Plant and Soil 69, 353–364 (1982). 0032-079X/82/0693-0353$01.80. Ms. 4727

Available phosphate content of an alluvial soil as influenced by inoculation of some isolated phosphate-solubilizing micro-organisms

S. BANIK
University College of Agriculture, Calcutta University, Calcutta 7000 19, India

and B. K. DEY
Department of Agricultural Chemistry and Soil Science, Bidhan Chandra Krishi Viswa Vidyalaya, Kalyani 741 235, India

Received 7 April 1981. Revised March 1982

Key words Available phosphorus Inoculation Manure Rock phosphate Phosphate solubilizers

Summary Among several phosphate-solibilizing micro-organisms isolated from an alluvial soil (Fluvaquent) in sucrose-$Ca_3(PO_4)_2$ agar plates, two fungal strains, ACF_2 (*Aspergillus candidus*) and ACF_1 (*A. fumigatus*) two bacterial strains, ACB_5 (*Bacillus firmus* B-7650) and ACB_6 (*B. firmus* B-7651) and one actinomycete strain, ACS_6 (*Streptomyces* sp.) were efficient solubilizers, solubilizing 297.0, 288.3, 49.0, 45.8 and 29.0 µg of P as free PO_4^{-3}, respectively, containing 15 mg insoluble P from $Ca_3(PO_4)_2$ in broth. Solubilization was lesser from $AlPO_4$ and $FePO_4$. The isolates producing oxalic and tartaric acids without or with citric acid showed higher ability of solubilizing insoluble inorganic phosphates.

All the above isolates possessed the ability of solubilizing rock phosphate in considerable amounts, ACF_1 (*A. fumigatus*) being the highest (31.5 µg), while ACB_6 (*B. firmus* B-7651) and both the aspergilli also possessed cellulose-decomposing ability in addition.

Inoculation of the isolates, in a flask culture experiment, had no significant effect on the accumulation of available phosphorus in soil even when amended with rock phosphate (RP), farm yard manure (FYM), (FYM + RP), rice straw (RS) and (RS + RP). Nevertheless, the overall performance of ACF_2 (*A. candidus*) and ACB_6 (*B. firmus* B-7651) was better than that of the others, in this respect, while ACB_5 (*B. firmus* B-7650) and ACF_1 (*A. fumigatus*) intensified the enhancing effect of FYM and RS. Partial sterilization, by autoclaving, of the soil had no significant effect on available phosphorus content of the soil irrespective of any inoculation.

Introduction

Many common soil micro-organism can dissolve insoluble inorganic phosphates known to occur in soil[13]. The major microbiological means by which insoluble phosphates are solubilized by the production of organic acids[2,13]. As phosphorus compounds in Indian alluvial soils is predominantly inorganic[6], chiefly locked as $Ca_3(PO_4)_2$, the group of phosphate solubilizing micro-organisms dissolving $Ca_3(PO_4)_2$ appears to have an implication in Indian agriculture. Since these are chemoheterotrophs, addition of carbonaceous organic manures greatly enhances their growth and activity in soil, and especially when supplemented with rock phosphate[8,20]. In the present investigation,

* Part of the Ph.D. thesis submitted by the Senior Author to the University of Calcutta in 1979.

several phosphate-solubilizing organisms were isolated, from an alluvial soil (Fluvaquent[9]), identified and characterised in terms of their ability of solubilizing inorganic phosphates and production of organic acids in artificial media and an attempt has been made to obtain information regarding the accumulation of available phosphorus in the same soil as influenced by the inoculation of some efficient $Ca_3(PO_4)_2$, solubilizing isolates in absence and presence of rock phosphate, farm yard manure, and rice straw, either alone or in combinations taking a manure and rock phosphate together, under partially sterilized and non-sterilized conditions in a flask culture experiment.

Materials and methods

The soil – a Gangetic alluvial one (Fluvaquent)[9] – was collected from the Calcutta University Experimental Farm, Baruipur, District 24 Parganas, West Bengal, India. Air-dried 2-mm sieved soil samples were used in the present investigation. General characteristics of the soil were determined in accordance with the methods described by Jackson[14] (Table 1).

Enumeration of total micro-organisms and $Ca_3(PO_4)_2$-solubilizing micro-organisms, together with isolation, identification upto generic level and estimation of phosphate-solubilizing power of and organic acid production by the $Ca_3(PO_4)_2$-solubilizing micro-organisms were done in accordance to the method followed by Banik and Dey[4]. The bacteria solubilizing appreciable amount of insoluble inorganic phosphate were identified upto specific level with the help of Commonwealth Mycological Institute, Kew, Surrey, England and fungi with the help of Mycological Laboratory, Department of Botany, University of Calcutta.

The isolates showing ability of solubilizing quite appreciable amount of inorganic phosphate were selected for inoculation in soil in the flask culture experiments. These were ACB_5 and ACB_6 (*Bacillus firmus* B-7650 and B-7651), ACS_6 (*Streptomyces* sp.), ACF_1 (*Aspergillus fumigatus*) and ACF_2 (*A. candidus*).

As rock phosphate, and organic manures containing appreciable amount of cellulosic materials

Table 1. General characteristics of the soil

Water holding capacity (%)	54.0
pH	7.4
Conductivity (mmho/cm)	0.082
Organic C (%)	0.603
Total N (%)	0.061
Total P (%)	0.065
Available P (%)	0.0007
Total Ca as CaO (%)	1.001
Total Mg as MgO (%)	1.028
Total Fe (%)	5.9
CEC (meq/100 g)	22.05
Exchangeable Ca (meq/100 g)	12.7
Exchangeable Mg (meq/100 g)	7.65
Sand (%)	9.84
Silt (%)	35.04
Clay (%)	55.12
Texture	Silty clay

were among the treatments in the experiment with soil, the cultures were tested for their ability of solubilizing rock phosphate and decomposing cellulose. In addition they were also tested for their ability of producing H_2S, as this is one of the means of liberating phosphate from ferric phosphate present in soil. The ability of solubilization of rock phosphate, decomposition of cellulose and production of H_2S, by the micro-organisms inoculated, were estimated according to the methods adopted by Banik and Dey[5].

Antibiotic-producing activity of the selected micro-organisms were tested on some pathogenic species according to method described by Waksman[22]. However, none of the organisms succeed to do so.

Available phosphorus content of soil as affected by inoculation of the isolates

The alluvial soil, from where the organisms were isolated, were taken in 100 ml conical flask in ten g lots with the following treatments: (a) untreated control, (b) rock phosphate (RP) – 30 kg P ha^{-1}, (c) farm yard manure (FYM) – 40 kg N ha^{-1}, (d) FYM – 40 kg N ha^{-1} + RP – 30 kg P ha^{-1}, (e) rice straw (RS) – 40 kg N ha^{-1} and (f) RS – 40 kg N ha^{-1} + RP – 30 kg P/ha^{-1}. The moisture level was kept at 100% water holding capacity as the subsequent experiment was proposed to be done on rice rhizosphere under flooded conditions. For each treatment, 36 flasks were kept. Half of these flasks were autoclaved at 15 lb per sq inch steam pressure for 30 minutes on two consecutive days to have the soils inside partially sterilized, with a view to compare the activity of the inoculated isolates under a minimal microbial competition. Those flasks containing partially sterilized and non-sterilized soils, per manurial treatment, were inoculated with each isolate in a similar manner as described by Banik and Dey[5]. The flasks were incubated at 30 ± 1°C. The moisture level was maintained by adding sterile distilled water aseptically and shaken in a rotatory shaker half an hour for aeration and homogenisation on every alternate day. After 15 days incubation, the soils were analysed for available phosphorus content following Olsen *et al.*[17] method.

Results

It can be seen from Table 2 that, in the alluvial soil studied, 14.3, 35 and 3 per cents of the total bacterial, actinomycetes and fungal population, respectively,

Table 2. Total and phosphate-solubilizing microbial population of soil before and after partial sterilization*

Soil	Number (× 10^4) per g dry soil**			
	Bacteria	Actinomycetes	Fungi	Total
	Total micro-organisms			
Before autoclaving	840.0	100.0	100.0	1040.0
After autoclaving	2.0	3.0	0.5	5.5
	Phosphate solubilizing micro-organisms P source: $Ca_3(PO_4)_2$			
Before autoclaving	120.0	35.0	2.5	157.5
After autoclaving	2.0	0.0	0.0	2.0

* Partial sterilization done by means of soil autoclaving in thin layers at 15 lb steam pressure for 30 minutes in two consecutive days.

** Average of triplicate sets.

Table 3. Phosphate-solubilizing power of micro-organisms isolated in sucrose tricalcium phosphate agar plates after 7 and 10 days

Organisms		P-solubilized in µg/15 mg insoluble P/0.15 g sucrose (Average of duplicate sets)									Mean for three phos-phates
Coded as	Identified as	$Ca_3(PO_4)_2$			$AlPO_4$			$FePO_4$			
		7	10	Mean	7	10	Mean	7	10	Mean	
ACB_1	*Micrococcus* sp.	0.0	0.3	0.2	0.0	3.8	1.9	0.0	0.3	0.2	0.8
ACB_2	*Arthrobacter* sp.	28.3	30.0	29.2	7.5	8.5	8.0	0.3	0.3	0.3	12.5
ACB_3	Bacillus sp.	21.0	35.0	28.0	0.0	1.3	0.7	0.0	0.0	0.0	9.6
ACB_4	– do –	35.8	42.3	39.1	1.8	5.5	3.7	0.0	1.3	0.7	14.5
ACB_5	*Bacillus firmus* (B-7650)**	35.0	49.0	42.0	4.5	22.3	13.4	1.3	1.8	1.6	19.0
ACB_6	*Bacillus firmus* (B-7651)**	40.5	45.8	43.2	0.0	5.3	2.7	0.0	1.5	0.8	15.6
ACS_1	*Streptomyces* sp.	3.8	3.5	3.7	7.0	17.0	12.0	1.0	0.0	0.5	5.4
ACS_2	– do –	4.5	3.5	4.0	11.3	1.3	6.3	0.5	0.3	0.4	3.6
ACS_3	– do –	2.3	4.5	3.4	0.0	13.5	6.8	1.5	0.0	0.8	3.7
ACS_4	– do –	20.0	18.0	19.0	1.5	8.0	4.8	3.0	0.0	1.5	8.4
ACS_5	– do –	10.0	24.0	17.0	0.0	0.0	0.0	0.0	0.0	0.0	5.7
ACS_6	– do –	15.5	29.0	22.3	4.8	17.5	11.2	1.3	0.0	0.7	11.4
ACS_7	– do –	8.5	8.0	8.3	0.0	8.0	4.0	0.0	0.0	0.0	4.1
ACS_8	– do –	4.5	10.0	7.3	6.0	13.0	9.5	1.3	0.0	0.7	5.8

Table 3 (*continued*)

Organisms		P-solubilized in µg/15 mg insoluble P/0.15 g sucrose (Average of duplicate sets)									Mean for three phos-phates
Coded as	Identified as	$Ca_3(PO_4)_2$			$AlPO_4$			$FePO_4$			
		7	10	Mean	7	10	Mean	7	10	Mean	
ACF_1	*Aspergillus fumigatus*	212.0	288.3	250.2	13.8	18.8	16.3	21.0	21.5	21.3	95.9
ACF_2	*Aspergillus candidus*	176.0	297.0	236.6	11.0	24.0	17.5	50.0	21.5	35.8	96.6
ACF_3	*Aspergillus* sp.	56.3	93.8	75.1	17.8	56.0	36.9	20.0	26.0	23.0	45.0
ACF_4	– do –	24.0	37.5	30.8	6.8	10.5	8.7	0.0	0.0	0.0	13.2
ACF_5	– do –	5.5	18.8	12.2	2.8	16.5	9.7	0.0	0.0	0.0	7.3
ACF_6	Penicillium sp.	59.8	95.0	77.4	0.0	20.0	10.0	0.0	2.3	1.2	29.5
Mean		38.2	56.7	47.5	4.8	13.5	9.2	5.1	3.8	4.8	20.4
BKM*	*Bacillus megatherium* var phosphaticum-847	54.5	80.5	67.5	0.0	1.5	0.8	2.5	6.0	4.3	24.2

* Obtained from 'Academy of Sciences' Moscow, USSR.
** CMI code numbers.

	L.S.D. at 5%	L.S.D. at 1%
For phosphate source (P)	4.8	11.4
Organisms (O)	10.4	14.8
Interaction (P × O) – two P at same O	17.9	25.7
Interaction (P × O) – two O at same or different P	18.6	28.1

possesed the ability of solubilizing $Ca_3(PO_4)_2$. There was a significantly positive correlation between the number of the above phosphate solubilizers and total number of soil micro-organisms ($r = +0.96$) showing a linear relationship with the regression equation $y = -4 + 0.14x$. Partial sterilization, by autoclaving, eliminated more than 99% of total bacteria and fungi and 97% of the total actinomycetes, while eliminating more than 98% of the phosphate-solubilizing bacteria and all the actinomycetes and fungi active in the process.

The phosphate solubilizers isolated included bacteria of the genera Bacillus, Micrococcus and Arthrobacter, fungi of the genera Aspergillus and Penicillium and actinomycetes of the genus Streptomyces. The isolates solubilizing appreciable amount of insoluble inorganic phosphates were tabulated in Table 3. It is evident from Table 3 that the highest amount of insoluble inorganic phosphate was solubilized by ACF_2 (*Aspergillus candidus*) followed by ACF_1 (*A. fumigatus*), ACB_5 (*Bacillus firmus* B-7650), ACB_6 (*B. firmus* B-7651) and ACS_6 – (*Streptomyces* sp.). In general, the solubilization was highest from $Ca_3(PO_4)_2$ followed by that from $AlPO_4$ and $FePO_4$ excepting for the aspergilli which solubilized more from $FePO_4$ than from $AlPO_4$. All the cited isolates excepting ACB_5 (*B. firmus* B-7650) produced oxalic acid (Table 4), highest being by ACF_1 (*A. fumigatus*). In addition citric and tartaric acids were produced by ACF_1, tartaric acid by ACF_2 and succinic and an unidentified one by ACB_6. ACB_5 produced succinic acid and a lesser amount of 2-ketogluconic acid. USSR superstrain *B. megatherium* var. phosphaticum (BKM) produced the cited unreported organic acid only.

Ability of solubilizing rock phosphate (containing 4.85% phosphorus) was in the order by ACF_1, ACF_2, ACB_5, ACB_6 and ACS_6 and that of cellulose decomposing ability by ACB_6, ACF_1 and ACF_2 as evidenced from the deterioration of the filter paper strips (Table 5). The presented sequence in the text would denote the order. ACB_5 gave a mucoid growth, ACB_6 a turbid and mucoid, ACS_6 submerged pellicle, and the ACF_1 and ACF_2 a mycellial pad with black spore in sucrose-rock phosphate broth. None produced H_2S or possessed any antibiotic activity.

The available phosphorus content of partially sterilized soil was more than that of the non-sterile soil under control untreated and uninoculated series (Table 6). Presence of FYM or RS significantly increased the available phosphorus content of both the soils irrespective of any inoculation. This was true for RS + RP in the case of non-sterile soil. The effect of FYM in non-sterile soil was enhanced with the inoculation of ACB_5 (*B. firmus* B-7650) and ACF_1 – (*A. fumigatus*); that of RS in partially sterilized soil with the inoculation of ACF_2 (*A. candidus*), ACF_1, ACB_5 and ACB_6 (*B. firmus* B-7651); and that of RS + RP in non-sterile soil with the inoculation of ACF_2, ACB_6 and ACF_1. A significantly increased amount of available phosphorus was also accumulated in partially sterilized soil under RS + RP when inoculated with ACF_2. RP alone or with FYM decreased the available phosphorus content of the soil which was reversed

Table 4. Organic acids produced in sucrose-calcium phosphate broth after ten days incubation by the micro-organisms

Organisms		Organic acids produced	
Coded as	Identified as	Identified as	Amount in mg/0.15 g sucrose
ACB_1	*Micrococcus* sp.	Oxalic	6.750
ACB_2	*Arthrobacter* sp.	Oxalic	4.950
		Malonic	2.925
ACB_3	*Bacillus* sp.	Oxalic	4.875
		Succinic	3.188
		Unidentified-I	–
ACB_4	– do –	Oxalic	4.613
		Succinic	4.238
		Unidentified-I	–
ACB_5	*Bacillus firmus* (B-7650)**	2-Ketogluconic	4.500
		Succinic	4.725
ACB_6	*Bacillus firmus* (B-7651)**	Oxalic	5.100
		Succinic	5.363
		Unidentified-I	–
ACS_1	*Streptomyces* sp.	2-Ketogluconic	0.300
		Unidentified-I	–
ACS_2	– do –	2-Ketogluconic	1.500
		Tartaric	0.300
ACS_3	– do –	Oxalic	4.725
ACS_4	– do –	– do –	4.538
ACS_5	– do –	– do –	3.713
ACS_6	– do –	– do –	2.288
ACS_7	– do –	Oxalic	5.250
		Succinic	2.175
		Unidentified-I	–
ACS_8	– do –	Oxalic	3.750
		Tartaric	1.350
ACF_1	*Aspergillus fumigatus*	Oxalic	6.063
		Tartaric	2.625
		Citric	3.225
ACF_2	*Aspergillus candidus*	Oxalic	1.613
		Tartaric	1.575
ACF_3	*Aspergillus* sp.	Oxalic	1.650
		Citric	4.575
ACF_4	– do –	Oxalic	6.975
ACF_5	– do –	– do –	3.900
ACF_6	*Penicillium* sp.	– do –	5.400
BKM*	*Bacillus megatherium* var *phosphaticum*	Unidentified-I	–

Unidentified-I – bromocresol green positive substance – Rf between 2-Ketogluconic acid and Tartaric acid, – Amount could not be measured; * and ** See Table 3.

with the inoculation of ACS_6 (*Streptomyces* sp.) in partially sterilized soil supplemented with FYM + RP. Although, comprising all the manurial treatments, inoculation of the isolates alone had no overall significant effect on the available phosphorus content of the soil, the performance of ACF_2 (*Aspergillus candidus*) and ACB_6 (*Bacillus firmus* B-7651) was better than the others, in this respect.

Discussion

The proportion of soil bacteria capable of solubilizing $Ca_3(PO_4)_2$ were well within the range reported earlier[2] (Table 2). The greater heat resistance of the actinomycetes, as compared to the other microflora, was evidenced from the greater survival of the general actinomycetes after partial sterilization by autoclaving (Table 2). But it was not so with the phosphate-solubilizing ones which succumbed to the process along with the earlier reports[5]. The phosphate-solubilizing bacteria which survived were sporeformers. Like earlier observations[1,19] species of Aspergillus and Penicillium among the fungi, Bacillus, Arthrobacter and Micrococcus among the bacteria and *Streptomyces* among the actinomycetes were active in the conversion.

The solubilization by the isolates was not restricted to $Ca_3(PO_4)_2$, on which they grew in agar plates, as aluminium and ferric phosphate were also solubilized, of course to a lesser extent, by them, (Table 3), suggesting a relatively more adaptive nature of the enzymes responsible for solubilizing the latter phosphates. In conformity with the views of Sen and Paul[19], some native Indian strains of soil microflora, *Aspergillus candidus, A. fumigatus* quite capable of solubilizing adequate amount of insoluble phosphates, next to which come *Bacillus firmus* (B-7650, and B-7651). The higher potentiality of the molds in a soil having a neutral reaction (Table 1) might appear to be curious but not surprising as they could make the environment favourably acidic by producing greater amount of polybasic organic acids (Table 4).

The isolates producing oxalic acid and tartaric acids with or without citric acid showed higher ability of solubilization. In the case of bacterial isolates, those producing succinic acid in addition to oxalic or 2-Ketogluconic acid were efficacious. These depict along with earlier reports[10,15,16], that organic acid with dissimilar carbon atoms possessed better activity of phosphate solubilization and oxalic acid and 2-Ketogluconic acid were active by virtue of their ability to form chelate with Ca^{+2} ion in addition to producing H^+ ions. However, a lack of correlation between the organic acid production and phosphate solubilization suggests that there might be some undeterminable factors for solubilizing inorganic insoluble phosphatic compounds other than production of organic acids which has been thought to be the major microbial means for execution process.

Table 5. Phosphate solubilizing power in sucrose-rock phosphate broth and cellulose decomposing power of the test micro-organisms

Organisms inoculated		P-solubilized in µg/15 mg in soluble P/0.15 g sucrose used (Average of duplicate sets)			Growth type in broth medium	H_2S-producing ability	Cellulose-decomposing power
Coded as	Identified as	Incubation in days					
		7	10	Mean			
ACB_5	*Bacillus firmus* B-7650	17.0	23.0	20.0	Mucoid	–	–
ACB_6	*Bacillus firmus* B-7651	19.0	15.5	17.3	Turbid + Mucoid	+	+ + +
ACS_6	*Streptomyces* sp.	14.0	18.5	16.3	Submerged pellicle	+	–
ACF_1	*Aspergillus fumigatus*	25.0	31.5	28.3	Mycellial pad with black spore	–	+
ACF_2	*Aspergillus candidus*	19.0	23.0	21.0	– do –	–	+

+ positive, – nil.

Solubilization of rock phosphate by the bacterial isolates appeared to be related with their growth type in broth, as ACB_5 (*Bacillus firmus* B-7650) producing an orthodox mucoid growth found to be a better solubilizing agent than ACB_6 (*B. firmus* B-7651) producing a turbid and mucoid growth (Table 1). Contrary to the earlier findings[5,21], solubilization of phosphorus by the fungi, ACF_1 (*Aspergillus fumigatus*) and ACF_2 (*A. candidus*) was intensified with sporulation, which was also true for ACS_6 (*Streptomyces* sp.). This may be ascribed to the greater demand of phosphorus during sporulation and hence more solubilization.

The universally higher average accumulation of available phosphorus, in the non-sterile as compared to that in the partially sterilized soil, in the inoculated series (Table 6) indicates that the soil organisms were basically proto cooperative possibly in providing some growth factors[2] beneficial for the phosphate solubilizers. FYM is known to supply not only the nutrients to the soil organisms, but also other growth substances which was appeared to be sure for RS too, after decomposition in soil. The higher accumulation of available phosphorus under these treatments were, therefore, indeed significant[7]. Although the inoculated organisms, in general, could not essentially enhance the accumulation of available phosphorus in soil, supporting earlier findings with Indian soils[3,12], inoculation of native aspergilli and *Bacillus firmus* provided some promising expectation, especially with FYM. This might presumably be due to an increase in the proliferation and activity of the phosphate solubilizers with the manure[11,20]. The ability of the inoculated organisms of utilizing cellulosic materials could not initiate accumulation of available phosphorus in the series under RS. It can be surmised along with Rao and Mikkelson[18] that toxic organic acids or other toxic metabolites liberated during decomposition of rice straw (RS) by soil microorganisms might have produced adverse effect on the activity of phosphate solubilizers as an increased accumulation of available phosphorus was obtained in the similar series under partially sterilized condition. The decrease in accumulation in the series under rock phosphate (RP) with or without FYM remained obscure. However, it may be presumed that the impurity in the RP variety caused liberation of some toxic metal ions which might have produced adverse effect on solubilization of phosphate from soil by the microorganisms.

From the foregoing discussion, it may be concluded that judicial use of manurial amendments supplemented with inoculation of efficient native phosphate-solubilizing organisms would be able to improve the available phosphorus status of soil even in the absence of vegetation.

Acknowledgement The authors thank the Council of Scientific and Industrial Research, India for providing necessary financial facilities.

Table 6. Available phosphorus content of soil on inoculation of some isolated micro-organism $^+$

Organisms inoculated		Soil	Available phosphorus content in kg/ha (Average of three sets)							
Coded as	Identified as		Control	RP	FYM	FYM + RP	RS	RS + RP	Mean	Mean for 2 sets
C	Uninoculated	I*	15.03	10.86	29.16	8.62	19.20	11.00	15.64	16.23
	Control	II	11.16	10.57	36.30	7.14	22.90	12.94	16.83	
ACB_5	*Bacillus firmus* (B-7650)	I	13.84	10.86	26.80	10.10	20.82	11.46	15.64	16.03
		II	11.00	11.75	38.08	7.59	17.55	12.64	16.43	
ACB_6	*Bacillus firmus* (B-7651)	I	11.25	10.71	29.02	10.71	19.78	11.46	15.48	16.30
		II	9.07	9.96	36.30	11.89	21.69	13.92	17.13	
ACS_6	*Streptomyces* sp.	I	12.50	9.21	26.80	12.64	17.55	11.46	15.02	15.04
		II	10.41	9.82	32.58	7.14	18.14	12.05	15.07	
ACF_1	*Aspergillus fumigatus*	I	13.53	11.46	25.58	11.89	20.82	11.46	15.79	16.10
		II	11.30	9.51	36.90	7.43	19.64	13.68	16.41	
ACF_2	*Aspergillus candidus*	I	14.14	8.93	26.80	8.53	21.71	16.82	16.15	16.68
		II	10.41	9.68	35.41	8.32	21.10	18.44	17.22	
Mean		I	13.38	10.33	27.36	10.41	19.98	12.27	15.62	16.06
		II	10.55	10.21	35.92	8.25	20.22	13.94	16.51	
Mean for 2 sets			11.96	10.27	31.64	9.33	20.10	13.10	16.06	

$^+$ Estimated after 15 days incubation

	LSD at 5%	LSD at 1%
For manurial treatment (M)	0.81	1.18
Interaction soil (S) × (M) two M at same S	1.40	2.05
– do – (S × M) two S at same or different M	1.67	2.84
– do – (M) × Organism (O) two M at same O	2.31	3.38
– do – (M × O) two O at same or different M	2.26	3.23

Available phosphorus of initial soil – 12.5 kg/ha

* I – Partial sterilized soil – autoclaved in thin layers for 2 consecutive days at 15 lb steam pressure for 30 minutes.
II – Natural nonsterile soil.

References

1 Agnihotri V P 1970 Solubilization of insoluble phosphates by some soil fungi isolated from nursery seed beds. Can. J. Microbiol. 16, 877–880.
2 Alexander M 1977 Introduction to Soil Microbiology. John Wiley and Sons, Inc. New York and London.
3 Bajpai P D and Rao M V B S 1971 Phosphate-solubilizing bacteria. Part 2. Extracellular production of organic acids by selected bacteria solubilizing insoluble phosphate. Soil Sci. Pl. Nutrition 17, 44–45.
4 Banik S and Dey B K 1981 Phosphate-solubilizing microorganisms of a lateritic soil. I. Solubilization of inorganic phosphates and production of organic acids by micro-organisms, Isolated in sucrose, calcium phosphate. agar plates. Zbl. Bakteriol. Abt. 136, 478–486.
5 Banik S and Dey B K 1981 Phosphate-solubilizing micro-organisms of a lateritic soil. II. Effect of inoculation of some tricalcium phosphate-solubilizing micro-organisms on available phosphorus content of soil. Zentrlbl. Bakteriol. II. Abt. 136, 487–492.
6 Basak M N and Bhattacharyya R 1962 Phosphate transformation in rice soils. Soil Sci. 94, 258–262.
7 Bhattacharyya B K and Debnath N C 1977 Studies on phosphate solubilizing efficiency of some organic manures. Indian Agric. 21, 207–210.
8 Dey B K, Banik S and Nath S 1976 Residual effect of organic manures on the microbial population and phosphate solubilizing power of wheat (*Triticum austivium* L.) rhizosphere soils. Indian Agric. 20, 245–249.
9 Dolui A K 1977 Characteristics and Genesis of different alluvial Soils of West Bengal. Ph.D. thesis, Calcutta University.
10 Duff R B, Webley D M and Scott R O 1963 Solubilisation of minerals and related minerals by 2-Ketogluconic acid producing bacteria. Soil Sci. 95, 105–114.
11 Fardeau J C and Guiraurd G 1971 Mobility of phosphorus in a soil receiving farm yard manure for thirty five years. Comp. Rend, 57, 1598–1605.
12 Gaur A C 1972 Role of phosphate-solubilizing micro-organisms and organic matter in soil productivity. Symposium on soil productivity, Natu. Acad. Sci. India, 259–268.
13 Hayman D S 1975 Phosphorus cycling by soil micro-organisms and plant roots. pp 67–92. *In* Soil Microbiology. Ed. N Walker. Butterworths, London.
14 Jackson M L 1967 Soil chemical Analysis. Prentice-Hall of India Pvt. Ltd., New Delhi.
15 Johnston H W 1954 The solubilisation of insoluble phosphate. II A quantitative and comparative study of the action of selected aliphatic acids on tricalcium phosphate. N.Z.J. Sci. Tech. 36, 49–55.
16 Louw H A and Webley D M 1959 The bacteriology of the root region of the oat plant grown under controlled pot-culture conditions. J. Appl. Bacteriol. 22, 216–226.
17 Olsen S R, Cole C V, Watenabe F S and Dean L A 1954 Estimation of available phosphorus in soils by extraction with sodium bicarbonate. USDA Circ. 939.
18 Rao D N and Mikkelsen D S 1977 Effect of rice straw addition on production of organic acids in flooded soil. Plant and Soil 47, 303–311.
19 Sen A and Paul N B 1957 Solubilization of phosphates by some common soil bacteria. Curr. Sci. 26, 222.
20 Sinkha M K 1970 Effect of composting soil with straw on mobility and uptake by plants of difficulty soluble soil phosphates. Izv. fimiryazev. sel'-Khoz. Akad. 6, 222–225, from Soils Fert. 34, 1897.
21 Swaby R J and Sperber J 1959 Phosphate dissolving micro-organisms in the rhizosphere of legumes. pp 289–294. *In* Nutrition of Legumes. Ed. E G Hallsworth, Butterworths, London.
22 Waksman S A 1967 The Actinomycetes. Ronald Press Co., New York.