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Biodiversity in *Aspergillus nidulans* group on the Basis of Lipases Profile

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Abstract: ***Thermophilic fungi are highly specific group of organism due to their adaptability of higher temperature and presence of thermostable proteins which contain lipids of variable lengths. In the present study various strains of Aspergillus nidulans group were diversified on the basis of their lipid degrading potency. A total of 27 strains were tested using a chromogenic lipid substrate of p-nitrophenol group attached to variable length of carbon chains for rapid detection of extracellular production of lipases at different temperatures. Almost all the test fungi showed extracellular lipase C_{18} activity at all higher temperature i.e., ranging between 35^0C to 60^0C temperatures. In most of the cases minimum activity of this enzyme was recorded at 35^0C and 60^0C temperatures, while its optimum activity was recorded at 45^0C. Different strains of the same species differ in their optimum temperature requirements for extracellular lipase C_{18} production. Reddy and Reddy (1983) reported increases lipase production with the age of fungal culture while studying the effect of temperature, pH and age of the cultures on lipase. Fungal strains which showed poor activity at 16^0C temperature includes strains of A. nidulans (TH133), Emericella nidulans (TH 6 B) after two weeks of incubation, A.nidulans (TH 2 and TH 15)and E. quadrilineata (TH 37 and TH 69) took three weeks of incubation and A.nidulans (TH 6A ,TH 21 and TH 68). E. nidulans var. echinulata (TH11) E.quadrilineata (TH 12, TH 81, TH 134 andTH135) required four weeks of incubation for the initiation of lipase C_{18} activity.***

Keywords: Thermophilic fungi, *Aspergillus nidulans*, *Emericella nidulans*, extracellular lipases, Biodiversity

1. Introduction

The term Lipid implies to the substances which show their sparing solubility in water and more in organic solvent and represent a heterogenous compounds, which includes acyl glycerol, waxes, phospholipids sphingolipids, terpenoides, carotenoids and steroids. In fungi, natural lipids are the secondary source of carbon then carbohydrates. It is assumed that lipid is hydrolyzed by enzymes lipase and esterase before utilization. The structural genes (*fad gene*) encoding the enzymes of fatty acid degradation map at various distinct loci on the fungi gene map and encode at least five enzyme involved in the transport, acylation and β-oxidation of medium(ranging from C_6-C_{10}) and long chain (ranging from C_{12}-C_{18}) fatty acid . The fungi produced a number of unusual lipid structures that contain ether linkage. This group of organisms is divided into three major subgroups i.e. Mesophiles, Thermophiles and Thermotolerant (Magan and Lacey 1987, Lode and Pederson 1970). As suggested by their names, these organisms thrive in extreme environments that uninhabitable by any other living forms, because these unusual lipid and other structural and biochemical factors aid in their survival , and function under these condition .

At higher temperature an increased amount of saturated fatty acid we reported in some fungi. This indicates increase lipid metabolism in these fungi at higher temperature. In the present investigation, to find out the strains of *Aspergillus nidulans* group, are capable of producing lipases that is responsible for the deterioration of Medium Chain Fatty acid (MCFA) and Long Chain Fatty acid (LCFA), at a wide range of temperature.

Several methods are known for the screening of extra cellular lipases from fungi. The method described by Yeoh et.al. (1986) was adopted for the assay of fungal lipases using Chromogenic lipid substrates of p- nitrophenyl group , is most suitable for the rapid detection of extra cellular lipases. During the survey of thermophilous fungi , *Aspergillus nidulans* group was found of common occurrence and in this present investigation , 27 fungal strain belonging to *Aspergillus nidulans* groups identified and deposited in Commonwealth Mycological Institute Kew, England , UK, were tested for the production of extracellur lipases using Chromogenic lipid substrate.

2. Materials and Methods

Extra cellular production of lipolytic enzymes by the storage fungi was determined the following methods describe by Yeoh *et. al.* (1986) and modified by Shukla (1991,2009). The details of method followed are as under:

2.1 Chromogenic lipid substrate(s)

In the present investigation six chromogenic lipid substrates of p- nitrophenyl group viz. p-nitrophenyl caprylate (c_8), p – nitrophenyl caparate (c_{10}) , p- nitrophenyl laurate (c_{12}), p-nitrophenyl myristate (c_{14}), p- nitrophenyl palmitate (c_{16}) and p- nitrophenyl stearate (c_{18}), were used to determine the extra cellular production of lipolytic enzymes by 27 isolates of different fungi belonging to Aspergillus nidulans group which were isolated from stored wheat and sorghum grains.

2.2 Preparation of assay tubes

For the preparation of assay tubes, the lipid substrates, (0.1%w/v) was dissolved in dimethyl sulphoxide (DMSO) and sterilized by Millipore filtration. Except lipid substrate(s), all the other reagents were sterilized by autoclave. Five ml.of 4.5 %w/v czapek dox agar medium was allowed to solidify in a culture tube (16x160 mm). the medium was then overlaid with 0.5 ml of a mixture of 0.8 ml

lipid substrate solution, 8.0 ml of 100 mm phosphate buffer pH 7.0 and 8.0 ml of 4.5%w/v czapek dox agar medium A set of tubes of having 5.0 ml of czapek dox agar medium was

2.3 Enzyme Assay

2.3.1 Production of extra cellular lipases

To study of extra cellular lipases production , a set of assay tubes (having a mixture of lipid substrate in buffered medium) were inoculated with a loopful of spore suspension (10^4 spore / ml) of each test organism . Tubes were incubated at 45^0C and observation was recorded after third day of incubation for appearance of yellow coloration in the tubes agar .Un-inoculated tubes of each substrate were served as control. Tubes having 5.0 ml czapek dox agar were inoculated with each test organism and served as fungal control to observed the pigmentation during fungal growth on this medium.(Table: 1&2).

2.3.2 Effect of temperature on lipase (C_{18}) production

Another set of experiment was also run to determine the effect of temperature on the extra cellular production of lipases C_{18} using p-nitrophenyl stearate as lipid substrate. The experiment was run in the same way , except that a set of tubes of each organism was incubated at $16^0C\pm 1^0C$, $35^0C\pm1^0C$,$40^0C\pm1^0C$, $45^0C\pm1^0C$, $50^0C\pm1^0C$, $55^0C\pm1^0C$, $60^0C\pm1^0C$ and $65^0C\pm1^0C$ in incubators (showing $\pm2^0C$ variation adjusted at higher temperature). The tubes were observed after 5 days for the appearance of yellow coloration in the tubes agar. In such cases where lipase activity was not recorded even after 5 days of incubation, the tubes were further incubated at the same temperature and observed after 1,2,3 and 4 weeks of incubation. The results obtained, are recorded in table: (3&4) .The day of incubation of yellow colorations in the tubes agar recorded in such cases.

3. Result & Discussion

In the experiment lipolytic activity was indicated by the appearance of yellow coloration in the tubes agar due to the liberation of p-nitrophenol from the chromogenic lipid substrate used in the present study. Fungal lipases showing affinity for this substrate have acted on ester linkages between the p-nitrophenol group and fatty acid molecule and thus resulted in the appearance of yellow coloration of p-nitrophenol at pH 7-0. The ability to hydrolyzed different lipid substrate is not of wide occurrence in fungi. However, some fungi are known to produce lipases (Jenson, 1974; Fedrici, 1982; Yeoh et.al., 1986) while others are also reported not to possess lipase activity (Trigiano and fegus, 1979).

In the present study most of the test strains of *A. nidulans* group were found weak to strong producer of lipases that responsible for hydrolysis of ester linkage in 8 carbons to 16 carbon containing fatty acid asters. One strain of *A. nidulans* (TH103) was found to lipase C_{12} and C_{14} activity. The same activity was also recorded with one strain of *Emericella nidulans* (TH12). It is suggested that some time short chain fatty acid inhibit the growth of some fungi. They are believed to act by decreasing the internal pH of the cells (Lode and Pederson, 1970).While on the other hand, long carbon chain fatty acids are reported to induce leakage of the cell contents (Soumalaines and Oura, 1955; Kurtzman, 1976).One strain of each including *A. nidulans*(TH4) and *E.quadrilineata* (TH81) showed their moderate activity with p-nitrophenyl caprylate and p-nitrophenyl caparate in comparison to p-nitrophenyl laurate. Two test strains i.e.,TH21 and TH68 have also showed greater activity of lipase C_{10} then lipase C_8.

Besides these, some strains were found with strong lipase activity with short carbon chain p-nitrophenyl fatty acid esterase then long carbon chain fatty acid ester. This might be due to their selective nature preferring short carbon chained fatty acid esters for their nutrition.

Emericella quadrilineata ver. acrustata (TH131) was found as strong producer of extra cellular lipases when tested using different lipid substrates. The highest lipolytic ability of this strain indicates its good enzyme potential for the deterioration of natural lipid substrates .Another isolates of *A. nidulans* (TH23) was also found to be strong lipase producer. In the present study, strains of test species of A. nidulans group showed a difference in their enzyme producing ability, this shows that there are some possible intracellular genes , which are determine the enzyme producing potential of individual strain . Robert et. al. (1987) have worked on a number of fungal strain for lipase production . He has also found intrageneric and intraspecific variability in lipase production occurring amongst their test strain.

In the present investigation, p-nitrophenyl stearate was used as lipid substrate, the effect of temperature on the extracellur production of lipase C_{18} by the test fungi. Almost all the test fungi showed extracellular lipase C_{18} activity at all higher temperature i.e., ranging between 35^0C to 60^0C temperatures. In most of the cases minimum activity of this enzyme was recorded at 35^0C and 60^0C temperatures, while its optimum activity was recorded at 45^0C. Different strains of the same species differ in their optimum temperature requirements for extracellular lipase C_{18} production. Reddy and Reddy (1983) reported increases lipase production with the age of fungal culture while studying the effect of temperature, pH and age of the cultures on lipase production by *Macrophomina phaseolina* and *Phoma nedulosa* associated with seasam (*Sesamum indicum* Linn.)seeds.

At 16^0C temperature very slow growth of some test organism was observed, in such cases lipase C_{18} activity was noted after a long incubation period. Fungal strains which showed poor activity at 16^0C temperature includes strains of ***A. nidulans*** (TH133***), Emericella nidulans*** (TH 6 B) after two weeks of incubation***, A.nidulans*** (TH 2 and TH 15)and ***E. quadrilineata*** (TH 37 and TH 69) took three weeks of incubation and ***A.nidulans*** (TH 6A ,TH 21 and TH 68). ***E. nidulans var. echinulata*** (TH11) ***E.quadrilineata*** (TH 12, TH 81, TH 134 andTH135) required four weeks of incubation for the initiation of lipase C_{18} activity.

Some fungal strains showed variation in their optimum temperature requirement for maximum lipase activity. From the data presented in **table : 1 and table: 2**, it is apparent

that the test fungal strains have a wide range of lipolytic enzymes as well as higher temperature requirement for greater production of these enzymes. Lipolytic activity of the present test fungi showed their greater deteriogenic potential for oil seeds and also for the cereal grains especially the germ and seed lost their viability and grains lost their nutritional quality.

Table 1: Lipolytic activity in Aspergillus nidulans group after 72 hrs of incubation at 45^0C ±2^0C temp

Organisms	*Isolate No.*	*IMI No.*	*Liberation of p-nitrophenol from lipid substrate**					
			Caprylate	*Caparate*	*Laurate*	*Myristate*	*Palmitate*	*Stearate*
Aspergillus nidulans	TH4	317908	++	++	+++	+	+	+
A. nidulans	TH5	317909	++	+++	++	++	++	++
A. nidulans	TH6A	317910	+++	+++	++	++	++	+
A. nidulans	TH13	317911	+++	+++	+++	+++	+	+
A. nidulans	TH20	317912	++	++	++	+	+	+
A. nidulans	TH21	317913	++	+++	+++	++	++	++
A. nidulans	TH23	317914	+++	+++	+++	+++	+++	+
A. nidulans	TH35	317915	++	++	++	++	+	+
A. nidulans	TH38	317916	+++	+++	+++	+++	++	++
A. nidulans	TH68	317917	++	+++	+++	+++	+	+
A. nidulans	TH103	317919	+	+	++	++	+	-/*
A. nidulans	TH133	317921	+++	+++	++	++	+	+

Table (2): Lipolytic activity of Emericella nidulans group after 3 days of incubation at 45^0C ±2 temp

Organisms	*Isolate No.*	*IMI No.*	*Liberation of p-nitrophenol from lipid substrate**					
			Caprylate	*Caparate*	*Laurate*	*Myristate*	*Palmitate*	*Stearate*
Emericella nidulans	TH2	321210	+++	+++	++	++	++	+
E. nidulans	TH15	321213	+++	+++	+++	+	+	++
E. nidulans	TH24	321214	+++	++	++	++	+	+
E.nidulans var.echinulate	TH11	321212	++	++	++	+	+	+
E. quadrilineata	TH68	317906	++	++	+	+++	++	+
E. quadrilineata	TH12	317907	+	+	+++	++	+	+
E.quadrilineata	TH37	317923	+++	+++	+++	++	++	++
E.quadrilineata	TH69	317909	++	++	++	++	++	++
Equadrilineata	TH81	321215	++	++	+++	+	+	+
E. quadrilineata	TH134	325133	++	++	++	++	+	-/*
E. quadrilineata	TH35	325136	++	++	++	++	++	+
E. quadrilineata state of A.nidulans var. acrustatus	TH131	325134	+++	+++	+++	+++	+++	+++
E.quadrilineata state of A.nidulans var.dentatus	TH132	325132	++	++	++	+++	+	+
E. rugulosa	TH128	325131	+++	+++	+++	+++	++	++

Note: * Liberation of p-nitrophenol from lipid substrate :p-nitrophenyl caprylate (C_8),p-nitrophenyl caparate (C_{10}),p-nitrophenyl laurate(C_{12}),p-nitrophenyl myristate (C_{14}),p-nitrophenyl palmitate(C_{16}) and p-nitrophenyl stearate (C_{18}).
Symbols : +++ strong, ++Moderate,+ Poor ,* Doubtful, -NILL.

Table 3: Lipase C_{18} activity at various temperatures recorded after 120 hrs of incubation

Organisms	Isolate No.	IMI No.	Liberation of *p*-nitrophenol from p-nitrophenyl stearate incubated at different temperature*							
			16^0C	35^0C	40^0C	45^0C	50^0C	55^0C	60^0C	65^0C
Aspergillus nidulans	TH4	317919	(4)-	+	++	+++	++	++	+	+
A. nidulans	TH5	317908	(4)-	+	++	+++	++	++	+	+
A. nidulans	TH6A	317911	(4)+	++	++	+++	++	++	++	+
A. nidulans	TH13	317912	(3)+	++	++	++	++	++	+	+
A. nidulans	TH20	317913	(3)+	++	++	++	++	++	+	+
A. nidulans	TH21	317914	(4)+	++	++	+++	++	++	++	+
A. nidulans	TH23	317915	(4)-	+	++	+++	+++	+++	++	+
A. nidulans	TH35	317916	(3)+	++	++	+++	++	+	+	+
A. nidulans	TH38	317917	(2)+	++	++	+++	+++	+++	+	+
A. nidulans	TH68	317918	(4)+	++	+	+++	++	+	+	+
A. nidulans	TH103	317921	(4)-	+	+	++	++	++	*/+	+
A. nidulans	TH133	317922	(4)-	+	++	+++	++	++	++	++

Table 4: Lipase C_{18} activity at various temperatures recorded after 120 hrs of incubation

Organisms	*Isolate No.*	*IMI No.*	*Liberation of p-nitrophenol from p-nitrophenyl stearate incubated at different temperature*							
			16^{0}C	*35^{0}C*	*40^{0}C*	*45^{0}C*	*50^{0}C*	*55^{0}C*	*60^{0}C*	*65^{0}C*
Emericella nidulans	TH2	321210	(3)+	++	++	+++	+++	+++	+	+
E. nidulans	TH24	321213	(4)-	+	+	+++	+++	+++	++	+
E. nidulans	TH80	321214	(4)-	+	++	+++	++	++	+	+
E. nidulans var. echinulata	TH11	321212	(4)+	++	++	+++	++	++	+	+
E.quadrilineata	TH6B	317906	(2)+	++	++	+++	++	+	+	+
E. quadrilineata	TH12	317907	(4)+	+++	+++	+++	+++	+++	+	+
E. quadrilineata	TH37	317923	(3)+	++	++	+++	++	++	+	+
E. quadrilineata	TH69	317909	(3)+	++	++	+++	++	+	+	+
E. quadrilineata	TH81	321215	(4)+	+++	++	++	++	++	+	+
E. quadrilineata	TH134	325133	(4)+	+	+	++	++	++	++	+
E. quadrilineata	TH135	325136	(4)+	+	+	++	+++	+	+	+
E. quadrilineata state of E. nidulans var. achinulate	TH132	325132	(4)-	+	++	+++	+++	++	+	+
E. rugulosa	TH28	325131	(4)-	+	+	+++	+++	+++	++	+

Note: the number given within brackets represent number of weeks after which observation was recorded at 16^0C
Symbols: +++,Strong; ++Moderate; +,Poor;*Doubtful; - Nill

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