



Screening selection identification production and optimization of bacterial lipase isolated from industrial rejection of gas station

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ABSTRACT

Production of extracellular lipase in submerged culture of *Bacillus licheniformis* has been investigated. Bacterial Lipase producers were isolated from industrial rejection of gas station. One of the sixty isolated strain exhibited a higher lipase activity was selected and identified based on their morphological and biochemical characteristics. The effect of incubation time, medium pH, temperature, carbon source and nitrogen source for the lipase production was studied. The lipase production was maximum at pH 8, temperature 40°C and incubation time 48 hours by the lipase producing bacteria *Bacillus licheniformis*. With a selected carbon source, olive oil and glucose were suitable substrate to maximize lipase production (1.5 U/ml). The optimized concentration of olive oil and glucose was 1% and 1%, respectively. The effect of nitrogen source on lipase production indicated that the yeast extract was suitable substrates for accelerating lipase production (1.47 U/ml).

Keywords: lipase, *Bacillus*, screening, production, and olive oil.

INTRODUCTION

Lipases or triacylglycerol acyl ester hydrolases are carboxylesterases that catalyze both hydrolysis and synthesis of esters formed from glycerol lipases can hydrolyze long chain water-insoluble triglycerides into diglycerides, monoglycerides, glycerol and fatty acids [1-2]. Lipases are ubiquitous enzymes which are widely distributed in plants, animals and microbes [3]. The ability of lipases to perform very specific chemical transformation (biotransformation) has made them increasingly popular in the food, detergent, cosmetic, organic synthesis, and pharmaceutical industries [4-5]. A wide range of microorganisms (bacteria, fungi and yeast) can produce lipases with different enzymological properties and substrate specificities [6]. In particular, lipases from fungi are important in industrial applications [7]. A relatively smaller number of bacterial lipases have been well studied compared to plant and fungal lipases. Microbial lipases have gained special industrial attention due to their stability, selectivity, and broad substrate specificity [8-9]. Microbial enzymes are also more stable than their corresponding plant and animal enzymes and their production is more convenient and safer [10].

Many microorganisms are known as potential producers of extracellular lipases, including bacteria, yeast, and fungi [11]. A variety of extracellular lipases of bacterial origin with different properties and specificities have been described and characterized. Extracellular lipase was isolated from many different bacterial species, including *Bacillus* [12]. Microbial lipases are mostly extracellular and their production is greatly influenced by medium composition besides physicochemical factors such as temperature, pH, and dissolved oxygen. The major factor for the expression of lipase activity has always been reported as the carbon source, since lipases are inducible enzymes. These enzymes are generally produced in the presence of a lipid such as oil or any other inducer, such as triacylglycerols, fatty acids, hydrolysable esters, Tweens, bile salts, and glycerol [13-14]. However, nitrogen sources

and essential micronutrients should also be carefully considered for growth and production optimization. The objective of this study was the production of lipase by *Bacillus.sp* and the optimization of the temperature, pH conditions, carbon sources and nitrogen sources for obtaining higher lipase activity

EXPERIMENTAL SECTION

Microorganism

Total 16 bacterial isolates were screened out as lipase producer from several locations. The bacterial isolate used in present investigation designated as BH1, was obtained from industrial rejection of gas station. It was identified as *Bacillus licheniformis* based on morphological and biochemical properties.

Screening for lipase Producer

The bacterial isolates were obtained by suspending the various samples in medium containing (%v/v) yeast extract 0.1; NaCl 0.25; MgSO₄.7H₂O 0.05; CaCl₂.2H₂O 0.01; K₂HPO₄ 0.07 ; KH₂PO₄ 0.03 (pH 7) and olive oil 1.0 (%v/v). All the isolates were grown at 37°C for 24 h with agitation (100 rpm). Efficient lipase producers were screened out by estimating the enzyme activities at 24 h. The lipolytic activity of each bacterial strain was determined by titrimetric method. The strain with the higher activity was used for further study.

Lipase assay

The crude enzyme used for assay was the culture broth after separation of cells and particles. The enzyme was normally stored at 4°C until used. Lipase activity was measured by a titrimetric method (olive oil emulsion method) [15]. One unit of enzyme activity is defined as the amount of enzyme required to liberate 1 µmol of equivalent fatty acid under the standard assay conditions.

Lipase Enzyme production

The composition of production medium used in this study was: (% w/v) yeast extract 0.1; NaCl 0.25; MgSO₄.7H₂O 0.05; CaCl₂.2H₂O 0.01; K₂HPO₄ 0.07 ; KH₂PO₄ 0.03; olive oil 1.0 (%v/v) ; pH 7.0. Overnight cultures were suspended in 5ml of sterile deionised water and used as the inoculum for pre culture to obtain an initial cell density to adjust the turbidity of 0.5 McFarland standards. Submerged microbial cultures were incubated in 500 ml Erlenmeyer flasks containing 100 ml of liquid medium on a rotary shaker (100 rpm) and incubated at 37°C. After 72 hours of incubation, the culture was centrifuged at 10,000 rpm for 20 min at 4°C and the cell free culture supernatant fluid was used as the sources of extracellular enzyme. The lipase activity in the supernatant was determined by the titrimetric method.

The effects of various factors like inoculums size, carbon sources, nitrogen sources, pH an temperature on the production of lipase were studied while optimization of the medium.

Optimization of Media Ingredients for lipase Production

Effect of carbon sources on lipase production

The effect of carbon sources on lipase production was tested by using five different carbon sources, namely soy bean oil, tween 80 and tween 20. They were tested individually by replacing the olive oil present in the basal medium at the concentration of 10 g/L. Then the maximum enzyme-producing carbon source was further optimized by varying its concentration (5, 10 and 50 g/L). In addition, the combination of olive oil with glucose was also used

Effect of nitrogen sources on lipase production

To test the effect of nitrogen sources on lipase production, tri different nitrogen sources such as yeast extract, casein, and urea were used. They were individually tested by replacing the yeast extract present in the basal medium at the concentration of 1 g/L.

Optimization of Physical Parameters for lipase Production

The effect of pH and temperature of the fermentation medium for lipase production was performed by varying pH of the medium from 5 to 9 whereas the other parameters were unaltered. For selection of optimum temperature for the production of lipases, the temperatures varying from 20 °C to 60°C were selected by keeping the remaining parameters same.

RESULTS AND DISCUSSION

Screening lipase producer

Among the total 16 bacterial isolates, six potential strains were screened out as potential was designated as BH1, gave high lipolytic activity among all 6 bacterial isolates (Fig.1). The fu isolate BH1 was identified as *Bacillus licheniformis* based on morphological and biochemical characteristics.

Effect of carbon sources on lipase production

The production of lipase was more significant in culture medium added with lipids as the carbon source than in the culture medium without lipids. It was demonstrated that the lipase activity is induced by the presence of lipid substrates in the medium. Extracellular lipase production by different microorganisms on lipids has been extensively reported [16]. Among the different carbon sources studied, maximum enzyme production (Table 1) was with olive oil at 1% after 48h.

Different behavior was observed for the lipase production in the presence of two carbon sources: glucose and olive oil. The best lipase activity, 1.5 IU/ml, was obtained when olive oil and glucose were added to the medium at 1 % (Table 1)

Effect of nitrogen sources on lipase production

Different sources of nitrogen were tested in order to determine their influence on the synthesis of lipase. Yeast extract (1g/l) produced maximum enzyme activity (1.47 U/ml) (Table 2) among all nitrogen sources studied, followed by urea and casein. Similar results were reported for organic nitrogen source by *Bacillus* sp [17].

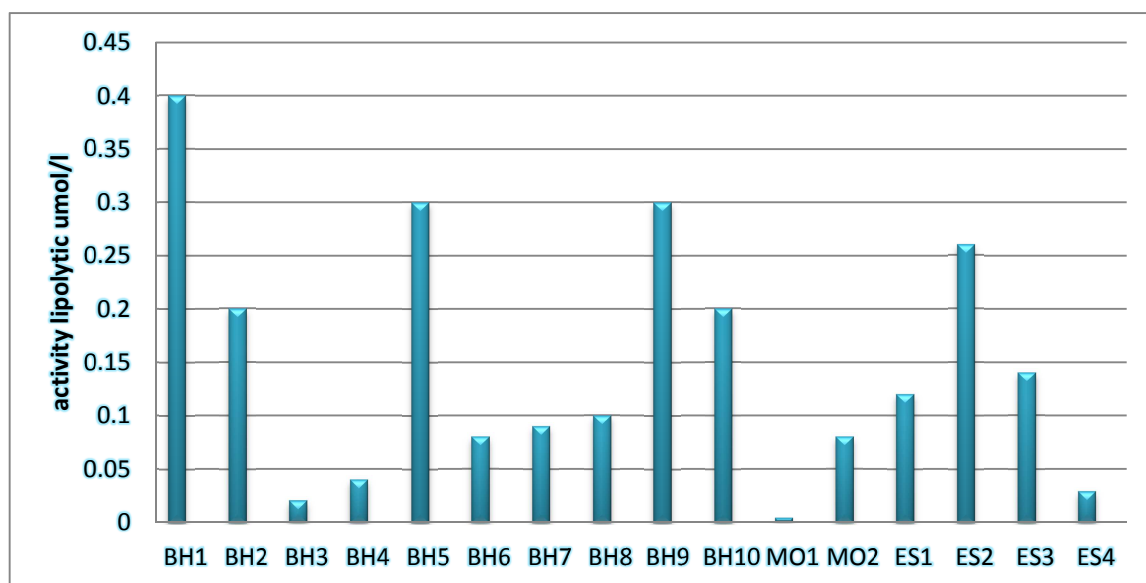


Figure 1: Microbial screening of selected bacterial isolates for lipase production using olive oil as carbon source. They were grown on mineral salt medium with tested substrate (10 g/L) and incubated at 37 °C for 24 h

Effect of pH on lipase production

As the pH plays an important role in all the biological processes, so lipase production was tested within a broad range of pH (5, 6, 7, 8 and 9). The study of the effect of different pH reveals that the enzyme secretion is greatly influenced by varying pH of the environment. The maximum enzyme was produced at pH 8 (0.83 U/ml) then followed by pH 7 (0.65 U/ml) and minimum production at pH 5 (Fig 2). The variation in the enzyme production at different pH value could be due to the strain specificity.

Table 1: Production of extracellular lipase by *Bacillus* sp on different carbon sources after 48 h

Carbon sources	Activity lipolytic U/ml
Olive oil (1%)	1.2
Soja oil (1%)	0.9
Tween 20 (1%)	0.6
Tween 80 (1%)	0.4
Olive oil (0.5%)	0.25
Olive oil (5%)	0.7
Olive oil (1%) + glucose (1%)	1.5
Olive oil (1%) + glucose (2.5%)	0.7
Olive oil 1% + glucose (5%)	0.1

Cultures were grown in Erlenmeyer flasks 500ml containing 100ml of medium (pH 8) with yeast extract 0.1% at 40°C for 48 h.

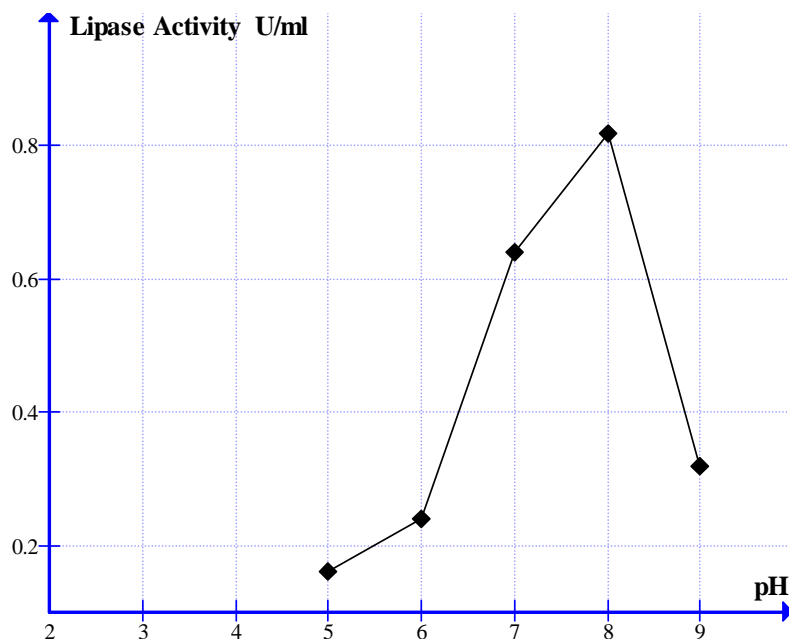


Figure 2 : Effect of pH on lipase activity

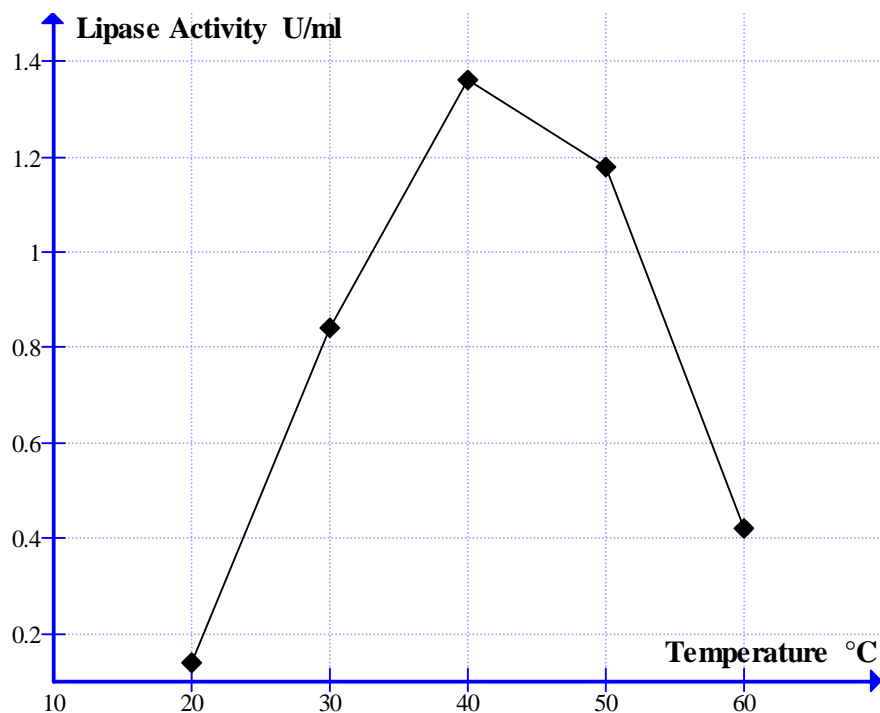


Figure 3: Effect of temperature on lipase activity

Effect of temperature on lipase production

Temperature is a critical parameter that has to be controlled and it varies from organism to organism. Temperature influences secretion of extra cellular enzymes by changing the physical properties of the cell membrane. Studies conducted for the optimization of temperature shows that the bacteria produces lipase in wide range of temperature from 20 °C to 60°C (Fig 3).The lipase enzyme produced at different range of temperature was from 0.12 U/ml to 1.34U/ml. The optimum temperature for lipase enzyme production was at 45°C (1.34U/ml) and the enzyme production was affected and decreased after increase of temperature above 45°C to 60°C.Similar result was reported that the maximum lipase production was at 45°C by *Humicola lanuginosa* [18].

Table 2 : production of extracellular lipase by *Bacillus. sp* on different nitrogen sources

Nitrogen sources	Activity lipolytic U/ml
Yeast extract	1.47
Urea	0.24
Casein	0.13

Cultures were grown in Erlenmeyer flasks 500ml containing 100ml of medium (pH 8) with olive oil 1% and glucose 1% at 40°C for 48 h.

CONCLUSION

The bacterial are important sources of bio-molecules with biotechnological potential as enzymes. The production of extracellular lipase by *Bacillus sp* was optimized using an experimental design, as well as the temperature, pH, carbon sources and nitrogen sources of the activity.

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