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SOLUBILIZATION OF INORGANIC PHOSPHATES BY BACTERIA FROM COCONUT PLANTATION SOILS*

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ABSTRACT

Studies on the distribution of phosphate-solubilizing bacteria in coconut plantation soils revealed that clayey soils harboured less population than laterite, alluvial and sandy soils. *Pseudomonas* sp., *Micrococcus* sp., *Micrococcus roseus*, *Bacillus subtilis*, *Corynebacterium* sp. and *Alcaligenes* sp. were the phosphate-solubilizing bacteria encountered in coconut soils. *In vitro* estimation of the phosphate-solubilizing ability of the isolates revealed solubilization of 19.5 to 54 per cent of the insoluble phosphates supplied in the culture broth. Inoculation of soils with efficient phosphate-solubilizing bacteria after addition of farm yard manure and rock phosphate released more available P from insoluble P sources. , *M. roseus* and *B. subtilis* possessed better capacity to survive in unamended soils, as compared to the other phosphate-solubilizing bacteria tested and have a great potential as inoculants in crop plants for better utilization of insoluble P-sources.

INTRODUCTION

Solubilization of insoluble inorganic phosphates by several soil micro-organisms and yield increases and higher phosphorus content of the crop plants due to inoculation with phosphate-solubilizing micro-organisms have been reported (Garretsen, 1948; Sundara Rao and Sinha, 1963; Taha et al. 1969). Failure to obtain response to inoculation in some soils is also attributed to the natural occurrence of a large population of phosphate-solubilizing micro-organisms in such soils or due to the failure of inoculated cultures to survive and express their activity in soil (Chhonker, 1979). The application of 320 g of P_2O_5 per palm per year is recommended (Anonymous, 1982) for the coconut palm (*Cocos nucifera* L.). The occurrence of phosphate-solubilizing micro-organisms will contribute to the dissolution of phosphate from insoluble P sources in soil and the palm gets a costant supply of available P. This would enable the application of low-cost phosphatic fertilizers like rock phosphate in coconut cultivation. Thomas, Shantaram and Saraswathy (1985) reported the activity and competitive saprophytic ability of the phosphate-solubilizing fungi isolated

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from coconut soils. The present investigation was undertaken to study the occurrence of phosphate-solubilizing bacteria in soils under coconut plantation and to isolate and test their ability to dissolve insoluble phosphates. The efficient phosphate-solubilizers were also studied with regard to their ability to enhance the available P concentration of soil and their survival in soil.

MATERIALS AND METHODS

Enumeration of phosphate-solubilizing bacteria

The soils used were surface (0–30 cm) samples drawn from 32 locations in the coconut growing tracts of Kerala (India) using 25 mm diameter core sampler. Coconut monocropped plots were only selected for sampling. Samples were obtained from eight locations from each of the four major soil types *viz.*, laterite, sandy, alluvial and clayey. The number of phosphate-solubilizing bacteria in soil was determined by dilution plate technique using Sperber's agar (Sperber, 1957) containing tri-calcium phosphate of 100–150 mesh as insoluble P source. Active phosphate-solubilizing bacteria were isolated from colonies which showed larger clearing zones of solubilization.

In vitro phosphate-solubilizing activity

Pikovskaya broth (Pikovskaya, 1948) was taken in 250 ml EM flasks in 50 ml lots and insoluble tricalcium phosphate ($Ca_3(PO_4)_2$) was added to obtain a concentration of 50 mg P per flask and autoclaved. The flasks were inoculated with 0.5 ml of 48h old culture of bacterial isolates grown in nutrient broth. After incubation for 1, 2, 4, 6, 8, 10 and 14 days at 30 ± 1°c, duplicate flasks were withdrawn at each of the incubation period. The bacterial cells and the insoluble phosphate were separated by centrifugation at 15000 rpm for 15 min. The soluble P content of the supernatent was determined calorimetrically by vanadomolybdic acid yellow colour method (Jackson, 1973). The titratable acidity was determined by titration with 0.01 N NaOH. The pH of the culture broth was also determined. The identification of the efficient bacterial cultures was confirmed with the help of Commonwealth Mycological Institute, England. The isolates which solubilized more than 24 per cent of the insoluble P were selected for inoculation and survival studies in soil.

Effect of inoculation on available P content of soil

Air dried, 2mm sieved laterite and sandy soils were taken in 100g lots in 250ml EM flasks and amended with farm yard manure at the rate of 5g per flask. Rock phosphate was added to provide 100 mg P in each flask. The flasks were inoculated with 5 ml culture broths of the bacterial cultures containing 10^8 cells ml^{-1} and incubated at 30 ± 1°C at 60 per cent water holding capacity. Control flasks were also maintained without bacterial inoculation. After 15 days of incubation, the soil samples were analysed for available P content.

Survival in soil

Ten gram portions of laterite and sandy soils were taken in test tubes and autoclaved at 1.1 kg cm^{-2} for one hour on each of the three consecutive days. To ascertain whether sterility has been achieved, suspensions of soil were plated on nutrient agar. The moisture level was adjusted to 60 per cent water holding capacity with sterile water and inoculated with one ml of bacterial suspension containing 10^8 cells ml^{-1}. The soil was thoroughly mixed and incubated at $30 \pm 1°C$ for 30 days. The number of cells survived was determined by plating serial dilutions of soil on nutrient agar.

RESULTS AND DISCUSSION

The data on the population of phosphate - solubilizing bacteria in coconut soil types are presented in Table I. The population did not show much variation in laterite, sandy and alluvial soil types whereas clayey soils harboured lower populations as compared to the other three soil types. Bacteria capable of solubilizing phosphates could not be detected from few locations within each soil type. Maximum phosphate - solubilizing bacterial count of 4.54×10^4 c. f. u./g dry soil was recorded from a location in the laterite soil type. The comparatively low numbers of bacteria capable of solubilizing insoluble phosphates in clayey soils may be due to the unfavourable conditions prevailing in such soils for the survival of bacteria due to the interaction of a number of physicochemical factors.

Table I. *Population of phosphate-solubilizing bacteria in coconut plantation soils*

	Population $\times 10^4$ c. f. u./g^{-1} dry soil	
	Mean$\pm$SE*	Range
Laterite	1.38$\pm$0.51	0–4.54
Sandy	1.77$\pm$0.58	0–4.50
Alluvial	1.60$\pm$0.40	0–3.12
Clayey	0.70$\pm$0.32	0–2.52

* *Standard error*

The percentage of phosphate solubilized and changes in pH and titratable acidity at different periods during the growth of phosphate solubilizing bacteria in culture broth are presented in Fig. 1. On the basis of variation in the colony morphology and clearing zones on solid media, phosphate-solubilizing bacteria in coconut soils were divided into six groups and representative isolates from each group were selected for detailed studies. The isolates varied considerably in their ability to solubilize phosphate and in the optimum period of incubation for maximum solubilization. Percentage solubilization of insoluble phosphate by bacterial isolates varied between 19.5 to 54.0. The tribasic calcium phosphate used in this study is regarded as a model compound for measuring potential and relative rates of bacterial solubilisation of insoluble inorganic phosphate compounds (Craven and Haysaka, 1982). In addition, the insoluble calcium phosphates form a major portion of insoluble phosphates in soil (Devi and Narasimham, 1978). The phosphate-solubilizing bacteria in coconut soils were identified as *Pseudomonas* sp., *Micrococcus* sp., *Micrococcus roseus*, *Bacillus subtilis*, *Corynebacterium* sp., and *Alcaligenes* sp. Maximum solubilization of 54% of the insoluble P was recorded with *M. roseus*, followed by 52.5 and 50% solubilization by *Micrococcus* sp., and *B. subtilis*, respectively. The groups of phosphate-solubilizing bacteria observed in coconut soils are not different from those reported in soil and

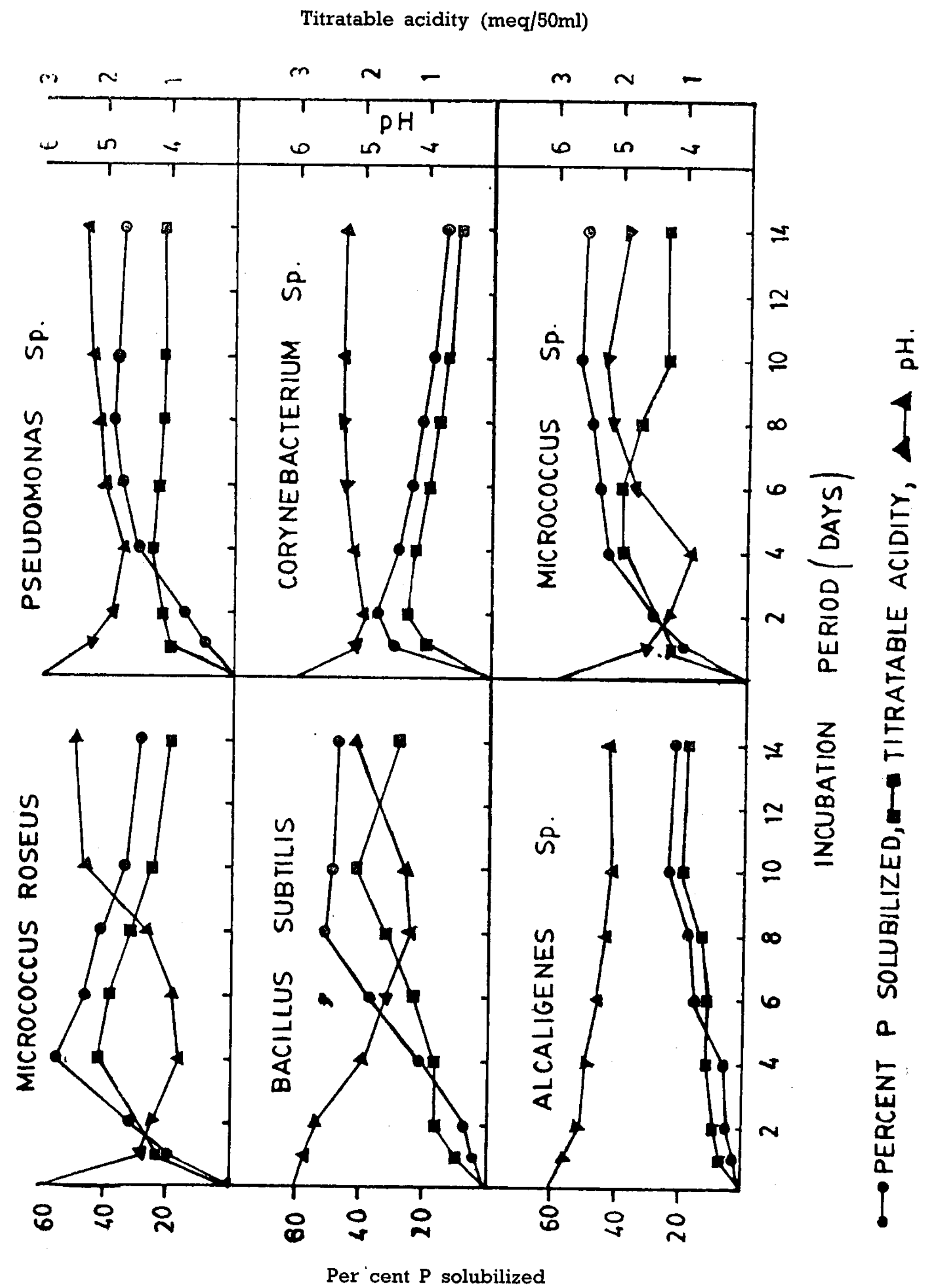


Fig. 1. *In vitro* solubilization of insoluble phosphate by bacterial isolates

rhizospheres of various crops (Sundara Rao and Sinha, 1963; Swaby and Sperber, 1958; Taha et al. 1969; Chhonker, 1979; Banik and Dey, 1982).

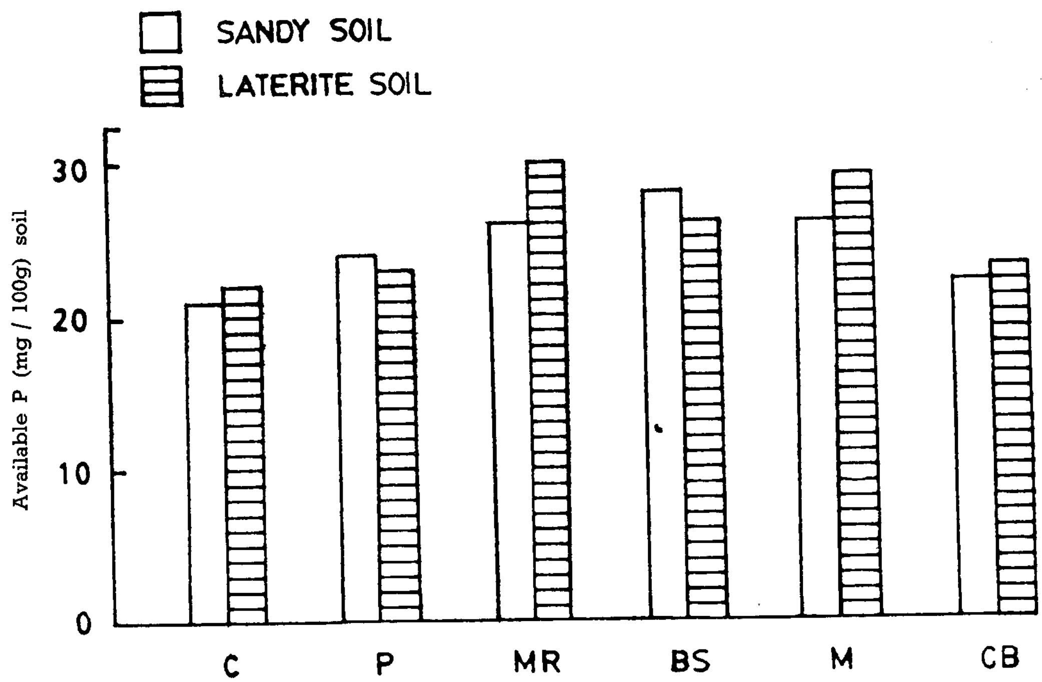
There was an increase in the quantity of phosphate – solubilized with the increase in incubation period till maximum solubilization was attained and then it decreased with further incubation. The optimum period of growth for maximum solubilization was 4, 10 and 8 days in case of efficient bacteria like *M. roseus*, *Micrococcus* sp. and *B. subtilis*, respectively. Taha et al. (1969) reported maximum solubilization of insoluble P by bacteria within 10 days of incubation. A decrease in available P concentration was observed after the optimum period of incubation. The reduction in available P content after attaining the maximum solubilization may be due to the transformation of acidic constituents to neutral products resulting in a decrease in the acidity responsible for phosphate solubilization. The pH values decreased gradually till maximum solubilization was attained and then it began to rise with further incubation.

A decrease in pH and increase in titratable acidity were always observed with the increase in solubilization. The lowest recorded pH of 3.8 was observed when *M. roseus* exhibited maximum solubilization of 54 per cent of the insoluble P supplied in the medium. The pH the culture broth was 4.3 when *B. subtilis* showed 50% P–solubilization. A highest titratable acidity of 2.1 m eq/50 ml was also observed at the maximum solubilization. There was significantly positive correlation between phosphate–solubilization and titratable acidity ($r=+0.92$) showing a linear equation $Y=124.30-19.74\ X$. Solubilization of phosphate also showed a significant inverse correlation ($r=-0.69$) with pH and a linear equation of $Y=-6.19+28.70\ X$ was obtained. The present results are in agreement with similar studies of Swaby and Sperber (1958), Louw and Webley (1959), Raghu and MacRae (1966) and Arora Gaur (1979). Soils are well buffered against large variations in pH observed in culture media.

There was an increase in the available phosphorus content of soil when inoculated with efficient phosphate-solubilizing bacteria after addition of rock phosphate and farm yard manure (Fig. 2). The maximum P–solubility was observed due to inoculation with *B. subtilis* in a sandy soil whereas inoculation with *M. roseus* resulted in the maximum increase in laterite soil. Banik and Dey (1982) reported improvement in available P content of soil due to inoculation of native *Aspergilli* and *Bacillus firmus* with the addition of farm yard manure and rock phosphate. Singh and Pareek (1985) also observed 11–35% increase in the available soil phosphorus when inoculated with single or composite cultures of phosphate dissolving microbes. The farm yard manure supplied in the present study might have provided the nutrients and growth factors for the multiplication of micro-organisms in soil.

The data on the survival of bacterial isolates in soil are presented in Table II. There was significant variation in the survival percentage of bacterial isolates

Fig. 2. Effect of bacterial inoculation on available phosphorus content of soil



C–*Control*, P–*Pseudomonas* sp., MR–*Micrococcus roseus*, BS–*Bacillus subtilis*, M–*Micrococcus* sp., CB–*Corynebacterium* sp.,

Table II. *Survival of bacterial isolates in soil.*

Organism	Initial count c. f. u. $\times 10^6$	Per cent survial	
		Sandy soil	Laterite soil
Pseudomonas sp.	134	2.86 (9.73)	0.82 (5.20)
Micrococcus roseus	155	6.41 (14.66)	9.08 (17.54)
Bacillus subtilis	110	18.16 (25.22)	10.97 (19.34)
Micrococcus sp.	90	2.41 (8.93)	8.52 (16.98)
Corynebacterium	128	0.84 (5.26)	1.42 (6.84)
LSD (P=0.01)		8.11	7.80

Figures in parentheses are values obtained by $\sin^{-1}\sqrt{x}$ transformation where x is the per cent survival.

but the difference in the percentage of survival in the two soil types was not significant. *B. subtilis* and *M. roseus* survived in greater numbers and a population density of 10^7 cells per gram of soil was recorded for these two isolates in both the soil types after 30 days of incubation. Although a number of studies showed significant response on crop growth due to inoculation with phosphate-solubilizing microorganisms there were also reports indicating poor or no response to inoculation (Chhonker, 1979). It may be possible that the organisms which show high *in vitro* activity in culture media fail to express their activity under field conditions due to several ecological factors which ultimately affect the growth and multiplication of an actively P-solubilizing micro-organism in soil. The information on the activity and survival of phosphate-solubilizing micro-organisms in soil is important not only for choosing bacteria for inoculation trials but also relevant in recognising the relative role of different heterotrophic bacteria in P-solubilization in cocon soil. The bacteria which possess bette capacity to survive and express thei activity in soil can be tested as inoculants to better the utilization of insolubl P sources like rock phosphate as a fertilizer nutrient for coconut and other plantation crops.

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