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SCREENING & OPTIMIZATION OF PHYSIOLOGICAL PARAMETERS FOR PRODUCTION OF LIPASE USING DE-OILED SEED CAKE AS A SUBSTRATE.

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ABSTRACT

The present work was conducted in the Laboratory of Biotechnology, Department of Biotechnology, Gulbarga University Kalaburagi, Karnataka, with the objective of process optimization studies on physiological parameters in the production process namely pH and temperature. It deals with the screening of microorganisms with different substrates- pongamia seed cake, jatropha seed cake, simarouba seed cake. The optimum parameters for maximum lipase production were carried out for four days of incubation, inoculums size 0.25ml to 1ml, pH 5.0-10.0, moisture content and 37°C-60°C incubation temperature. Our results suggested that solid state fermentation process is a tool to utilize the low-cost substrate like simarouba seed cake, jatropha seed cake, pongamia seed cake for the production of industrially important enzyme such as lipase.

KEYWORDS: Lipase, Simarouba seed cake, Optimization and characterization.

INTRODUCTION

Enzymes, which may be used in a diverse array of industrial applications, are important biocatalysts. There is always a demand for new enzymes that may offer better properties for specific applications in ever-changing industrial activities (Konarzycka-Bessler and Jaeger, 2006).

Lipases are a particular group of enzymes with many uses such as in textile, food, biomedical, petrochemical, pharmaceutical, detergent, and many other industries (Hasan et al., 2006). Lipases (EC 3.1.1.3, glycerol ester hydrolases) are enzymes that catalyze both the hydrolysis and synthesis of insoluble or poorly soluble long-chain triacylglycerols with an acyl chain length of ≥ 10 carbon atoms based on the presence or absence of water (Jeager et al., 1999; Gupta et al., 2004). In the absence of water, they play a role in esterification, transesterification, acidolysis, and aminolysis reactions (Jeager et al., 1994).
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) (Sharma et al., 2001).

Microbial lipases have already established their vast potential regarding to their usage in different industries. The interest in microbial lipase production has increased in the last decades. Due to the versatility of the molecular structure and catalytic properties, these enzymes have potential biotechnological application in different industrial sectors such as food, waste water treatment, cosmetics, oleo chemical, pharmaceutics, detergents and in the fuel sector, which applies lipase as catalyst for synthesis of esters and for trans esterification of the oil for the production of bio diesel [Sharma R et al., 2001].

Generally, the enzymes of industrial interest are produced in the presence of inducers in the case of lipases, the presence of triacyl glycerol, surfactants, vegetable oils, oil industry wastes or their hydrolysis products in the culture medium have, in most cases, an inducible effect on lipase production [Damaso MCT et al., 2008]. It was under taken to optimize the key process variables, including incubation time, inoculum level, initial moisture content, carbon level, and nitrogen level of the medium for the production of lipase using the de-oiled seed cake.

MATERIALS AND METHODS

Isolation & screening of lipase producing microorganism from soil

The bacteria used in this study were isolated from a contaminated garage soil sample at Kalaburagi, Karnataka, India. The isolates were identified on the basis of various morphological, physico-chemical, and biochemical characteristics. The isolates obtained were screened for the lipolytic activity using lipid hydrolysis in nutrient broth containing 1% of Tributyrin. After autoclaving, 1% of Tributyrin was then added to the medium at 90°C

with continuous stirring for 1hr, and then poured (20-25ml) into 100mm petri-dishes. The samples were enriched at 45°C in the nutrient broth.

Lipolytic isolates were selected on Tributyrin agar medium using tributyrin as substrate. The isolates produce a zone of clearance surrounding the colony; isolates having a higher ratio of clearing zone were used for the further study. The colony producing the large clearance zones were further grown in Nutrient broth and incubated at 37°C. Later the level of lipase production was determined from the cell free culture supernatant fluid.

Biochemical Characterization of Lipase

Effect of Additives: The carbon and nitrogen sources, additives used in the present study were dextrose, peptone, yeast extract, tributyrin, sucrose and Tween 80. About 30ml of MSS_5 media was dispensed into different six conical flask then about 0.05ml (1%) of dextrose, peptone yeast extract, tributyrin, sucrose, tween 80 were added to different conical flask and about 5g of Simarouba cake was added to them and about 0.25ml of culture media was added to different conical flasks and incubated for 24 h in an incubator. After 24 h enzyme assay was done as said before.

Effect of Temperature: About 5ml of MSS_5 media was taken in 3 different conical flasks and to that about 5g of Simarouba cake was added and autoclaved and after cooling about 0.250 ml of inoculum was added and kept one conical flask in room temperature (37^oC) one in incubator (45^oC) and one in water bath (55^oC) for 24 h. After 24 h of incubation enzyme assay was carried out.

Effect of Incubation Time: About 15ml of MSS_5 media was prepared and about 5ml was dispensed into different conical flasks and about 5g of Simarouba cake was added to the conical flasks and autoclaved. After cooling, 0.25ml of inoculum was added and all three conical flasks were incubated. From three conical flasks each flasks was assayed for 24, 48 and 72h.

Effect of Moisture: Four conical flasks were taken about 2.5ml of the MSS_5 media was added, to one conical flask 5ml, to another 10ml and 20ml to respective conical flasks with a ratio of 1:0.5, 1:1, 1:2 and 1:4. To the flasks, 5g Simarouba cake was added and autoclaved. After cooling, 0.25ml of culture was added and incubated for 24 h. After incubation the conical flasks containing the Simarouba cake was subjected to lipase assay.

Effect of Inoculum: About 5ml of MSS_5 media was taken in four different conical flasks and to that 5g of Simarouba cake was added and autoclaved. After cooling one conical flask was added with 0.25ml of inoculum and second with 0.5ml of inoculum and third with 0.75ml and fourth with 1ml of inoculum and incubated for 24 h. After 24 h enzyme assay was done as said before.

Optimum pH and temperature studies: Extracellular lipases optimum pH was determined over a pH values from 5.0 to 10.0. Sodium Citrate buffer 25mM was used for pH 4.0, Acetate buffer was used for pH 5.0 to 6.0, Phosphate buffer for pH 7.0, Tris-HCl buffer for pH 8.0, Glycine-NaOH for pH 9.0, 10.0. The effect of temperature of the crude lipase was evaluated by assaying at temperatures ranging from 37 to 60°C in 25mM of Tris-HCl buffer (pH 8.0).

Effect of additives on activity of the purified enzyme: The effects of various metal ions were examined on the activity of the purified lipases at a concentration of 10mM and then the activity was determined. The additives used were SDS (1%), NaCl (1M), PMSF (phenylmethane sulphonyl fluoride), EDTA and 1-10 phenanthroline (2 mM each) and H_2O_2 (1%).

Effect of substrate concentration: To examine the effect of the different concentration of the substrate on the enzyme activity, the substrates were used in a concentration ranging from 1mM to 1M.

Kinetic studies of characterization of the enzyme: In many cases lipases appear to obey Michaelis-Menten kinetics. Michaelis-Menten kinetics is characterized by two parameters, K_m and V_{max}. The initial velocity was evaluated K_m and V_{max} and read using Lineweaver-Burk plots.

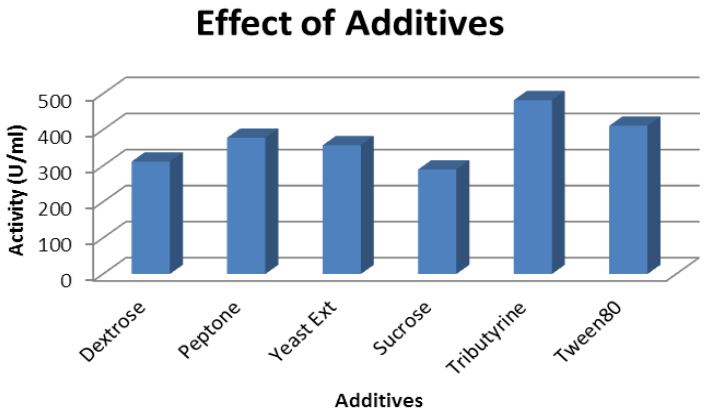
RESULTS AND DISCUSSION

After carrying out several sequential procedures such as screening, isolation of bacteria for production of lipase from enriched soil sample, the results of the study are as follows.

Effect of Additives

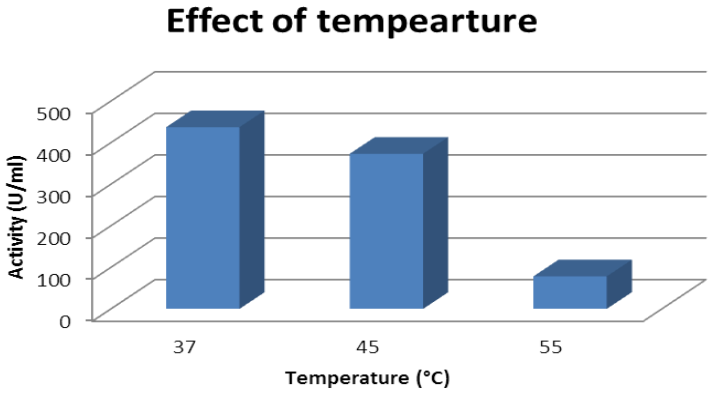
Different carbon and nitrogen sources, additives were added into the media to study their effect on production of lipase. The data suggested that addition of tributyrin has induced the production of lipase better than other additives.

Additives	Lipase activity U/ml
Dextrose	312
Peptone	378
Yeast Ext	357
Sucrose	290
Tributyrin	482
Tween 80	411



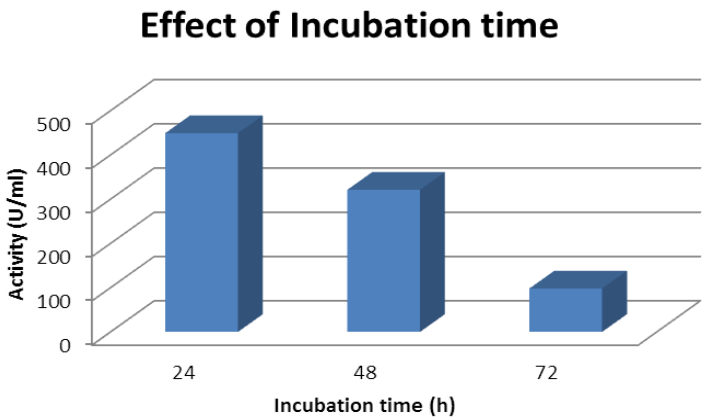
Effect of Temperature: The effect of temperature on growth of isolate and production of lipase was monitored and 37^{o}C was found suitable as culture turbidity was more and activity of lipase was also recorded maximum.

Temperature (°C)	Lipase activity U/ml
37	437
45	373
55	78



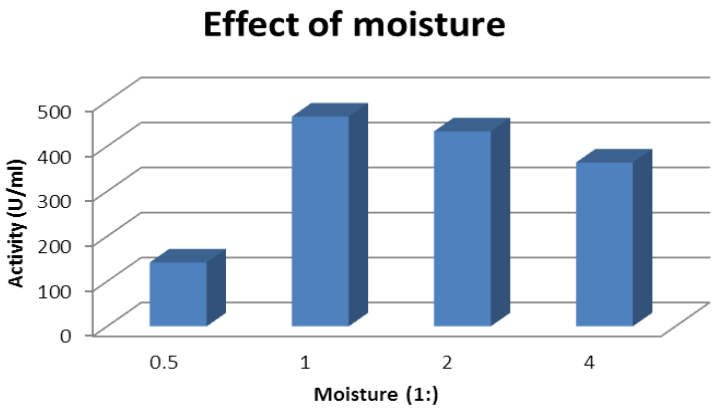
Effect of Incubation Time: The optimum production time was screened by incubating the flasks for different time. The maximum production was found in 24 h which declined at 48 h and a sharp decrease was found at 72h.

Incubation time (h)	Lipase activity U/ml
24	449
48	321
72	98



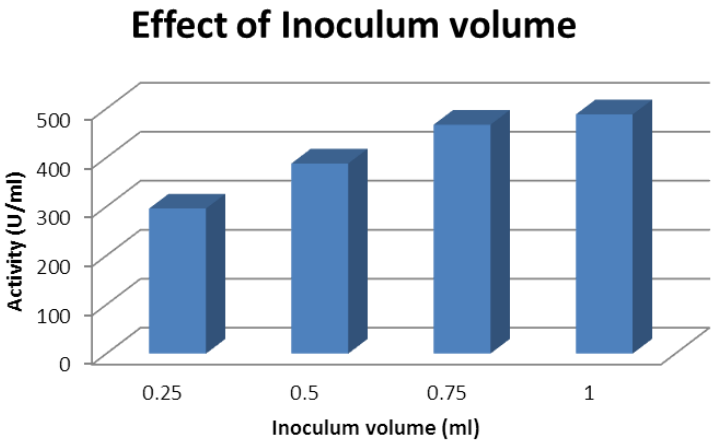
Effect of Moisture: 5 g of seed cake was moistened with different volumes of media so that the effect of moisture on production of lipase could be monitored. Initial level of moisture was not effective, later the increased moisture levels have induced the production of enzyme, though a decline was noted at 1:2 levels.

Moisture level	Lipase activity U/ml
01:0.5	142
01:01	466
01:02	433
01:04	364



Effect of Inoculum: Different volumes of inoculums were added into the production mixture to monitor the effect on production of lipase.

Inoculums volume (ml)	Lipase activity U/ml
0.25	296
0.5	388
0.75	467
1	488



Optimization of pH and temperature: The partially purified lipase was studied for the optimum pH by assaying the enzyme in different pH. The results (Fig. 5) show that the lipase was alkaline with optimum pH of 8.0. To find the optimum temperature the enzyme was assayed at different temperatures, which show that (Fig.6) enzyme was active at 35^0C.

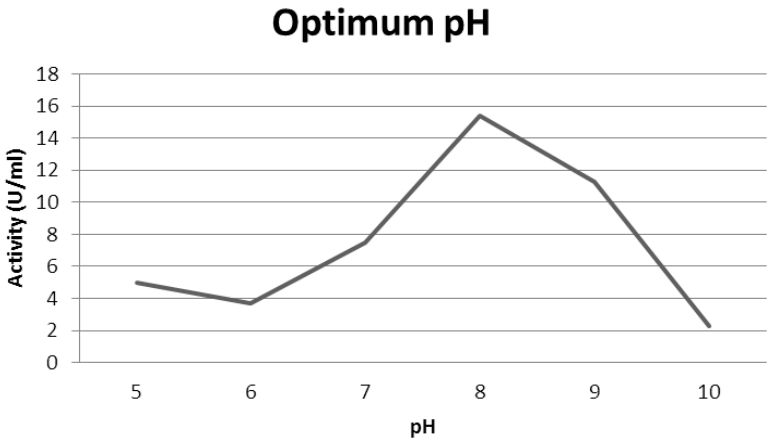


Fig.5- Determination of optimum pH

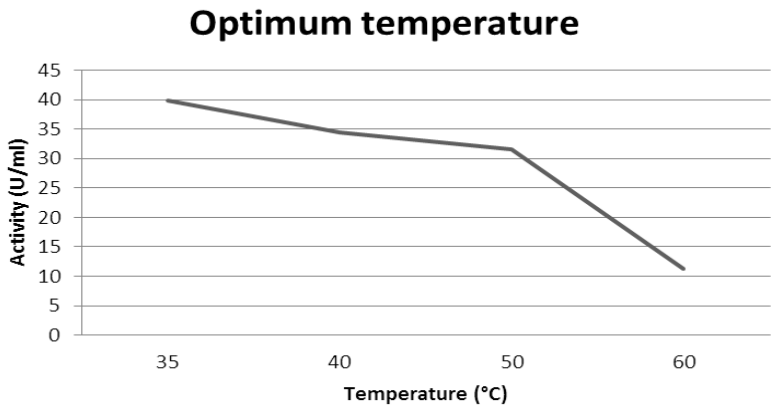


Fig.6- Determination of optimum temperature

Kinetic Studies: The effect of different substrate concentrations on enzyme activity was studied to find the Km and Vmax of the lipase. The (figure 7) shows that the enzyme had a Vmax of 8.0 and Km of 5.26.

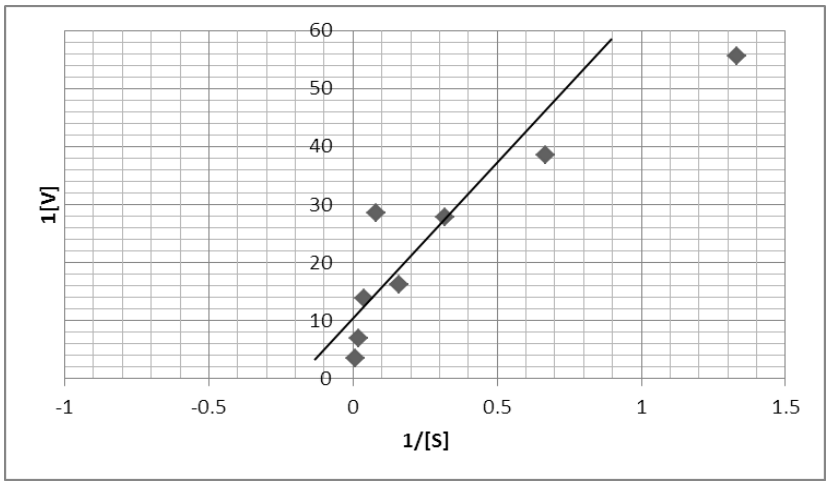


Fig.7- Determination of Km and Vmax

Effect of metal ions and inhibitors: Various additives were used to find their effect on the enzyme activity. The results are presented in Table.2.

Table-2: Effect of additives on lipase activity.

Additives	Activity (U/ml)	Residual Activity (%)
Control	29.6	0
PMSF	42.6	143.91
EDTA	36.4	122.97
1-10,Phenanthroline	21.6	72.97
NaCl	38.1	128.71
H_2O_2	36.3	122.63
SDS	24.7	83.44

The inhibition in presence of 1-10 phenanthroline indicates that the enzyme is a metallo enzyme and inductions in presence of PMSF indicate the possible absence of serine in the active site of the enzyme. The enzyme is found to be tolerant to salt, detergent and bleaching agent.

SUMMARY AND CONCLUSION

Lipases have been extensively purified and characterized in terms of their activity and stability profiles relative to pH, temperature, and effects of metal ions and chelating agents. Fermentation time of 5 days, temperature of 35°C, pH 8.0, 5th day old culture, 15% inoculum level and 60% initial moisture content were found to be the optimum conditions for lipase production and the maximum lipase activity was found to be 57.25 U/ml. Different carbon and nitrogen sources were used as supplements at 5% (w/w) and 3% (w/w) respectively, to determine their effect on enzyme yield. Maltose (60.25 U/ml) and Peptone (63.35 U/ml) supplements improved enzyme activity.

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