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ENZYMATIC STUDY OF SOME POROID FUNGUS

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Comparative study of electrophoretic mobility of two fungal enzymes i.e. esterase and acid phosphatase , of some poroid fungus has been done .It has been found that the result so obtained established similarity as well as dissimilarity among these poroid fungi. The analysis of esterase enzyme profile showed maximum co-efficient of similarity i.e.43 % between species *F. brasiliensis* and *T. cingulata* whereas minimum similarity exhibited between species *P.grammocephalus* and *T. cingulata* and also between species *F. brasiliensis* and *G.lucidum* . *D.flavida* shared none of its bands with *P. zonatus, F. brasiliensis* and *T. cingulata* which support its generic identity. The analysis of acid phosphatase bands showed 59% co-efficient of similarity between *F. brasiliensis* and *P. zonatus* . *F. brasiliensis* also showed closeness with *T. cingulata* having 42% co-efficient of similarity. Thus electrophoretic study of esterase and acid phosphatase enzyme might be taken as an additional parameter that helps in establishing co-relation between species of poroid fungus .

Key words : electrophoresis , enzymatic study , poroid fungus, Enzymatic study of some poroid fungus.

Fungi are expert at digesting and decomposing nutrient sources. It has been estimated that 40–60% of woody plant material is made up of cellulose, while lignin constitutes between 20% - 30%. Floudas *et al*. 2012 reported that fungal species causing white-rot are among the most efficient lignin decayers in the biosphere. Though it has been variously reported that enzymes are the chief weapons with which poroid fungi invade the plant cells and degrade wood but only few poroid fungi have been studied with regard to their enzymes.

The potentialities of enzyme in the taxonomy of living entity is recognized and a large number of publications are available. Concurrently, mycologists were using electrophoretic analysis of enzyme in identifying Bent grass cultivars (Freeman and Yoder,1990) and in identification of Banana variety (Mandal *et al* 2001) .This approach has been successfully employed to identify fungal strains from a number of different groups, such as ectomycorrhiza (El Karkouri *et al.* 1996; Sims *et al*.1999), deuteromycetes (Buddie *et al*. 1999) and basidiomycetes (Liu *et al.*1990, Zervakis *et al.* 2001). Tsung and Lay (1988) proposed that esterase to be one of the marker enzyme useful for the identification of *Ganoderma* sp.. On the basis of his finding the enzyme gel of *G.tsugae* and *G.lucidum* were easily distinguished. Recently on the basis of molecular data [Koukol O, Kotlaba F, Pouzar Z. (2014), Kout and Vlasak (2011) and Ryvarden and Melo 2014)] *Daedaleopsis tricolor* has given the rank of variety**:** *D. confragosa* var. *tricolor*.Previously it was treated as a separate species based on macro-morphological feature.

MATERIALS AND METHODS

For electrophoretic study of enzyme the following reagents were prepared- Monomer A contains 36.6 gm Tris-buffer added to distilled water to make it 100 ml. Monomer B contain 5.98 gm Tris buffer added to distilled water to make it 100 ml. Monomer C contain 30 gm acrylamide and 1 gm bis-acrylamide dissolve with distilled water to make it 100 ml.

Monomer D prepared by adding 10 gm acrylamide and 2.5 gm bis- acrylamide dissolve with distilled water to make it 100 ml. Monomer E prepared by adding 140 mg Ammonium per sulphate with 100 ml distilled water.

Running gel -- Running gel was prepared by adding One/fourth part of pre-prepared Tris-

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HCl Buffer, two/fourth part of pre-prepared Acrylamide –Bisacrylamide solution and one /fourth part distilled water. pH 6.8 was maintain by adding N-HCl. Then Ammonium per sulphate solution was mixed.

Electrode Buffer -- For filling in both of the chambers of electrophoretic apparatus Buffer was prepared by adding 72 gm Glycine and 15 gm Tris ,pH 8.3 maintained and by adding distilled water make it 1000 ml .Just before use 5 gm SDS was added and again distilled water was added in the ratio 1:4 to dilute it.Take up to 1000 ml of GDW . Now diluted the above solution in the ratio of 1 : 10 .

Stacking gel -- Stacking gel was prepared by adding 1.95 ml solution of Acrylamide and Bis-acrylamide, 0.75 ml SDS, 3.75 ml Tris-HCl Buffer for maintaining pH 6.5-6.8 and distilled water.#Lastly 15 ml TEMED was added before its use.##

Procedure:

For the study of enzyme at first the fruiting bodies were blended in phosphate buffer. Homogenate of each fungal species was prepared as 100 mg fungal tissue with 1 ml of enzyme extraction buffer at 4^0c. The prepared homogenate of each sample was then centrifuged at 4,000 rpm at 4^oc for 10-15 minutes . After centrifugation,cell debris were removed and the clear supernatant was collected and stored at 4^oc. For enzymatic study the extracted samples of polypores were loaded in acrylic gel tubes PAGE system was followed.

Procedure for Esterase enzyme activity :

After completion of run gels were at first transferred in staining solution prepared by adding 1 ml 1% Naphthyl acetic acid and 100 mg fast blue RR in 100 ml of 0.1 M phosphate buffer (pH 6.2). Staining technique for esterase was carried out using specific method of Hall *et al*.1969 and incubation has been done at 35^0 c for 20 minutes. Within 20- 25 minutes sharp bands of enzymes were appeared in each gel.

Procedure for Acid Phophatase enzyme activity:

For the study of acid phosphatase after completion of run at first the runned gels were washed with .1 M Acetate buffer with maintaining pH 5.0.The gels were transferred in staining solution prepared by adding 100 mg Naphthyl phosphate, 100 mg fast blue RR and 2 mg NaCl in 100 ml of 0.1 M acetate buffer (pH5.0) with few drop of 10% $MgCl_2$ Staining technique following Hopkinson *et al* 1964.Then incubation has been done at 25^0c for 30-35 minutes. Within 20- 25 minutes sharp bands of enzymes were appeared in each gel.

The enzyme bands of each polypore species were measured according to their mobility and were assigned with R_m value. Relative mobility of subsequent bands are calculated as.

OBSERVATIONS

Esterase analysis -The esterase enzyme profile of *P.grammocephalus* showed four uncommon bands at R_m .24 mm, R_m .31,R_m .48 and R_m .69 (table 1). It shared bands at R_m .37 mm and R_m.64 mm with *D.flavida* , bands at R_m .53mm with *F. brasiliensis* and band at R_m .61mm with two poroid fungi i.e. *G.lucidum* and *P.zonatus*..*P.zonatus* showed five uncommon bands (fig.1). It shared two esterease bands at R_m .29 mm and R_m .46 mm with *F. brasiliensis* and also with *G. lucidum* . Esterase enzyme band at Rm.29 mm and .46 mm were common in between *F.brasiliensis*, *T.cingulata* and *P.zonatus*. It was observed that *D.flavida* shared none of its enzyme band with *P.zonatus , F. brasiliensis* and *T.cingulata*. Esterase enzyme band at R_m .64 and R_m .37 were common in between *P.grammocephalus* and *D.flavida*. *D.flavida* shared only a single esterase enzyme band with *G.lucidum* (Fig.1) *T.cingulata* also shared a single band with *G.lucidum* but so far co-efficient of esterase enzyme band similarity was concerned *T.cingulata* showed maximum similarity with *F.brasiliensis*.

Acid phosphatase analysis : Analysis of acid phosphatase enzyme gels showed *P.grammocephalus* has only two uncommon acid phosphatase band *i.e.*at R_m .01 mm and R_m .23 mm . *P.zonatus* shared five acid

phosphatase band out of seven band with *F.brasiliensis* (Fig. 2). *P.zonatus* also shared acid phosphatase bands at R_m.58 mm and R_m .69 mm with *D.flavida* ,at R_m .48 mm and R_m .55 mm with *T.cingulata* and at R_m .31 mm and R_m .55 mm with *P.grammocephalus* depicting a family feature. No uncommon band was found in *P.zonatus* (table 3) .Out of seven acid phosphatase band appeared in *D.flavida* ,only one band at R_m .52 mm was uncommon whereas it shared two different acid phosphatase bands each with *G.lucidum* at R_m .35 mm and R_m .46 mm, with *P.zonatus* at R_m .58 mm and .69 mm ,with *F. brasiliensis* at R_m .30 mm and R_m .58 mm and with *T.cingulata* at R_m .30 mm and .64 mm (table3).

DISCUSSION AND CONCLUSION

After analysis of observed bands of esterase enzyme it can be concluded that no species specific esterase bands has been found in any poroid species under investigation which is helpful in delimitating species .So far the data regarding similarity or dissimilarity has been concerned a lots of esterase enzyme bands can help in family or generic level discrimination. Electrophoretic study of esterase enzyme of species under investigation reflected a distinct enzyme band at relative mobility .61mm found in three poroid species viz.,*P.grammocephalus, G.lucidum* and *P.zonatus* which shows resemblance between these three species (Table 1). Presence of band at relative mobility .29 mm and .46 mm exhibit that *P.zonatus* is more closer to *F. brasiliensis* and *T.cingulata*. Thus a generic relation could be established among these species. Here one more band at R_m.66 mm is common in *F. brasiliensis* and *T.cingulata.* Thus it has been concluded that species *F. brasiliensis* with 43% co-efficient of similarity is more closer to *T.cingulata* than *P.zonatus* (table2) whereas distant relation exhibited due to absence of common esterase enzyme band

Figure 1-Lane A to F-*Polyporus sp., Ganoderma sp., Polyporus sp., Daedalea sp., Favolu*s sp. and *Trametes sp.* respectively showing esterase enzyme bands after PAGE

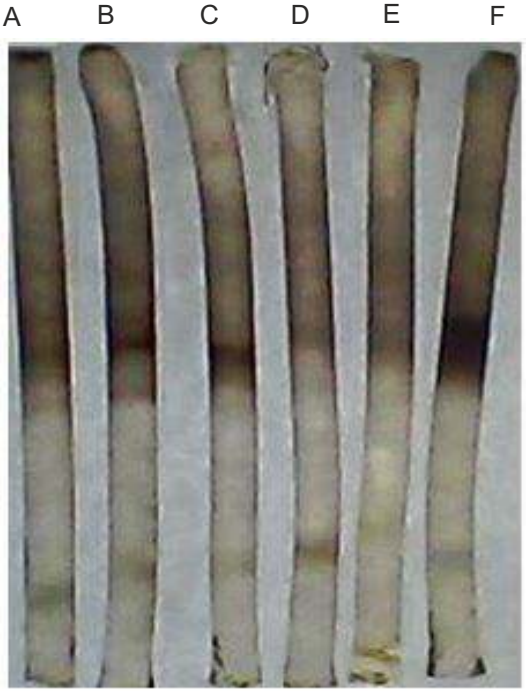

Figure 2- Lane A to F-*Polyporus sp., Ganoderma sp.,Polyporus sp., Daedalea sp., Favolu*s sp.and *Trametes sp*. respectively showing acid phosphatase enzyme bands after PAGE.

Table 1 - Showing relative mobility of esterase enzyme of poroid fungi visible in PAGE

Name of poroid fungus	Relative mobility of esterase in mm							
	R_m	R_m	R_m	R_m	R_m	R_m	R_m	R_m
P.grammocephalus	0.24	0.31	0.37	0.48	0.53	0.61	0.64	0.69
G.lucidum	0.01	0.28	0.56	0.61	0.67	0.70	--	--
P.zonatus	0.29	0.32	0.46	0.55	0.61	0.63	0.72	0.77
D.flavida	0.01	0.15	0.19	0.37	0.45	0.64	--	--
F.brasiliensis	0.29	0.34	0.41	0.46	0.53	0.58	0.66	--
T. cingulata	0.26	0.29	0.46	0.56	0.59	0.66	0.74	--

Table 2 - Comparison of % of coefficient of similarity (above) and comparison of number of bands (below) with maximum number of bands denoted diagonally in middle (underlined) visible in PAGE of esterse enzyme of six poroid fungi

S. No.	Name of poroid fungal species	*P.grammocephalus*	*G.lucidum*	*P.zonatus*	*D.flavida*	*F.brasiliensis*	*T.cingulata*
1	*P.grammocephalus*	8	14	13	29	13	--
2	*G.lucidum*	1	6	14	17	--	15
3	*P.zonatus*	1	1	8	--	27	27
4	*D.flavida*	2	1	--	6	--	--
5	*F.brasiliensis*	1	--	2	--	7	43
6	*T. cingulata*	--	1	2	--	3	7

Table 3 - Showing relative mobility of acid phosphatase enzyme of six poroid fungi visible in PAGE

Name of poroid fungus	Relative mobility of Acid Phosphatase (in mm)									
	R_{m1}	R_{m2}	R_{m3}	R_{m4}	R_{m5}	R_{m6}	R_{m7}	R_{m8}	R_{m9}	R_{m10}
P.grammocephalus	0.01	0.23	0.31	0.35	0.40	0.48	0.55	0.57	0.62	-
G.lucidum	0.21	0.25	0.28	0.35	0.40	0.46	0.53	0.57	0.66	-
P.zonatus	0.21	0.31	0.33	0.43	0.55	0.58	0.69	-	-	-
D.flavida	0.30	0.35	0.46	0.52	0.58	0.64	0.69	-	-	-
F.brasiliensis	0.16	0.21	0.25	0.30	0.33	0.43	0.51	0.55	0.58	0.62
T. cingulata	0.20	0.25	0.30	0.38	0.43	0.48	0.55	0.59	0.64	-

Table 4 - Comparison of % of coefficient of similarity (above) and comparison of number of bands (below) with maximum number of bands denoted in middle (in red) visible in PAGE of acid phosphatase of six poroid fungus.

S. No.	Name of Poroid species	*P.grammocephalus*	*G.lucidum*	*P.zonatus*	*D.flavida*	*F.brasiliensis*	*T.cingulata*
1	*P.grammocephalus*	**9**	33	25	13	21	22
2	*G.lucidum*	3	**9**	13	25	21	11
3	*P.zonatus*	2	1	**7**	29	59	25
4	*D.flavida*	1	2	2	**7**	24	25
5	*F.brasiliensis*	2	2	5	2	**10**	42
6	*T. cingulata*	2	1	2	2	4	**9**

between polypore species *P.grammocephalus* and *T. cingulata* and also between species *F. brasiliensis* and *G.lucidum* . *D.flavida* shared none of its esterase enzyme bands with *P. zonatus, F. brasiliensis* and *T. cingulata* (table 2) which support its generic identity.Due to absence of uncommon band in *P.zonatus* it is suggestive that it may be merged among other poroid fungus if supported by other taxonomic feature.

The analysis of acid phosphatase enzyme profile depicts with 59% co-efficient of similarity showed maximum resemblance between *F. brasiliensis* and *P. zonatus. F. brasiliensis* also showed closeness with *T. cingulata* having 42% co-efficient of similarity (table4). Bands showing relative mobility at .55mm appeared in four species *viz.*, *P.grammocephalus* , *P.zonatus*, *F. brasiliensis* and *T. cingulata.* Thus it may be justifiable to be considered these four species in specified sub family group. Resemblance of bands at R_m .43 mm and R_m .55 mm in *P. zonatus. F. brasiliensis* and *T. cingulata* differentiate them with *P. grammocephalus*. Also sharing of five common bands between *P. zonatus* and *F. brasiliensis* depict their generic relationship. Three bands at relative mobility .35 mm, .40 mm and .57 mm showed closeness between *P. grammocephalus* and *G. lucidum*. *D. flavida* shared two bands each with *P. zonatus*, *G.lucidum* and *T. cingulata* ; of which maximum co-efficient of similarity ie. 29% appeared with *P.zonatus*. Thus to keep *D.flavida* and *P.zonatus* in the same family can be justified and due consideration if the sub family rank given to *D. flavida* , *G. lucidum* and *T. cingulata* supporting texture similarity of basidiocarp as all possess tough texture . A band at relative mobility .52 mm has given *D.flavida* a species specific identity.

By the analysis of Acid phosphatase bands it could be concluded that the mode of decaying of wood by *F. brasiliensis* and *P. zonatus* could be similar. Acid Phosphatase profile of the species under investigation also exhibited differentiation between species *P. grammocephalus* and *F. brasiliensis* (table 4) depicting different mode of decaying of wood. Thus observation so obtained shows that generic or specific separation can be possible when enzyme bands are taken into account. It was observed that while performing the process, an inherent problem encountered that some bands cannot be resolved due to standardization of buffer system or the electrophoretic procedure which is needed for

comparing it with standard data. It could be proved a helpful marker if taken together with some other parameter to establish similarity ,dissimilarity, identity and taxonomic position of such species.

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