Middle East Journal of Applied Sciences 4(4): 1080-1089, 2014
ISSN 2077-4613

Influence of Salinity on Phosphate Solubilization by Fungi

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ABSTRACT

Nearly 95 - 99% of phosphorous in soil is present in the insoluble form and hence cannot be utilized by plants. Fungi have been reported to possess greater ability to solubilize insoluble phosphates than bacteria. This study aimed to isolate a high-efficient level of phosphate-solubilizing fungus from rhizosphere salinity soil samples and apply it in solubilization of rock phosphate (RP) and tricalcium phosphate (TCP). Out of all the 20 fungi isolated from the salinity soils, only two fungi showed significant zone of P solubilization (PS2 and TCP10). The conventional methodologies were carried out for morphological fungus characterization and the analysis of 18s rRNA sequence. Then the effects of time, temperature, initial pH, RP and TCP concentration, shaking speed and salinity concentration on the content of soluble P released by this isolate were investigated. Based on their 18S rRNA gene sequences and phylogenetic positions, the isolate SP2 and TCP10 were designated as *Penicillium oxalicum* strain Po-5 and *Penicillium expansum* strain J1 No. HQ73298, respectively. The Maximum solubilization of RP and TCP was observed after 6 and 7 days of incubation by *P. oxalicum* and *P. expansum*. *P. oxalicum* and *P. expansum* showed different levels of phosphate solubilization under different saline conditions tolerating maximum salinity up to 5% NaCl concentrations

Keywords: Phosphate-solubilizing fungus, rock phosphate (RPs), tricalcium phosphate (TCP), salinity soil.

Introduction

Natural phosphate rocks have been recognized as a valuable alternative for P fertilizers. In recent years, the possibility of practical use of rock phosphates (RP) as fertilizers has received significant interest. Unfortunately, RP is not plant available in soils with a pH greater than 5.5 to 6.0 and, even when conditions are optimal, plant yields are lower than those obtained with soluble phosphate (Khasawneh and Doll, 1978). Conventionally, RP is chemically processed by reacting with sulphuric acid or phosphoric acid into soluble phosphate fertilizer. The process increases fertilizer cost and makes the environment worse (Reddy *et al.,* 2002; Chuang *et al.,* 2007; Xiao *et al.,* 2008).

Microorganisms play a critical role in natural phosphorus cycle (Biswas and Narayanasamy, 2006; Vassilev *et al.,* 2006) and recently, microbial-based approach can improve the agronomic value of RPs to a certain extent. Microbial solubilization of RPs is gaining great attentions in agriculture. Some organisms have been isolated for the purpose (Coutinho *et al.,* 2011; Gupta *et al.,* 2012*;* Luetal, 2012). These phosphate solubilizers can be used directly as biofertilizers in the soil or bioreactors for the bio-processing of RPs. This approach not only compensates for higher cost of manufacturing phosphate fertilizer in industry but also reduces environment pollution caused by traditional chemical process. An alternative has been the use of microorganisms with the capability to solubilize RP and release soluble P through the production of organic acids, chelating oxo acids from sugars, reduction of pH and production of enzymes. Several reports have indicated that some microorganisms are capable of solubilizing insoluble RP and releasing soluble P. However, few reported microorganisms represent a high potential to release soluble P from RP and TCP and this seriously restrains the biosolubilization of RP and TCP and its use as biofertilizer, hence, isolation and application of new and potential phosphate solubilizing microorganisms are significant and necessary (Son *et al,* 2006; Achala *et al.,* 2007; Xiao *et al.,* 2008). Filamentous fungi are widely used as producers of organic acids, particularly black *Aspergilli* and some species of *Penicillium,* these species have been tested for solubilization of RP and TCP and have been reported for various properties of biotechnological importance, such as, biocontrol, biodegradation, phosphate solubilization and P fertilizer (Chuang *et al.,* 2007; Richa *et al.,* 2007; Pandey *et al.,* 2008).

Under the situation, many phosphate-solubilizing microorganisms have been isolated from different environments and phosphorus availability to plants (Yu *et al.,* 2012) through the inoculation of phosphate-solubilizing microorganisms has been widely studied under pot and field conditions (Duponnois *et al*., 2005; Valverde *et al*., 2006). However, in fact, these microorganisms are only a small percentage of the total microbial population and few of them present a high potential to solubilize RPs under natural conditions which seriously restrains the application of this microbial-based technique. Moreover, a main problem, indeed, is that how to remain a high destiny and activity of the introduced microorganisms (Van Veen *et al.,* 1997). Normally, many

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isolates present high phosphate-solubilizing capability in growth medium, but when they are inoculated to natural environment, they are often in a form characterized by non-growth and/or low phosphate-solubilizing capability (Xiao *et al.,* 2008). Therefore, it is significant and necessary to isolate new and potential phosphate-solubilizing microorganisms.

Therefore, this study was conducted to isolate fungi capable of solubilizing rock (RP) and tricalcium phosphate (TCP) and studying the solubilization process, especially, under stress conditions as well as salinity.

Materials and Methods

Tricalcium phosphate (TCP) and Rock Phosphate (RP):

Tricalcium phosphate (TCP) and Rock Phosphate (RP) were kindly obtained from Soils, Water and Environment Research Institute, Agric. Res. Center, Giza, Egypt and analyzed for its P content (7.97%). Rock phosphate was added to the growth medium instead of TCP in the amount equivalent to 50 mg $P_2O_5$100 mL^{-1} (Reddy *et al*., 2002).

Isolation and Identification of Phosphate Solubilizing Fungi (PSF):

Isolation and identification of phosphate-solubilizing fungus: The fungus strain was isolated from rhizosphere soil samples collected from the various agricultural fields. Various serial dilutions of soil samples with 100 uL aliquots were plated on a modified Pikovskaya's agar (MPA) medium (glucose, 10 g; $(NH_4)_2SO_4$, 0.15 g; KC1, 0.2 g; $MgCl_2.6H_2O$, 0.5 g; $MgSO_4.7H_2O$, 0.25 g; agar, 20 g; distilled water, 1000 mL) supplemented with ampicillin (50 ug mL^{-1}) and streptomycin (30 ug mL^{-1}) and containing TCP $Ca_3(PO_4)_2$, (10.0 mg mL^{-1}) or RP was added to the growth medium instead of TCP in the amount equivalent to 50 mg $P_2O_5$100 mL^{-1} (Reddy *et al*., 2002). Here TCP and RP was chosen as sole P source for selectively screening strains which have the capability to solubilize unsoluble-P and release soluble P from TCP or RP. After incubation for 3 days at 28°C, the strain developed clear zones around colonies.

Zone measurement is done as:

Phosphate solubilized = (Z-C)/C * 100

S.E = (Z-C)/C X 100

Z= Solubilizations zone + Colony diameter

C = Radius of fungal colony inside zone i.e. colony diameter.

Colonies with clear zones were further purified by replanting on MPA medium. The screened phosphate-solubilizing fungus was selected and stored for further work. The isolate was identified on the basis of sequence of 18S rRNA gene according to the methods described by White *et al*., (1990) and Tamura *et al*., (2007).

Culture medium and solubilizing conditions:

P solubilization experiments were carried out in flasks with 100 mL (in 500 mL conical flask) of MPA's medium (without agar) and TCP or RP as sole P source 5.0 or 10g respectively, (Yadav *et al*., 2011). Each flask was inoculated with the 1×10^6 spores of the fungus spore suspension. Flasks were shaken under 160 rpm at 28°C for 7 days. The pH of the culture medium was periodically adjusted and maintained at 7.0. All experiments were performed supplemented with triplicate.

Optimization of cultural parameters of P solubilization isolates:

The investigated parameters were carried out in MPA's medium (without agar) as follows: (1) incubation time (1 to 9 days), (2) shaking speeds (100 to 220 rpm), (3) incubation temperature (10 to 40°C), (4) initial culture pH (4.5 to 8.5, using HC1 or NaOH), (5) carbon sources at 1% (Glucose, Fructose, Sucrose, Xylose, Arabinose, Galactose, Maltose and Mannitol), (6) nitrogen sources (Ammonium sulphate , Ammonium chloride, sodium nitrate, potassium nitrate, calcium nitrate), (7) RP or TCP concentration (1.0 to 5.0 gL^{-1}), (8) NaCl stress (0.5 to 5%).

After incubation, suspended mycelium was carefully harvested from the medium by centrifugation (4000 rpm) and the supernatant was collected to be analyzed for pH (with glass electrode) and soluble phosphate was determined by chlorostannous reduced molybdophosphoric acid blue method (Jackson, 1967) and expressed as P_2O_5. The uninoculated autoclaved medium with phosphate substrate was incubated under similar conditions to serve as the control.

Statistical analysis:

Capability of RP and TCP solubilization was determined by the content of soluble P in the supernatant liquid. Values are given as means ± SD. for triplicate samples. Data were analyzed by analysis of variance (ANOVA) and the means were compared with Duncan's Multiple Range Test at $p<0.05$ level.

Results and Discussion

Isolation and Identification of PSF:

Fungal isolates with the ability to solubilize insoluble P were isolated from soil characterized with high level of salinity. Out of all the 20 fungi isolated from the salinity soils, only two fungi showed significant zone of P solubilization. The two isolates that displayed the highest ratio of clear zone/colony diameter were selected as RP2 for RP and TCP10 for TCP. The zone of P solubilization appeared on third day of incubation on MPA's agar. Continuous observation of the halo zone formation indicates phosphate solubilizing ability which was in increasing order up to the 7th day. The appearance of a clear halo zone around the colony indicated phosphate solubilization by the fungus (Kang *et al*., 2002; Gupta *et al*., 2007). The advantage of using natural phosphate solubilizers over the genetically manipulated or ones that have been isolated from a different environmental sets-up is the easier adaptation and succession when inoculated into the medium containing RP (Xiao *et al*., 2008).

Identification of the isolates and phylogenetic tree analysis:

The isolates were identified based on 18S rRNA gene sequences. The 18S rRNA gene sequences comparison revealed that the isolate RP2 had 98% similarity with *Penicillium oxalicum* and isolate TCP10 had 97% similarity with *Penicillium expansum*. Based on their 18S rRNA gene sequences and phylogenetic positions, the isolate RP2 was designated as *Penicillium oxalicum* strain Po-5 No. HQ680452 and the isolate TCP10 was designated as *Penicillium expansum* strain J1 No. HQ73298 (Fig. 1).

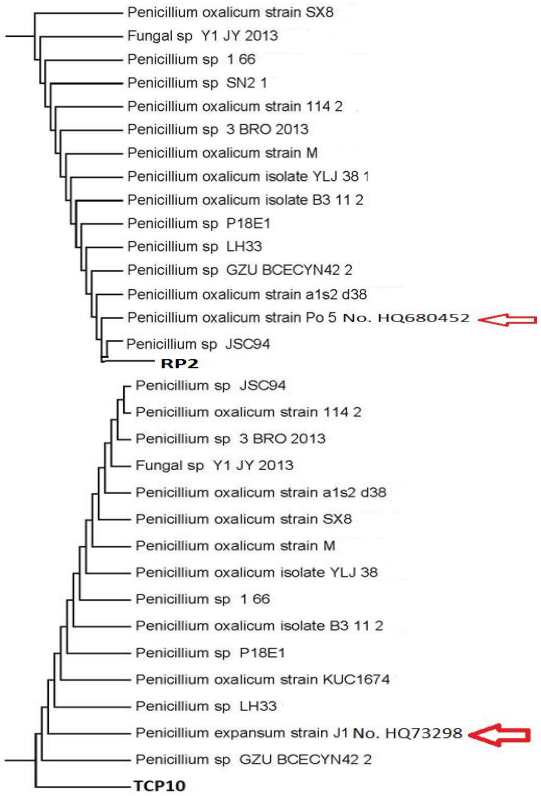

Fig. 1: Phylogenetic tree of fungus isolates RP2 and TCP10 relationship among the selected strains based on sequencing analysis and the most closely related fungus species.

Optimization of incubation time on RP and TCP solubilization:

The results (Fig. 2) of the periodic solubilization of RP and TCP show that the solubilization of RP and TCP were achieved earlier at the third and fourth day of incubation on MPA's broth medium supplemented with RP or TCP as a sole P source, respectively. Maximum solubilization of RP and TCP were observed after 6 and 7 days of incubation by *P. oxalicum* (67.0 mg L^{-1}) and *P. expansum* (46.2 mg L^{-1}), respectively. After that, there was no additional solubilization of RP or TCP. Maximum P solubilization by *P. oxalicum* occurred at the end of logarithmic growth phase, whereas, it occurred at the beginning of stationary phase of *P. expansum* growth. The decrease in solubilization activity after a particular incubation period might be due to the availability of soluble form of phosphate in the nutritive media that has an inhibiting effect on further phosphate solubilization (Narsian *et al*., 1995). The decrease in P concentration at the beginning stages of experiment is related to the findings of Seshadre *et al*. (2000), who stated that existing P is utilized for growth and development of the organism during this period. Vyas *et al*. (2007) found significant increase with the prolongation of incubation period from 3 to 9 days, followed by a significant decline after 12 day of incubation. Narsian and Patel (2000) reported maximum release of P from China and Udaipur RPs and Sonrai and Hirapur RPs by *A. aculeatus* after 8 and 14 days of incubation, respectively, they added that variation of time in solubihzation of RP may have been due to the nature and quantity of organic acids secreted in the medium. The effect of powerful and efficient phosphate-solubilizing capability was not reported previously (Keyes, 1990; Gleddie *et al.,* 1993; Achala *et al*., 2007; Collavino *et al*., 2010; Coutinho *et al*., 2011; Gupta *et al*., 2012; Lu *et al*., 2012). Longer or less than the optimal time, the amount of soluble P decreased.

Generally, it might be due to the increasing of the production of organic acid metabolites by different phosphate-solubilizing microorganisms and these metabolites can convert the insoluble RP into soluble P (Illmer and Schnner, 1995; Rashid *et al*., 2004; Kim *et al*., 2005).

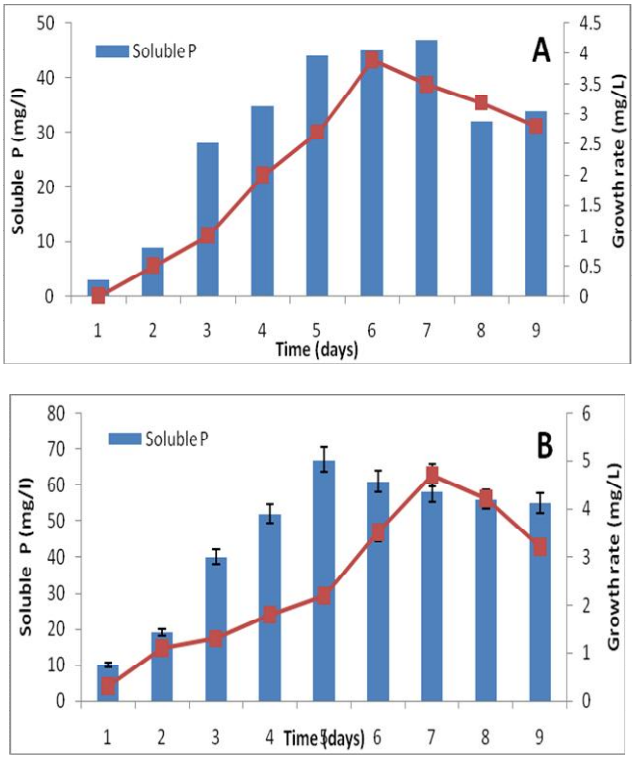

Fig. 2: Time course of RP and TCP solubilization by the isolated fungi, (a) *P. oxalicum* and (b) *P. expansum.*

Optimum Shaking Speeds for Solubilization of RP and TCP:

The amount of soluble P released by the two fungal isolates at different shaking speeds ranging from 80-200 rpm is shown in Fig. 3. The content of soluble P increased with the increase of shaking speed from 100 up to 160 rpm. The maximum contents of soluble P released were 80.5 mg L^{-1} (*P. oxalicum*) and 63 mg L^{-1} (*P. expansum*). However, the amount of soluble P was decreased, when the shaking speed increased from 160 to

220 rpm in RP and TCP solubilization. These results are in consistent with the findings of Xiao *et al*. (2008), who reported that at the excessive shaking speeds, the growth of the isolates was often weakened by the shear stress resulting from the strong stirring, that was why the released soluble P decreased when the shaking speed increased from 160 to 220 rpm.

At the different shaking speed, decrease in the final pH of the medium of both isolates showed variable values (Fig. 3a, b) and *P. expansum* was recorded slightly lower pH than that recorded by *P. oxalicum.* Such decrease in final pH was accompanied by increasing P solubility. Many authors (Cerezine etal, 1988; Vassilev *et al.,* 1995; Vassileva *et at.,* 1998; Barroso and Nahas, 2005) reported reduction in the final culture pH with the increasing of soluble P. However, no significant relationship could be established between the quantities of phosphate solubilized and drop in pH (Narsian and Patel, 2000).

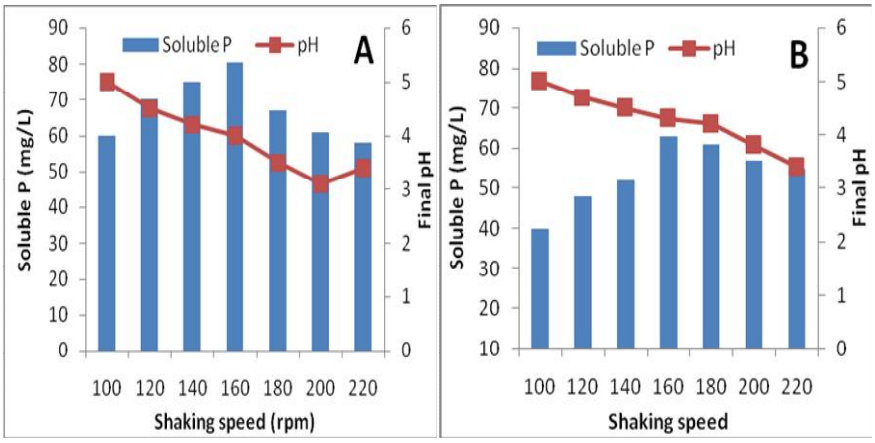

Fig. 3: Relationship between shaking speed and pH on soluble P content.

Optimum temperature and initial pH for solubilization of RP and TCP:

Results of the effect of temperature and initial pH on P solubilization were factors had significant influence on P solubilization.

The optimum temperature and initial pH for RP solubilization were investigated as shown in Figures 5 and 6. Temperature and initial pH had significant effects on the solubilization of RP and TCP (Figures 4 and 5). The maximum content of soluble P was recorded at 30°C, which were 83.5 and 77.31 mg L^{-1} released by *P. oxalicum* and *P. expansum*., respectively (Fig. 4). Vyas *et al*. (2007) reported maximum biosolubilization of RP at 36°C.

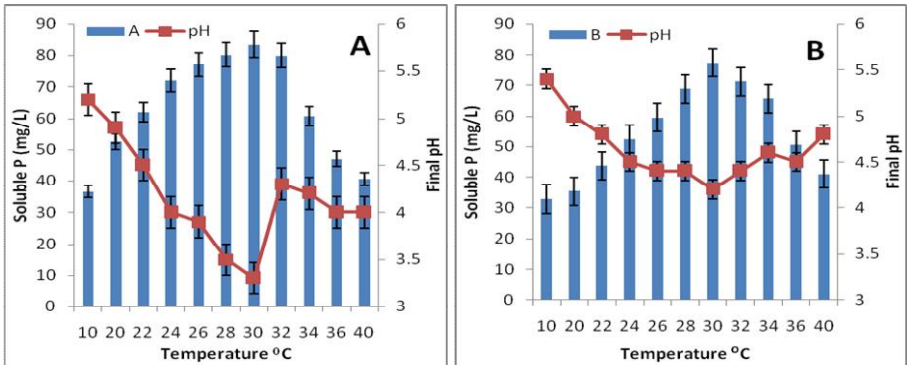

Fig. 4: Optimization of temperature for soluble P content and Relation to final pH.

As far as initial pH is concerned, there were differences between the two fungi (Fig. 5). The maximum content of soluble P was recorded at 99.3 mg L^{-1} released by *P. oxalicum* at initial pH 6.5, while it was recorded at 87.2 mg L^{-1} released by *P. expansum* at initial pH 7.0.

Higher or lower than the optimal temperature and initial pH, the content of soluble P decreased. In addition, there was remarkable reduction in final pH for both isolates during the investigations on temperature and initial pHs. The results are in similar to those of Barroso and Nahas (2005) and Xiao *et al*., (2008).

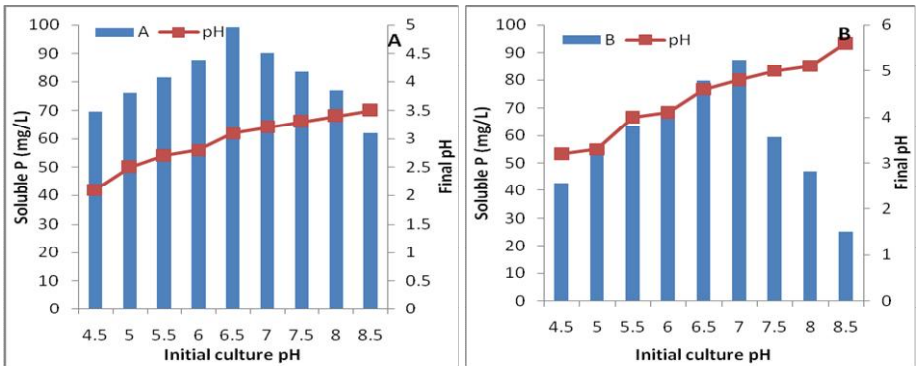

Fig. 5: Optimization of initial culture pH for soluble P content and Relation to final pH.

Effect of different sources of carbon on RP and TCP solubilization:

To find out the best source of carbon that can achieve the highest phosphate solubilization, different sources of carbon were added individually in growth medium inoculated with *P. oxalicum* or *P. expansum* (Table1). The obtained results showed that glucose was the best followed by fructose and xylose for the two tested fungi in phosphate solubilization. While, the other tested carbon sources showed lower values. Analysis of variance showed the differences in phosphate solublization between different sources of nitrogen (Table 1). According to Cerezine *et al*. (1988), glucose and fructose are the most frequent and abundant sugars detected in plant exudates that possibly affect the microbial population which solubilizes insoluble phosphates.

Table 1: Effect of different carbon sources on P solubilization from RP and TCP by *P. oxalicum* or *P. expansum.*

Carbon source	*P. oxalicum*		*P. expansum*	
	P soluble (mg L^{-1}	Final culture pH	P soluble (mg L^{-1}	Final culture pH
Glucose	95.00[a]*	3.1	82.66[a]	3.2
Fructose	85.97[b]	4.0	75.14[b]	4.3
Sucrose	65.24[c]	4.7	41.45[c]	5.0
Xylose	58.72[d]	5.0	43.00[c]	5.1
Arabinose	37.55[f]	6.0	29.60[e]	6.2
Galactose	40.25[e]	4.8	27.2e[f]	5.1
Maltose	50.50[d]	6.2	25.31[f]	6.0
Mannitol	41.33[e]	5.5	37.00[d]	5.6

*Means followed by the same letter within a columns are not significantly different at P= 0.05 (Duncan's)

Effect of nitrogen sources on phosphate solubilization:

Both ammonium and nitrate salts have been used as N sources in P solubilization studies. Ammonical N was best in reducing culture pH and promoting P solubilization (Pradhan and Shukla, 2005; Cunningham and Kuiack, 1992). The result showed that maximum solubilization of RP and TCP occurred when ammonium sulphate was used. (Table 2) and reduced the pH of the medium. This could be due to the production of inorganic acids by proton exchange mechanism in presence of NH_4^+ that cause accelerated phosphate solubilization.

Table 2: Effect of different nitrogen sources on P solubilization from RP and TCP by *P. oxalicum* or *P. expansum.*

Nitrogen source	*P. oxalicum*		*P. expansum*	
	P soluble (mg L^{-1})	Final culture pH	P soluble (mg L^{-1})	Final culture pH
Ammonium sulphate	92.00[a]*	3.0	87.66[a]	3.2
Ammonium chloride	81.97[b]	3.4	72.14[b]	4.3
sodium nitrate	45.24[d]	5	37.45[d]	5.0
potassium nitrate	55.72[c]	4.5	48.00[c]	5.1
calcium nitrate	47.55[d]	5.5	39.60[d]	5.2

*Means followed by the same letter within a columns are not significantly different at P= 0.05 (Duncan's)

Optimum of RP and TCP concentrations for P solubilization:

It is clearly obvious from data shown in Table 3 that both *P. oxalicum* and *P. expansum* showed positive responses in solubilization efficiency to RP and TCP concentrations but with various degrees (Table 3). Generally, a continuous increase in RP and TCP solubilization was observed by increasing the concentration of

RP and TCP added to the growth medium until 2.0 and 2.5 g L^{-1}, respectively. This concentration was the optimum in yielding the maximum soluble P. The corresponding values were 96.3 mg L^{-1} for *P. oxalicum* and 87.0 mg L^{-1} for *P. expansum*. It was also observed that as the concentration of added RP or TCP decreased from 2-5 g L^{-1}, there was a slight increase in RP and TCP solubilization capability as the content of soluble P increased. But as the concentration of added RP and TCP increased from 5-8 g L^{-1}, there was no increase but a visible decrease in the content of soluble P.

Table 3: Effect of different concentration of RP and TCP on soluble P and final pH.

RP concentrations	*P. oxalicum*		TCP concentrations	*P. expansum*	
	Soluble P (mgL^{-1})	Final pH		Soluble P (mgL^{-1})	Final pH
1.0	77.5^{d}*	3.7	1.0	37.8^{h}	5.5
1.5	88.5^{b}	3.2	1.5	66.5^{d}	5.1
2.0	96.2^{a}	2.7	2.0	77.2^{b}	4.8
2.5	80.2^{c}	2.8	2.5	87.0^{a}	4.3
3.0	75.3^{d}	3.0	3.0	70.1^{c}	4.4
3.5	69.0^{e}	3.3	3.5	65.0^{d}	4.6
4.0	60.7^{f}	3.8	4.0	59.8^{e}	5.0
4.5	55.4^{g}	4.2	4.5	45.3^{f}	5.3
5.0	50.1^{h}	4.5	5.0	32.9^{g}	5.8

*Means followed by the same letter within a columns are not significantly different at P= 0.05 (Duncan's)

The solubilization of RP have been reported to depend on their structure complexity, particle size and quantity of organic acid secreted by microorganisms (Pradhan and Sukla, 2005). Consequently, P is released from mineral phosphate by proton substitution from Ca_2^+ (Goldstein, 1994), while, Illmer and Schinner (1995) mentioned that RP solubilization is the result of release of protons accompanying respiration or NH_4^+ assimilation.

At all concentrations of RP and TCP, the two tested fungi showed reduction in the final pH, *P. oxalicum* showed priority in this respect. The results are in accordance with those obtained by Reddy *et al*. (2002), who reported that phosphate solubilization was not always accompanied by a drop in pH, but always showed the largest production of acids.

Abdel-Hafez (1966), Vassilev *et al*. (1995), Vassileva *et al*. (1998) and Barroso and Nahas (2005) explained the solubilization of phosphate on the base of acid production. The significant relationship detected between pH and TA shows that the fall in pH may possibly have been the consequence more of acid production than of selective ion absorption by the fungus (Cerezine *et al*., 1988; Gupta *et al*., 2007). Indeed, *P. oxalicum* is characterized by the production of large amount of acids such as oxalic and citric acids (Vassilev *et al*., 1995; Rashid *et al*., 2004). Such organic acids have been also, recognized for phosphate solubilization by several species of *Penicillium*, namely, *P. bilaii, P. citrinum, P. janthinellum, P. oxalicum* and *P. purpurogenum* (Cunningham and Kuiack, 1992).

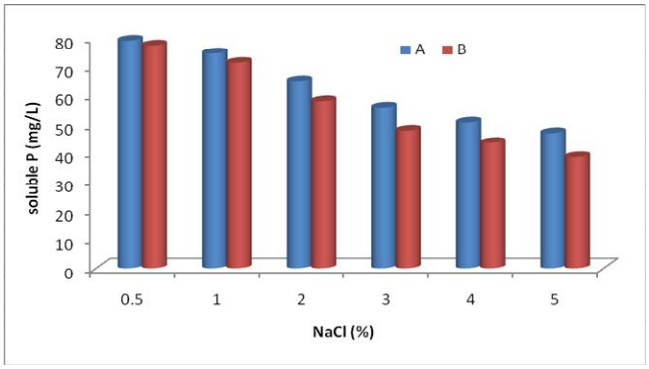

Fig. 6: Effect of salinity (NaCl %) on P solubilization by *P. oxalicum* (A) and *P. expansum* (B).

Effect of salinity on P solubilization:

P. oxalicum and *P. expansum* shows P solubilization up to 5% NaCl concentration (Fig. 6). Similarly, the phosphate solubilizing ability of *Fomitopsis* sp. PS 102 was enhanced in the presence of 1% NaCl (Kang *et al*., 2002). The strains *P. oxalicum* and *P. expansum* can thus be of great benefit in maintaining the available phosphate levels for crops in saline alkaline soils. The fungus strain *P. oxalicum* and *P. expansum* can thus be utilized in land reclamation of the saline regions along with biological nitrogen fixers. However, since

conditions in the soil are far more complex, further study is required to assess the ability of the fungal isolates, in vivo. Kang *et al.* (2002) and Kim *et al*. (1997) reported the enhancement of solubilization in presence of 1% sodium chloride. Johri *et al*. (1999) reported eighteen bacterial isolates out of fifty seven isolates in presence of 5% sodium chloride while two bacterial isolates lost the ability of phosphate solubilization in plate assay in absence of sodium chloride.

Conclusion:

A high efficient level of phosphate-solubilizing fungus was isolated from rhizosphere salinity soil samples, identified as *Penicillium oxalicum* strain Po-5 No. HQ680452 and *Penicillium expansum* strain J1 No. HQ73298 in GeneBank. The study also demonstrated the capability of solubilizing of RP and TCP obviously varies from the resource of P (RP and TCP). In addition, the RP2 and TCP10 are the powerful RP and TCP solubilizer in modified Pikovskaya's agar (MPA) medium, suggesting a high soluble P releasing ability and good adaptation. The optimum conditions for the *P. oxalicum* and *P. expansum* releasing soluble P from RP and TCP, respectively were shaking speed from 100 up to 160 rpm, incubation time 40 and 50 hours, temperature at 30°C; initial pH, 7.0; RP and TCP concentration, 2 and 5 g L^{-1}, respectively and approximately close to the growth conditions of this fungus. The maximal content of soluble P can be achieved when RP or TCP as the sole P source in the medium inoculated with the *P. oxalicum* and *P. expansum*, respectively. Moreover, the two isolates showed intolerance with increasing concentration of salinity to 5% NaCl.

The action of microorganisms leading to solubilization of minerals is recognized as direct and indirect action also showed diverse levels of phosphate solubilization under different growth conditions with an additional ability to solubilise phosphate in saline conditions. Hence, there is need to develop the strain of fungi as a phosphate solubilisezr and application of the biofertilizer prepared by fungi should be helpful to reduce the salinity of soil by neutralization phenomenon. However, the solubilizing mechanism by the *P. oxalicum* and *P. expansum* should be further studies for crops in saline alkaline soils. Thus, it could be recalled that, *P. oxalicum* and *P. expansum* could serve as phosphate solubilizers in RP or TCP amended soil.

References

Abdel-Hafez, A.M., 1966. Some studies on acid producing microorganisms in soil and rhizosphere with special reference to phosphate dissolvers. Ph.D. Thesis, Fac. Agric. Ain Shams Univ., Cairo, Egypt.

Achala, V., V.V. Savantb and M.S. Reddya, 2007. Phosphate solubilization by a wild type strain and UV-induced mutants of *Aspergillus tubingensis.* Soil Biol. Biochem., 39: 695-699.

Barroso, C.B. and E. Nahas, 2005. The status of soil phosphate fractions and the ability of fungi to dissolve hardly soluble phosphates. Appl. Soil Ecol., 29: 73-83.

Biswas, D.R. and G. Narayanasamy, 2006. Rock phosphate enriched compost: An approach to improve low-grade Indian rock phosphate. Bioresour. Technol., 97: 2243-2251.

Cerezine, P.C., E. Nahas and D.A. Banzatto, 1988. Soluble phosphate accumulation by *Aspergillus niger* from fluorapatite. Applied Microbiol. Biotechnol., 29: 501-505.

Chuang, C.C., Y.L. Kuo, C.C. Chao and W.L. Chao, 2007. Solubilization of inorganic phosphates and plant growth promotion by *Aspergillus niger.* Biol. Fert. Soils, 43: 575-584.

Collavino, M.M., P.A. Sansberro, L.A. Mroginski and O.M. Aguilar, 2010. Comparison of *in vitro* solubilization activity of diverse phosphate-solubilizing bacteria native to acid soil and their ability to promote *Phaseolus vulgaris* growth. Biol. Fertil. Soils, 46: 727-738.

Coutinho, F.P., M.A.Q. Cavalcanti and A.M. Yano-Melo, 2011.Phosphate-solubilizing fungi isolated from a semiarid area cultivated with melon *(Cucumis melo* L. cv. goldmine). ActaBot. Bras., 25: 929-931.

Cunningham, J.E. and C. Kuiack, 1992. Production of citric and oxalic acids and Solubilization of calcium phosphate by *Penicillium bilaii.* Appl. Environ. Microbiol., 58: 1451-1458.

Duponnois, R., A. Colombet, V. Hien and J. Thioulouse, 2005. The mycorrhizal fungus *Glomus intraradices* and rock phosphate amendment influence plant growth and microbial activity in the rhizosphere of *Acacia holosericea.* Soil Biol. Biochem., 37: 1460-1468.

Gleddie, S.C., D. Schlechte and G. Turnbull, 1993. Effect of inoculation with *Penicillium bilaji* (provide) on phosphate and yield of canola in Western Canada. Proceedings of the Alberta Soil Science Workshop, February, 19-20. Edmonton, Alberta, pp: 155-160.

Goldstein, A.H., 1994. Involvement of the quinoprotein glucose dehydrogenase in the solubilizations of exogenous phosphates by gram-negative bacteria. In: Torriani-Gorini A, Yagil E, Silver S (edi). Phosphate in microorganisms.

Gupta, M., S. Kiran, A. Gulati, B. Singh and R. Tewari, 2012. Isolation and identification of phosphate solubilizing bacteria able to enhance the growth and aloin-A biosynthesis of *Aloe barbadensis* Miller. Microbiol. Res., 167: 358-363.

Gupta, N., J. Sabat, R. Parida and D. Kerkatta, 2007. Solubilization of tricalcium phosphate and rock phosphate by microbes isolated from chromite, iron and manganese mines. ActaBot. Croat., *66:* 197-204.
Illmer, P. and F. Schinner, 1995. Solubilization of inorganic calcium phosphates-solublization mechanisms. Soil Biol. Biochem., 27: 257-263.
Jackson, M.L., 1967. Soil Chemical Analysis. 1st Edn. Prentic Hall Inc., New Delhi, USA.
Johri, J.K., S. Surange and C.S. Nautiyal, 1999. Occurrence of salt pH and temperature –tolerant phosphate solubilizing bacteria ion alkaline soils; Curr. Microbiol, 39(2): 89-93.
Kang, S.C., G.C. Ha, T.G. Lee and D.K. Maheshwari, 2002. Solubilization of insoluble inorganic phosphates by a soil inhabiting fungus sp. Ps 102; Curr. Sci., 79(5): 439-442.
Kang, S.C., C.G. Ha, T.G. Lee and D.K. Maheshwari, 2002. Solubilization of insoluble inorganic phosphates by a soil-inhabiting fungus *Fomitopsis* sp. PS 102. Curr. Sci., 82: 439-441.
Keyes, D.O., 1990. *Penicillium bilaji:* Interactions with barley or canola, growth in rhizosphere soil and overwinter survival. M.Sc. Thesis, University of Alberta, Edmonton, Canada.
Khasawneh, F.E. and E.C. Doll, 1978. The use of phosphate rock for direct application to soils. Adv. Agron., 30: 159-206.
Kim, Y.H., B. Bae and Y.K. Choung, 2005. Optimization of biological phosphorus removal from contaminated sediments with phosphate solubilizing microorganisms. J. Biosci. Bioeng., 1: 23-29.
Kim, K.Y., D. Jordan and H.B. Kirshanan, 1997. *Rahnella aquatilis*, a bacterium isolated from soyabean rhizophere, can solubilize hydroxyapatite; FEMS Micobiol. Lett., 153 : 273-277.
Lu, J., X. Gao, Z. Dong and L. An, 2012. Expression of mitochondrial malate dehydrogenase in *Escherichia coli* improves phosphate solubilization. Ann. Microbiol, 62: 607-614.
Narsian, V. and H.H. Patel, 2000. Aspergillus aculeatus as a rock phosphate solubihzer. Soil Biol. Biochem., 32: 559-565.
Narsian, V., J. Thakker and H.H. Putei, 1995. Mineral phosphate solubilization by *Aspergillus aculeatus*; Ind. J Exp.Biol., 33: 91-93.
Pandey, A., N. Das, B. Kumar, K. Rinu and P. Trivedi, 2008. Phosphate solubilization by *Penicillium* spp. Isolated from soil samples of Indian Himalayan region. World J. Microbiol. Biotechnol, 24: 97-102.
Pradhan, N. and L.B. Sukla, 2005. Solubilization of inorganic phosphates by fungi isolated from agriculture soil. Afr. J. Biotechnol., 5: 850-854.
Rashid, M., S. Khakil, N. Ayub, S. Alam and F. Latif, 2004. Organic acids production and phosphate solubilization by Phosphate Solubilizing Microorganisms (PSM) under *in vitro* conditions. Pak. J. Biol Sci., 7: 187-196.
Reddy, M.S., S. Kumar, K. Babita and M.S. Reddy, 2002. Biosolubilization of poorly soluble rock phosphates by *Aspergillus tubingensis and Aspergillus niger.* Bioresour. Technol., 84: 187-189.
Richa, G., B. Khosla and M.S. Reddy, 2007. Improvement of maize plant growth by phosphate solubilizing fungi in rock phosphate amended soils. World J. Agric. Sci., 3: 481-484.
Seshadre, S., R. Muthukumarasamy, C. Lakshminarasimhan, S. Ignaacimuthu, 2000. Solubilization of inorganic phosphate by *Bradyrhizobium*. Indian J.Exp. Biol., 29: 28-31.
Son, H.J., G.T. Park, M.S. Cha and M.S. Heo, 2006. Solubilization of insoluble inorganic phosphates by a novel salt- and pH-tolerant *Pantoea agglomerans* R-42 isolated from soybean rhizosphere. Bioresour. Technol., 97: 204-210.
Tamura, K., J. Dudley, M. Nei and S. Kumar, 2007. MEGA4: Molecular Evolutionary Genetics Analysis (MEGA) software version 4.0. Mol. Biol. Evol, 24: 1596-1599.
Valverde, A., A. Burgos, T. Fiscella, R. Rivas, E. Velazquez, C. Rodriguez and J.M. Igual, 2006. Differential effects of co inoculations with *Pseudomonasjessenii~PS06* (aphosphate solubilizing bacterium) and *Mesorhizobium ciceri* c-2/2 strains on the growth and seed yield of chickpea under greenhouse and field conditions. Plant Soil, 287: 43-50.
Van Veen, J.A., L.S. Van Overbeek and J.D. Van Elsas, 1997. Fate and activity of microorganisms introduced into soil. Microbiol. Mol. Biol. Rev., 61: 121-135.
Vassilev, N., A. Medina, R. AzconandM. Vasslev, 2006. Microbial solubilization of rock phosphate media containing agro-industrial wastes and effect of the resulting products on plant growth and P uptake. Plant Soil, 287: 77-84.
Vassilev, N., M.T. Baca, M. Vassileva, I. Franco and R. Azcon, 1995. Rock phosphate solubilization by *Aspergillus niger* grown on sugar-beet wastemedium. Appl. Microbiol. Biotechnol, 44: 546-549.
Vassileva, M., N. Vassilev andR. Azcon, 1998. Rock phosphate solubilization by *Aspergillus niger* on olive cake-based medium and its further application in a soil-plant system. World. J. Microbio. Biotechnol., 14: 281-284.
Vyas, P., P. Rahi, A. Chauhan and A. Gulati, 2007. Phosphate solubilization potential and stress tolerance *oiEupenicillium parvum* from tea soil. MycologicalRes., Ill: 931-938.
Wei, J.C., 1979. Handbook of Fungal Identification. Shanghai Scientific and Technical Publisher, Shanghai,

China, pp: 406-649.
White, T.J., T. Bruns, S. Lee and J. Taylor, 1990. Amplification and Direct Sequencing of Fungal Ribosomal RNA Genes for Phylogenetics. In: PCR Protocols: A Guide to Methods and Applications, Inms, M.A., D.H. Gelfand, J.J. Smnsky and T.J. White (Eds.). Academic Press, New York, USA, pp: 315-322.
Xiao, C.Q., R.A. Chi, X.H. Huang, W.X. Zhang, G.Z. Qiu and D.Z. Wang, 2008. Optimization for rock phosphate solubilization by phosphate-solubilizing fungi isolated from phosphate mines. Ecol. Eng., 33: 187-193.
Yadav, J., J.P. Verma, S.K. Yadav, and K.N. Tiwari, 2011. Effect of Salt Concentration and pH on Soil Inhibiting Fungus Penicillium Citrinum Thom for Solubilization of Tricalcium Phosphate, Microbiolology Journal, Volume 1, No. 1, pp. 25-32.
Yu, X., X. Liu, T.H. Zhu,G.H. Liu and C.Mao, 2012. Co-inoculation with phosphate-solubilzing and nitrogen-fixing bacteria on solubilization of rock phosphate and their effect on growth promotion and nutrient uptake by walnut. Eur. J. Soil Biol., 50: 112-117.