Effect of Bioparameters in The Production of Lipase From *Streptomyces aurofaciens*

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Abstract

Lipases are enzymes, which are widely used in many industries. The actinomycetes member *streptomyces aurofaciens* strain was purchase from MTCC 325 (Microbial type culture collection – Chandigarh), which was having lipase coding gene. In the present study, several factors affecting the activity of lipase were investigated. Olive oil was the best substrate for enhancing the enzyme activity. Due to high cost, instead of using olive oil, sunflower oil and palm oil were used as a substrate for lipase production and the enzyme activity was determined by titrimetric method. In this study, olive oil was using as a (standard), while sunflower oil and palm oil as a (test). The maximum lipase activity was achieved at 48 & 24 h of incubation period and the enzyme activity was 47.61 and 52.85U/ml by using sunflower oil and palm oil as a substrate. The incubation period was very short for obtaining maximum lipase by using palm oil than sunflower oil. No significant changes obtained in the pH ranges from 6 to 9.

Key words: *Streptomyces aurofaciens,* Olive oil, sunflower oil, palm oil, titrimetic method

Introduction

Lipases (triacyl glycerol acyl hydrolase) catalyse the hydrolysis and the syntheses of ester formed from glycerol and long-chain fatty acids. Lipases are synthesized by microorganisms which grow on fats or oils. Lipase is a potential enzyme employed in industries for decades to hydrolyse fats and catalyse a number of useful reactions including esterification, transesterification and leather industries Chowdary *et al.,* 2001. The enzyme catalyses the above said chemical reations, helps to minimize the environmental hazards.

Use of lipases in oleochemical processing saves energy and minimizes thermal degradation during alcoholysis, acidolysis, hydrolysis, and glycerolysis Lynn Peacock *et al.,* 2003. Lipases find promising applications in organic chemical processing, detergent formulations, Synthesis of bio surfactants, the oleo chemical industry, the dairy industry, the agrochemical industry, paper manufacture, nutrition, cosmetics, and pharmaceutical processing Akram kashmiri *et al.,* 2006; Fariha hasan *et al.,* 2006; Haki.G.D and Rakshit.S.K 2003.

In the present study a potent lipase production from *S. aurofaciens* was determined by using olive oil, palm oil and sunflower oil of its yield's also tabulated. Optimization of pH for enzyme production was also analyzed.

Materials and method

Source of Microorganisms

The organism required for the lipase production is *S. aurofaciens* which was purchased from MTCC-325. Stock culture of *S. aurofaciens* was maintained by periodic subculture and kept in refrigerator at 4°C throughout the investigation.

Production media

The basal medium for lipase production contained olive oil (standard) 7 % (v/v), Dextrose 1 % (w/v), Peptone 3 % (w/v) pH 7 to 8.6. 100 ml of portion of the medium was utilized with 2 % of cell suspension and incubated at 37°C in orbital shaker with the shaking speed of 100 rpm for 96 hrs. The study samples were withdrawn regularly for every 24 h. Samples were filtered by Whitman No -1 filter paper. Cells were separated and culture filtrate was used for the study of enzyme activity Lyn peacock *et al.,* 2003; Sailas Benjamin and Ashok Pandey, 1996. On the other hand, palm oil and sunflower oil 7 % (v/v) were considered as tests which are added with dextrose and peptone, instead of adding olive oil and inoculate 2% cell suspension into it Alessandro D'Annibale and Giovanni Giovannozzi Sermanni, 2006.

Enzyme assay by titrimetric method

The enzyme assay was performed with the cell free supernatant of fermented broth as the crude enzyme source (EC 3.1.1.3). One ml sample solution was added to the assay substrate containing 10 ml of 10 % homogenized olive oil in 10 % gum acacia, 2 ml of 0.6 % Cacl$_2$ solution and 5 ml of 0.2 mol/l phosphate buffer pH 7.0 Karl-Erich Jaeger *et al.,* 1994.The enzyme substrate mixture was incubated on an orbital shaker with a shaking speed of 100 rpm at 37°C for 1 hr. To stop the react 20 ml ethanol acetone mixture (1:1) was added to the reaction mixture Burkert *et al.,* 2004; Dongwang *et al* 2008.Liberated fatty acids were titrated with 0.1 mol/l NaOH. Endpoint is an appearance of pink color. Enzyme

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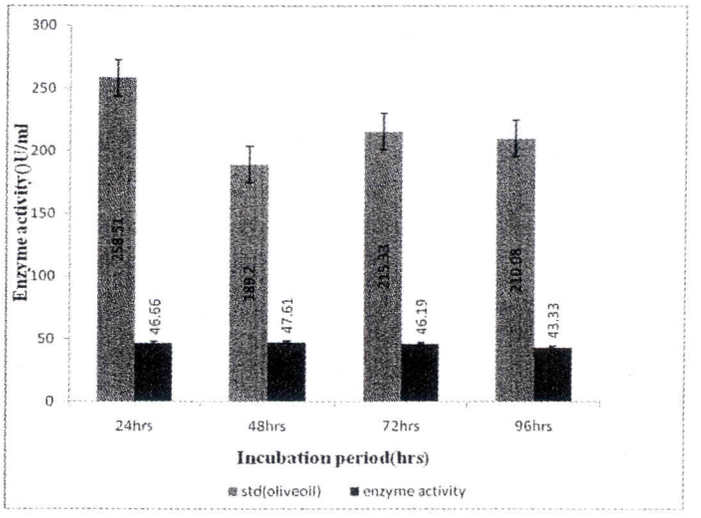
for the test also proceeded with same titrimetric method as ıtioned above.

Characterization of lipase

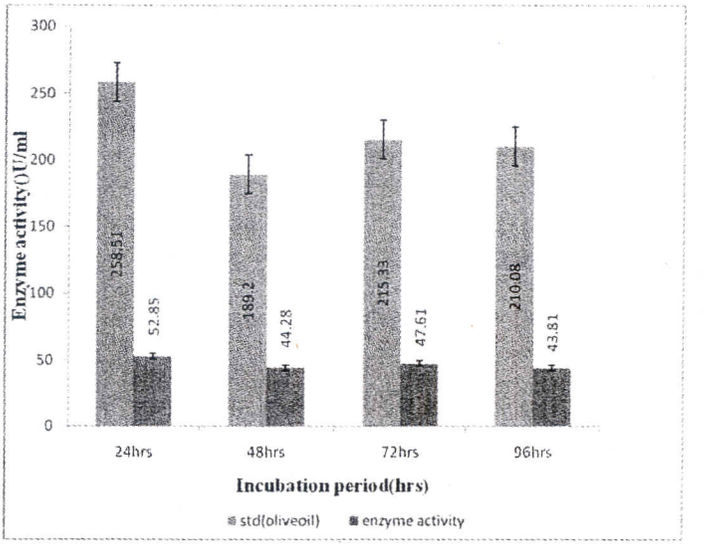
Lipase activity was determined at different time intervals such as 24 hrs, 48 hrs, 72 hrs and 96 hrs of incubation Sztajer. H and Maliszewska, 1988; Zullo and Iride. A, 2006. The optimum pH required for the lipase production and growth of microorganism was determined by adjusting the pH of the fermentation medium at pH ranges between 6 to 10 separately in 250 ml conical flasks and incubated at 37°C for 4 days in shaker incubator (200 rpm). Culture were studied every 24 hrs and assayed by titrimetric method as explained above.

Results and Discussion

The organism required for the lipase production was *S. aurofaciens* purchased from MTCC 325. The basal medium for lipase production also prepared separately for standard and test and enzyme assay were calculated by titrimetric method. Optimization of pH for enzyme production also analyzed and tabulated in tables 1-4 and the effect of incubation period on lipase production was graphically represented in Figures 1and 2. The rate of oils (100 ml) and its yield also are tabulated in Table 5.



Graph No: 1 Effect of different pH on lipase production from *S.aurofaciens* at different incubation period using sunflower oil as a substrate.



Graph No: 2 Effect of different pH on lipase production from *S.aurofaciens* at different incubation period using palm oil as a substrate.

pH	Standard (olive oil)	Sunflower oil	Palm oil
6	164.29	47.14	51.42
8	162.26	45.71	54.29
9	164.27	47.14	52.86

Table No: 1 Effect of pH on lipase production from *S.aurofaciens* after 24 h of incubation using sunflower oil and palm oil as a substrate.

pH	Standard (olive oil)	Sunflower oil	Palm oil
6	188.57	45.71	44.29
8	190.53	48.57	42.86
9	188.51	48.57	45.71

Table No: 2 Effect of pH on lipase production from *S.aurofaciens* after 48 h of incubation using sunflower oil and palm oil as a substrate.

pH	Standard (olive oil)	Sunflower oil	Palm oil
6	215.71	47.14	45.71
8	213.62	44.29	48.57
9	216.68	47.14	48.57

Table No: 3 Effect of pH on lipase production from *S.aurofaciens* after 72 h of incubation using sunflower oil and palm oil as a substrate.

pH	Standard (olive oil)	Sunflower oil	Palm oil
6	211.43	44.29	42.86
8	208.41	42.86	44.29
9	210.4	42.86	44.29

Table No: 4 Effect of pH on lipase production from *S.aurofaciens* after 96 h of incubation using sunflower oil and palm oil as a substrate.

OIL	Amount (ml)	Rate (Rs)	Lipase yield (%) *S. aurofaciens*
Olive oil	100	45.00	82
Sunflower oil	100	6.00	47.14
Palm oil	100	3.00	54.29

Table 5 Lipase yield obtained by using different oil source

Lipase activity was analysed by adjusting the pH ranges from 6 to 9. But, there is no significant changes obtained in the pH range. So, the maximum lipase activity at the particular pH could not be specified. Another important parameter for lipase activity was incubation period. The lipase yields were optimum at 24 h by using olive oil as a substrate and its yield was 258.51U/ml. By using sunflower oil as a substrate, the maximum lipase activity was found to be 47.61U/ml during 48 h of incubation period.

The optimum yields obtained at 24 h of incubation period by using palm oil as a substrate and its yield was 52.85U/ml. So, both standard and test were given maximum lipase at 24h of incubation period. The previous study investigated, in the fungal strain of *Penicillium simplicissimum* could grow on babassu cake, an abundant residue of the oil industry, which showed maximum lipase production at pH 7 for 72h and its activity was 45.36U/ml.

Statistical analysis

The results were expressed as the mean±SD for each group. Statistical differences were evaluated using a one way analysis of variance (ANOVA). Results were considered to be statistically significant at $P<0.01$.

Considered the rate of olive oil, sunflower oil & palm oil (each 100 ml) were 45 RS, 6RS & 3RS respectively. The yields given by those oil were 82%, 47.14%, 54.29%. Olive oil gave the optimum yield, but by considering the rate, was highly expensive. So, in an industry increasing the contents of sunflower oil & palm oil, the lipase yields also become increasing two folds with low cost. Also they are the cheapest substrate and available in higher amount.

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