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Comparative assessment of phosphorus solubilizing enzymes production by three *Aspergillus spp.*

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Abstract

The rhizospheric soil of *Leptadenia pyrotechnica* from saline (Pachpadra) and non-saline (Jodhpur) site was collected to isolate fungi and to assess the efficiency in P solubilization. Three common fungal species *viz., Aspergillus fumigatus, A. flavipes* and *A. niger* were assessed for acid phosphatase, alkaline phosphatase and phytase enzyme production for P solubilization at both intracellular and extracellular conditions. All three fungi from saline soil produced significantly higher amount of acid and alkaline phosphatase, whereas in non-saline soil phytase production was higher. All fungi showed higher level of intracellular enzyme activity than extracellular condition in both type of soils.

Keywords: *Leptadenia pyrotechnica, Aspergillus fumigatus, A. flavipes, A. niger*, acid phosphatase, alkaline phosphatase, phytase

INTRODUCTION

Saline soil distributed throughout the world especially in the arid and semi arid regions where agriculture performs under irrigation (Vassileva *et al.*, 1998). High salinity affects plant growth through the osmotic effects; toxicity of salt ions and the changes in the physical and chemical properties of soil (Abd Alla, 1994). Phosphorus (P) is one of the essential macronutrients after nitrogen required for plant growth and development (Illmer and Schinner, 1992). Phosphate in soil mostly exists in insoluble (bound) forms and thus the content of soluble phosphate in soil is very low (Rodriguez and Fraga, 1999). Salinity affects the rate of P mineralization from organic matter decomposition.

Phosphorous is taken up from the soil in the form of soluble orthophosphate ions; $H_2PO_4^1$, HPO_4^{-2} and PO_4^{-3} and generally the availability of these ions to the plants is in the order of $H_2PO_4^-$

^{1}> HPO_4^{-3}>PO_4^{-3}. Two forms of phosphorus *viz.*, organic and inorganic forms occur in soils and both are important to plants as source of phosphorus. The importance of soil organic P as a source of plant available P depends on its rate of solubilization and the rate of inorganic P release. There are groups of soil microorganisms including bacteria, fungi, actinomycetes and arbuscular mycorrhiza have the ability to solubilize the precipitated phosphates, converting them into soluble forms; $H_2PO_4^{-1}$ and HPO_4^{-2} that are available to plant (Coutinho *et al.,* 2012).In soil P solubilizing bacteria constitute 1-50% and fungi 0.1-0.5% of the total respective population (Zaidi *et al.,* 2009). The strains from the genera *Aspergillus* and *Penicillum* are among the most powerful phosphate solubilizers. Microbial solubilization of insoluble phosphate occurs by different mechanisms, such as acidification, chelation, ion-exchange reactions and polymeric substances formation (Delvasto *et al.,* 2006). Different enzymes like phosphatases and phytases play important role in dephosphorylation of organic phosphorus. Phosphatases in the rhizosphere may arise from plant roots or from soil microorganisms (Hubel and Beck, 1993; Tarafdar, 1995; Richardson and Hadodas, 1997 and Hayes *et al.,*1999). In soil, the hydrolysis of organic P is predominantly mediated by the activity of soil microorganisms, although plant roots also possess phosphatase and phytase activity (Tarafdar and Junk, 1987 and Li *et al.,* 1997).

In present comparative study aims to assess three common fungi's, isolated from saline and non-saline areas of Rajasthan, efficiency in phosphorus solubilizing enzyme production under *in vitro* culture condition.

MATERIALS AND METHODS

1. Collection of sample

Rhizospheric soils of *Leptadenia pyrotechnica* were collected from two sites, one from Pachpadra(saline) and other from Jodhpur(non-saline). In Pachpadra soil was taken from near the salt pit. The soil preserved in refrigerator for enzymatic analyses and their physico-chemical properties i.e. EC and pH were assessed using 1:5 Soil: water solution.

2. Isolation and identification of fungi

Fungi were isolated from both the soil samples by dilution series method in two different media i.e. Rose Bengal and Czapek Dox Agar media. The plates were incubated at 28± 2°C and total fungal colony and number of species was counted. The colonies were purified by further culturing in slants. Then pure cultures were grown in liquid media for further experiments. The fungal species were identified on the basis of morphology.

3. Estimation of enzymes

The pure cultures were grown in triplicates for three different stages initial, mid and final on the basis of fungal growth. Each culture media flask contained 100ml media and inoculated with 5ml pure culture. After this as per the growth of different species experiments were done for estimating intracellular and extracellular enzyme levels and the selected enzymes are: acid phosphatase, alkaline phosphatase (Tabatabai and Bermner, 1969; Eivazi and Tabatabai, 1977) and phytase (Ames, 1966).

4. Statistical analysis

The data were subjected to analysis of variance using random block design (Gomez and Gomez, 1984).

RESULTS

1. Physico-chemical characteristics and mycoflora of the soil

The pH, EC and mycoflora of rhizospheric soil of saline and non-saline was shown in Table 1. Colony forming unit in three seasons was counted which shows that in non-saline soil colony count was more as compare to saline in all three seasons.

Table 1: Physico-chemical characteristics and mycoflora of the rhizospheric soil of *Leptadenia pyrotechnica* plant

Season	SUMMER		RAINY		WINTER	
	Saline	Non saline	Saline	Non saline	Saline	Non saline
pH	8.66	7.59	8.41	8.91	9.32	8.09
EC	0.84	0.71	0.72	0.48	0.71	0.65
CFU ($X10^5$)	7	12	7	13	7	10
No. of Species	3	5	3	5	3	4

1. Fungal growth

Growth of fungi collected from rhizospheric soils of *Leptadenia pyrotechnica* of saline and non-saline soils was estimated which shows the increase in biomass as incubation period increase in both type of soil. And except *Aspergillus fumigatus* there was more biomass in nonsaline soil as compare to saline soil (Table 2).

Table 2: Growth (g FW) of rhizospheric fungi associated with *Leptadenia pyrotechnica* plant at different stages

Rhizospheric Fungi	Saline			Non Saline		
	Initial	Medium	Final	Initial	Medium	Final
Aspergillus fumigatus	4.166	5.299	6.607	3.646	4.767	6.545
Aspergillus flavipes	3.499	4.009	5.95	4.698	5.813	8.269
Aspergillus niger	3.6	4.972	6.662	3.955	6.600	8.098

2. Enzyme estimation

Acid phosphatase: In general, intracellular enzyme activity in saline (0.0318-0.1722) and non-saline (0.0084-0.1633) were higher than extracellular enzyme activity in saline (0.0041-0.1374) and non-saline (0.0036- 0.0427), which varies with fungi as in *A. niger* from saline soil showed more extracellular enzyme activity (0.0111- 0.0749) than intracellular (0.0356- 0.0677).

Enzyme activity decreased with increase in incubation period in *A. fumigatus, A. flavipes* (except saline intracellular) and in *A. niger* enzyme activity increased except non-saline

intracellular where activity decreased. *A. fumigatus* showed higher activity in saline intracellular (0.0318) and non-saline extracellular (0.0045) as compare to non-saline intracellular and saline extracellular. In *A. flavipes* and *A. niger*, higher enzyme activity was recorded in saline intracellular (0.1722 and 0.0677) and extracellular (0.0542 and 0.0749) than non-saline (Table 3). Acid phosphatase production was higher than alkaline phosphatase in both conditions and also acid phosphatase production was higher saline soil as compare to non-saline soil. Variation between fungi of different places are highly significant p>0.1.

Table 3: Acid phosphatase activity ($\mu g^{-1}h^{-1}$pi released FW basis) of *Leptadenia pyrotechnica* rhizospheric soil fungi at different intervals

	Saline		Non saline	
A. fumigatus	Intracellular	Extracellular	Intracellular	Extracellular
I	0.1127±0.0046	0.0331±0.0032	0.1633±0.0323	0.0377±0.0141
II	0.1363±0.0449	0.0464±0.0151	0.1255±0.0166	0.0205±0.0010
III	0.0318±0.0075	0.0041±0.0021	0.0154±0.0031	0.0045±0.0005
A. flavipes				
I	0.1230±0.0113	0.1374±0.0101	0.1124±0.0153	0.0427±0.0071
II	0.1408±0.0050	0.0573±0.0091	0.0880±0.0098	0.0190±0.0040
III	0.1722±0.0055	0.0542±0.0040	0.0452±0.0115	0.0101±0.0044
A. niger				
I	0.0356±0.0024	0.0725±0.0048	0.0216±0.0023	0.0070±0.0052
II	0.0569±0.0071	0.0111±0.0027	0.0140±0.0019	0.0036±0.0011
III	0.0677±0.0042	0.0749±0.0054	0.0084±0.0031	0.0180±0.0032

Alkaline phosphatase: In all fungi enzyme activity decreased as incubation period increased. Intracellular activity in saline (0.0344-0.1501) and non-saline (0.004-0.1218) were higher than saline (0.0053-0.1416) and non-saline (0.0029- 0.0998) extracellular activity, which varies with different fungal species.

In *A. fumigatus* maximum Pi release were recorded in saline intracellular (0.0609) and extracellular (0.0107) than non-saline intracellular and extracellular. *A. flavipes* showed higher enzyme activity in saline intracellular (0.0852) and extracellular (0.0867) as compare to nonsaline enzyme activity. In *A. niger* enzyme activity was higher in saline intracellular (0.0344) and non-saline extracellular (0.0085) than non-saline intracellular and saline extracellular (Table 4).

Alkaline phosphatase production was more in saline soil in all the three fungi *viz*., *A. fumigatus*, *A. flavipes* and *A. niger* than in non-saline soil (Table 3 and 4). Variation between fungi of different places are highly significant p>0.1.

Table 4: Alkaline phosphatase activity ($\mu g^{-1}h^{-1}$pi released FW basis) of *Leptadenia pyrotechnica* rhizospheric soil fungi at different intervals

	Saline		Non saline	
A. fumigatus	Intracellular	Extracellular	Intracellular	Extracellular
I	0.1173±0.0139	0.0834±0.0037	0.1218±0.0078	0.0998±0.0172
II	0.1138±0.0025	0.0637±0.0055	0.0910±0.0124	0.0603±0.0053
III	0.0609±0.0177	0.0107±0.0016	0.0142±0.0068	0.0029±0.0006
A. flavipes				
I	0.1501±0.0215	0.1416±0.0095	0.1091±0.0069	0.0839±0.0076
II	0.0996±0.0035	0.1114±0.0053	0.1273±0.0213	0.0720±0.0074
III	0.0852±0.0143	0.0867±0.0051	0.0344±0.0095	0.0138±0.0084
A. niger				
I	0.0483±0.0021	0.0644±0.0035	0.0486±0.0061	0.0297±0.0033
II	0.0828±0.0015	0.0328±0.0033	0.0251±0.0033	0.0153±0.0025
III	0.0344±0.0050	0.0053±0.0026	0.0049±0.0021	0.0085±0.0038

Phytase: Enzyme activity increased as incubation period increases in *A. niger* and *A. fumigatus* (from non-saline soil) whereas decreased in *A. flavipes* and *A. fumigatus* (from saline soil). Intracellular enzyme activities in both saline (0.0441-0.5469) and non-saline (0.1890-3.4284) were more than extracellular saline (0.0069-0.3463) and non-saline (0.0270- 3.4284).

A. fumigatus, *A. flavipes* and *A. niger* all showed maximum activity in non-saline intracellular (0.2099, 0.4899 and 3.4284) and extracellular (0.0737, 0.1624 and 3.4284) than saline intracellular and extracellular respectively, (Table 5). Variation between fungi of different places are highly significant p>0.1.

Table 5: Phytase activity ($\mu g^{-1}h^{-1}$pi released FW basis) of *Leptadenia pyrotechnica* rhizospheric soil fungi at different intervals

	Saline	Non saline

A.fumigatus	Intracellular	Extracellular	Intracellular	Extracellular
I	0.2365±0.0322	0.1347±0.0221	0.2030±0.0031	0.0376±0.0287
II	0.1046±0.0269	0.0788±0.0054	0.1890±0.0460	0.0270±0.0277
III	0.0441±0.0264	0.0069±0.0043	0.2099±0.0275	0.0737±0.0425
A.flavipes				
I	0.1092±0.0322	0.1156±0.0169	0.2508±0.0474	0.1816±0.0304
II	0.5469±0.0622	0.1947±0.0422	0.5887±0.0611	0.2636±0.0554
III	0.4406±0.0608	0.0309±0.0193	0.4899±0.0779	0.1624±0.0773
A. niger				
I	0.2702±0.0132	0.0356±0.0113	1.0391±0.0552	0.5490±0.0410
II	0.1876±0.0187	0.1153±0.0230	0.3694±0.0455	0.2510±0.0564
III	0.5277±0.0392	0.3463±0.0605	3.4284±0.1486	3.4284±0.1486

Discussion

The present investigations were made to isolate the effective phosphorus solubilizing fungi from saline soil of Pachpadra so as to compare with non-saline soil of Jodhpur, and accordingly, to evaluate their suitability for solubilizaton of phosphorus in different conditions. It may become the first report on the inland saline isolates (Pachpadra) that can be used for solubilizing the phosphate. Meyers and Reynolds (1959 a, b, 1960 and 1963), Rodriguese *et al.*(1970), Tiwari *et al.*(1989), Madhura and D'Souza *et al.*(1982), and Prabhakaran (1990) conducted studies on solubilizing activity of phosphate and interestingly only few saline fungi has been assessed so far and the dominant genera recorded was *Aspergillus spp*. Chuang *et al.* (2007), Onyia *et al.* (2015), Verma and Ekka (2015) and Elias *et al.* (2016) also isolated phosphate solubilzing fungi such as *Aspergillus niger* and *Penicillium* spp. from various rhizospheric soil samples. In the present study, P-solubilizing activity of *Aspergillus fumigatus*, *A. flavipes* and *A. niger* were isolated from rhizospheric soil of *Leptadenia pyrotechnica* from both saline and non-saline conditions were studied. This result also is in agreement with the earlier finding of Vassilev *et al.* (2006) and Richa *et al.* (2007) who also observed predominant occurrence of phosphate solubilizing fungi belonging to genus *Aspergillus* followed by *Penicillum* in rhizosphere of different plants.

All the three fungal spp. exhibited the P-solubilizing activity but there are differences in phosphate solubilizing capacity among same species from saline and non-saline rhizospheric soil. Lapeyrie *et al.* (1991) supported that P solubilization ability can vary even within the same fungal species. The enzyme activity decreases with increase of incubation period of all three enzymes. The decline activity of enzyme (acid phosphatase, alkaline phosphatase and phytase) is supported by Tarafdar *et al.*(2003) and Aseri *et al.* (2009) who have recorded decline in activity of enzyme after 21 day in acid phosphatase and 14 day for alkaline phosphatase. The reason may be due to onset of stationary phase in fungal culture. The decrease in P-solubilization at the end of incubation period was also observed by Mahamuni *et al.* (2012). Giand and Gaur (1989) reported that the drop in solubilization after a maximum value might be attributed to deficiency in nutrients in the culture medium. In non-saline soil, acid phosphatase production was higher than alkaline phosphatase which coincides with results of Rubio *et al.* (2015). Aseri *et al.* (2009) showed 2.35 times less production of alkaline phosphatase as compare to acid phosphatase. However, in saline soil alkaline phosphatase production was higher.

In present study, there is difference in the amount of enzyme activity in extracellular and intracellular phosphatase and phytase between different fungi and it found that intracellular enzyme activity was higher than extracellular in both saline and non-saline soil for all three fungi. In *L. pyrotechnica*, the extracellular activity was almost 2-12% less than intracellular for both types of soil. The observed results supported by Aseri *et al.* (2009) who recorded that fungi had higher intracellular acid phosphatase activity than the enzyme released extracellularly, Tarafdar *et al.* (2001b) considered that this difference may be due to fungal structure.

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