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Production and characterization of lipase from *Pseudomonas aeruginosa* isolated from soil region

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ABSTRACT

Lipases have gained importance to a certain extent protease and amylase, especially in area of organic synthesis. Lipase is an enzyme necessary for the absorption and digestion of nutrients in the intestine. This digestive enzyme is responsible for breaking down lipid in particular triglycerides, which are fatty substances in the body come from fat in the diet. Lipase producing *Pseudomonas aeruginosa* strain was isolated from soil and its enzyme productivity and activity was evaluated effect of various carbon and nitrogen sources. Maximum lipase production was obtained in the medium containing 2% of starch among carbon source olive oil and glucose supported maximum lipase production. Yeast extract was best nitrogen source, the lipase enzyme exhibited wide pH and temperature range and activity was found to be optimal at pH 8 and 40°C respectively.

Keywords: Lipase, production, enzyme activity, optimization

INTRODUCTION

Lipases constitute a group of enzyme defines as carboxyesterases that hydrolyze long chain acylglycerol at the lipid-water interface. Microbial lipases have been widely used for biotechnological applications in fat, food ingredients, detergents, daily and textile production of surfactants, hydrolyze a substrate that exists in solid form at room temperature. Thermophiliclipases show higher thermo stability, higher activity at elevated temperatures, and often shows more resistance to chemical denaturation. This makes them ideal tools in industrial and chemical process when relatively high reaction temperatures or organic solvents are used. The industrial demand for the thermo stable continues to stimulate the search for microorganisms produces of thermostable enzymes. Thermo stable enzymes are usually derived from thermophilic strains, which may be expected to produce intrinsically more heat stable enzyme. A small number of thermophilic lipase producing bacteria have been described in the last decades. In the present study lipase enzyme was produced from *Pseudomonas aeruginosa* isolated from soil region. After the production of enzyme lipase, its productivity, activity and characterization was done using various parameters.

MATERIALS AND METHODS

ISOLATION OF LIPASE PRODUCIING STRAIN FROM SOIL SAMPLE

Isolation of *Pseudomonas aeruginosa* from soil sample: Soil from the Paddy field is taken serially diluted by standard plate count and specific organism was isolated. *Pseudomonas aeruginosa* a pure isolate was screened using cetrimide agar and it was confirmed by using various biochemical reactions.The organism was inoculated in production medium in 100 ml conical flask and incubated at rotary shaker at 37°Cfor 24-48 hours.The growth condition of organism was determined by measuring O.D. value using calorimeter.

Preparation of Culture Supernatant: Culture were harvested by centrifugation in a high speed centrifuge at 10,000 rpm for 15 minutes and supernatant was carefully removed and stored at 20° C. this supernatant was assayed for lipase activity.

Tween 80 Medium: Tween 80 was homogenized with the nutrient agar with addition of calcium chloride and autoclaved at 15 lbs at 121°C for 15 mins and powered into petriplate and allowed to solidity with the help of sterile cork borer wells measuring 1 cm diameter.

Preliminary Screening for Lipase Production by Plate Assay: Culture filtrate (0.5ml) were pipetted oil into well of the Tween 80 medium under aseptic condition and incubated at 37° C for 24-48 hours. The area of clear zone of Tween 80around the well was an indication of lipolytic activity.

Lipase Assay: Lipase activity was assayed by the titrimetric method with the standard olive oil emulsion as substrate.

End Point: The lipase activity was indicated by the formation of pale pink.

Calculation

$$\textbf{Lipase activity was calculated using the formula} = \frac{(\mathbf{T}-\mathbf{C}) \times \mathbf{0.5} \times \mathbf{1000}}{\textbf{Period of incubation in hours}}$$

Characterization of Lipase

Effects of carbon source on lipase production - To study the effect of carbon sources on lipase production, soluble starch was replaced by 1% of different soluble sugar (Glucose, Maltose, Sucrose, Lactose and Olive oil) keeping the rest of the media, lipase in fermented broth was recorded after 24 hrs.

Effect of organic Nitrogen source on lipase enzyme - Ammonium sulphate, Ammonium carbonate was replaced with 1% different organic components as nitrogen source keeping the rest of the media compounds as same. The activity in the fermented broth was recorded after 24 hrs.

Effect of temperature on lipase activity -The effect of temperature of lipase was determined by incubating the cell-free supernatant fluid at different temperature in the Range from 10-50° C in the increment of 10° C.

Effect of pH on lipase activity - To study the effect of pH on lipase activity the culture was combined with sodium phosphate buffer was adjusted at different pH in the ranges from 5-9. The samples were then assayed for the residual lipase activity.

RESULTS

Isolation of lipase producing strain from soil sample: Soil sample which was collected from the agricultural field from 5-10 cm depth into sterile polythene bags was serially diluted using plate count agar from which *Pseudomonas* species was isolated. Isolate was further identified by physiological and biochemical method by described in Bergey's manual of systematic acteriology and coven and steel's manual for the identification of medical bacteria.The isolated organism was cultivated at 37° C in the production media in shaker. After incubation period, culture was collected and supernatant was separated which was used for lipolytic activity. Preliminary screen of lipase production by plate assay method - Supernatant which was stored at 4° C was used for lipolytic activity by using Tween 80 medium. Area of clear crystal zone when incubated almost 7mm hole was obtained which indicates has the capacity to produce lipase enzyme.

DISCUSSION

Pseudomonas species are good lipase produces, and are preferred in the food industry for enzyme production. The lipase production by *Pseudomonas* species was growth associate reaching a maximum production was around 20 hours(Wolfgang Stuer, 1986). This may be industrial property because it could allow harvesting of enzyme for shorter period of time. In addition of carbon source in the form of either monosaccharide, polysaccharide and olive oil may influence the production of enzyme. In nature the amount of carbon source in culture media is important for growth and production of extra cellular lipase in bacteria. In present study, olive oil supported maximum yield of lipase when compared to other carbon sources which were correlating with the work (Sugihara, 1991). Apart from the carbon source even the nitrogen source influence the production of lipase enzyme. Among nitrogen source yeast extract is found to be suitable substrate which was correlating with the results (Sharma, 2002). The temperature and pH has profound effect on the activity of lipase as every enzyme has an optimum temperature and pH and its structure is most stable and enzyme is most active. The activity of lipase enzyme from isolate *Pseudomonas aeruginosa* was detected over a wide range of assay pH and temperature. The activity of enzyme was optimal when assay was carried out at pH 6 and temperature 40° C respectively which was correlating with the work (Dannert, 1996, Dharmahiti and Schmidt Luchai, 1999).

Table.1.Effects of carbon source on lipase activity

Carbon source (0.5%w/v)	Test	Control	Enzyme activity (I.U.)
Glucose	27.5	15.2	20.5
Fructose	10.5	3.4	11.83
Sucrose	8.7	2.9	9.66
Lactose	8.8	3.4	9.0
Olive oil	16.8	4	23.3

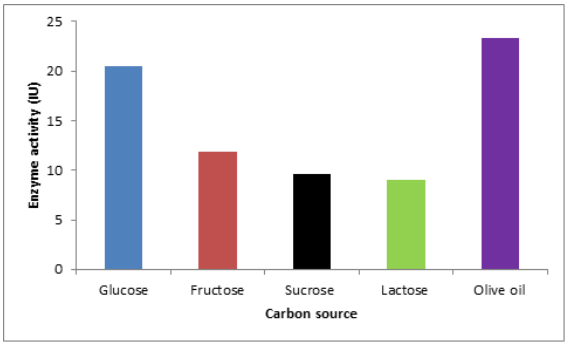


Figure.1.Effects of carbon source on lipase activity

Table.2.Effects of nitrogen source on lipase activity

Nitrogen Source (0.5% w/v)	Test	Control	Enzyme activity (I.U)
Yeast extract	13.3	7.5	9.6
Ammonium sulphate	12.8	7.2	9.3
Glycine	12.2	7	8.6
Potassium nitrate	11.2	5.5	9.5

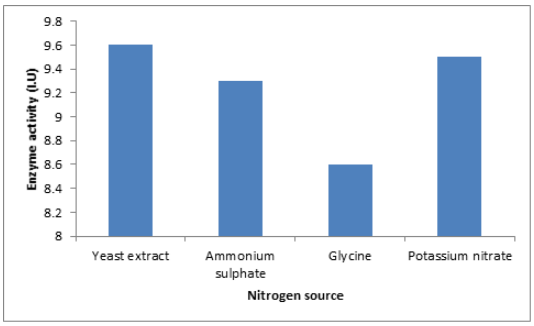


Figure.2.Effects of nitrogen source on lipase activity

Table.3.Effects of temperature on lipase activity

Temperature	Test	Control	Enzyme activity (I.U.)
10°C	2	1	1.0
20° C	2.5	1	2.5
30°C	3.7	1.5	3.6
40° C	4	1.0	5.0
50° C	3	2	1.6

Figure.3.Effects of temperature on lipase activity

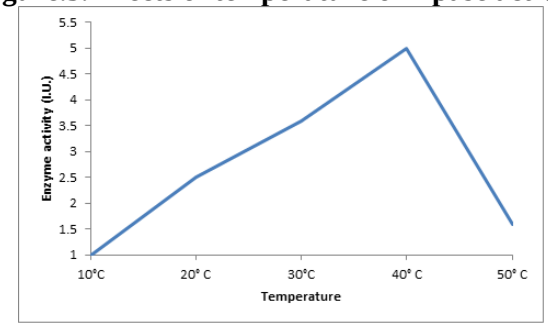
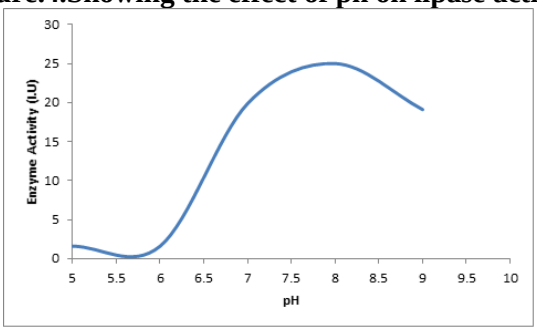


Table.4.Showing the effect of ph on lipase activity

Ph	Test	Control	Enzyme activity (I.U.)
5	5.5	3.4	1.6
6	11.6	9.5	1.6
7	24.1	12.2	19.87
8	24.5	9.5	25
9	22.5	11	19.1

Figure.4.Showing the effect of ph on lipase activity



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