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PHOSPHORUS SOLUBILIZATION BY SOME MARINE FUNGI

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ABSTRACT

Ten marine fungi were qualitatively screened for phosphorus solubilizing ability. The organisms were then tested quantitatively in liquid media containing tricalcium phosphate as a phosphorus source. The optimum pH for phosphorus solubilizing activity of fungi was from 6 to 6.5. An incubation period of 6 days was required for an appreciable quantity of phosphorus to be leached into the medium. A maximum amount of 18% phosphorus was obtained in the culture filtrate and about 54% phosphorus was found in the total cell mass with *Penicillium funiculosum*.

INTRODUCTION

Although enough phosphorus is found in the marine environment it is often not available as a nutrient, because of its presence in an insoluble form. The bones of fishes, rock bark of marine plants and polyphosphates in microorganism are not available as nutrients for most marine algae and edible seaweeds. In this study it has been shown that certain marine fungi present in sea water play an important role in solubilizing the unavailable phosphorus in nature and making it available as a nutrient for other forms. Although considerable work has been done on the physiology of phosphorus solubilizing microorganisms of the terrestrial origin (Wani, More and Patil, 1979; Goswani and Sen, 1962; Mehta and Bhide, 1970), the phosphorus solubilizing microorganisms of the marine environment have not been studied well. Naik and D'Souza (1980) have indicated that yeasts also play an important role in mobilising the phosphorus from the sea. In the present study marine fungi were screened for their phosphorus solubilizing ability.

MATERIAL AND METHODS

Ten species of fungi, namely *Aspergillus terreus* Thom Pale strain, *Aspergillus terreus* Thom, *Aspergillus candidus, Aspergillus fumigatus, Verticillium species, Penicillium funiculosum, Trichoderma koningii, Aspergillus terreus, Curvularia species* and *Stemphylium species* isolated by D'Souza, Araujo, Karande and Freitas (1979) were screened for phosphorus solubilization. The phosphorous solubilization by these

organisms was qualitatively tested on Czapek–Dox agar plates. The medium consisted of $NaNO_3$, 2 g; KCl, 0·5g; $MgSO_4$ 0·5 g; $FeSO_4 \cdot 7H_2O$, traces; glucose, 3% and 2% tricalcium phosphate as a source of phosphorus with pH 5·6. The plate were inoculated with a loopful of spores of the above mentioned organisms, incubated at 30°C for 3–4 days and the phosphorus solubilization was observed by the clear zone that developed around the colonies.

Quantitative determination of phosphorus solubilization was carried out in 100 ml of the above mentioned medium having 0·5% tricalcium phosphate as a substrate. The media were inoculated with the fungal spores (12 to 15 days old slants) in Czepek–Dox medium. The seeded flasks were then allowed to grow on a rotary shaker at 30°C. On the 5th day the cell mass was harvested by straining through a cheese cloth. The cells were then washed with 10% HCl to remove the unutilized tricalcium phosphate and finally washed with water, dried in an oven at about 80°C.

Phosphorus in cell mass and in the culture filtrate was then estimated by employing modified Molybdateascorbic acid method as described by Herbert, Phipps and Strange (1971). For the cultures producing pigments, 1 gm of activated charcoal was added to the culture filtrate and after shaking for a few minutes it was filtered (Mehta and Bhide, 1970). The concentration of phosphorus was found out by referring it to a standard curve prepared by using A. R. grade KH_2PO_4. The colour intensity of the phospho–molybdate complex was measured by spectrophotometer at 730 nm. Organisms showing maximum phosphorus solubilization were then selected for further study.

The effect of pH of the medium used for phosphorus solubilization by the selected isolate was studied as follows: 100 ml portions of Czepek–Dox media with pH values ranging from 5·6 to 8 and containing the same amount of tricalcium phosphate (0·5%) were inoculated with the spores of *Penicillium funiculosum* and allowed to grow on a rotary shaker at 30°C. On the 5th day the cells were separated by straining through a cheese cloth. The filtrate was centrifuged at 6000 × g for 20 minutes and the amount of phosphorus in the culture filtrate was estimated.

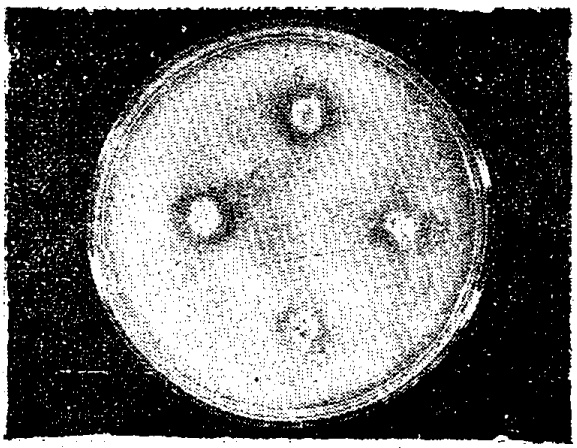

Fig. 1. Plate showing phosphorus solubilization by *Penicillium funiculosum.*

To study the effect of incubation period on phosphorus solubilization 100 ml portions of sterilized Czepek–Dox media pH6 having 0·5% tricalcium phosphate as a substrate were inoculated with spores of *Penicillium funiculosum* and incubated at 30°C. At every 24–hour interval the amount of inorganic phosphorus formed in the culture filtrate was determined adopting the procedure mentioned above.

RESULTS AND DISCUSSION

From the results given in Table I, it can be seen that 7 out of 10 marine fungi used, possess the ability to solubilize phosphorus. One of the isolates (*Penicillium funiculosum*) showed the maximum ability as it had produced a clear larger zone around the colonies (Fig. 1).

Results obtained from the experiments on the effect of pH showed that the optimum pH for better phosphorus solubilization was 6. Phosphorus solubilization was poor at pH 5.6 and 8.0 (Fig. 2). Wani, More and Patil (1979) and Naik and D'Souza (1980) observed the optimum pH for phosphorus solubilizing activity lies between 6 and 7 for fungi; between 7 and 8 for bacteria and between 6 and

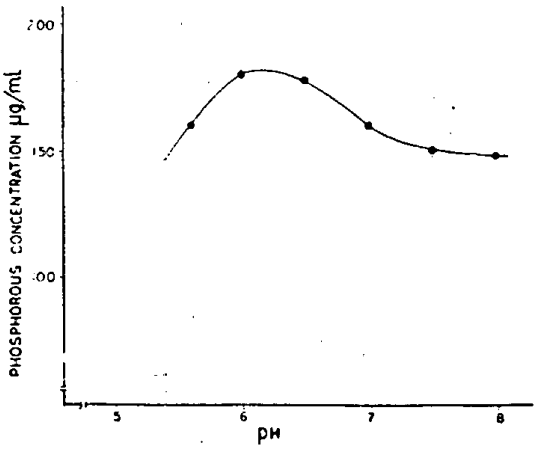

Fig. 2. Effect of pH on phosphorus solubilization.

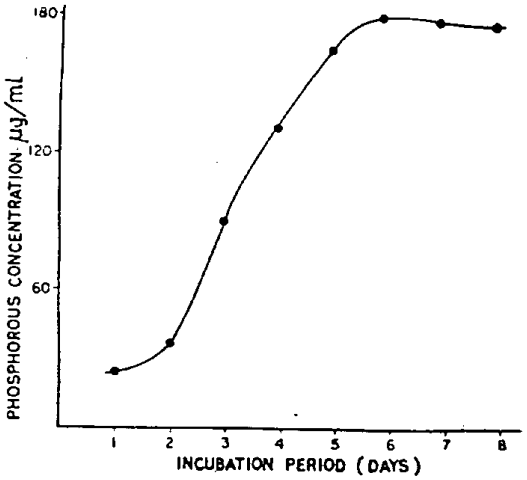

Fig. 3. Effect of incubation period on phosphorus solubilization.

Table I. Qualitative and quantitative screening of fungi for phosphorus solubilization.

Organism	Qualitative	Quantitative			
	Phosphorus solubilizing ability	Phosphorus concentration in culture filtrate mg/ml	Dry weight of cell mass mg	Percent of phosphorus in dry cell mass	Total phosphorus culture filtrate & cell mass mg/100 ml
Aspergillus terreus Thom Pale strain	+++	0·098	1620	3·2	60·64
Aspergillus terreus Thom	+	0·020	1600	2·72	46·12
Aspergillus terreus	–	0·010	1250	2·75	33·37
Verticillium species	–	0·012	1050	2·6	26·30
Penicillium funiculosum	++++	0·180	1700	3·2	70·4
Aspergillus candidus	+	0·010	1300	2·7	40·0
Aspergillus fumigatus	+	0·020	1500	2·68	42·2
Curvularia species	+++	0·110	1650	2·90	46·80
Stemphylium species	–	0·010	1210	2·60	31·46
Trichoderma koningii	++	0·025	1580	3·02	46·70
Control	–	0·010	–	–	–

– = Negative + = Poor ++ = Moderate +++ = Good ++++ = Very good

6.5 for yeasts. Although pH is an important factor, no major change in the pH of the media was observed during the growth of the organisms.

The effect of incubation period showed that the fungi grew fast in the medium resulting in the formation of a thick suspension with a slow release of phosphorus from the medium. Maximum growth was reached on the 6th day (Fig. 3). Goswani and Sen (1962) have shown that maximum solubilization of insoluble phosphate occurred within 15 days under controlled conditions.

The isolate *Penicillium funiculosum* released about 18% phosphorus in the culture filtrate with a good amount of dry cell mass which had utilized about 54% of phosphorus (Table I), thus indicating that fungi play a very important role in mobilizing phosphorus in an aquatic environment and thereby channelising the growth of plants by making the phosphorus available.

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