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RESEARCH **P**APER

Micro-organism isolation and process optimization for lipase production

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A highly lipase producing *Bacillus* sp. was isolated from soil under optimized culture conditions such as medium pH, temperature, incubation period, carbon sources, nitrogen sources, lipid sources and various surfactants at different concentrations. The medium pH of 7.0 and temperature of 40 °C were optimum for maximizing lipase production. The maximal yield of lipase production by *Bacillus* sp. was obtained after incubation periods ranging between 3 and 4 days. Casein produced maximum lipase (6.5±0.015) U/ml) as compared to others nitrogen sources and the medium containing starch was more suitable for maximum lipase (15.60±0.20) U/ml) production than other carbon sources. The gingily oil was found to be most suitable for maximizing the lipase production (20.52±0.20) U/ml). The studies on the influence of surfactants on lipase production revealed that maximum lipase production was induced by tween-20 ((27.10±0.01) U/ml).

Key words : Lipases, *Bacillus* sp., Hydrolysis, Optimization

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INTRODUCTION

Enzymes have a great industrial potential and are widely found in various sources like plants, animals and microbes. Lipase (carboxyl ester hydrolases; E.C. 3.1.1.3) is a fat-splitting enzyme widely distributed in the animal and vegetable worlds. Lipases have considerable industrial potential and find promising applications as additives in detergent (Arnold, 1996) and food additives for flavour enhancement in cheese ripening, baking etc. (Gerritse *et al.*, 1998). Lipases are used to synthesize chiral building blocks for pharmaceuticals and as component of personal care products (Kazlauskas and Bornscheuer,1998). In general, lipase supplements are thought to help the body absorb food more easily, keeping nutrients at appropriate, healthy levels throughout the body. The present study was carried out microorganism isolation and process optimization for lipase production.

RESEARCH METHODOLOGY

Sample collection :

Surface soil samples were collected from six different industrial (preferably oil mill effluent) areas in and around Raigad district, Taloja, Navi Mumbai.

Enrichment soil sample :

One gram of sample was suspended in 10 ml sterile normal saline. After shaking, 5 ml of the suspension was transferred into a 250 ml blue cap bottle containing 50 ml of enrichment medium. The culture was incubated at 60^0C under shaking condition (150 rpm) for 2 days.

Isolation and screening of lipase producing micro-organisms :

The enriched culture was streaked after serial dilutions on tributyrin agar plates. Plates were incubated at 60^0C.

Identification of the micro-organism using different tests :

The isolate was identified by help of various staining (Gram staining, Capsule staining, Endospore staining) and biochemical tests(Indole production, Methyl red, Voges Proskauer, Citrate utilization, Catalase, Caesin hydrolysis and Nitrate reduction) based on the key of Bergey,s manual given in the book of (Aneja, 2003).

Maintenance of the culture :

The organism isolated was maintained on nutrient agar slants containing 1 per cent tween 80 (polyoxyethylene sorbitan monooleate), pH 7.0.

Fermentative production of lipase :

Approximately 10 per cent of enriched culture (prepared earlier) was inoculated in 250 ml Erlenmeyer flask containing 45 ml of basal TS broth. The cells were then harvested by centrifugation at 10,000 rpm, 4^0C for 15 min. and the supernatant was used for further assay.

Optimization of lipase production :

The purified enzyme was optimized for the effect of pH and temperature on lipase production. The optimization of medium components with suitable nutrient sources were carried out at the optimum pH and temperature, by substituting components present in the basal medium and subsequent optimization.

RESEARCH FINDINGS AND ANALYSIS

Enrichment culture technique enabled the isolation of strains with lipolytic activity in tributyrin media plates. In total, 12 isolates (2 from each source) were collected from the soil sample and among them; four isolates showed high lipolytic activity. Colonies with clearing zones around them were isolated. The lipolytic microbes were further screened and characterized by their features and reactions. When it was grown on Rhodamine B lipase agar, orange fluorescence was observed under UV irradiation indicating lipase activity. The results of all the tests performed have been summarized in Table 1.

Bacterial culture capable of producing lipase was isolated by serial dilution method and it was isolated from the industrial effluent by spread plate technique. Individual colonies were picked up and streaked in nutrient agar plates. The colonies appeared as pale white, flat, irregular and lobate. Finally the morphological and biochemical test indicated that the suspected organism was *Bacillus* sp.

Effect of pH on lipase production :

The effect of the pH of the medium on lipase production indicated a linear increase from (1.18±0.012) to (4.0±0.01) U/ml corresponding to the increase of pH from 4 to 8. At the pH of 9 and 10, the lipase production decreased (3.83±0.07) and (2.71±0.03) U/ml), respectively.

Invariably, the lipase production by *Serratia marcescens* prefers slightly acidic pH (6.5–7) (Albertsson *et al.*, 1990) and *Pseudomonas aeruginosa*

Table 1: Identification of lipase-producing micro-organisms

Sr. No.	Test	Observation	Result
1.	Motility determination	Microorganisms were found motile	+
2.	Gram staining	Observed as purple cells	+
3.	Capsule staining	Non-capsulated	-
4.	Endospore staining	Spores were observed	+
5.	Indole production	No red colour was seen	-
6.	Methyl red	Red colour was observed at pH 4.0	+
7.	Voges Proskauer	Crimson to Ruby pink (red) colour seen	+
8.	Citrate utilization	Growth visible on surface with blue colour	+
9.	Catalase	Bubbles of oxygen were observed after addition of H_2O_2 within a minute	+
10.	Caesin hydrolysis	Clear area surrounding the growth was observed	+
11.	Nitrate reduction	Negative test was observed	-

MB prefers neutral pH. The present study revealed that the lipase production by the isolated *Bacillus* sp. required alkaline pH (7.5 ± 0.1) as shown in Fig. 1.

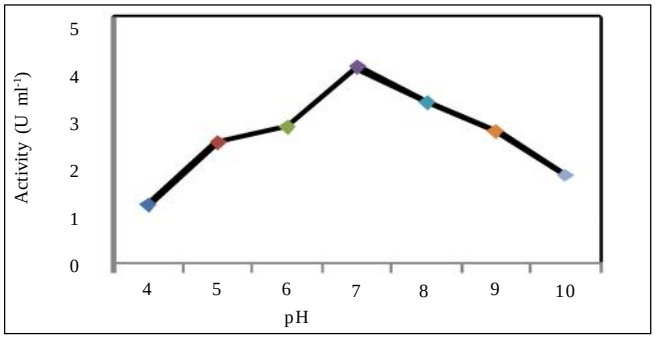

Fig. 1: Determination of optimum pH for lipase production

Effect of temperature on lipase production :

The effect of temperature indicated that the lipase production was maximum ((4.29±0.001) U/ml) at the optimum temperature of 40 °C. But below (10 to 30 °C) and above (50 to 80 °C) the optimum temperature, the lipase production recorded was low ((1.9±0.02) to (3.59±0.033) and (2.10±0.05) to (3.91±0.06) U/ml, respectively). Earlier studies regarding lipase production stated that optimum temperature for lipase production was 30–40 °C (Andersson *et al*., 1979), In the present study, lipase activity showed gradual increase with the increase of temperature from 10 to 40 °C and further increase of temperature, beyond 40 °C, decreased the production of lipase (Fig. 2).

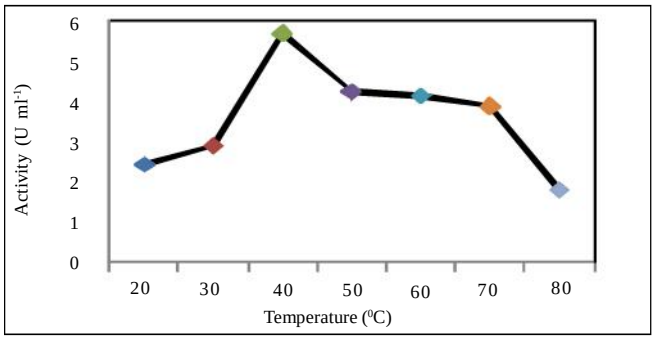

Fig. 2: Determination of optimum temperature

Effect of incubation period on enzyme production:

The effect of incubation period was conducted to follow lipase production during the fermentation process. Samples were taken daily to determine the proper time for the highest yield of lipase production. Lipase production started after the first day of fermentation period. Data revealed that 3 days of incubation period were optimum for maximal lipase production. These results are in accordance with Antonian (1996) who found that the maximal yield of lipase production by *Bacillus* sp. was obtained after incubation periods ranging between 3 and 4 days (Fig. 3).

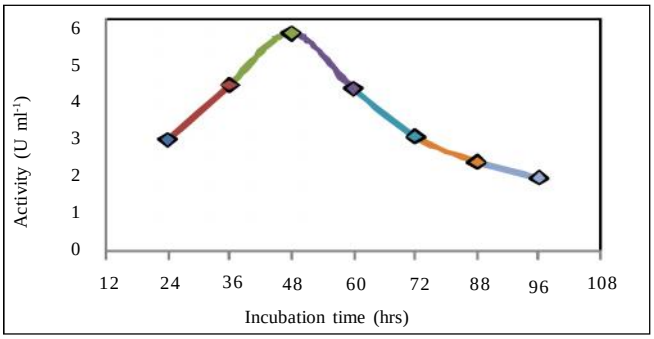

Fig. 3: Effect of incubation time on lipase production

Effect of nitrogen sources on lipase production :

In the present study different nitrogen sources (either organic or inorganic) were screened at a fixed concentration of 16 g/lit. The lipase production was greatly influenced by the tested nitrogen sources. Among these nitrogen sources, casein produced maximum lipase ((6.5±0.015) U/ml), compared to others (Fig. 4). After casein, higher lipase production was registered in tryptone-supplied basal medium, followed by others. The lowest lipase production ((2.93±0.05) U/ml) was registered in yeast extract-supplied medium. A possible mechanism may be that yeast extract is a complex nitrogen source and thus, requires the cells to secrete more protease for its enzymatic degradation before utilization. This might result in lower production and higher degradation of the extracellular lipase (Bradoo *et al*.,

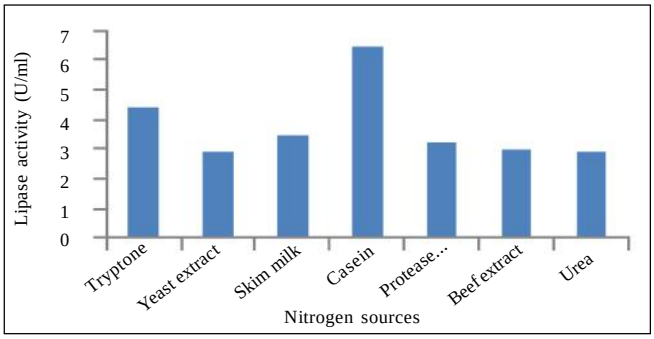

Fig. 4: Optimization of nitrogen source for lipase production

1999).

Lipase production was also influenced by the concentration of casein in the medium. In the medium with 18 to 36 g/lit of casein (added at the interval of 2 g), maximum lipase production ((9.95±0.015) U/ml) was registered at the concentration of 24 g/lit. But at low (18 to 22 g/lit) and high (26 to 36 g/lit) casein concentrations, the lipase production was lower (6.98±0.06) to (8.70±0.031) U/ml and (7.76±0.02) to (5.42±0.011) U/ml, respectively.

Effect of additional nitrogen sources :

Effect of additional nitrogen source on the production of lipase revealed that the basal medium supplied with soytone gave its maximum production ((9.40±0.02) U/ml) as shown Fig. 5. In consistence with the present study, Henderson *et al*. (1995) reported that the lipase production by *Pseudomonas* sp. S34 was maximum with two nitrogen sources, namely trypton and soytone, and the studies by Salleh *et al*. (1993) reported that lipase production by *Penicillium aurantiogriseum* was high when using inorganic nitrogen source, but a medium with two organic nitrogen sources displayed lipase production more or less the same to that of the medium containing one organic nitrogen source. In the present study, in addition to soytone, medium supplied with skim milk or tryptone also gave more or less similar production results. The effect of different concentrations of soytone on lipase production showed a positive linear increase ((9.9±0.034) to (14.8±0.03) U/ml) with respect to the increase in the concentration of soytone from 3.5 to 5.0 g/lit at 0.5 g interval. Further increase in the concentration of soytone to 6.5 g/lit resulted in decreased lipase production ((12.2±0.011) U/ml).

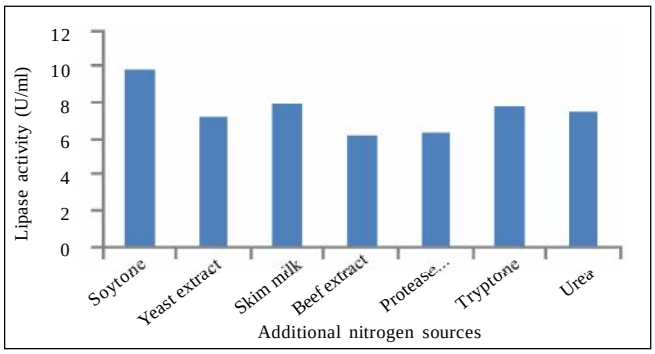

Fig. 5: Effect of additional nitrogen sources in lipase activity

Effect of carbon sources on lipase production :

A range of different carbon sources, mainly carbohydrates, were screened for their efficiency to support lipase production at the fixed concentration of 2.5 g/lit. On the basis of lipase activity, it was concluded that the medium containing starch was more suitable for maximum lipase ((15.60±0.20) U/ml) production than other carbon sources (Sugiura *et al.*, 1977) as shown in Fig. 6. In the medium with added starch, lipase production depended on its concentration. Among the tested starch concentrations in the range from 3.0 to 6.5 g/lit., at the interval of 0.5 g/lit., the lipase production was maximum (17.46±0.20) U/ml) at 4.0 g/lit. When low (3.0 to 3.5 g/lit.) or high (4.5 to 6.5 g/lit.) concentrations of starch were added, the lipase production was lower ((16.2±0.23) to (16.76±0.27) U/ml and (16.5±0.11) to (14.6±0.02) U/ml, respectively.

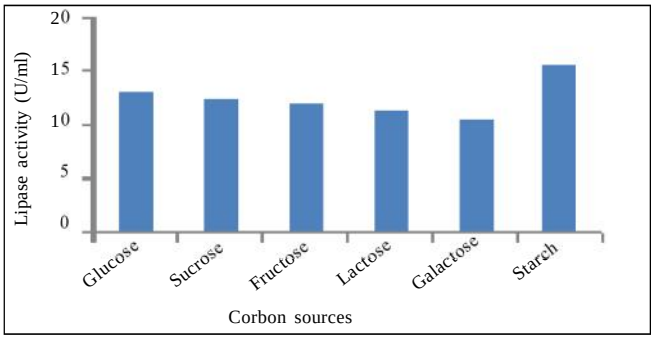

Fig. 6: Optimization of carbon source for lipase production

Influence of lipid sources on lipase production :

Lipid-induced lipase production was confirmed by the addition of lipids to the culture medium. Here, the lipase production in lipid-supplemented medium gave better production than the control medium. On the basis of the activity, the gingily oil was found to be suitable for maximizing the lipase production ((20.52±0.20) U/ml).This is because triglycerides are important substrates for lipase production as they can act as an inducer as well as an inhibitor. In the present study, all the tested triglycerides were found to induce the lipase synthesis with different level of enzyme production. This study is in agreement with the previous work on castor oil-induced lipase production by *Pseudomonas aeruginosa* KKA-5, sunflower oil and olive oil-induced extracellular lipase production by *Yarrowia lipolytica* and vegetable oil-induced lipase production by *Candida rugosa* (DSM

2031) (Surinenaite *et al*., 2002). Addition of gingily oil in the fraction range of 5 to 25 ml/lit. at an interval of 5 ml/lit. indicated that the lipase production was optimum (23.15±0.24) U/ml) in the medium containing 15 ml/lit. of gingili oil. At low (5 ml/lit.) and high (25 ml/lit.) fractions of gingily oil, the lipase production was reduced (18±0.05) and (19.98±0.15) U/ml) as shown in Fig. 7.

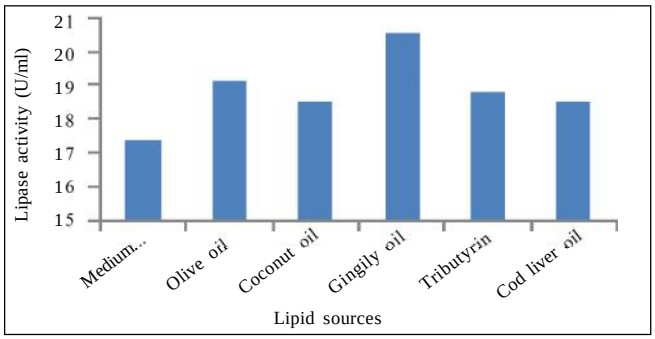

Fig. 7: Optimization of lipid source for lipase production

tween-80 (Tobin *et al*., 2000). The production of lipase by *Rhizopus oligosporus* induced by tween 20 was reported, while surfactant-induced lipase production by *Yarrowia lipolytica* was studied.

From the present study it is also evident that the lipase production by micro-organisms could be induced by the addition of surfactants (Fig. 8).

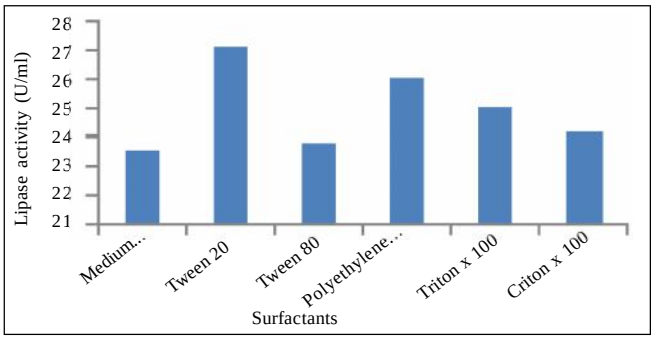

Fig. 8: Influence of nitrogen source for lipase production

Effect of surfactants on lipase production :

The studies on the influence of surfactants on lipase production revealed that maximum lipase production was induced by tween-20 (27.10±0.01) U/ml), followed by polyethylene glycol 300 (26.00±0.06) U/ml). Thus, all the tested surfactants showed positive influence on lipase production compared to the control. Similarly, the studies of lipase production by *P. aeruginosa* EF2 indicated the positive influence of

Addition of surfactants to the medium also showed that the lipase production depended on their fraction. Among the tested fractions of tween-20 from 3.0 to 10.0 ml/lit., at an interval of 1 ml/lit., the lipase production was maximum ((34.20±0.01) U/ml) in the medium with 6.0 ml/lit. The lipase activity was low ((30.93±0.04) to (32.66±0.03) U/ml and (30.46±0.045) to (33.53±0.013) U/ml) in medium supplemented with 3.0 to 5.0 and 7.0 to 10.0 ml/lit. of tween-20, respectively (Voordouw *et al*., 1974).

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