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Effects of Nutrients on the Extracellular Lipase Production by Mutant Strain of *Rhizopus oligosporous* T^{UV}-31

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Abstract: Mutant strain of *Rhizopus oligosporous* T^{uv}-31 was used for the production of extracellular lipase by solid-state fermentation. Maximum lipase activity (76.69 μ/g) was observed when different nutrients were added to the substrate. Among different carbon sources Tween 80 at 0.5% was found to be the best carbon source. The best organic and inorganic nitrogen sources for lipolytic activity were soybean meal (1%) and ammonium sulphate (0.4%) respectively. Addition of egg yolk to fermentation medium enhanced the lipase production.

Key words: Lipase, *Rhizopus* species, mutant, soybean

Introduction

Lipases are hydrolytic enzymes extensively used in the hydrolysis of fat as well as in the synthesis of glycerides. Fungi like *Aspergillus, Rhizopus, Mucor, Geotrichum, Penicillium, Candida* and *Syncephalostrum* are potential sources of lipase production (Toide *et al.*, 1996). Microbial lipases are both extracellular and intracellular (Haq *et al.*, 1998; Pastou *et al.*, 2000). Among different fungi, *Rhizopus oligosporous* shows higher productivity of lipase as reported by Toshihiko *et al.* (1989). Although lipases can be produced both by submerged and solid-state fermentation (Huang *et al.*, 1995; Rivera *et al.*, 1991; Pandey, 1992). Solid-state fermentation gave better results (Christen *et al.*, 1998).

Lipases are extremely versatile enzymes, showing many interesting properties for industrial applications. They are currently applied in detergent formulations, for the removal of fatty stains (Egmond *et al.*, 1996). Lipases can be applied in fur processing (Rezanka, 1991). The hydrolytic behaviour of lipases is applied in oil and fat hydrolysis (Bell *et al.*, 1981). Lipases are used in bakery products for enhancing taste and aromatic properties of milk, butter cheese and yogurt (Mohsen *et al.*, 1986). The specificity of lipases makes it possible to obtain compounds, which are difficult to prepare by conventional chemical methods (Rezanka, 1991). All these commercial applications make it a potential subject for this study.

Materials and Methods

Inoculum preparation: Spore suspension was used as inoculum. In this study, 5-7 days old culture was used and the spore suspension was prepared in sterilized 0.005% Monoxol. O.T. (Di-Octyl ester

of sodium sulphosuccinic acid). Haemocytometer slide was used for counting of spores. Production of fungal lipase was studied by solid-state fermentation (Korn and Fujio, 1997).

Lipase activity: Lipase activity in the fermented meal was determined titrimetrically on the basis of olive oil hydrolysis, as reported by Kundu and Pal (1970).

Results and Discussion

Effect of carbon sources: Different carbon sources were used for extracellular lipase production by mutant strain of *Rhizopus oligosporous* T^{uv}-31 (Table 1). One percent additive was added to the substrate of almond meal. Glucose, sucrose, starch, olive oil, mustard oil and Tween 80 were used as carbon sources. Tween 80 was found to be the best carbon source, as compared to others because it gives maximum lipase production (61.87μ/g), since it was miscible with water and did not generally inhibit fungal growth (Martinez *et al.*, 1993). Handelsman and Shoham (1994) also reported the production of extracellular lipase by addition of Tween 80 as best carbon source. Our studies are in accordance with Nahas (1988), highest yields of enzymes were obtained when Tween 80 were the carbon sources.

Effect of different concentration of Tween 80: Variation in the concentration (0.1-2%) of Tween 80 was also effective against lipase production. Maximum lipase level (65.17 μ/g) was obtained at 0.5% concentration of Tween 80 as it provided optimum amount of carbon (Table 2). Enzyme level however decreased with further increase in Tween 80 concentration. It might be due to the increase in fatty acid accumulation through hydrolysis of substrate, suppressing lipase synthesis. Sidhu *et al.* (1998) used 0.5% Tween 80 for the production of extracellular lipase. Espinosa *et al.* (1990) also reported that Tween 80 exerted a positive effect on enzyme production in a range that goes from 0.02 to 2.00%.

Effect of organic nitrogen sources: Different organic nitrogen sources were used for extracellular lipase production by mutant strain of *Rhizopus oligosporous* T^{uv}-31 (Table 3). For this purpose 1% of different organic nitrogen sources such as urea, peptone, yeast extract, soybean meal, nutrient broth and casein were added to the substrate. Soybean meal gave maximum (69.65μ/g) production of lipase, was found to be the best organic nitrogen source. As soybean meal supported good growth of *Rhizopus oligosporous,* thus there was an increased enzyme production. Results are also in accordance with Huang *et al.* (1995) and Nahas (1988).

Effect of inorganic nitrogen sources: Different inorganic nitrogen sources were used for extracellular lipase production by mutant strain of *Rhizopus oligosporous* T^{uv}-31. $(NH_4)_2SO_4$ gave maximum production (70.97μ/g) as compared to other inorganic nitrogen sources (Table 4). It might be due to the optimum growth of mycelium when $(NH_4)_2SO_4$ was used as nitrogen source. According

Table 1: Effect of different carbon sources on the production of lipase by mutant strain of *Rhizopus oligosporous* T^{uv}-31

Carbon sources (1%)	Extracellular lipase activity (μ/g)
Glucose	26.03
Sucrose	28.01
Starch	25.84
Olive oil	26.05
Mustard oil	23.09
Tween 80	61.87

Temperature = 30°C Incubation period = 48 hours. Substrate used = Almond meal.

Table 2: Effect of different amounts (%) of Tween 80 on the production of lipase by mutant strain of *Rhizopus oligosporous* T^{uv}-31

Tween 80(%)	Extracellular lipase activity (μ/g)
0.1	41.65
0.5	65.17
1.0	60.61
1.5	51.65
2.0	39.69

Temperature = 30°C Incubation period = 48 hours. Substrate used = Almond meal

Table 3: Effect of different organic nitrogen sources on the production of lipase by mutant strain of *Rhizopus oligosporous* T^{uv}-31

Organic nitrogen sources (1%)	Extracellular lipase activity (μ/g)
Urea	35.21
Peptone	45.63
Yeast extract	30.89
Meat extract	33.32
Soybean meal	69.65
Nutrient broth	42.34
Casein	39.20

Temperature = 30°C Incubation period = 48 hours. Substrate used = Almond meal.

to Gao and Breuil (1995), ammonium sulphate gave the best lipase production. 0.3% concentration of $(NH_4)_2SO_4$ was found to be the best for lipase production by T^{uv}-31.

Table 4: Effect of different inorganic nitrogen sources on the production of lipase by mutant strain of *Rhizopus oligosporous* T^{uv}-31

Inorganic nitrogen sources (1%)	Extracellular lipase activity (μ/g)
$NH_4H_2PO_4$	28.32
NH_4NO_3	31.65
$(NH_4)_2SO_4$	70.97
$(NH_4)_2C_2O_4$	30.07
NH_4Cl	25.45
$NaNO_3$	26.15

Temperature = 30°C Incubation period = 48 hours. Substrate used = Almond meal.

Table 5: Effect of different amounts of ammonium sulphate $(NH_4)_2SO_4$ on the production of lipase by mutant strain of *Rhizopus oligosporous* T^{uv}-31

Inorganic nitrogen sources (%)	Extracellular lipase activity (μ/g)
0.1	39.98
0.2	44.98
0.3	70.31
0.4	74.80
0.5	41.65

Temperature = 30°C Incubation period = 48 hours. Substrate used = Almond meal.

Table 6: Effect of addition of different amounts (%) of egg yolk on the production of lipase by mutant strain of *Rhizopus oligosporous* T^{uv}-31

Concentration of egg yolk %(v/w)	Extracellular lipase activity (μ/g)
0.5	32.40
1.0	49.01
1.5	76.69
2.0	45.30

Temperature = 30°C Incubation period = 48 hours. Substrate used = Almond meal.

Effect of different amounts of ammonium sulphate: The data of Table 5 shows that the effect of different amounts (0.1-0.5%) of $(NH_4)_2SO_4$ on the production of lipase. Maximum lipase production (74.80μ/g) was observed when 0.4% $(NH_4)_2SO_4$ was added in the substrate as an additional inorganic nitrogen source. Ammonium sulphate (0.4%) may fulfill the nutritional needs of organism, presumably because the enzyme activity was associated with cell growth (Handelsman and Shoham, 1994).

Effect of addition of egg yolk: Effect of different concentration (0.5-2.0%) of egg yolk (v/w) has been used (Table 6). 1.5% (v/w) egg yolk gave the maximum production (76.69 μ/g) of lipase. As egg yolk contained 32.5% lipids, which include cholesterol as well as glycerides. Similarly an augmenting effect of fat and cholesterol on lipase production has been reported by Valero *et al.* (1988). They reported an increase in growth and lipase secretion in the presence of fat and sterol.

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