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Production of Phophatase by mutated fungal strains

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Abstract

Phosphate solubilizing microorganisms were isolated from rhizosphere soils of rice fields around Mannachanalur area, Tiruchirappali district of Tamilnadu. The isolated fungal cultures were identified as *Aspergillus niger, Aspergillus fumigatus* and *Penicillium sp.* The efficacy of Phosphatase activity of fungal strains was carried out. The fungal strains were subjected to random mutational studies such as UV and chemical mutation. The Phosphatase assay was carried out after random mutagenesis using wild and mutated cultures. The efficacy of Phosphatase activity of the wild strains were observed in the descending order of *Aspergillus niger* > *Penicillium* sp. > *Aspergillus fumigatus.* Among the chemical treated fungal strains, *Aspergillus niger* (ANsa120) was the predominant fungal strain for Phosphatase activity. Glucose and Ammonium sulphate were found to be the best carbon and nitrogen source respectively for both phosphate solubilization and phosphatase production. Significance of the findings is discussed in detail.

Keywords: Phosphate solubilizing fungi, Mutagenesis, Enhancement, Mutant strains, Phosphatase activity.

Abbreviation: SA: Sodium azide, EMS: Ethyl methane sulphonate, UV: Ultra Violet, ANsa: Sodium azide treated *Aspergillus niger,* AFsa: Sodium azide treated *Aspergillus fumigatus,* PEsa: Sodium azide treated *Penicillium.* ANems: Ethyl methane sulphonate treated *Aspergillus niger*, AFems: Ethyl methane sulphonate treated *Aspergillus fumigatus,* PEems: Ethyl methane sulphonate treated *Penicillium,* ANuv: Ultra Violet treated *Aspergillus niger,* AFuv: Ultra Violet treated *Aspergillus fumigatus* and PEuv: Ultra Violet treated *Penicillium.*

INTRODUCTION

Many soils are deficient in readily available forms of phosphorus for plant uptake (Hinsinger *et al*., 2003) and may require the application of phosphate based fertilizers to remain productive. Plants take phosphate in the form of soluble orthophosphate ions but due to the presence of Ca^{++}, Mg^{++}, K^{+}, Na^{+}, Al^{+} and Fe^{2+} ions in soil, the soluble orthophosphate is converted to insoluble form. Because of this process plants utilize very little amount of phosphate, even though phosphorus containing fertilizers are added (Vivekkumar *et al.,* 1999). It is thought that part of this phosphosphate is made available for uptake through acidification of rhizosphere soil and release of organic anions (Hinsinger, 2001; Hinsinger *et al*., 2003). Another possible mechanism to liberate P from soil is the release of phosphatase enzymes into the environment by many microorganisms.

MATERIALS AND METHODS

Microorganisms and isolation

The fungal strains were isolated from rhizosphere soils of Paddy field, Mannachanallur, one of the largest paddy producing taluk located in Tamil Nadu State, India. The soil samples were screened for phosphate solubilizing fungi in Pikovskaya's agar medium by conventional dilution plate count method. The fungal strains showing halo formation around their colonies were isolated and grown on Sabouraud agar slants at 27ºC for 3 days and kept in the refrigerator at 4^0C until further use (Elliaiah *et al*., 2002).

Identification

The fungal cultures were identified based on the colony morphology and spore structure (Pradhan and Sukla. 2005).

Random mutational studies

Mutagenesis by UV

72 hrs old fungal cultures were scraped off from agar slants and suspended in 5ml sterile distilled water and then diluted with 45 ml of sterile distilled water containing Tween 80 (1:4000). Sterile glass beads were added to the suspension and kept on rotary shaker for 30 min to break the hyphal mycelium. The suspension was filtered to remove the mycelium. The spore suspension was prepared in phosphate buffer (pH7.0) containing 10^6 spores per ml. Five ml quantities of the spore suspension were transferred aseptically into sterile petri dishes and exposed to UV light (2600 A^o) at a distance of 15cm away from the center of the Germicidal lamp for various time intervals (10, 20, 30, 40, 50, 60, 70 and 80 min). The suspension was agitated by gently rotating the plates in between the time intervals (Elliaiah *et al*., 2002).

The UV exposed spore suspensions were stored overnight in dark to avoid photo reactivation. After overnight incubation, irradiated spore suspensions were serially diluted by using phosphate buffer (pH 7.0) and plated on Sabouraud's Dextrose agar medium. The plates were incubated for 5 days at 27°C. The colonies were selected on the basis of their morphological characters and were given the code numbers ANuv30, ANuv40, ANuv50, ANuv60,

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AFuv30, AFuv40, AFuv50, AFuv60, PEuv30, PEuv40, PEuv50 and PEuv60.

Mutagenesis by Chemicals

Chemical mutagenesis was performed using Sodium azide and Ethyl methane sulphonate (EMS) for the strain improvement of phosphate solubilizers. Spore suspensions of fungal strains were prepared by using phosphate buffer pH 7.0 as described earlier (Ellaiah *et al*., 2002). To 9 ml of each spore suspension, 1 ml of sterile solution of Sodium azide (250 μg ml^{-1} in phosphate buffer) was added. Similar procedure was adopted for Ethyl methane sulphonate (EMS) (150 μg ml^{-1} in phosphate buffer). The reaction was allowed to proceed. Control tube was also kept without any chemical mutagen. Samples were withdrawn from the reaction mixture at an interval of 30, 60, 90, 120 and 150 min. and centrifuged for 10 min. at 5000 rpm. The cells were washed three times with sterile distilled water and again re-suspended in 10 ml sterile buffer. The samples were serially diluted in the same buffer and plated on Sabouraud's Dextrose agar medium. The selected sodium azide treated mutants were given the code numbers ANsa30, ANsa60, ANsa90, ANsa120, AFsa30, AFsa60, AFsa90, AFsa120, PEsa60, PEsa90, PEsa120 and PEsa150.

The EMS treated mutants were given the code numbers ANems30, ANems60, ANems90, ANems120,AFems30, AFems60, AFems90, AFems120,PEems60, PEems90, PEems120 and PEems150 (Bapiraju et al., 2004).

Submerged fermentation

Inoculation (Sudhansupal, 1999; Varshanarishan *et al.,* 1995)

The pure fungal cultures from Sabouraud agar slants were transferred to Sabouraud agar plates. After four days incubation at 27°C, two 8 mm disc were cut from the plates of all three fungal plates and were inoculated into 100 ml Pikovskaya broth. The flasks were incubated at 27°C. Uninoculated Pikovskaya broth served as control in each case. Each experiment was done in triplicate set.

Acid phosphatase assay (Eileen ingham *et al.*, 1979)

The enzyme Acid phosphatase was assayed using para-nitrophenyl phosphate (PNP-P) as a substrate. The reaction mixture contained 2.5 ml (0.1M) sodium acetate buffer (pH 5.8), I ml (1mM) Magnesium chloride, 0.5 ml 1% PNP-P and 0.5ml of a suitable dilution of enzyme preparation. One ml of the reaction mixture was transferred to 2ml of 0.2M Sodium Hydroxide before and after 15 min incubation at 37°C to stop the reaction. The Sodium Hydroxide solution added before incubation act as a control sample for each analysis. The amount of Para- nitro phenol (PNP) liberated was measured by recording the absorbance at 420 nm using an appropriate calibration curve. Activity is expressed as μmol PNP liberated min^{-1}. The blank was run in a similar manner using distilled water.

Preparation of Standard curve

Dissolve 1.0 g of Para- nitro phenol in water, and dilute the solution to 1000 ml. This is standard Para nitro phenol solution. Store the solution in a refrigerator. To prepare the standard graph, dilute 1 ml of the standard Para- nitro phenol solution to 100 ml in a volumetric flask and mix the solution thoroughly. Then pipette 0, 1, 2, 3, 4 and 5-ml aliquots (equaling to 0, 10, 20, 30, 40 and 50 μg of Para- nitro phenol) of this diluted standard solution into 5 ml volumetric flask and made up to 5 ml by addition of water, and proceed as described above. The O.D value (Y axis) and corresponding Para nitro phenol concentration (X axis) were plotted in a graph.

Effect of Different Carbon Sources

To study the effect of different carbon sources on Phosphatase activity, the carbon source of Pikovskaya broth i. e. Glucose was replaced by Lactose, Sucrose and Mannitol. Then the mutated cultures which were superior in Acid Phosphatase were inoculated, incubated and Acid Phosphatase assay was carried out as described earlier.

Effect of Different Nitrogen Sources

To study the effect of different nitrogen sources on Phosphatase activity, the nitrogen source of Pikovskaya broth i.e. Ammonium Sulphate was replaced by Potassium Nitrate, Sodium Nitrate and Urea. The mutated cultures which were superior in Acid Phosphatase activity in Pikovskaya broth were inoculated, incubated and Acid Phosphatase assay was carried out as described earlier.

RESULTS AND DISCUSSION

The efficacy of Phophatase activity of fungal strains treated by ultraviolet rays, sodium azide and Ethyl methane sulphonate (EMS) are presented in the Tables 1 to 3, 4 to 6 and 7 to 9 respectively. The influence of different carbon and nitrogen sources on phosphate solubilization of efficient fungal mutants are reported in the Tables 10 to 15. Figure- 1 shows the comparsion of efficacy of Phosphatase activity between wild strains and Figure-2 shows the comparsion of efficacy of Phosphatase activity between chemically treated fungal strains.

The Phosphatase production capacity of isolated fungal cultures were analysed by inoculating the fungal isolates into Pikovskaya broth. In the present study, para nitro phenyl phosphate was used as a substrate for determining the Acid Phosphatase activity. The earlier reports were available for the utilization of para nitro phenyl phosphate as a substrate for Acid Phosphatase assay (Prada *et al.* 1996;Puruchothaman, 1994).

In the present study, the efficacy of Phosphatase production by the wild strains were observed in the descending order of *Aspergillus niger> Penicillium* sp. > *Aspergillus fumigatus.* Among the UV irradiated fungal strains, ANuv60 was found to be the best Phosphatase producing strain followed by ANuv50, ANuv30, ANuv40, AFuv50, PEuv50, AFuv60, PEuv40, PEuv60, PEuv30, AFuv40 and AFuv30.

Among the chemical treated fungal strains, ANsa120 was the predominant fungal strain followed by ANems120, PEems150, ANsa90, PEsa150, ANems90, PEsa120, AFsa120, PEems120, PEems60, PEems90, ANems60, AFsa120, AFsa90, PEsa90, ANsa30, AFems120, ANems30, AFems90, PEsa60, AFsa60, AFems60, AFems30 and AFsa30. The results of the present study is in agreement with Relwani *et al.* (2008) who reported that the enzyme activities such as Acid Phosphatase and Phytase was increased significantly in mutants of *A. tubingensis* when compared to the wild type. Achal *et al.* (2007) reported that Acid Phosphatase production by UV induced mutants of *Aspergillus tubingensis,* might be the reason for highest phosphate solubilization by mutant strains. The production of the Acid Phosphatase was favoured by low pH values.

Table 1. Efficacy of phosphatase activity of UV treated *Aspergillus niger*

UV irradiated *Aspergillus niger* strains	*Phosphatase activity µmol min^{-1} (Day)			Increased % of phosphatase activity (Day)		
	3rd	6th	9th	3rd (%)	6th (%)	9th (%)
ANuv30	0.121±0.04	0.223±0.05	0.345±0.04	24.74	16.15	13.86
ANuv40	0.103±0.02	0.218±0.04	0.341±0.03	6.19	13.54	12.54
ANuv50	0.134±0.03	0.260±0.05	0.399±0.05	38.14	35.42	31.68
ANuv60	0.165±0.02	0.275±0.05	0.424±0.06	70.10	43.23	39.93
Wild type	0.097±0.05	0.192±0.03	0.303±0.04			

*Mean value ± Standard Deviation of triplicates

Table 2. Efficacy of phosphatase activity of fungal strains UV treated *Aspergillus fumigatus*

UV irradiated *Aspergillus fumigatus* strains	*Phosphatase activity µmol min^{-1} (Day)			Increased % of phosphatase activity (Day)		
	3rd	6th	9th	3rd (%)	6th (%)	9th (%)
AFuv30	0.171±0.05	0.203±0.06	0.221±0.06	9.62	14.04	13.86
AFuv40	0.160±0.05	0.205±0.05	0.232±0.08	2.56	15.17	12.54
AFuv50	0.195±0.04	0.227±0.07	0.269±0.04	25.00	27.53	31.68
AFuv60	0.173±0.05	0.224±0.04	0.265±0.03	10.90	25.84	39.93
Wild type	0.156±0.04	0.178±0.04	0.197±0.06			

*Mean value ± Standard Deviation of triplicates

Table 3. Efficacy of phosphatase activity of *Penicillium* strains

UV irradiated *Penicillium* strains	*Phosphatase activity µmol min^{-1} (Day)			Increased % of phosphatase activity (Day)		
	3rd	6th	9th	3rd (%)	6th (%)	9th (%)
PEuv30	0.180±0.04	0.228±0.06	0.239±0.06	6.51	7.04	1.27
PEuv40	0.195±0.05	0.245±0.08	0.264±0.04	15.38	15.02	11.86
PEuv50	0.209±0.05	0.237±0.07	0.267±0.06	23.67	11.27	13.14
PEuv60	0.178±0.05	0.234±0.04	0.257±0.05	5.33	9.86	8.90
Wild type	0.169±0.04	0.213±0.06	0.236±0.06			

*Mean value ± Standard Deviation of triplicates

Table 4. Efficacy of phosphatase activity of Sodium azide treated *Aspergillus niger* strains

Sodium azide treated *Aspergillus niger* strains	*Phosphatase activity µmol min^{-1} (Day)			Increased % of phosphatase activity (Day)		
	3rd	6th	9th	3rd (%)	6th (%)	9th (%)
ANsa30	0.107±0.04	0.218±0.04	0.335±0.06	10.30	13.54	10.56
ANsa60	0.110±0.05	0.234±0.03	0.401±0.03	13.40	21.87	32.34
ANsa90	0.152±0.03	0.268±0.03	0.421±0.06	56.70	39.58	38.94
ANsa120	0.184±0.03	0.340±0.03	0.474±0.05	89.69	77.08	56.43
Wild type	0.097±0.05	0.192±0.03	0.303±0.04			

*Mean value ± Standard Deviation of triplicates

Table 5. Efficacy of phosphatase activity of Sodium azide treated *Aspergillus fumigatus* strains

Sodium azide treated *Aspergillus fumigatus* strains	*Phosphatase activity µmol min^{-1} (Day)			Increased % of phosphatase activity (Day)		
	3rd	6th	9th	3rd (%)	6th (%)	9th (%)
AFsa30	0.165±0.04	0.189±0.04	0.213±0.06	5.76	6.18	8.12
AFsa60	0.174±0.03	0.203±0.05	0.241±0.05	11.53	14.04	22.33
AFsa90	0.185±0.06	0.229±0.04	0.351±0.05	18.59	28.65	78.17
AFsa120	0.232±0.04	0.274±0.03	0.365±0.05	48.71	53.93	85.27
Wild type	0.156±0.04	0.178±0.04	0.197±0.06			

* Mean value ± Standard Deviation of triplicates

Table 6. Efficacy of phosphatase activity of Sodium azide treated *Penicillium* strains

Sodium azide treated *Penicillium* strains	*Phosphatase activity µmol min^{-1} (Day)			Increased % of phosphatase activity (Day)		
	3rd	6th	9th	3rd (%)	6th (%)	9th (%)
PEsa60	0.181±0.06	0.233±0.05	0.261±0.04	7.10	9.39	10.59
PEsa90	0.191±0.04	0.249±0.04	0.349±0.04	13.01	16.90	47.88
PEsa120	0.200±0.05	0.261±0.05	0.400±0.06	18.34	22.53	69.49
PEsa150	0.210±0.04	0.276±0.06	0.420±0.08	24.26	29.57	77.96
Wild type	0.169±0.04	0.213±0.06	0.236±0.06			

*Mean value ± Standard Deviation of triplicates

Table 7. Efficacy of phosphatase activity of Ethyl Methane Sulphonate (EMS) treated *Aspergillus niger*

EMS treated *Aspergillus niger* strains	*Phosphatase activity µmol min^{-1} (Day)			Increased % of phosphatase activity (Day)		
	3rd	6th	9th	3rd (%)	6th (%)	9th (%)
ANems30	0.099±	0.210±	0.316±	2.06	9.37	4.29
ANems60	0.104±0.03	0.222±0.05	0.365±0.04	7.21	15.62	20.46
ANems90	0.125±0.04	0.253±0.06	0.412±0.03	28.86	31.77	35.97
ANems120	0.131±0.05	0.273±0.05	0.452±0.07	35.05	42.18	49.17
Wild type	0.097±0.05	0.192±0.03	0.303±0.04			

*Mean value ± Standard Deviation of triplicates

Table 8. Efficacy of phosphatase activity of Ethyl Methane Sulphonate (EMS) treated *Aspergillus fumigatus*

EMS treated *Aspergillus niger* strains	*Phosphatase activity µmol min^{-1} (Day)			Increased % of phosphatase activity (Day)		
	3rd	6th	9th	3rd (%)	6th (%)	9th (%)
AFems30	0.175±0.04	0.197±0.06	0.214±0.05	12.17	10.67	8.62
AFems60	0.189±0.05	0.212±0.04	0.231±0.07	21.15	19.10	17.25
AFems90	0.206±0.05	0.236±0.07	0.261±0.05	32.05	32.58	32.48
AFems120	0.219±0.05	0.258±0.04	0.318±0.04	40.38	44.94	61.42
Wild type	0.156±0.04	0.178±0.04	0.197±0.06			

*Mean value ± Standard Deviation of triplicates

Table 9. Efficacy of phosphatase activity of Ethyl Methane Sulphonate (EMS) treated *Penicillium* strains

EMS treated *Penicillium* strains	*Phosphatase activity µmol min^{-1} (Day)			Increased % of phosphatase activity (Day)		
	3rd	6th	9th	3rd (%)	6th (%)	9th (%)
PEems60	0.197±0.05	0.275±0.05	0.370±0.05	16.56	29.10	56.78
PEems90	0.227±0.05	0.325±0.05	0.369±0.05	34.32	52.58	56.35
PEems120	0.245±0.06	0.324±0.03	0.371±0.05	44.97	52.11	57.20
PEems150	0.278±0.04	0.381±0.07	0.425±0.07	64.49	78.87	80.08
Wild type	0.169±0.04	0.213±0.06	0.236±0.06			

*Mean value ± Standard Deviation of triplicates

Table 10. Efficacy of phosphatase activity of *Aspergillus niger* (ANems120) grown on different Carbon sources

Carbon sources vs ANems120 Strains	*Phosphatase activity µmol min^{-1} (Day)		
	3rd	6th	9th
Glucose	0.131±0.05	0.273±0.05	0.452±0.07
Sucrose	0.107±0.04	0.206±0.05	0.279±0.04
Mannitol	0.083±0.05	0.167±0.03	0.229±0.05
Lactose	0.094±0.04	0.182±0.07	0.257±0.04

*Mean value ± Standard Deviation of triplicates;

Table 11. Efficacy of phosphatase activity of *Aspergillus niger* (ANems120) grown on different Nitrogen sources

Nitrogen sources vs ANems120 strains	*Phosphatase activity µmol min^{-1} (Day)		
	3rd	6th	9th
Ammonium Sulphate	0.131±0.05	0.273±0.05	0.452±0.07
Sodium Nitrate	0.074±0.04	0.122±0.06	0.173±0.08
Potassium Nitrate	0.102±0.04	0.200±0.05	0.271±0.03
Urea	0.109±0.07	0.203±0.05	0.275±0.06

*Mean value ± Standard Deviation of triplicates

Table 12. Efficacy of phosphatase activity of *Aspergillus niger* (ANsa120)grown on different Carbon sources

Carbon sources vs ANsa120 Strains	*Phosphatase activity µmol min^{-1} (Day)		
	3rd	6th	9th
Glucose	0.184±0.03	0.340±0.03	0.474±0.05
Sucrose	0.186±0.05	0.248±0.07	0.330±0.06
Mannitol	0.076±0.07	0.094±0.04	0.134±0.08
Lactose	0.165±0.04	0.187±0.08	0.213±0.06

*Mean value ± Standard Deviation of triplicates;

Table 13. Efficacy of phosphatase activity of *Aspergillus niger* (ANsa120) grown on different Nitrogen sources

Nitrogen sources vs ANsa120 Strains	*Phosphatase activity µmol min^{-1} (Day)		
	3rd	6th	9th
Ammonium Sulphate	0.184±0.03	0.340±0.03	0.474±0.05
Sodium Nitrate	0.08±0.02	0.103±0.06	0.125±0.07
Potassium Nitrate	0.108±0.08	0.203±0.08	0.254±0.04
Urea	0.112±0.07	0.216±0.07	0.273±0.06

*Mean value ± Standard Deviation of triplicates

Table 14. Efficacy of phosphatase activity of *Penicillium* sp.(PEsa150) grown on different Carbon sources

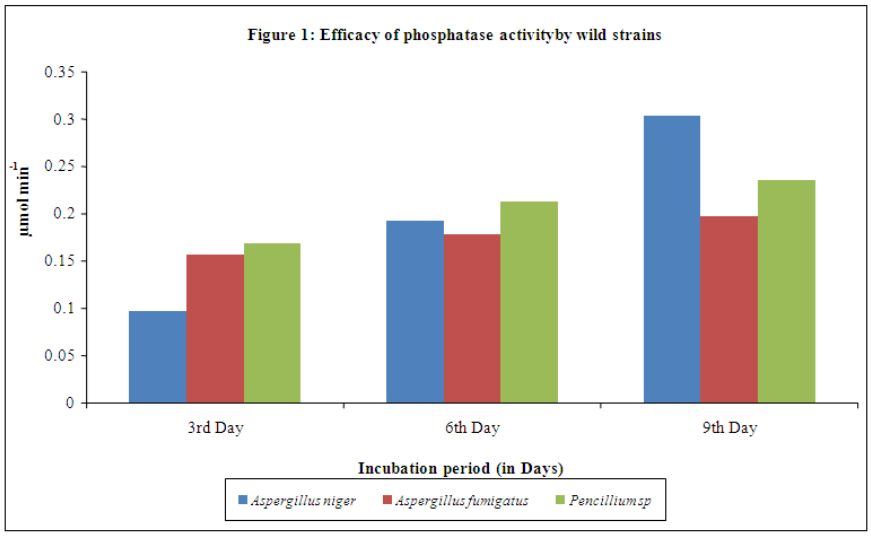
Carbon sources PEsa150	*Phosphatase activity µmol min^{-1} (Day)		
	3rd	6th	9th
Glucose	0.210±0.04	0.276±0.06	0.420±0.08
Sucrose	0.171±0.08	0.288±0.06	0.342±0.03
Mannitol	0.162±0.04	0.204±0.04	0.261±0.04
Lactose	0.168±0.05	0.214±0.06	0.253±0.04

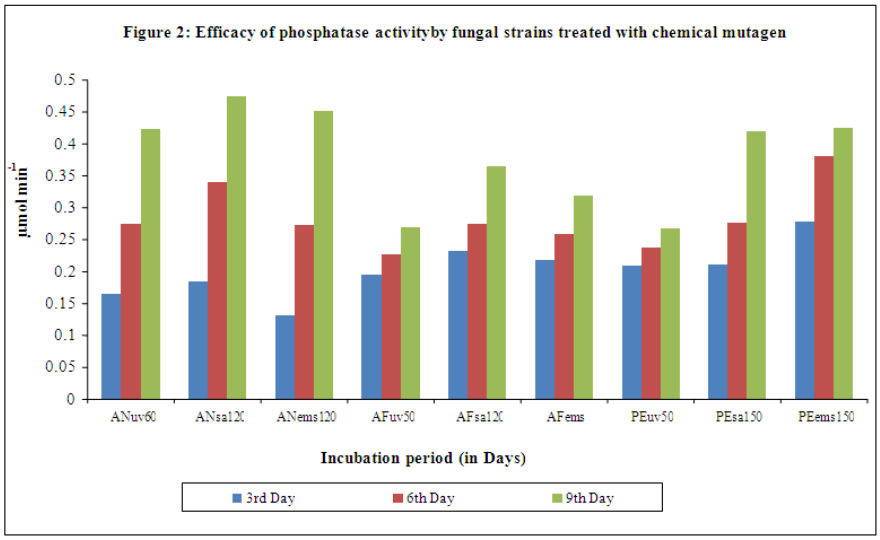
*Mean value ± Standard Deviation of triplicates

Table 15. Efficacy of phosphatase activity of *Penicillium* sp.(PEsa150) grown on different Nitrogen sources

Nitrogen sources PEsa150	*Phosphatase activity µmol min^{-1} (Day)		
	3rd	6th	9th
Ammonium Sulphate	0.210±0.04	0.276±0.06	0.420±0.08
Sodium Nitrate	0.163±0.05	0.209±0.03	0.229±0.05
Potassium Nitrate	0.165±0.04	0.218±0.04	0.261±0.05
Urea	0.205±0.05	0.223±0.07	0.270±0.03

*Mean value ± Standard Deviation of triplicates





Among all physical and chemical treated fungal strains, the best Phosphatase producing strains such as ANsa120, ANems120 and PEems150 were demonstrated in the presence of various carbon and nitrogen sources in the present study. The effect of different carbon sources on Phosphatase production by fungal strains were found to be in the ascending order of Mannitol< Lactose < Sucrose < Glucose. Andrea *et al.* (1990) reported that *Pseudomonas aeruginosa* and *Rhizobium meliloti*, utilized several choline derivatives as the sole carbon and nitrogen source and increased the production of Acid Phosphatase activity. Spicers and McGill (1979) demonstrated an increase in Phosphatase activity during incubation of soil amended with Glucose and Ammonium Sulphate.

The effect of different nitrogen sources on Phosphatase production by fungal strains were found to be in the ascending order of Sodium Nitrate < Potassium Nitrate < Urea < Ammonium Sulphate in the present study. The results are in agreement with Semenova *et al.* (1986) who stated that the synthesis of the secreted enzyme depended on the source of carbon and nitrogen nutrition. The enzyme activity was highest in a medium with Sucrose as a carbon source and Ammonium salt as a nitrogen source. The secretion of Acid Phosphatase is stimulated by an increase in the sugar content and a deficiency of the nitrogen source in the medium.

Sudha and Purushothaman (2000) reported that *Pseudomonas* strain isolated from the freshwater pond system showed repressive effect on phosphatase production. Casida (1959) isolated several species of *Aspergillus* sp. capable of producing Acid Phosphatase active against organic phosphate in soils. Smith *et al.* (1973) reported the addition of gradient amount of Potassium phosphate to Phosphatase test broth exhibited the least inhibition to Phosphatase activity by *Candida tropicalis.* Sarapatka *et al.* (2004) stated that increasing the amount of available phosphate increased the Phosphatase activity in cereal roots. Pedregosa *et al.* (1991) reported that *Aspergillus* strains produce non repressible, repressible and phosphate inducible Phosphotases. It is accepted that culture condition may cause fluctuations and multiplicity of fungal Phosphatases. Ponmurugan and Gopi (2006) stated that there was a positive correlation between phosphate solubilizing capacity and Phosphatase enzyme activity. These earlier reports are in agreement with present results which show that the increase in inorganic phosphate concentration due to phosphate solubilization does not repress the Phosphatase production by all strains used in this study. But this is contrary with the earlier reports of Semenova *et al.* (1986) who studied that Acid Phosphatase production from *Saccharomyces cerevisiae* in presence of phosphate concentration of 10 mM which showed repression of the Phosphatase production. Ohta *et al.* (1968) stated that the production of Phosphatase by the black koji fungus *Aspergillus awamori* is a repressible enzyme.

The earlier reports explained that there are two systems of regulation in Phosphatase induction in microorganisms. This may be the reason for repressible and non repressible nature of Phosphatases by inorganic phosphate. Singleton and Sainsberry (1988) explained that the phosphate controlled genes or *pho* genes is the gene cluster, consisting of *Pho A* encoding Phosphatase*, Pho S* encoding inorganic phosphate binding site*, PhoE* encoding porin *and Pho B* encoding the regulator gene as like the operon model. Wagner *et al.* (1995) reported that *Synechoccus* strain system of phosphate irrepressible Acid Phosphatase in addition to the normal repressible system.

The present study exhibited the phosphatase activity of fungal cultures were increased by random mutagenesis. This was in agreement with Rajeshkumar and Ilyas (2011) who stated that the lipase activity of *Aspergillus niger* was increased by random mutagenesis when compared to wild strains, The phosphate solubilization efficacy of fungal cultures were improved by physical and chemical mutagen (Rajeshkumar and Ilyas, 2010 a) and The lipase activity of mutant strain of *Pseudomonas* sp using chemical mutagen was 2 fold higher than the wild strain (Rajeshkumar and Ilyas, 2010 b).

CONCLUSION

Many microorganisms are able to produce Phosphatase in liquid broth containing various phosphate substrates. The present study concluded that the treatment with physical and chemical mutagenic agents increased the phosphatase production efficacy of the fungal strains. This study also strengthened the idea that addition of carbon and nitrogen sources favors the phosphatase production to a certain extent.

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