from the coconut based cropping systems (average of 3 replications).**

Isolate No.	Bacteria	P-solubilizing ability (%)	pH
22	<i>Bacillus subtilis</i>	40.8	3.0
23	<i>Bacillus sp.</i>	31.7	4.2
24	<i>Bacillus polymyxa</i>	35.7	3.1
25 BB	<i>Bacillus subtilis</i>	29.1	2.8
29 A	<i>Bacillus sp.</i>	18.3	5.0
29 B	<i>Bacillus cereus</i>	28.8	3.2
37	<i>Pseudomonas sp.</i>	31.3	2.9
41	<i>Micrococcus sp.</i>	21.8	4.0
46	<i>Pseudomonas striata</i>	43.3	2.7
49	<i>Pseudomonas sp.</i>	28.5	3.7
55	<i>Bacillus polymyxa</i>	45.2	2.8
57	<i>Bacillus subtilis</i>	36.7	3.3
60	<i>Bacillus sp.</i>	23.3	2.9

solubilized the tricalcium phosphate to the extent of 40.8% and 36.7% respectively (Table II.). The *Bacillus polymyxa* (45.2%) and *Pseudomonas striata* (43.3%) have shown greater P-solubilizing ability. The pH of the culture filtrate of efficient P-solubilizing bacteria decreased from the initial pH and *Pseudomonas striata* recorded lowest pH of 2.7. The P-solubilizing bacteria has been reported in the root zone of coconut growing in sandy, alluvial, clayey and laterite soil types (Thomas and Shantaram, 1986) and in coconut-cocoa mixed cropping system (Nair and Subba Rao, 1977).

The phosphate solubilizing ability, pH and mycelial biomass production of seven fungal isolates are given in Table III. Among these, *Aspergillus niger* (2 isolates) and *Penicillium citrinum* have shown greater P-solubilizing ability as compared to the other isolates of fungi tested. The reduction in pH of the culture filtrate and higher mycelial biomass were recorded in the efficient P-solubilizing isolates of fungi. The P-solubilizing ability of fungi ranged from 15.2 to 42.3%.

Out of the six bacteria tested for the survival in sandy loam soil, *Bacillus subtilis* (18.2%), *Bacillus polymyxa* (20.3%) and *Pseudomonas striata* (18.7%) showed better survival (Table IV.). The bacteria which possess better capacity to survive combined with higher P-solubilizing ability can be used as inoculum for coconut.

The data on competitive saprophytic ability of fungi *in-vitro* are presented in Table V. Among the nine fungi tested, *Aspergillus niger* (2

**Table III. *In vitro* phosphate solubilizing ability, pH and mycelial biomass of fungi isolated from coconut based cropping systems (average of 3 replications)**

Isolate No.	Fungi	P-solubili zing ability (5)	pH	Mycelial biomass (mg/50 ml)
20	<i>Aspergillus niger</i>	34.8	2.6	0.21
21	<i>Penicillium sp.</i>	15.2	4.6	0.11
28	<i>Aspergillus terreus</i>	28.2	3.2	0.17
29	<i>Aspergillus fumigatus</i>	30.5	3.6	0.14
32	<i>Aspergillus sp.</i>	27.8	4.3	0.20
35	<i>Aspergillus niger</i>	42.3	2.9	0.24
39	<i>Penicillium citrinum</i>	34.7	3.7	0.15

**Table IV. Percentage of survival of P-solubilizing bacteria in sandy loam soil**

Organisms	Initial count cfu (x 10 <sup>6</sup> )	Percent survival
<i>Bacillus polymyxa</i>	124	20.3
<i>Bacillus subtilis</i>	145	18.2
<i>Bacillus</i> sp.	158	12.2
<i>Micrococcus</i> sp.	88	3.5
<i>Pseudomonas striata</i>	128	18.7
<i>Pseudomonas</i> sp.	112	7.4

**Table V. Competitive saprophytic ability of P-solubilizing fungi**

Organisms	Percentage of straw bits colonised*				Efficiency Class**
	50:50	80:20	90:10	98:2	
<i>Aspergillus niger</i>	100	100	80	76	++
<i>Aspergillus fumigatus</i>	100	80	52	44	+
<i>Aspergillus niger</i>	100	92	60	52	++
<i>Aspergillus niger</i>	84	56	28	28	+
<i>Aspergillus terreus</i>	100	64	32	28	+
<i>Aspergillus</i> sp.	64	32	24	0	-
<i>Fusarium</i> sp.	56	44	20	12	-
<i>Penicillium citrinum</i>	100	100	82	68	++
<i>Penicillium</i> sp.	68	48	28	16	-

\* - soil : inoculum ratio

\*\* (-) less than 25% colonisation at 98:2 inoculum potential (lp)

(+) - more than 25% colonisation at 98:2 IP

(++) - more than 50% colonisation at 98:2 IP

isolates) and *Penicillium citrinum* have shown better competitive saprophytic ability as revealed by its ability to colonise the straw bits in 98:2 soil : inoculum ratio. The reasons for the variation in response of the P-solubilizing micro-organisms under *in-vitro* and field conditions can be due to several ecological factors which ultimately affect the survival and multiplication of these micro-organisms in soil.

A positive correlation between the incidence of P-solubilizing micro-organisms and available phosphorus content in soil of coconut-cocoa mixed cropping system was reported (Nair and Subba Rao, 1977). Sundara Rao (1965) reported significant increase in yield due to phosphobacterin inoculation in certain field trials conducted with different crops. The addition of low grade rock phosphate coupled

with the inoculation of phosphate solubilizing micro-organisms may give better response in coconut crop. Therefore further studies are needed to evaluate the performance of P solubilizers under field condition using the coconut seedling.

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