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Effect of amino acids on production of lipase from Fusarium sp. SM-9

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Abstract
The effect of different amino acids added to basal medium was observed with the aim of improving the enzyme production. Supplementation with L-valine, L-leucine, L-asparagine, L-lysine increased lipase production from *Fusarium* sp. SM-9 upto two-fold. This enhancement in yield of extracellular lipase has mainly been correlated with the chemical structure of amino acids.

Keywords: Amino acids, *Fusarium*, lipase, agitation

Introduction
Lipases (triacyl glycerol acyl hydrolases E.C. 3.1.1.3.) are one of the most important classes of industrial enzymes. They hydrolyse esters preferentially at the interface between lipid and water in heterogenous systems. Commercial preparations of microbial lipases are produced by fermentation of different fungi, yeast and bacteria. Fungi are well known as lipase producers and some species belonging to the genera *Fusarium* have been widely recognized as lipase producers [1]. Increased productivity of lipase during fermentation process is of great importance, since lower costs of production could promote new industrial applications. The productivity of lipase is affected by different environmental factors such as temperature, pH, nitrogen, carbon sources, agitation, dissolved oxygen concentration and presence of lipid sources as inducers. Lipase production is usually coordinated with, and dependent on the availability of triglycerides. Besides this, free fatty acids, hydrolysable esters, bile salts, detergents, solvents, ions, glycerol and amino acids also stimulate lipase production [2, 3, 1]. In the same line the present study has been carried out with as an objective of enhancing the production of lipase from, a potent lipase producing fungus *Fusarium sp.* SM-9, using various amino acids and their analogues.

Materials and Methods
Microorganism and culture conditions: The fungal strain, Fusarium sp. SM-9 was isolated locally from curd by an enrichment culture method using sunflower oil as the carbon source in (Czapek Dox agar medium containing0.5% (v/v) tributyrin and chloramphenicol (100µg/ml) and maintained on (Czapek-Dox agar slants at 4ºC. The basal medium used for lipase production was composed of (g/l): $NaNO_3$, 5.0, $MgSO_4.7H_2O$, 0.5, KCl, 0.5, KH_2PO_2, 1.0, Na_2HPO_4, 3.0, $ZnSO_4.7H_2O$, 0.01, $MnSO_4H_2O$, 0.01, 2%(v/v) olive oil, pH 5.5. The stock solutions of amino acids and their analogues were filter sterilized and added to the sterilized medium at variable concentration between 1 and 10 mM. An aqueous spore suspension containing $4x10^7$ spores/ml was used as an inoculum. 1.0ml of spore suspension was added to 50ml of medium in 250ml erylenmeyer flasks and incubation at 30ºC on a rotary shaker (160 rev/min). After 72h of growth, the fermented broth was separated by filtration for mycelium and the filterate was used for the enzyme assay. The mycelium was washed with acetone followed by 10ml of distilled water and dried at 80ºC to constant weight.

Lipase assay
The lipase assay mixture comprises of 5ml olive oil emulsion, composed of 25ml of olive oil and 75ml of 2.0% (w/v) polyvinyl alcohol solution, 4ml 0.1M sodium phosphate buffer pH 7.0, 1ml of 110mM $CaCl_2$ and 1ml of enzyme solution was incubated at 37ºC for 10min. Immediately, after the incubation, the emulsion was destroyed by the addition of 20ml of acetone-ethanol (1:1) mixture and liberated free fatty acid was liberated 1µmol of fatty acid in 1min under the assay conditions. 1ml of 0.02N sodium hydroxide is equivalent to 100µmol of fatty acid.

Result and Discussion

The results of effect of amino acids on lipase production are presented in Table 1. When the culture media were supplemented with amino acids, the enzyme production was much higher than those same media without amino acids (Table 1). This increase in production was upto 2.10 fold. The presence of valine, leucine, asparagine, lysine and 2-amino-n-butyric acid increased the yield of extracellular lipase, but the cell growth was reduced when compared to the same media without the above amino acids, which indicated that lipase production is not a direct function of cell growth. Lipases mostly are inducible enzyme and inducers such as oils are necessary for lipase productions [1]. So possibly there might be some switching on and off phenomenon present in the organism for lipase production which is not only operating in presence of olive oil but also in the presence of some specific amino acids. The stimulatory effect of amino acids in this study for the *Fusarium* sp SM-9 is in accordance to certain extent with the results observed for the lipase of *Pseudomonas sp*., where a mixture of lysine, arginine and glutamic acid in the basal medium was observed to be effective for lipase production [2]. The present study revealed that the carbon chain length, position of –CH3 and –NH2 groups and increased substitution in α-R group have a significant role is the stimulation of lipase production. These results may be interpreted to mean, but by no means prove as how the lipase stimulation and its localization is correlated with the chemical structure-function relationships of amino acids. The mechanism of this amino acid specific-effects remains to be establish.

Table 1: Effect of amino acids on production of lipase from *Fusarium sp*. SM-9 at pH 5.5 and 30ºC under shaking (160rev/min) conditions.

Amino acids (5mM)	Biomass(g/L)	Lipase yield (U/ml)	Yield index
Control	12.1+0.9	20.7+0.6	1.00
L-Glycine	13.0+0.6	25.2+0.1	1.21
L-Alanine	10.6+0.7	31.6+0.6	1.52
L-Valine	9.4+0.6	36.5+0.8	1.76
L-Leucine	6.8+0.4	39.8+1.2	1.92
L-Lysine	6.5+0.3	43.5+2.1	2.10
L-Methionine	11.6+0.6	29.7+0.9	1.43
L-Aspartic acid	8.4+0.3	36.9+1.3	1.78
L-Asparagine	7.2+0.1	40.5+1.8	1.95
L-Glutamic acid	9.2+0.3	37.4+0.9	1.80
L-Phenyl alanine	9.6+0.3	29.7+0.6	1.43
L-Proline	9.0+0.9	31.6+2.2	1.52
L-Tyrosine	8.3+0.4	30.5+1.4	1.47
2-Amino-n-butyric acid	6.2+0.1	39.8+2.1	1.92

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