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Occurrence and activity of phosphate-solubilizing fungi from coconut plantation soils*

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Summary The occurrence of phosphate-solubilizing fungi in coconut plantation soil types was investigated. The laterite, alluvial and clayey soils harboured more of the P-solubilizing fungi than the sandy soils. The isolated P-solubilizing fungi solubilized 26 to 74 per cent of the tri-calcium phosphate in 5 to 15 days. The competitive saprophytic ability of the active P-solubilizing fungi in soil varied between the isolates. Eight fungi with high P-solubilizing capacity and high competitive saprophytic ability were recognised. They have better capacity to survive in soil and express their role in P-solubilization.

Introduction

Many soil micro-organisms are known to solubilize insoluble forms of inorganic phosphatic compounds. *In vitro* studies with microbial isolates from soil indicated that fungi were more efficient in the solubilization of inorganic phosphates as compared to bacteria[2,3,4,13]. Efficient P-solubilizing fungi could be used as inoculants to better the utilization of low-cost insoluble P sources like rock phosphate as fertilizer nutrient for crop plants. Failure to obtain response to inoculation in some soils is attributed to the natural occurrence of a large population of P-solubilizing micro-organisms in such soils or due to the poor competitive saprophytic ability of the inoculated cultures[4].

The application of 320 g of P_2O_5 per palm is recommended in coconut cultivation[1]. Wahid *et al.*[14] reported that P application to coconut could be withheld for a few years when sufficient P reserves are built up in soil due to fertilization. But McLean and Logan[8] reported that the applied fertilizer phosphorus is converted to insoluble forms in soil. This would again call for an investigation on the role of micro-organisms in mobilizing insoluble forms of P in soil.

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The objectives of the present study were to estimate the population of P-solubilizing fungi in several coconut plantation soils of Kerala (India) and to isolate efficient P-solubilizers and study their P-solubilizing efficiency. The ability of the P-solubilizing fungi to survive and establish in soil was also investigated.

Materials and methods

Population estimates of P-solubilizing fungi

Surface 0–30 cm depth soil samples were collected from 32 locations under different agro-climatic conditions in the coconut growing tract of Kerala. The samples were obtained using 25 mm diameter core sampler and transported to the laboratory in sealed polythene bags. The samples were stored in refrigerator at 5°C and analysed as early as possible. Eight samples were obtained from each of the four soil types *viz.* lateritic, sandy, alluvial and clayey. The basic properties of the different soils are presented in Table 1.

Table 1. General characteristics of soils

	Sandy	Laterite	Alluvial	Clayey
pH	6.3	5.9	5.6	5.5
Organic carbon %	0.24	0.61	0.90	0.82
Water holding capacity %	19.2	38.1	42.4	46.4
Total P %	0.041	0.062	0.068	0.077
Available P %	0.0011	0.0007	0.0010	0.0008

The P-solubilizing fungal population in soil was estimated by dilution plate counting technique using Sperber's agar[12] modified with streptomycin and rose bengal. The medium contained tricalcium phosphate of 100–150 meshes as insoluble P source. Active P-solubilizers were recognised by clear zones of solubilization around the colony after four days incubation. The diameter of the clearing zones produced by different fungi ranged from 1.5 to 3.5 cm on agar media. Thirty seven representative isolates were obtained from the different soil types.

In vitro *P-solubilizing activity of fungal isolates*

Aliquots of 50 ml Pikovskaya medium[11] were apportioned into 250 ml EM flasks and insoluble tricalcium phosphate was added to obtain a concentration of 50 mg phosphorus per flask and autoclaved at 10 lb for 30 min. The flasks were inoculated with 9 mm diameter mycelial discs drawn through a cork borer along the margin of actively growing cultures of fungi on potato dextrose agar. At the end of 5, 10 and 15 days incubation at 30 ± 1°C duplicate flasks of each fungus were withdrawn, the contents were filtered and the soluble P-content in the filtrate was analysed calorimetrically following the vanadomolybdic acid yellow colour method[5]. The percentage solubilization of P by the fungal isolates was estimated after subtracting the amount of P solubilized by autoclaving. Titratable acidity was determined by volumetric titration procedure with 0.1 *N* NaOH. The pH of the filtrate and dry weight of the mycelium (dried at 70°C to constant weight) were also recorded. The relative efficiency of fungal isolates to solubilize insoluble tricalcium phosphate was determined with reference to a standard inoculant strain of phosphate-solubilizing culture of *Aspergillus niger* obtained from the Division of Microbiology, Indian Agricultural Research Institute, New Delhi.

Survival of P-solubilizing fungi in soil

The competitive saprophytic ability (CSA) of those fungi whose P-solubilizing efficiency equalled or excelled the standard *A. niger* strain was determined by following Cambridge method[7] with slight modifications. The fungal inocula were prepared on rice bran fortified

with 0.2% urea and 0.1% potassium phosphate (w/w). The inoculum was added to a fertile garden soil to obtain the following ratios of soil:inoculum-50:50, 80:20, 90:10 and 98:2 and filled into plastic boxes. The physico-chemical characteristics of the soil were: sand 58.5%, silt 11.2%, clay 30.3%, organic C 0.82% total N 0.08%, available P 0.0010% and pH 5.5. One cm long rice straw bits were implanted in the different soil-fungal inoculum mixtures and moisture content of the soil was adjusted to 60 per cent of water-holding capacity. After 15 days of incubation at 35°C the straw bits were examined for the colonization of fungi microscopically. Sporulation of inoculated fungi within straw bits was considered as positive for successful colonization. Twenty five bits were examined at each inoculum dilution and the colonization of maximum number of bits at a low inoculum potential was considered as the criterion for better competitive saprophytic ability.

Results

The data on the population of P-solubilizing fungi in soil is presented in Table 2. The fungi occurred in most soils examined and the lateritic, alluvial and clayey soils had higher populations than sandy soils. The populations varied considerably among the different locations in each soil type. A maximum population of 23.69×10^3 was recorded from a lateritic soil type and no P-solubilizing fungi were recorded from one location each in sandy and clayey soils.

Table 2. Population of phosphate solubilizing fungi in coconut plantation soils

	Population $\times 10^3$/g oven dry soil									
Soil type	1*	2	3	4	5	6	7	8	Mean	S.E.**
Sandy	4.08	3.67	1.35	3.15	0.00	2.77	1.77	1.07	2.23	0.50
Alluvial	6.59	2.72	0.68	4.04	0.40	4.28	11.58	14.46	5.59	1.79
Clayey	11.63	9.44	7.90	0.00	4.81	6.12	1.09	9.18	6.27	1.45
Lateritic	5.00	23.69	0.67	5.15	3.43	3.09	0.36	0.70	5.26	2.72

* Locations
** Standard error of the mean

The data on P-solubilizing capacities and changes in p^H and titratable acidity of the culture broth and dry weight of mycelium during fungal growth are presented in Table 3. The period of growth for optimum solubilization and extent of solubilization varied between the different fungi. The quantity of insoluble P solubilized varied from 26 to 74 per cent among the different fungi. A number of fungi exhibited maximum solubilization after 5 days of growth, while others needed 10 or 15 days for maximum solubilization. The standard *A. niger* strain solubilized 68 per cent of the insoluble P supplied in 15 days time. The P-solubilizing fungi belonged to three genera *viz*; Aspergillus, Penicillium and Phialotubus, the dominant ones being the former two genera. Aspergillus group were more widely distributed in coconut plantation soils studied and exhibited on an average a better activity in P-solubilization (62 per

Table 3. *In vitro* solubilization of Tricalcium phosphate by fungal isolates

Isolate No.	Organism	Percent P solubilized *	pH	Titratable acidity (meq/50 ml)	Mycelial dry wt (mg/50 ml)	Relative efficiency+
1	*Aspergillus* sp.	69.5 (5)	2.55	3.51	294.5	102.2
2	*Aspergillus* sp.	72.0 (5)	2.45	3.54	260.5	105.9
3	*Aspergillus* sp.	62.5 (5)	4.00	2.08	304.5	91.9
4	*Aspergillus* sp.	46.5 (10)	4.05	2.48	300.0	68.4
5	*Aspergillus* sp.	65.0 (5)	4.30	2.07	235.0	95.6
6	*Penicillium* sp.	39.5 (10)	4.60	1.63	337.0	58.1
7	*Penicillium* sp.	26.0 (15)	5.30	1.16	259.0	38.2
8	*Aspergillus* sp.	51.0 (5)	4.60	2.06	256.5	75.0
9	*Aspergillus* sp.	54.0 (15)	5.00	1.83	341.5	79.7
10	*Penicillium* sp.	54.0 (10)	4.70	1.68	264.5	79.4
11	*Aspergillus* sp.	64.5 (15)	2.95	2.61	382.0	94.9
12	*Penicillium* sp.	42.5 (15)	4.85	2.61	396.0	62.5
13	*Penicillium* sp.	45.0 (5)	4.15	2.20	166.0	66.2
14	*Penicillium* sp.	41.0 (10)	5.10	1.71	382.5	60.3
15	*Penicillium* sp.	49.0 (15)	5.10	1.56	277.0	72.1
16	*Penicillium* sp.	38.5 (10)	5.50	1.44	375.0	56.6
17	*Penicillium* sp.	47.0 (15)	4.80	1.69	333.0	69.0
18	*Aspergillus* sp.	65.0 (15)	2.55	2.43	251.0	95.6
19	*Aspergillus* sp.	74.0 (15)	2.50	2.50	246.0	108.8
20	*Aspergillus* sp.	68.0 (10)	2.90	2.12	260.0	100.0
21	*Penicillium* sp.	65.0 (15)	2.50	2.33	315.0	95.6
22	*Penicillium* sp.	68.0 (10)	2.70	2.07	231.5	100.0
23	*Phialotubus* sp.	56.0 (15)	4.40	2.00	308.0	82.4
24	*Penicillium* sp.	69.0 (15)	2.90	2.28	241.0	101.5
25	*Aspergillus* sp.	47.5 (5)	4.50	1.65	266.5	64.7
26	*Penicillium* sp.	40.0 (5)	3.80	2.68	219.0	58.8
27	*Penicillium* sp.	67.0 (5)	3.00	2.68	315.0	98.5
28	*Aspergillus* sp.	63.0 (10)	4.95	1.92	300.0	92.7
29	*Aspergillus* sp.	55.0 (10)	5.45	1.83	313.5	80.9
30	*Aspergillus* sp.	50.0 (5)	4.05	2.75	243.0	73.5
31	*Penicillium* sp.	34.0 (10)	4.35	1.85	273.0	50.0
32	*Aspergillus* sp.	71.0 (10)	3.65	2.53	290.0	104.4
33	*Penicillium* sp.	48.0 (5)	3.80	2.63	186.5	70.6
34	*Aspergillus* sp.	51.0 (15)	4.15	2.05	324.0	75.0
35	*Penicillium* sp.	53.5 (5)	3.70	2.81	283.0	77.9
36	*Aspergillus* sp.	71.0 (10)	2.30	2.92	349.0	104.4
37	*Aspergillus* sp.	72.0 (10)	3.10	2.61	281.0	105.9
38	*Aspergillus niger* (I.A.R.I.)	68.0 (15)	2.55	2.80	303.0	100.0

* Figures in parenthesis indicate incubation period (days) for optimum solubilization.
+ In relation to inoculant strain of *Aspergillus niger* isolate No. 38.

cent) as compared to the mean activity of isolates of Penicillium (49 per cent).

A decrease in pH and a rise in the titratable acidity always accompanied the growth of P-solubilizing fungi in broth culture. The more efficient phosphate solubilizers were also found to decrease the

pH of the culture medium to a significant extent. Correlation studies with the changes in pH, titratable acidity and P-solubilization showed a significant inverse relationship with pH and positive relationship with titratable acidity (Table 4). Mycelial dry weight did not correlate well with per cent P solubilized.

The results obtained on the competitive saprophytic ability of the fungal isolates are presented in Table 5. Isolate numbers 19 and 36 were efficient in their CSA as they extensively colonized the rice straw even at the low inoculum potential of two per cent. Isolate 19 was competitively more active and also was the most efficient P-solubilizer. A few other fungi (Isolates 2, 5, 24, 27, 32, 37) also were saprophytically competent as they colonized more than 25 per cent of the straw bits at a low inoculum level of two per cent.

Table 4. Correlation of per cent P-solubilized with pH and titratable acidity

P-solubilizing fungi	Correlation coefficient	
	% P-solubilized *vs* pH	% P-solubilized *vs* titratable acidity
P-solubilizing fungi	-0.75^{**}	0.57^{**}
Aspergillus spp.	-0.67^{**}	0.41^{**}
Penicillium spp.	-0.81^{**}	0.50^{**}

** Significant at 1% level.

Table 5. Competitive saprophytic ability of efficient P-solubilizing fungi

Isolate* number	Organism	Percentage of straw bits colonised				Efficiency class***
		50:50**	80:20	90:10	98:2	
1	*Aspergillus* sp.	40	28	28	16	–
2	*Aspergillus* sp.	48	40	28	28	+
5	*Aspergillus* sp.	100	100	60	32	+
18	*Aspergillus* sp.	100	80	32	12	–
19	*Aspergillus* sp.	100	76	80	68	++
20	*Aspergillus* sp.	100	64	0	0	–
21	*Penicillium* sp.	40	48	40	40	+
22	*Penicillium* sp.	56	56	32	24	+
24	*Penicillium* sp.	100	100	48	28	+
27	*Penicillium* sp.	40	28	52	28	+
32	*Aspergillus* sp.	100	68	80	44	+
36	*Aspergillus* sp.	100	100	80	80	++
37	*Aspergillus* sp.	100	56	64	48	+
38	*Aspergillus niger* (Inoculant strain)	100	84	76	52	++

* Isolate number with reference to Table 3.
** Soil : inoculum ratio
*** (–) Less than 25 per cent colonisation at 98 : 2 inoculum potential (IP)
(+) More than 25 per cent colonisation at 98 : 2 I.P.
(++) More than 50 per cent colonisation at 98 : 2 I.P.

Discussion

The population of P-solubilizing fungi varied with different soils examined. The lower population of P-solubilizing fungi observed in sandy soils as compared to laterite, alluvial and clayey soils is possibly due to the low organic matter content of the sandy soils. Also the moisture retentive capacity of these sandy soils was low (Table 1).

The P-solubilizing fungi in coconut soils mainly belonged to the *Aspergillus-Penicillium* group. The previous reports of several other workers also showed the dominance of these two genera of fungi among the P-solubilizing fungal isolates from soil[3,4,6,10]. The recognition of Phialotubus as an active P-solubilizer is reported for the first time. As many as nine fungi were more efficient than the *A. niger* strain used as the standard P-solubilizer and five others were comparable to the standard strain. The efficient P-solubilizing fungi were isolated from all the four soil types, but limited to some locations.

The extent of reduction in pH correlated well with the P-solubilization obtained. In broth culture a fall in pH of the medium during the growth of P-solubilizing microorganisms has already been reported[2,3]. The optimum pH for solubilization of phosphorus from insoluble phosphorus compounds has been found to be 4.0 for fungi such as Penicillium[2]. In the present study it was found that pH of the medium fell within a range of 2.5 to 4.5 from the initial pH of 6.0 when the fungi expressed active solubilization of P. The growth of many of the Aspergilli and some Penicillia led to a significant reduction of pH to 2.3–3.0. A highly significant correlation between the reduction in pH and P-solubilization and increase in titratable acidity and P-solubilization was evident from these studies thereby stressing the role of the formation of acidic constituents like organic acids in P-solubilization in broth culture. A pH change of this magnitude does not take place in soil due to its buffering capacity.

Studies on the competitive saprophytic ability indicated that a few of the fungi were very competitive and established well even at a low inoculum potential of 2 per cent. This is perhaps the first report on the competitive saprophytic ability of the P-solubilizing fungi in soil. Literature on the effects of inoculation of P-solubilizing micro-organisms on crop growth shows that in a few instances the response was significant, while in others there was no response to inoculation[4]. *In vitro* activity of these organisms in solubilizing insoluble phosphate need not always lead to a response under field conditions. One of the principal reasons for the variation in response of the P-solubilizing micro-organisms under *in vitro* and field conditions is due to the operation of several ecological factors which ultimately affect the growth and

multiplication of an actively P-solubilizing micro-organism in the soil. The soils low in organic matter may not provide a favourable environment for the multiplication of inoculants of P-solubilizers. In addition, most of the soils are well buffered against large variations in pH observed in culture broth during the growth of P-solubilizing micro-organisms. The P-fixation capacity of soil also might play a role in limiting the effect of inoculation of P-solubilizers by converting newly released soluble P to insoluble forms thereby controlling the plant-available P concentration in soil.

The information on CSA is important in assigning relative role of different heterotrophic fungi in P-solubilization in soil. From this study it could be postulated that in the natural soil ecosystem it is these fungi endowed with a better capacity to survive and multiply in soil, which are likely to be more actively involved in solubilization of phosphate. The fungi possessing high P-solubilizing capacities coupled with a high competitive saprophytic ability will have a better chance of surviving and expressing their role in P-solubilization. Studies are in progress to evaluate the effect of inoculation of the active P-solubilizing fungi recognised from this study on the growth and phosphate nutrition of coconut seedlings.

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