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EFFECT OF CARBON AND NITROGEN SOURCES ON THE INORGANIC PHOSPHATE SOLUBILIZATION BY DIFFERENT ASPERGILLUS NIGER STRAINS

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Abstract
The Mineral phosphate solubilization (MPS) was studied in five Aspergillus niger strains. MPS activity was measured in solid (Pikovskaya's medium) as well as liquid media using different phosphate sources (dicalcium phosphate, tricalcium phosphate aluminium phosphates, and Rock phosphate). All the strains showed a zone of clearance of tricalcium phosphate in Pikovskaya's medium in plates and solubilized tricalcium phosphates in broth efficiently. Solubilization was lower in aluminium phosphates and Rock phosphate. Among the carbon sources the fungi preferred sucrose for higher P solubilization. Nitrogen in the form of nitrate was very effective in solubilizing inorganic phosphates. Xylose and urea were the poorest sources of carbon and nitrogen for all the strains of Aspergillus. Phosphate release was associated with reduction in pH.

Keywords: Aspergillus Niger; Phosphate Solubilization; Carbon Source; Nitrogen Source.

Introduction

Microorganisms play a significant role in solubilizing P sources and making phosphate available to plants by bringing about favorable changes in the soil microenvironment leading to solubilization of inorganic phosphate sources (Jones et al., 1991). The solubilization of inorganic P-bearing materials has been attributed to two different mechanisms, acidification by proton extrusion associated with ammonium assimilation and organic acid production (Roos and Luckener, 1984; Cunningham and Kuiack, 1992; Vassilev et al., 1996). Phosphate solubilization was usually measured using glucose [Asea et al, 1988, Kucey, 1983] or sucrose [Cunningham, 1992] as the sole source of carbon. Furthermore, in most studies ammonium was found to be a better nitrogen source than nitrate [Asea et al, 1988]. These results suggest that phosphate solubilization was affected by various carbon and nitrogen sources [Narsian and Patel 2000, Reys et al, 1999]. Acid phosphatases and phytases secreted by these microorganisms also have an important role in phosphate solubilization (Achal, 2007, Richardson et al, 2000). These observations indicate that P solubilization is a complex phenomenon which depends on many factors such as the nutritional, physiological, and growth conditions of the cultures (Cunningham, 1992). In the present study, an attempt has been made to screen *Aspergillus niger* strains isolated from soils of Sambalpur district, Odisha, India for the solubilization of inorganic phosphates and its performance on different carbon and nitrogen sources.

Materials and Methods

Microorganisms

Fungal strains were isolated from the rice field soil of Sambalpur, Odisha, India after serial dilution of soil solution on Potato Dextrose Agar plates. Isolated, predominant, morphologically distinct colonies were selected, purified by repeated culturing and maintained on PDA slants at 4°C. Isolates were identified by their colony characteristics, spores morphology and microscopic observations.

Phosphate Solubilization

Five strains exhibiting better clearing zone around the colonies, from a lot of 78 isolates were selected and used for P solubilization studies on Pikovskaya's agar (Pikovskaya, 1948). For broth culture studies, 100mL of Pikovskaya's broth was distributed in a conical flask (250 mL) and sterilized at 120ºC for 20 minutes. The flasks were inoculated with the inoculum blocks. Uninoculated medium was maintained as control. All treatments and control were incubated at room temperature (30ºC) and maintained as stationary cultures. Determination of soluble phosphate was carried out by Molybdenum blue method Jackson. (1967). The cultures were harvested after 15 days of incubation, centrifuged at 15,000 rpm for 10 minutes at 4ºC to remove the cells and debris, and then subjected to analysis. The pH of the media was also measured simultaneously.

Phosphate sources (0.5%), tricalcium and aluminium phosphate (HiMedia, India), , and Rock phosphate obtained from Rajasthan(11% total P, 75 mesh) , carbon sources (1%; HiMedia, India), Xylose, Glucose, Galactose, Fructose, Maltose and Sucrose and nitrogen sources (0.05%; HiMedia, India), ammonium sulfate, ammonium chloride, ammonium nitrate, sodium nitrate, potassium nitrate, and urea, were amended individually to evaluate the performance of fungi in P solubilization.

Results and Discussion

Solubilization of Inorganic Phosphates

Aspergillus niger was reported to be capable of solubilizing insoluble phosphates (Agnihothri, 1970, Bojinova et al., 1998). In the present study, all the strains solubilized inorganic phosphates in solid and liquid media (Tables 1 and 2). However, as reported earlier, difference among strains in P solubilization was noticed in this study also (Illmer and Schinner, 1992; Narsian et al., 1993). The diameter of the zone of phosphate solubilization in solidified Pikovskaya's medium by *A. niger* strains ranged from 0.4-1.4 cm on the second day, 1.5-3.6 cm on the fifth day, and 3.3-6.8 cm on the thirteenth day to 6.2-7.8 cm on the fifteenth day. Among the strains, PSF34 produced the maximum solubilization zone (7.8cm).

In liquid medium the strain PSF22 effected maximum P solubilization as well as pH reduction (Table 2). The strains PSF34 and PSF55 were poor in P solubilization and pH reduction. In liquid media assays, all the strains solubilized P from all the phosphate sources: tricalcium, dicalcium, ferric and aluminium phosphates, and Rock phosphate. The solubilization pattern by different strains of different phosphate sources is of the order DP < TP < AP < RP. Some strains (PSF5 and PSF12, PSF22) were more effective on AP than RP, a phenomenon that was also reported earlier (Banik and Dey, 1982). In this study higher solubilization of DP than other phosphates by *A. niger* was observed.

Table 1 Clearing Zone (in Cm) Produced By *A. niger* Strains On Petriplates

Strains	Days				
	3	5	9	13	15
PSF5	0.6	1.2	3.1	6.2	7.1
PSF12	0.9	2.5	3.8	6.9	7.8
PSF22	1.2	3.4	5.6	7.1	8.4
PSF34	1.4	3.6	6.8	7.8	9.1
PSF55	0.4	1.4	4.8	6.4	7.6

Values are the mean from three replications. P source is tri calcium phosphate

Table 2 Inorganic Phosphate Solubilization by *A. niger* Strains In Pikovskaya's Broth

Strains	Tricalcium		Dicalcium		Aluminium		Rock Phosphate	
	Soluble P	pH	Soluble P	pH	Soluble P	pH	Soluble P	pH
PSF5	854±1.65	3.96	876±1.03	3.85	761±1.81	4.58	420±1.25	4.84
PSF12	840±2.72	3.64	980±2.25	3.42	781±2.87	4.11	546±0.84	4.58
PSF22	1121±0.01	3.35	1160±1.23	3.21	960±0.05	3.82	628±3.01	3.86
PSF34	484±0.03	4.21	552±1.78	3.96	312±0.47	4.64	94±2.05	4.85
PSF55	376±0.01	4.34	438±2.05	4.11	206±0.02	4.62	86±0.01	4.93

Values are the means ±SD from six replications, scored after 15 days.

The pH of the culture filtrate turned acidic with all the cultures. The reasons for the pH reduction by microbes, the mechanism of acid production, as observed by earlier workers (Agnihothri, 1970; Banik and Dey, 1982; Asea et al., 1988; 1989; Cunnigham and Kuiack, 1992; Illmer and Schinner, 1992; Narsian et al., 1993; Bojinova et al., 1998; Reyes et al., 1999), would have resulted in reducing the pH in this study also. A drastic reduction in pH was registered in TCP, DP, AP amendments, while in the RP amended media the pH reduction was negligible.

Effect of Carbon and Nitrogen on P Solubilization

Among the carbon sources, sucrose was the best, followed by glucose, for insoluble phosphate solubilization (Tables 3). Different carbon sources in relation to phosphate solubilization activity were in the following order: sucrose >glucose >galactose >lactose >fructose>Mannitol>Xylose. Mannitol and glucose were reported to be the best sources for A. niger to solubilize P (Shesadri et al 2004). Reyes et al. (2000) showed that sucrose was the best carbon source for *P. rugulosum* for solubilization of hydroxylapatite and $FeSO_4$. Narsian and Patel (2000) reported maximum P solubilization by Aspergillus aculeatus with arabinose and glucose. In the present study, sucrose and glucose significantly increased P solubilization compared to other carbon sources. These studies suggest that different fungi use different carbon sources, and depending on the carbon source, the fungi use alternative metabolic pathways to produce organic acids.

Potassium nitrate promoted P solubilization better than other nitrogen sources in this study (Table 4). P solubilization in relation to nitrogen sources was in the following order: KNO_3 > $(NH_4)_2SO_4$ >NH_4NO_3 >NaNO3>NH_4Cl >Urea. Acidification of the culture media was significantly reduced in the case of $(NH_4)_2SO_4$ in all the isolates compared to KNO_3, though maximum soluble P was observed when KNO_3 was used as the nitrogen source. The pH reduction was minimal in the presence of KNO_3 compared to other nitrogen sources, where it decreased from an initial pH of 7.5 to 4.7. (Table 4). Seshadri et al. (2004) reported that A. niger showed maximum phosphate solubilization in the presence of NH_4NO_3. Narsian and Patel (2000) showed that $(NH_4)_2SO_4$ was the best nitrogen source for *A. aculeatus*. The isolate of *P. rugulosum* grows better on nitrate than on ammonium when relatively insoluble phosphates are used (Reys et al, 1999). The pH was drastically reduced when $(NH_4)_2SO_4$ was used as the nitrogen source, whereas the pH did not decrease significantly when KNO_3 was used, though more soluble P was recorded with it. The reduction in pH in the case of $(NH_4)_2SO_4$ indicates the possibility of the operation of a NH_4/H^+ exchange mechanism acidifying the medium as reported by Roos and Luckener (1994). Hence acidification due to NH_4^+ is more evident rather than NO^{3-} because the acidification of the medium is a result of H+ efflux from hyphae during NH_4^+ uptake (Jacobs 2002). Phosphate solubilization was greatly affected by nitrogen sources, because fungi acidify their nutrient medium during growth and are dependent on the nitrogen source (Whitelow 1999).

Table3. Influence of Carbon Sources on Tricalcium Phosphate Solubilisation by *A. niger* (PSF22)

Carbon Source	Control		A. niger (PSF22)	
	pH	Soluble	pH	Soluble P
Xylose	5.87	192±1.70	4.11	620±2.68
Glucose	6.12	215±1.55	2.96	1121±2.05
Galactose	5.97	202±1.25	3.34	1076±2.62
Fructose	5.88	189±1.49	3.45	843±3.85
Lactose	6.02	196±2.05	3.56	879±2.08
Sucrose	5.96	220±4.20	2.89	1228±1.63
Mannitol	6..1	194±3.94	3.67	743±2.05

Values are the means±SD from six replications, scored after 15 days.

Table4. Influence of Nitrogen Sources on Tricalcium Phosphate Solubilisation by *A. niger* (PSF22)

Nitrogen Source	Control		A.niger (PSF22)	
	pH	Soluble P	pH	Soluble P
Ammonium	5.72	231±1.25	2.96	1121±1.24
Ammonium	5.88	227±1.70	3.61	976±1.67
Ammonium	5.78	236±3.09	3.35	1078±2.19
Sodium nitrate	5.86	234±1.63	3.54	1054±2.16
Potassium nitrate	6.02	238±2.49	4.71	1248±1.24
Urea	6.11	224±2.05	3.67	743±3.26

Values are the means±SD from six replications, scored after 15 days.

Bojinova et al. (1997) reported that the kind of strain of *A. niger*, and kind of nutritive medium significantly influence the process of conversion of phosphates from a non-utilizable to a water soluble and utilizable form. The result from the above experiments clearly indicates the existence of strain-level difference in P solubilization and suggests the use of effective native strains for agricultural inoculation purposes.

Conclusion

From the results it is evident that all the *A. niger* strains possess mineral phosphate solubilizing potential. However, the results vary according the phosphate source used. The results obtained in nitrogen supplementation have to be taken into account while using these strains in agriculture as biofertilizer or in vitro biotechnological processing of phosphates, where supplementation of nitrate was more effective than urea in phosphate solubilization. Further studies on identification of

structural and regulatory genes responsible for mineral phosphate solubilization activity in *A. niger* will be helpful in cloning and expression of specific genes in heterologous systems.

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