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ISOLATION OF PHOSPHATE SOLUBILIZERS FROM DIFFERENT SOURCES

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SOILS generally contain adequate amount of inorganic and organic phosphorous but most of these remain unavailable to plants. The efficiency of water soluble phosphorous is usually low and its recovery does not exceed 20% because soluble phosphorous when added to soil form $Ca_3(PO_4)_2$ in calcareous and alkaline soils and $FePO_4$ and $AlPO_4$ in acidic soils[1]. Several workers[2–4] have proposed the utilization of low grade phosphorous by the use of phosphate dissolving micro-organisms.

In India there are large deposits of phosphatic rock estimated about 140 million tonnes distributed in different states. Most of these deposits are of low grade (<25% P_2O_5)[5]. Keeping this in view, the present investigation was carried out to isolate the phosphate solubilizers from different sources and their effects on Mussoorie rock phosphate solubilization.

Table 1 *Screening of isolates for tricalcium phosphate solubilization*

Source	No. of isolate	Organism	pH after growth	P_2O_5 solubilization (mg/50 ml)
Compost material	4	*A. niger*		
	As1		3.1	22.5
	As2		3.3	23.7
	As3		3.0	26.9
	As4		3.5	22.8
Wheat rhizosphere	2	*A. niger*		
	As5		3.2	4.9
	As6		3.5	10.5
Garden soil	2	*Trichoderma* sp.		
	Ts1		3.7	3.5
	Ts2		3.9	6.2
Gram rhizosphere	2	*Penicillium fumiculosum*		
	Pf1		3.3	3.4
	Pf2		3.5	7.1
Gram rhizosphere	3	Gram negative bacteria		
	1		5.5	3.7
	2		5.3	3.2
	3		5.1	2.8
Control (without inoculum)	—	—	5.0	—
CD at 5%	—	—	0.31	2.62

Table 2 *Effect of incubation period on tricalcium phosphate and rock phosphate solubilization by A. niger As3*

Incubation period (days)	Tricalcium phosphate			Rock phosphate		
	Control pH	pH after growth	P_2O_5 solubilization (mg/50 ml)	Control pH	pH after growth	P_2O_5 solubilization (mg/50 ml)
3	5.0	3.35	6.80	5.0	4.35	5.10
7	4.9	3.20	12.40	4.8	3.65	7.20
10	4.8	3.10	9.50	4.8	3.80	10.20
13	4.8	3.20	8.10	4.7	4.0	8.50
CD at 5%	NS	0.065	0.392	NS	0.082	0.561

Cultures were isolated from different samples of rhizospheres of wheat, gram, garden soils and compost materials (Instructional Farm, N. D. University of Agriculture and Technology) and they were grown on solid medium containing $Ca_3(PO_4)_2$ as described by Pikovskaya[6]. Of several isolates, only ten fungal and three bacterial cultures were isolated on the basis of clear zone formation around the colonies. Different isolates were characterized and designated as As1, As2, As3, As4, (from compost), As5, As6, (wheat rhizosphere), Ts1, Ts2, (garden soil), Pf1, Pf2 (gram rhizosphere) and gram negative bacteria 1 and 2 from gram rhizosphere.

All the cultures were screened in liquid medium of Pikovskaya using tricalcium phosphate (0.25 g/50 ml) and incubated for seven days at 28 ± 1°C. An uninoculated control was also kept. Among the efficient isolates, *Aspergillus niger* As3 was found to be most effective for maximum solubilization of tricalcium phosphate (table 1).

A separate experiment was conducted to evaluate the effect of *A. niger* As3 on phosphate solubilization of rock phosphate and tricalcium phosphate equivalent to 50 mg P_2O_5 were added separately in Pikovskaya's broth (pH 5) and incubated for 13 days at 28 ± 1°C. Supernatants were collected by filtration through Whatman No 42 filter paper and soluble phosphate was determined by the methods described by Jackson[7]. The percentage of phosphorous was calculated considering P_2O_5 content of rock phosphate as 20%. All the experiments were repeated four times with six replications.

Among the 13 isolates, *A. niger* As3 was found most efficient and solubilized 26.9 mg P_2O_5/50 ml that is equivalent to 23.5%. Tricalcium phosphate was preferred over rock phosphate by this isolate (table 2). Maximum content of phosphate solubilization enhanced by incubation period (7 to 10 days) in both the cases. However, organic acids production by *A. niger* in growth medium resulted decrease in pH (from 5 to 3.7 on average). Organic acids production and lowering of pH attributed to the phosphate solubilization[8]. However, lowering of pH is not a major factor for phosphate solubilization as evident by data presented in table 1. There is a need to isolate better strains which can solubilize more of phosphate and also able to grow during summer seasons (around 37–45°C).

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BIOSORPTION POTENCY OF HEAVY METALS BY SOME FUNGI

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MICROBES tolerating heavy metals and capable of sequestering them under natural conditions have