33

MICROBIAL SOLUBILISATION OF INSOLUBLE PHOSPHATES

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ABSTRACT

Phosphate solubilising micro-organisms from rubber growing soils of 10 different regions were enumerated and compared with the total bacterial population. The phosphate solubilising bacteria in rubber growing soils ranges from 1 to 2.8 per cent. The population of phosphate solubilising fungi and actinomycetes are very small in rubber growing soils which are acidic in nature. The solubilisation of rock phosphate, aluminium phosphate and ferric phosphate by the soil micro-organisms as well as the effect of various carbon and nitrogen sources on the solubilisation of rock phosphate were studied. The results showed that rock phosphate is solubilised by all groups of micro-organisms. The fungi acted on rock phosphate more effectively in the presence of simple sugars and different nitrogen sources. The possibility of using *Asperigillus* spp. for phosphate solubilisation in rubber growing soils is discussed.

INTRODUCTION

Insoluble soil phosphates are rendered available either by plant roots or by soil micro-organisms through secretion of organic acids (Banik and Dey, 1983). Acid soils, in which rubber is green, are poor in calcium and therefore phosphate are precipitated in the form of ferric or aluminium compounds which are not so easily amenable to solubilisation by plant roots or soil micro-

organisms (Subba Rao, 1977). Rock phosphate, an insoluble form of phosphate is recommended for rubber plants as a source of phosphorus (Pushpadas and Ahammed, 1980). Therefore, phosphate solubilisation is a very important aspect in the nutrition of rubber plants.

Soil becteria, fungi and actinomycetes were reported to solubilise phosphate (Ortuno et al., 1979; Rao et al., 1982) and they differ to the extent of solubilisation (Arora and Gaur, 1979). All these phosphate solubilisers were found to be more in the rhizosphere of higher plants and solubilise native as well as applied insoluble phosphates. Besides, phosphate solubilisation, the micro-organisms produce vitamins and phytohormones (Baya et al., 1981) which favour the growth of plants. Hence, soil samples from a few rubber growing areas were tested for the presence of phosphate solubilising micro-organisms and the effect of different carbon and nitrogen sources on the phosphate solubilisation by micro-organisms is broth culture were studied.

MATERIALS AND METHODS

Soil samples (0 to 5 cm) were collected from ten rubber growing areas and the microbial population were estimated by plate count method of Timonin (1940) using soil extract agar for total bacterial population and special soil extract apatite agar medium (Speeber, 1958) for phosphate solubilising micro-organisms. The population of micro-organisms in soil were expressed on oven dry soil weight basis. The pH of the soil was estimated.

Solubilisation of different phosphates

Two isolates each of bacteria and fungi and one actinomycete isolate showing more phosphate solubilising capacity were selected and studied for their ability to solubilise rock phosphate, aluminium phosphate and ferric phosphate. Two hundred and fifty mg of sterilised phosphates were added to 50 ml of sterile Pikovskaya's liquid medium. The flasks were inoculated with the test cultures and incubated for 10 days (Subba Rao, 1977). After incubation the cultures were filtered through a bacteriological filter and the cell free filterates were analysed for phosphate content following the colorimetric method (Jackson, 1962).

The effect of carbon and nitrogen sources on the solubilisation of insoluble phosphates

Glucose, fructose, xylose and mannitol equivalent to 500 mg of glucose were added to 50 ml of Pikovskaya's broth medium, so as to maintain the carbon content in the sugars at the same level. Ammonium sulphate, sodium nitrate and urea equivalent to five mg of nitrogen were added and tested for their effect on solubilisation of rock phosphate as described earlier.

RESULTS AND DISCUSSION

The results of the enumeration of total bacteria in rubber growing soils show that bacterial population is maximum in soils having pH around 5.5 (Table 1). Gray and Williams (1975) also

Table 1. Population of total bacteria and phosphate solubilising bacteria in soils of varying pH

Sl. No.	Location	pH	Bacterial population × 10^3	
			Total bacteria	Phosphate solubilising bacteria
1.	Chethackal	5.2	1128.1	19.9 (1.8)
2.	Chithalvetty	5.5	1610.4	22.9 (1.4)
3.	Kaliar	5.3	982.0	23.8 (2.4)
4.	Karikatoor	5.8	452.3	12.6 (2.8)
5.	Kinalur	6.4	518.6	12.3 (2.3)
6.	Lahai	5.3	356.5	5.4 (1.5)
7.	Mundakayam	6.1	868.4	8.5 (1.0)
8.	Nagercoil	5.5	1186.1	23.3 (2.0)
9.	Thirumbadi	6.2	710.3	8.5 (1.2)
10.	Vellanikkara	6.4	413.6	6.7 (1.6)

Figures in parentheses indicate the percentage of phosphate solubilising bacteria.

reported that optimum pH level for bacteria is five. The phosphate solubilising becteria in rubber growing soils vary from 1 to 2.8 per cent. The differences of the pH of the soil did not cause marked variation in the population of phosphate solubilising bacteria. Besides bacteria a few fungi and actinomycetes were also observed to solubilise phosphates. The results reveal that soil bacteria are major phosphate solubilisers in rubber growing acid soils. Subba Rao (1977) also reported that phosphate solubilising bacteria were more in cultivated soils.

Solubilisation of insoluble phosphates by soil micro-organisms

Table 2 shows that *Asperigillus niger* solubilised more rock phosphate when compared to other isolates of fungi, bacteria and actinomycetes. Soil micro-organism differ widely in the solubilisation of insoluble phosphates. The fungi are found to solubilise more rock phosphate and this may be due to the production of more organic acids by these micro-organisms (Banik and Dey, 1983). Among becteria, *Bacillus* sp. II solubilised more rock phosphate than *Bacillus* sp. I. *Streptomyces* sp., was also found to be a good phosphate solubiliser as seen from the Table 2.

Table 2. Solubilisation of insoluble phosphates by micro-organisms (μg P_2O_5/ml of culture filtrate)

Micro-organism tested	Phosphates tested		
	Rock phosphate	Aluminium phosphate	Ferric phosphate
Aspergillus niger Van Tieghum	21.0	19.5	4.0
Aspergillus flavus Link	19.0	7.0	—
Bacillus sp. I	4.0	4.8	4.5
Bacillus sp. II	19.0	—	4.0
Streptomyces sp.	17.0	—	1.4

Micro-organisms solubilise more rock phosphate than other types of insoluble phosphates. Similar results were reported by Banik and Dey (1983). Acid soils are not rich in *Aspergillus* population as evident in the present investigation. Hence inocula-

tion of *A. niger* in rock phosphate before its application to the soil may augment the phosphate uptake by the plants.

Among different cultures of micro-organisms tested, *A. niger* is found to solublise more aluminium phosphate followed by *A flavus*. *Bacillus* sp. I. A poor rock phosphate solubiliser also solubilised aluminium phosphate while *Bacillus* sp. II which was found to be a good rock phosphate solubiliser failed to solubilise aluminium phosphate. Therefore, it is clear that micro-organisms differ very much in their capacity to solubilise different forms of insoluble phosphates. All the micro-organisms except *A. flavus* had a little action on ferric phosphate. The solubilisation of ferric phosphate by *A. niger* may be due to acid production, while the solubilisation of ferric phosphate by the bacteria, which produce less acid, may be due to the production of hydrogen sulphide (Bromfield, 1953). The results show that *A. niger* is capable of solubilising more quantity of insoluble rock phosphate and bound aluminium and ferric phosphates compared to other micro-organisms.

Effect of carbon sources on the solubilisation of rock phosphate by micro-organisms

The results of the study on the effect of different carbon sources in solubilising rock phosphate reveal, that *A. niger* is capable of solubilising more phosphate when fructose is added in the medium (Table 3). Fructose was found to be a good carbon source for the phosphate solubilisation by micro-organisms, except

Table 3. Effect of different sugars on the solubilisation of rock phosphate (μg P_2O_5/ml of culture filtrate)

Micro-organism tested	Carbon source			
	Glucose	Fructose	Mannitol	Xylose
Aspergillus niger Van Tieghum	21.0	34.7	24.7	2.0
Aspergillus flavus Link	19.0	17.7	6.2	7.5
Bacillus sp. I	4.0	17.7	14.0	2.0
Bacillus sp. II	19.0	5.4	20.0	—
Streptomyces sp.	17.0	22.7	2.2	26.0

Bacillus sp. II, which gave good results when glucose or mannitol was present in the medium. Mannitol was also found to favour micro-organisms in solubilising phosphates, while xylose was a poor carbon source. However, *Streptomyces* sp. solubilised more rock phosphate when xylose was used as carbon source. From the results, it is evident that simple sugars like glucose and fructose favour the micro-organisms for the effective solubilisation of rock phosphate. This may be due to more acid production with these sugars (Banik and Dey, 1983). It is also interesting to note that *A. niger* and *Bacillus* sp. I and II solubilise more rock phosphate in the presence of mannitol, which indicates sugar alcohol is also preferred by soil micro-organisms in solubilising insoluble rock phosphate.

Effect of nitrogen sources on the solubilisation of rock phosphate

Asperigillus niger and *A. flavus* solubilised compartively more rock phosphate in all the nitrogen sources tested (Table 4). Maximum solubilisation was recorded in *A. niger* inoculated medium containing sodium nitrate. *Streptomyces* sp. solubilised more rock phosphate in the presence of ammonium sulphate followed by sodium nitrate.

Table 4. Effect of nitrogen sources on the solubilisation of rock phosphate (μg P_2O_5/ml of culture filtrate)

Micro-organism tested	Nitrogen source		
	Ammonium sulphate	Sodium nitrate	Urea
Aspergillus niger Van Tieghum	21.0	31.2	29.5
Aspergillus flavus Link	19.0	26.2	22.5
Bacillus sp. I	4.0	11.7	4.7
Bacillus sp. II	19.0	17.2	9.5
Streptomyces sp.	17.0	6.2	—

This result shows that soil fungi like *A. niger* and *A. flavus* are capable of solubilising rock phosphate and bound phosphate like aluminium and ferric phosphates utilising simple sugars and various nitrogen sources. As the population of phosphate solubi-

lising fungi in acid soils is less, it is possible to augment the phosphate solubilisation by soil inoculation using phosphate solubilising fungi.

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DISCUSSION

Q : Have you done any work on the solubilisation of rock phosphate by mycorrhiza?

Ans: The present study does not include this aspect. A separate study on mycorrhiza is in progress.