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Production of lipase from *Streptomyces griseus* and evaluation of Bioparameters

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Abstract: Lipases are enzymes, which are widely used in many industries. The actinomycetes member *streptomyces griseus* strain was purchase from MTCC 4734(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 titrimetic method. In this study, olive oil was using as a standard (standard), while sunflower oil and palm oil as a (test). The maximum lipase activity was achieved at 24 & 48 h of incubation period and the enzyme activity was 51.9 and 51.9U/ml by using sunflower oil and palm oil as a substrate. The incubation period was very short for obtaining maximum lipase by using sunflower oil than palm oil. No significant changes obtained in the pH ranges from 6 to 9.
Key words: *streptomyces griseus,* Olive oil, sunflower oil and 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.[1] The enzyme catalyse 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. 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.[2,3,4]

In the present study a potent lipase production from *S.griseus* 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.griseus* which was purchased from MTCC 4734. Stock culture of *S. griseus* 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.[5, 6] On the other hand, palm oil and sunflower oil 7% (v/v) were considered as a test which are added with dextrose and peptone, instead of adding olive oil and inoculate 2% cell suspension into it.[7]

Enzyme asaay by titrimetic method

The enzyme assay was performed with the cell free supernatant of fermented broth as the crude enzyme source.[8] 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.[9] 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.[10, 11] Liberated fatty acids were titrated with 0.1 mol/l NaOH. Endpoint is an appearance of pink color. Enzyme assay for the test also proceeded with same titrimetric method as mentioned 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.[12, 13] 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 h and assayed by titrimetric method as explained above.

Results

The organism required for the lipase production was *S.griseus* purchased from MTCC 4734. 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 table No 1 to 4 and the effect of incubation period on lipase production was graphically represented in graph No 1and 2. The rate of oils (100 ml) and its yield also tabulated in Table No 5.

Discussion

Present study revealed that the, Optimization of enzyme production and to determined the yields at different incubation period by using different oil source as a substrate. To analyse the enzyme activity by adjusting the pH between 6 and 9 by using 0.1N Hcl and 0.1N NaOH.

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 72 h by using olive oil as a substrate and the enzyme activity was found to be 117.88U/ml. At the same time sunflower oil & palm oil were used as a substrate and the maximum yields were obtained at 24h & 48hour's of incubation period and their enzyme activity were 51.90 and 51.90 U/ml respectively. But, the incubation period was very short for obtaining maximum lipase by using sunflower oil than palm oil.

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 75.71%, 50%, 48.57%. Olive oil gave the optimum yield.

But, by considering the rate, Olive oil was highly expensive. So, in an industries increasing the contents of sunflower oil& palm oil, the lipase yields also become increasing two folds with low cost. Because they were the cheapest substrate & available in higher amount.

The previous study investigated, in the bacterial strain of *Bacillus subtilis* could grow on media containing olive oil mill waste (OMW) which showed maximum lipase production at pH 6 for 96h and the extra cellular lipase activity was 106.2U/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 $P<0.01$.

Table No: 1 Effect of pH on lipase production by *S.griseus* at 24 h

pH	Standard(olive oil)	sunflower oil	palm oil
6	75.71	50	45.71
8	75.69	52.86	48.57
9	75.7	52.86	48.57

Table No: 2 Effect of pH on lipase production by *S.griseus* at 48 h.

pH	Standard(olive oil)	sunflower oil	palm oil
6	91.4	50	50
8	91.43	52.86	52.86
9	90.89	50	52.86

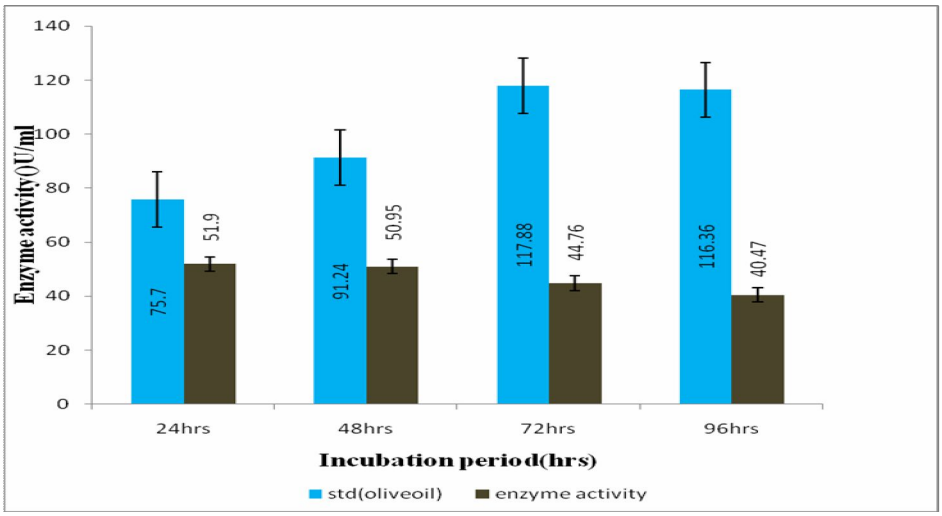
Table No: 3 Effect of pH on lipase production by *S.griseus* at 72 h.

pH	Standard(olive oil)	sunflower oil	palm oil
6	118.57	42.86	44.29
8	116.54	45.71	44.49
9	118.55	45.71	41.42

Table No: 4 Effect of pH on lipase production by *S.griseus* at 96 h.

pH	Standard (olive oil)	sunflower oil	palm oil
6	115.71	41.42	34.29
8	115.7	38.57	34.29
9	117.68	41.42	31.43

Graph No: 1 Effect of incubation period on lipase production by *S.griseus* (Sunflower oil)



Graph No: 2 Effect of incubation period on lipase production by *S.griseus* (palm oil)

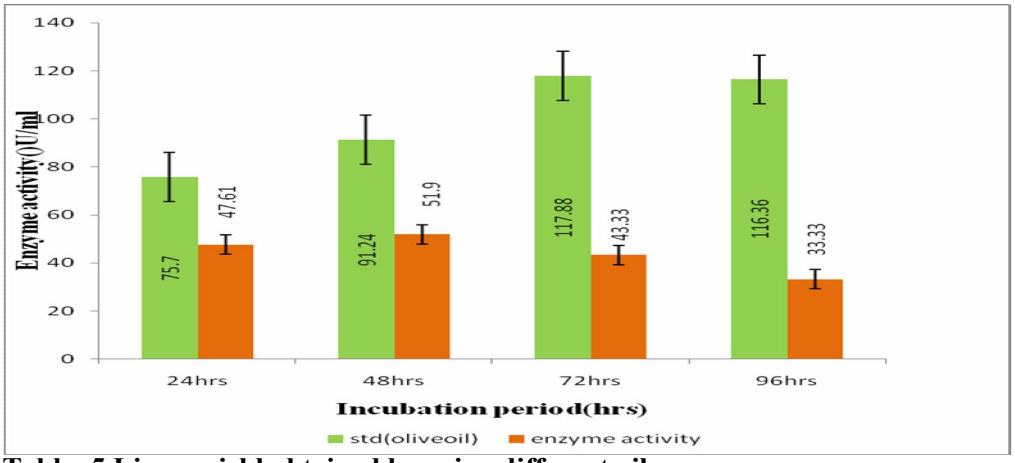


Table: 5 Lipase yield obtained by using different oil source:-

OIL	Amount (ml)	Rate (Rs)	Lipase yield (%) *S.griseus*
Olive oil	100	45.00	75.71
Sunflower oil	100	6.00	50
Palm oil	100	3.00	48.57

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References

1. Chowdary, Hamsaveni , Production, purification, characterization, and applications of lipases, *Biotechnology Advances*(2001), Volume 19, Pages 627-662.

2.Akram kashmiri.M, Ahmad Adnan and Beenish Waseem Butt-production, purification and partial characterization of lipase from *Trichoderma Viride*, *African Journal of Biotechnology* (2006) Volume 5, pages 878 -882 .

3.Fariha Hasan , AamerAli Shah and Abdul Hameed, Industrial applications of microbial lipases, *Enzyme and Microbial Technology*(2006), Volume 39, Pages 235-251.

4.Haki.G.D, Rakshit.S.K, Developments in industrially important thermostable enzymes: a review, *Bioresource Technology*(2003), Volume 89, Pages 17-34.

5. Lynn Peacock, JohnWard, Colin Ratledge, Mark Dickinson .F and AndrewIson, How *Streptomyces lividans* uses oils and sugars as mixed substrates, *Enzyme and Microbial Technology*(2003), Volume 32, Pages 157-166.

6.Sailas Benjamin and Ashok Pandey, optimization of liquid media for lipase production by *candida rugosa*, *bioresource technology*(1996), volume 55, pages 167-170.

7.Alessandro D'Annibale,Giovanni Giovannozzi Sermanni, Olive-mill wastewaters: a promising substrate for microbial lipase production, *Bioresource Technology*(2006), volume 97, Pages 1828–1833.

8. Enzyme assay of LIPASE,(EC 3.1.1.3), VELIPA01, Revised:04/30/97, Sigma quality control procedure.

9. Karl-Erich Jaeger, StéphaneRansac, Bauke, Dijkstra.W, Charles Colson, Margreet van Heuvel and Onno Misset, Bacterial lipases, *FEMS Microbiology Reviews*(1994), Volume 15, Pages 29-63.

10.Burkert. J. F. M, Maugeri.F and Rodrigues.M.I, Optimization of extracellular lipase productions by *Geotrichum* sp. using factorial design, *Bioresource Technology*(2004), Volume 91, Pages 77-84.

11.DongWang, Yan Xu, Tianyu Shan, effects of oils and oil-related substrates on the synthetic activity of membrane-bound lipase from *rhizopus chinensis* and optimization of the lipase fermentation media, *biochemical engineering journal* (2008), volume 41, pages 30-37.

12. Sztajer.H and Maliszewska.I , Production of exogenous lipases by bacteria, fungi, and actinomycetes, *Enzyme and Microbial Technology*(1988), Volume 10, Pages 492-497.

13.Zullo.B.A and Iride.A, Lipase production by yeasts from extra virgin olive oil, *Food Microbiology*(2006), Volume 23, Pages 60-67.

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