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Optimization of Process Parameters for the Production of Lipase in Submerged Fermentation by *Yarrowia lipolytica* NCIM 3589

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Abstract: The production of extra cellular lipase in submerged fermentation of *Yarrowia lipolytica* NCIM 3589 has been investigated. Several compounds have been added to the culture medium in order to assess their efficiency as inducers of lipase production. The effect of triglycerides (olive oil, sunflower oil and tributyrin) has been studied. The highest lipase activity was obtained with sunflower oil (2.5 U mL^{-1}) in four days.

Key words: Lipase, submerged fermentation, triglycerides, *Yarrowia lipolytica*

INTRODUCTION

Lipases (triacylglycerol acylhydrolases, EC 3.1.1.3) are one of the most important classes of industrial enzymes. They hydrolyse triglycerides into diglyceriods, monoglycerides, glycerol and fatty acids. Lipases occur widely in nature, but only microbial lipases are commercially significant. Commercial preparations of microbial lipases are produced by fermentation of different bacteria, fungi, yeast and actinomycetes (Sharma *et al*., 2001).

In recent years, there has been an increasing interest in the study of lipases, mainly due to their potential applications as medicines (digestive enzymes), food additives (flavour modifying enzymes), clinical reagents (glyceride-hydrolysing enzymes) and cleaners (detergent additives) (Sugiura, 1984). Additionally, a promising application field for lipases in the biodegradation of plastics such as polyhydroxyalkanoates (PHA) and polycaprolactone (PCL) (Jager *et al*., 1995; Mochizuki *et al*., 1995). These enzymes are able to catalyze not only hydrolysis but also synthesis reactions (i.e., esterification, transesterification) in media with low water content (Schmitt-Rozieres *et al*., 1999; Osorio *et al*., 2001). More over, they usually retain their structure and activity in organic solvents and at high temperature (Khmelnitsky and Rich, 1999). Also they have several advantages over chemical catalysts: substrate specificity, region- and enatio- selectivity, lower temperature and pressure requirements.

In the present study, several compounds have been added to submerged fermentation of *Yarrowia lipolytica* NCIM 3589 in order to evaluate their ability to stimulate lipase secretion. The effect of the addition of triglycerides has been studied and the most potent lipase inducer has been selected.

MATERIALS AND METHODS

Microorganism

Yarrowia lipolytica NCIM 3589 obtained from National Chemical Laboratory, Pune, India, was used throughout the study.

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Growth Medium and Growth Conditions

The culture was maintained on MGYP slants having the composition (%): malt extract 0.3, glucose 1.0, yeast extract 0.3, peptone 0.5 and agar agar 2.0. The pH of the medium was adjusted to 6.4-6.8 and culture was incubated at 30°C for 48 h. Sub culturing was carried out once in every 2 weeks and the culture was stored at 4°C.

Inoculum Preparation

The yeast *Yarrowia* strain was cultivated in a medium containing peptone 5 g, yeast extract 3 g and sodium chloride 3 g L^{-1} of distilled water. The cells were cultivated in this medium at 30°C on a shaker at 200 rpm for 24 h (Oswal *et al*., 2002).

Production Media

The composition of the basal medium for lipase production was as follows (g L^{-1}): Urea,2; KH_2PO_4,1; $MgSO_4.7H_2O$,0.5; $CaCl_2$,0.1; NaCl,0.1; H_3BO_3,0.0005; $CuSO_4 5H_2O$, 0.00004; KI,0.0001; $FeCl_3 4H_2O$,0.0002; $ZnSO_4 7H_2O$,0.0004; $MnSO_4 H_2O$,0.0004; myo-inositol,0.0000004 and d-biotin, 0.0000008 (Corzo and Revah, 1999). The medium without vitamins was sterilized at 121°C for 15 min. After cooling, the vitamins previously sterilized by filtration were added to the basal medium. A cell suspension of inoculum was added to the basal medium. The flasks were incubated in an orbital shaker at 160 rpm and 30°C for 96 h.

Optimization of Medium Parameters

The strategy adopted was to optimize one particular parameter at a time and then include it at its optimum value in the next optimization step, if found beneficial. The parameters optimized were: (1) carbon level, (2) salt solution concentration, (3) incubation time, (4) temperature, (5) nitrogen level and (6) effect of triglycerides.

Lipase Assay

The activity of lipase was determined as described in the literature (Winkler and Stuckmann, 1979) with the following modifications: 1 mL of isopropanal containing 3 mg of p-nitrophenyl palmitate (pNPP) was mixed with 9 mL of 0.05 M Tris-HCl buffer (pH 8.0), 40 mg of Triton X-100 and 10 mg of gum arabic. Liberation of p-nitrophenol at 28°C was detected at 410 nm. One enzyme unit was defined as 1 μmol of p-nitrophenol enzymatically released from the substrate per minute (Bruno *et al*., 2004).

RESULTS AND DISCUSSION

Effect of Carbon Source

The carbon sources were selected based on earlier reports that show increase in lipase activity in other microorganisms, such as *Beauveria bassiana* (Hegedus and Khachatourians, 1988) *Rhiozopus oligosporus* (Nahas, 1988) *Candida* and *Yarrowia* (Novotny, 1988) *Humicola lanuginose* (Omar *et al*., 1987) and *Acremonium strictum* (Okeke and Okolo, 1990). The lipase activity by different carbon sources is presented in Table 1. The lipase activity was not observed on the culture

Table 1: Effect of the different carbon sources on lipase activity

Carbon source	Lipase activity (U mL^{-1})
Glucose	1.1
Olive oil	0.8
Corn oil	0.7
Glycerol	Not detected

medium containing glycerol because it was an end product of the hydrolysis of triacylglycerols and it might repress lipase induction. Olive oil and corn oil have increased the yield of extra cellular lipase in microorganisms such as *Candida rugosa* (Benjamin and Pandey, 1996), *Aspergillus niger* (Hatzinikolaou *et al*., 1996) and *Aspergillus flavus* (Long *et al*., 1996). Among all the carbon sources, the maximum lipase activity was observed with the glucose. The highest lipase activity in the supernatant of the culture was observed at 2%(w/v) of glucose concentration as shown in Fig. 1. These results were in accordance with the observed lipase production by *Yarrowia lipolytica* 681 (Corzo and Revah, 1999).

Effect of Salt Solution Concentration

Salt solution of the medium plays a vital role for microbial growth and biochemical activities in submerged fermentation. The maximum yield was obtained at 15% salt solution concentration as shown in Fig. 2. At higher salt solution concentration, lipase production was reduced due to the inhibitory effects of salts.

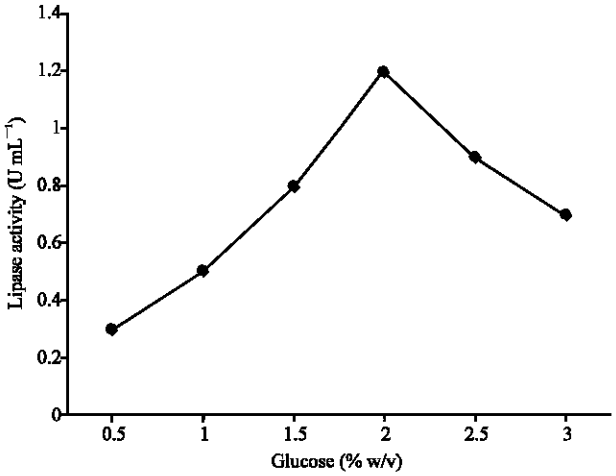

Fig. 1: Effect of glucose concentration on lipase activity

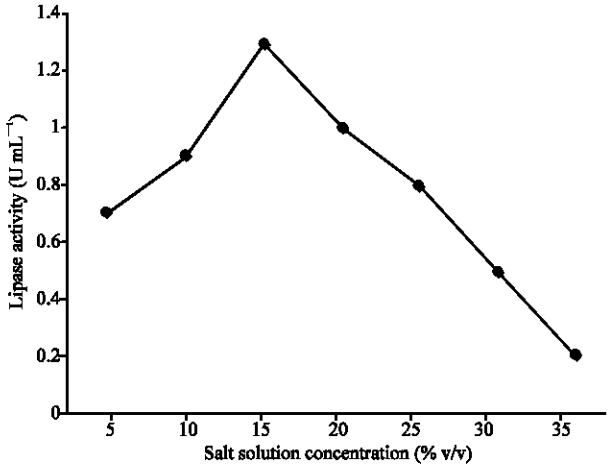

Fig. 2: Effect of salt solution concentration on lipase activity

Effect of Incubation Time

The amount of lipase produced was observed daily during a period of six days. The maximum lipase activity was observed on fourth day as listed in Table 2. After fourth day, it was reduced due to the consumption of nutrient materials.

Effect of Temperature

As yeast strains are very sensitive towards temperature, a slight change in temperature causes reduction in the yield. *Yarrowia lipolytica* produced maximum amount of lipase at 30°C is presented in Table 3.

Effect of Nitrogen Source

Nitrogen source mediated lipase production was well documented in submerged fermentation (Corzo and Revah, 1999). Table 4 depicts the role of different nitrogen sources on lipase production. Among all the nitrogen sources, urea yielded maximum lipase activity. Lipase production was not detected in the medium containing ammonium sulfate. Urea was the best nitrogen source for increasing lipolytic activity in six different yeast cultures (Novotny *et al*., 1988).

Effect of Triglycerides

Triacylglycerols and fatty acids have been reported to induce lipase secretion by various microorganisms (Obradors *et al*., 1993; Berto *et al*., 1997; Kulkarni and Gadre, 1999). The imperative role of different triglycerides on lipase production by this organism was elucidated by incorporating the selected triglyceride 1.5% (v/v) to the culture medium. The lipase production by different triglycerides are presented in Table 5. Among all the triglycerides, the maximum lipase activity was observed with sunflower oil.

Table 2: Effect of incubation time on lipase activity

Incubation time (h)	Lipase activity (U mL^{-1})
24	0.5
48	0.7
72	0.9
96	1.5
120	1.1
144	0.8

Table 3: Effect of temperature on lipase activity

Temperature (°C)	Lipase activity (U mL^{-1})
25	0.5
30	1.8
35	0.9

Table 4: Effect of different nitrogen sources on lipase activity

Nitrogen source	Lipase activity (U mL^{-1})
Urea	1.9
Yeast extract	1.1
Peptone	0.7
Ammonium sulfate	Not detected

Table 5: Effect of different triglycerides on lipase activity

Triglycerides	Lipase activity (U mL^{-1})
Sunflower oil	2.5
Olive oil	1.9
Tributyrin	1.7

CONCLUSIONS

The present study describes the production of extra cellular lipase in submerged fermentation of *Yarrowia lipolytica* NCIM 3589. The highest lipase activity (2.5 U mL^{-1}) was obtained with the addition of sunflower oil in four days. These results also implied the existence of significant differences between the behavior of *Yarrowia lipolytica* NCIM 3589 and other wild strains described in the literature.

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