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ISOLATION AND ACTIVITY OF LIPASE PRODUCING STORAGE FUNGI FROM MEDICINAL PLANTS OF MAHARASHTRA

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ABSTRACT:
The purpose of this study was to isolate and activity of lipase producing fungi from different some medicinal plant parts during storage condition.. A total six lipase producing fungal isolates were obtained from different medicinal plant parts. It was observed that six fungal isolates produced lipase. The medicinal plants are found to be heavily infected with variety of fungi in field and storage. These associated fungi are known to deteriorate the plant parts and its chemical contents. These associated fungi isolated from the different medicinal plant parts during storage in gunny bags in store houses.Isolated fungi were screened qualitatively and quantitatively for production of extracellular enzyme lipase, on both substrate and non-substrate media. Totally eleven fungi isolated from different drug plant parts were tested for their lipolytic nature and it was interesting to note that all the fungi showed production of lipase with more or less degree.

Key words : Lipase production, storage fungi, medicinal plants

I. INTRODUCTION:

Medicinal plants are considered as a rich source of ingredients which can be used in drug development. The plant parts after harvesting they stored in store houses or godowns in gunny bags. Theyare found to be heavily infested with variety of fungi, these associated fungi are known to deteriorate the plant parts and its contents. The isolation and identification of fungal pathogens on different medicinal plants and their plant parts. For this isolation of fungi was made from medicinal plants collected from different regions of Maharashtra at different age and different varieties of medicinal plants in field and in storage conditions. It was interesting to observe that, different medicinal plants and their plant parts specially *Withania somnifera, Rauwolfia serpentina, Glycyrrhiza glabra, Emblica officinalis, Asparagus racemosus, Chlorophytum borivilianum, Zingiber officinale* showed association of different fungi having different physiological behavior. During studies on survey of medicinal plant diseases it was interesting to note that following diseases were highly destructive to the crops in live field and storage conditions. Therefore six pathogenic moulds like *Aspergillus flavus, Curvularia lunata, Alternaria alternata, Fusarium oxysporum, Phytopthora sp., Rhizoctonia solani* were screened for their ability to produce amylase enzyme.In recent years, the uses of microorganisms have become a huge importance to food, textile, baking and detergent industries and sparked a large interest into the exploration of enzyme activity in microorganisms (Sivaramakrishnan *et al.*, 2006).

Regarding the effect of supplementation of carbohydrates on lipase production, the fungi showed variation in their selection for carbohydrates to stimulate amylase production. Role of extracellular enzyme lipase produced by stored drug plant parts fungi, during the process of deterioration of plants parts has been considered to be important ability to the fungi. Fazilath Uzma et al (2016) showed that endophytic fungal diversity and extracellular enzyme activity from the endangered plants.Molds are good lipase producers and

numerous fungal enzymes are utilized in various food industrial processes (Aravindan et al., 2007). Lipases with new properties have been widely used in several areas such as medicine, biotechnology, detergent, and bioremediation, still there are substantial interests in developing new microbial lipases. A considerable number of fungal and bacterial lipases have been commercially produced, but fungi are more preferable because fungi generally produce extracellular enzymes which facilitate recovery of the lipase (Reetz, 2002; Colen et al., 2006). During the process of biodeterioration extracellular hydrolytic enzymes plays very important role in the invasion and establishment of plant pathogen (Bateson and Miller,1966, Wood,1967). Regarding medicinal plant parts fungi veryscanty information is available about their role in biodeterioration of medicinal plant parts.

II. MATERIAL AND METHODS:

Lipase Enzymes of Fungal Pathogens:

a) Production :

Lipase production was studied by using liquid medium containing KNO3 – 0.25%, KH_2PO_4-0.1% and MgSO4.7H2O-0.05%, pH of medium was adjusted to 5.0. Twenty five ml of the medium was prepared in 100 ml Erlenmeyer flask and autoclaved at 15 lbs pressure for 30 minutes. The flasks on cooling were inoculated separately with 1 ml spore/mycelial suspension of test fungi prepared from 7 days old cultures grown on PDA slants. The flasks were incubated for 6 days at 25 ± 1 ^{0}C with diurnal periodicity of light . On 7th day the flasks were harvested by filtering the contents through WhatmanNo.1 filter paper. The filtrates were collected in presterilized bottles and termed as crude enzymes preparation.

b) Enzyme assay (cup – plate method) :

Determination of lipase activity was done with the help of cup plate method, where 20 ml of oil agar assay medium (oil 1% and agar 2%) were poured in each petriplate. On solidifying the medium, a cavity was made in the center with the help of a cork borer (No.4) and was filled with 1 ml culture filtrate (Crude enzyme). The plates were incubated at 28^{0}C for 24 hours then they were flooded with 1% phenolphthalein as an indicator kept it 20-40 minutes activity zones are clearly seen. The diameter zone was measured (mm) as lipase activity.

c) Composition of media used for lipase production :

The synthetic media were employed for the production of lipase (s) in the preliminary experiment. Composition of media is given below:

i) Substrate (Oil medium) :

Oil	-	1%
$KN0_3$	-	0.25%
KH_2PO_4	-	0.1 %
$MgSO_4.7H_{2O}$	-	0.05%

Dissolved in 100 ml of D. W.

ii) Non Substrate (Glucose nitrate):

Glucose	-	10 gm
KNO_3	-	2.5 gm
KH_2PO_4	-	1.0 gm
$MgSO_4$. $7H_2O$	-	0.5 gm

Dissolved in 100 ml of D. W.

III. RESULTS &DISCUSSION:

Table :01 Effect of carbohydrates on lipase production in medicinal plants fungi:

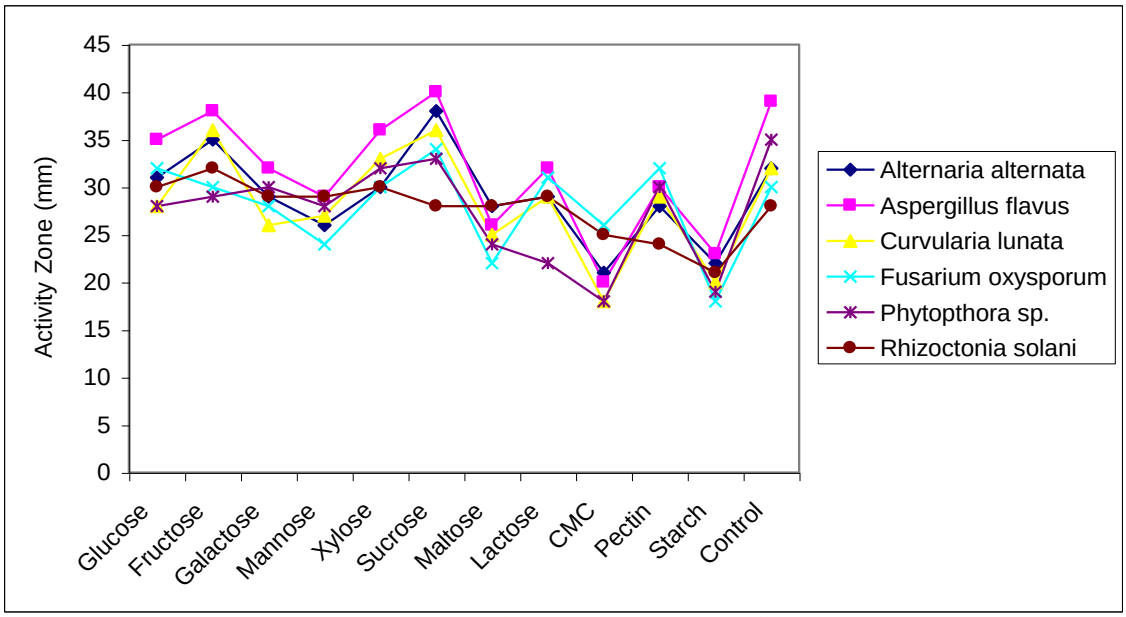
Carbohydrates (0.5 % conc.)	Fungi					
	Alternaria alternata	*Aspergillus flavus*	*Curvularia lunata*	*Fusarium oxysporum*	*Phytopthora sp.*	*Rhizoctonia solani*
	Activity zone (mm)					
Monosaccharides						
Glucose	31	35	28	32	28	30
Fructose	35	38	36	30	29	32
Galactose	29	32	26	28	30	29
Mannose	26	29	27	24	28	29
Xylose	30	36	33	30	32	30
Disaccharides						
Sucrose	38	40	36	34	33	28
Maltose	28	26	25	22	24	28
Lactose	29	32	29	31	22	29
Polysaccharides						
CMC	21	20	18	26	18	25
Pectin	28	30	29	32	30	24
Starch	22	23	20	18	19	21
Control	32	39	32	30	35	28

Aal – Alternaria alternata *Fox – Furarium oxysporum*
Asf – Aspergillus flavus *Phy – Phytopthora sp.*
Cul – Curvularia lunata *Rso- Rhizoctonia solani*

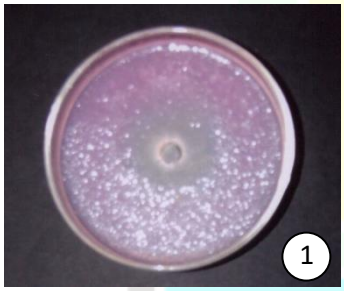
Among the eleven medicinal plant parts fungi screened as in Table 01 only six medicinal plant parts fungi were employed for further detail studies on lipase production. In order to study the effect of different nutrients on lipase production, ten carbohydrates other than glucose were tested by supplementing them individually in the oil medium of which five belong to monosaccharides, three belong to disaccharides and three polysaccharides were selected and the results are given in Table 01. It is clear from the results that, among monosaccharides, fructose and xylose stimulated lipase production in *Alternaria alternata*, *Aspergillus flavus*, *Curvularia lunata*, *Fusarium oxysporum* and *Rhizoctonia solani*, while sucrose was stimulated in case of *Aspergillus flavus* and *Alternaria alternata.* Galactose, mannose, xylose, lactose, Carboxy Methyl Cellulose (CMC) and starch were found to be inhibitory for lipase production in all fungi. Similarly in case of *Aspergillus flavus* only fructose and sucrose stimulated lipase production. while, other carbohydrates did not show any effect.

Regarding the effect of supplementation of carbohydrates on lipase production, it was seen (Table 01) that, the fungi showed variation in their selection for carbohydrates to stimulate lipase production. This indicates the specificity of carbohydrates during synthesis of fungal lipase. Among different carbohydrates, fructose, xylose and sucrose proved stimulatory for lipase prodcution in *Alternaria alternaria, Aspergillus flavus, Curvularia lunata, Fusarium oxysporum* and *Rhizoctonia solani*. Galactose, mannose, xylose, lactose, CMC and starch were found to be inhibitory for lipase production in all fungi. The effect of carbohydrates was studied by different workers, Glucsoe in case of *Candida regosa* (Valero et al, 1991), *Penicillum requefostii* (Petrovic et al, 1990), *Rhizopus nigricans* (Chander et al, 1981), *Mucor racemosus* (Chopra et al, 1981) , *Aspergillus wentii* (Chander et al, 1980). Maltose in case of *Rhizopus rhizopodiformis* (Samad et al, 1990), *Penicillium verrucosum* (Glenza and Jaballah, 1985). Fructose, Xylose, and Sucrose in case of *Alternaria carthami* (Sandikar and Mukadam, 1992), Fructose in case of *Syncephalastrum racemosum* (Chopra and Chander, 1983) were stimulatory for lipase production.

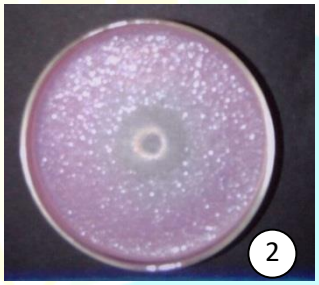
Fig. No1. Effect of carbohydrates on lipase production:



ZONE OF LIPASE ACTIVITY IN DIFFERENT FUNGI

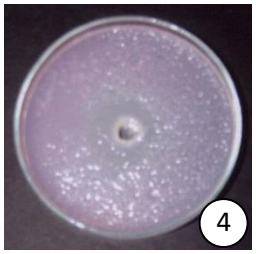


Glucose

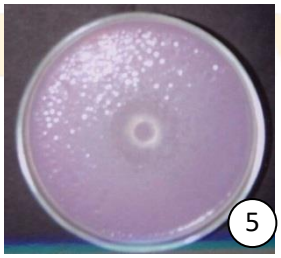


Fructose

Galactose

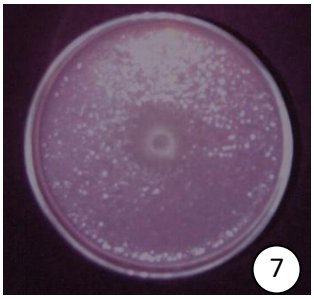


Mannose

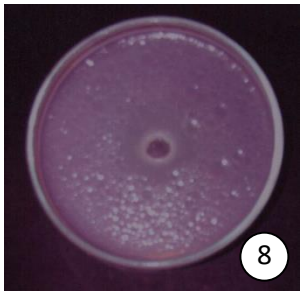


Xylose

Sucrose

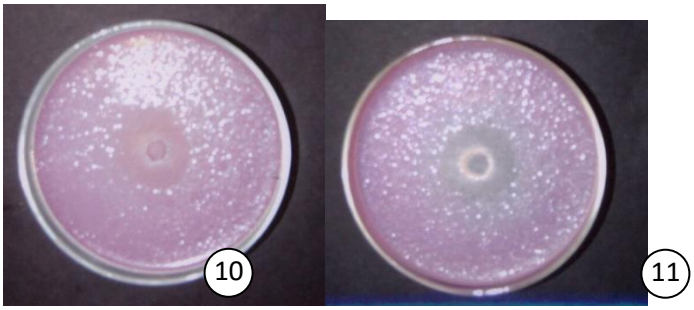


Maltose



Lactose

CMC



Pectin Starch

IV. CONCLUSION:

Among the total eleven fungi isolated from drug plants were capable to produced lipase enzyme.

1) All the medicinal plant parts fungi were able to produce lipase on both substrate and non-substrate media.
2) Totally eleven fungi isolated from different drug plant parts were tested for their lipolytic nature and it was interesting to note that all the fungi showed production of lipase with more or less degree.
3) Among the various carbohydrates, fructose, xylose and sucrose proved stimulatory for lipase production in *Aspergillus flavus*. While Carboxy Methyl Cellulose and starch were found to be inhibitory for lipase production in all fungi.

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