Optimization studies for the production and activity of lipase from new marine actinomycete isolate, ABT-206

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Abstract

Lipase production from a novel marine actinomycetes isolate, ABT – 206, was studied by optimizing various physical and chemical parameters. Maximum lipase production of 2.98 U/ml was obtained in the medium containing 1% (v/v) Palm oil as carbon source, 0.5% (w/v) Peptone as organic nitrogen source, 0.1% (w/v) NH₄H₂PO₄ as inorganic nitrogen source, 1.25 (g/L) NaCl, 0.2 (g/L) of MgSO₄.7H₂O and CaCl₂, 0.2 (g/L), pH 7.0, incubated at 30 ºC for 120h. The enzyme characterization studies showed that lipase enzyme of actinomycetes isolate, ABT – 206 was stable in the pH range of 8.0 to 10.0 and a temperature range of 35 ºC to 50 ºC with maximum activity at pH 8.0 and temperature of 45 ºC.

Keywords: Marine Actinomycete isolate, ABT – 206, Lipase production, Optimization, Characterization

1. Introduction

Lipases, (triacylglycerol acylhydrolases; EC 3.1.1.3.) are one of the most important classes of hydrolytic enzymes that catalyse both hydrolysis and synthesis of esters. Hydrolysis of a triacylglycerols by lipases yield di- and monoacylglycerols, glycerol and free fatty acids. Lipases were produced from microbial sources of actinomycetes, bacteria, and fungi [1]. These extracellular microbial lipases are high in demand due to their thermostability, stereo-specificity and lower energy consumption than conventional methods.

Lipases are produced from gram positive microorganisms such as Bacillus sps [2-4], Staphylococcus sps [5, 6], Lactobacillus sps [7], gram negative bacteria such as Pseudomonas sps [8], fungi such as Rhizopus sps [9, 10] and actinomycetes species [11, 12]. Marine actinomycetes have attracted great attention since they have unique metabolic and physiological capabilities that not only ensure survival in extreme habitats but also produce stable enzymes.

Some of the important industrial applications of lipase include developing flavors in milk, cheese and butter in dairy industry, and shelf life prolongation in bakery items and in beverages in food industry. Lipases also have promising applications in the industries of detergent, oleochemicals, leather, textiles, paper and pharmaceuticals [1].

Lipases can be obtained by solid state fermentation [13, 14], and submerged fermentation [15, 16]. But, most of the lipase productions from bacteria have been performed under submerged batch mode because of ease of handling and greater control of environmental factors such as temperature and pH. Optimization of media components and other physical parameters are important in the development of production process [17-19]. Lipases from different microorganisms have been characterized in terms of their activity and stability related to pH and temperature [5, 20].

Hence, the present study deals with the medium optimization process using varying carbon sources and nitrogen sources along with other physical process parameters for lipase production and also characterization of lipase from a novel marine actinomycete isolate, ABT-206

2. Materials and Methods

2.1 Microorganism and culture maintenance

Lipase producing marine actinomycete isolate, ABT – 206, isolated from sediments of the Bay of Bengal, Visakhapatnam, was used in the present study [21]. Culture was maintained on Yeast Extract Malt Extract agar (YEME) slants having the composition (g/L): Yeast extract 4.0, Malt extract 10.0, Glucose 4.0, and Agar 20. The pH of the medium was adjusted to 7.2, sterilized, inoculated with culture and incubated at 28 ºC for 3-4 days. The culture was preserved at 4 ºC and sub-culturing was carried out once in a month.

2.2 Preparation of seed culture

A loopful culture of marine actinomycete isolate, ABT-206 was inoculated into 100 ml of YEME medium and incubated at 28 ºC, 100 rpm for 48 h.

2.3 Optimization of lipase production by marine Actinomycete isolate, ABT-206 in submerged Fermentation

Various fermentation parameters were optimized by conventional method of optimizing one independent parameter at a time, while fixing the others at a certain value. The parameter optimized in one experiment was maintained in the subsequent experiments. The medium with following composition, 2% inoculum and incubation time of 5 days was selected from the previous results (unpublished work) for the production of lipase.
The production medium is prepared, pH adjusted to 7.0, sterilized and inoculated with 2% seed culture of marine actinomycete isolate, ABT-206. The flasks were incubated at 28°C, 120 rpm for 5 days.

2.3.1 Effect of Carbon source
Effect of carbon source on lipase production was analyzed by replacing olive oil in the production medium with 2% of different oil sources namely Olive oil, Palm oil, Sunflower oil, Sesame oil, groundnut oil, Mustard oil and Coconut oil. The best oil was selected and concentration of selected oil was optimized by varying the concentration from 1% - 4% (v/v) in production medium.

2.3.2 Effect of Organic nitrogen sources
Effect of nitrogen source on lipase production was analyzed by replacing peptone in the production medium with 0.5g of different organic nitrogen sources namely Yeast extract, Malt extract, casein, and urea. The best nitrogen source was selected and different concentrations of selected nitrogen source 0.25%, 0.5%, 1%, 2%, 4% were screened to find optimum concentration for production of lipase.

2.3.3 Effect of Inorganic nitrogen source
Effect of inorganic nitrogen source concentration (Ammonium dihydrogen phosphate) on lipase production was studied to find optimum concentration for production of lipase by varying the concentrations of NH₄H₂PO₄ such as 0.05%, 0.1%, 0.15%, 0.2% and 0.25% (w/v).

2.3.4 Effect of pH
Optimum pH for the production of lipase was determined by varying the pH of the medium from 5.0 to 10.0.

2.3.5 Effect of temperature
Optimum incubation temperature for lipase production was determined by incubating flasks at varying temperatures of 25 °C, 30 °C, 35 °C, 40 °C, and 45 °C for 5 days.

2.3.6 Effect of Salts concentration
The concentrations of the three salts used in the production medium are NaCl - 2.5g/L, MgSO₄.7H₂O - 0.4g/L and CaCl₂ - 0.4 g/L, (total salt concentration 3.3g/L). Media with different salt concentrations such as 0.825g/L, 1.65g/L, 3.3g/L, 6.6g/L, 13.2g/L, and 26.4g/L were prepared. The individual salt concentrations were given in table 2.

2.4 Lipase enzyme extraction
After 5 days of incubation, 2ml of samples were taken, centrifuged at 10,000 rpm for 10 min and supernatants obtained were used as crude lipase enzyme extracts.

2.5 Lipase assay
Lipase assay was carried out by estimating the amount of p-nitro phenol released by lipase from p-nitro phenyl palmitate substrate using spectrophotometry method. 2.4mL of substrate solution was added to each test tube. The test tubes were incubated at 45 °C for 2 min. 0.1mL of cell free fermentation broth was added to the test tube and incubated at 45 °C for 2min and formation of yellow color indicates lipase activity. Reaction was stopped by mixing 50µL of 30mM CaCl₂, 2H₂O and 50µL of phosphate buffer pH-7. Test tubes were incubated for another 2min and centrifuged at 10,000rpm. Absorbance was read at 410nm in UV-VIS spectrophotometer. One Unit of lipase activity is defined as the amount of enzyme that releases one micro mole of p-nitrophenol per minute per mL under standard assay conditions.

All the submerged batch cultivations are conducted in duplicates and the results were the average of duplicates.

2.6 Characterization of lipase produced by marine actinomycete isolate, ABT – 206
The optimal pH for lipase activity was determined by incubating the enzyme – substrate at various pH from 4 – 10 for 5 min at 45°C. Sodium acetate buffer for pH 4.0, 5.0, Phosphate buffer for pH 6.0, 7.0, 8.0 and Glycine – NaOH buffer for pH 9.0, 10.0 were used. The optimum temperature for lipase activity was determined by incubating the enzyme - substrate at various temperatures i.e. 25°C, 30°C, 35°C, 40°C, 45°C for 5 min at pH 9.0.

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<thead>
<tr>
<th>Total Salt Concentration (g/L)</th>
<th>Individual Salt Concentration of the medium</th>
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<tr>
<td></td>
<td>NaCl (g/L)</td>
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<td>0.825</td>
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3. Results and Discussion

3.1 Screening of different oils

Seven oils were screened to determine the best oil for the production of lipase by marine actinomycetes isolate, ABT – 206. The media containing Palm oil, Groundnut oil and Olive oil produced maximum lipase with insignificant differences followed by Coconut oil. Lipase production was found to be less in the medium containing Sunflower oil (Figure 1). Since palm oil is cheap compared to olive oil and ground nut oil, palm oil was selected for optimization of its concentration.

Various researchers have reported different oils for optimal lipase production. Sirisha et al., (2010) [22], screened Palm oil, Groundnut oil, Olive oil, Ghee, Coconut oil, Mustard oil and Sunflower oil, and reported maximum lipase production with palm oil for newly isolated Staphylococcus sp. Maximum lipase production with olive oil as carbon source was reported for Streptomyces griseus [15], and Pseudomonas sp. [19]. Patcha et al., (2013) [23] obtained maximum lipase production with cooking palm oil.

3.2 Effect of Palm oil Concentration

Effect of palm oil concentration on lipase production was studied by varying the concentration of oil from 1% to 4%. Lipase activity decreased gradually with increasing oil concentration. Maximum lipase activity of 2.669 U/ml was observed with 1% palm oil concentration (Figure 2). So, palm oil concentration of 1% was used in further analysis. Lipidic carbon sources are essential for obtaining high lipase yield. Lipase production from Rhodotorula glutinis was increased 12-fold in the medium containing 2% (v/v) palm oil [24]. Sirisha et al., (2010) [22], reported that new isolate Staphylococcus sp. has produced maximum lipase with 1% palm oil. Pseudomonas sp. exhibited maximum lipase production in medium containing 1% olive oil [19]. Maximum lipase production was obtained in a medium containing 1% (v/v) used cooking palm oil by Pseudomonas aeruginosa NA37 [23].

3.3 Screening of different nitrogen sources

Five different nitrogen sources namely peptone, yeast extract, malt extract, urea and casein were screened for lipase production by ABT-206. Maximum lipase production of 2.765 U/ml was obtained with peptone as best nitrogen source by the isolate ABT- 206 (Figure 3). So, peptone is selected as the organic nitrogen source for production of lipase by ABT-206. In agreement with our results, maximum lipase production with peptone as nitrogen source was reported for Candida cylindracea [25], Aspergillus wentii [26], Rhizopus oryzae [27], and Bacillus subtilis Y-IVI [18]. However, Bokhari et al., (2013) [28] obtained maximum lipase production with yeast extract.
3.4 Effect of Peptone concentration on the production of Lipase
Effect of peptone concentration on lipase production by Marine Actinomycete isolate, ABT-206 was studied by varying the peptone concentration from 0.25% to 4% in the medium. Maximum lipase production was observed at 0.5% peptone concentration and decreased with increasing concentration from 1% to 4%. (Figure 4). Aliyu et al., (2011) [25], reported that peptone in the range of 0.4% to 0.5% (w/v) was found to be more significant than yeast extract for the production of lipase. Sirisha et al., (2010) [22], have reported high amount of lipase activity in a medium containing 0.3% (w/v) of peptone.

Fig 4: Effect of Peptone concentration on lipase production by marine actinomycete isolate, ABT – 206

3.5 Effect of NH₄H₂PO₄ concentration on the production of Lipase
Effect of inorganic nitrogen source, ammonium dihydrogen phosphate concentration on lipase production by Marine Actinomycete isolate, ABT-206 was studied by varying the concentration from 0.05% to 0.25% in the medium. Lipase production increased with increasing concentration from 0.05% to 0.1% and thereafter decreased with increasing concentration. Maximum lipase production of 2.709 U/ml was observed at 0.1% of NH₄H₂PO₄ concentration (Figure 5). There are very few reports of effect of inorganic nitrogen source concentration on lipase production. Pau and Omar (2004) [29], have reported maximum production of lipase at 0.05% NH₄H₂PO₄ concentration. Tembhukar et al., (2012) [30], obtained maximum lipase in medium containing 0.1% (w/v) NH₄H₂PO₄ by Pseudomonas spp.

Fig 5: Effect of NH₄H₂PO₄ Concentration on lipase production by marine actinomycete isolate, ABT – 206

3.6 Effect of pH on the production of Lipase
Effect of pH on lipase production was studied by varying the pH of the medium with 4.0, 5.0, 6.0, 7.0, 8.0, 9.0 and 10.0. Among these, Lipase production is very low at pH- 4.0, low at pH 9.0, and 10.0, moderate at 5.0 and maximum at pH- 6.0, 7.0, and 8.0 (Figure 6). Among pH 6.0, 7.0 and 8.0, pH 7.0 was selected as the optimum pH for further studies. Gunalakshmi et al., (2008) [11] found that the new Actinomycete strain LE-11 produced maximum lipase at pH 7.0. This suggests that organism sustains at neutral ph. Maximum lipase production was reported at pH 6.5 for Pseudomonas sp. [31], pH 7.5 for Staphylococcus aureus [6] and for Rhizopus sps [9].

Fig 6: Effect of pH on lipase production by marine actinomycete isolate, ABT – 206

3.7 Effect of Temperature on lipase production
Effect of Temperature on lipase production by marine actinomycetes isolate, ABT-206 was studied by incubating the flasks at 25 °C, 30 °C, 35 °C, 40 °C and 45 °C for 5 days. Maximum lipase production was recorded with 30°C temperature. Lipase production decreased with increase in temperature from 35 °C to 45 °C (Figure 7). This result is in agreement with the results of other workers. Maximum lipase production at 30 °C was reported for Rhodococcus sp. [32], newly isolated marine actinomycetes strain [12] and also for Rhizopus sp [9].

Fig 7: Effect of Temperature of lipase production by marine actinomycete isolate, ABT – 206

3.8 Effect of Salts concentration on the production of Lipase
Effect of three salts concentrations namely, Sodium Chloride. Magnesium sulphate and Calcium chloride were optimized by
varying the total salt concentration from 0.825g/L to 26.4 g/L (Table 2). Lipase activity increased with increasing the concentration of the salts from 0.825g/L to 0.165 g/L. Maximum lipase activity of 2.98U/ml was reported at 0.165 g/L salt concentration (1.25g/L NaCl, 0.2g/L MgSO 4.7H2O, 0.2g/L CaCl2). Lipase production was decreased with increase in salt concentration from 3.3 g/L to 26.4 g/L (Figure 8). High accumulations of salts lead to destability of enzymes [33]. Very few researches have performed systematic optimization studies of salt concentration for lipase production. Maximum lipase production at 0.5% of salt concentration was reported for Bacillus sps. by Bokhari et al., (2013) [28], whereas, Bora et al., (2012) [34] have reported maximum production at 7% (w/v) NaCl for Bacillus sps LBN 02.

3.9 Characterization of lipase of marine actinomycete isolate, ABT – 206

3.9.1 Determination of optimum pH

Optimum pH for lipase activity of the marine isolate ABT – 206 was determined by carrying lipase assay at different pH using Sodium acetate buffer for pH 4.0 and 5.0, Phosphate buffer for pH 6.0, 7.0 and 8.0 and Glycine - NaOH for pH 9.0 and 10.0. From Figure 9, it was evident that maximum lipase activity of 2.915 U/ml at pH 8.0 and slight decrease in activity with increase in pH from 9.0 to 10.0. So the lipase enzyme of marine actinomycetes species ABT – 206 is stable at alkaline conditions. These results are in close agreement with Gunalakshmi et al., (2008) [11], who reported maximum lipase activity for newly isolated Actinomyctge Strain LE-11 at pH 8.0 and also for Trichoderma atroviride [35]. The lipase activity of Aerobacillus spp. was found to be stable in the pH range of 8.0-9.0 and showed maximum lipase activity at 8.0 [36]. Lactobacillus sp. showed maximum lipase activity at pH 9.0 [7].

3.9.2 Determination of Optimum temperature

From Figure 10, it is evident that the marine actinomycete isolate, ABT-206 showed maximum lipase activity of 2.84 U/ml at 45 ºC assay temperature. Our results are in agreement with the results of Kumar et al., (2012) [37] who also reported the maximum lipase activity at 45 ºC for marine actinomycetes. Tembhukar et al., (2012) [30], obtained maximum lipase activity at 50 ºC for Pseudomonas spp.

4. Conclusion

In the present study, the Lipase production by marine actinomycete isolate, ABT-206 was carried out in submerged fermentation. Optimization of media and physical parameters of fermentation conditions viz., effect of inoculum level, oil sources and oil concentration, organic nitrogen sources and organic nitrogen concentration, inorganic nitrogen source concentration, Salts concentration, pH and Temperature were studied to enhance lipase production. A maximum lipase production of 2.98 U/mL was obtained after 5 days of incubation with palm oil at 1% (v/v) concentration, 0.5% (w/v) Peptone, 0.1% (w/v) NH₄H₂PO₄, 1.25 (g/L) NaCl, 0.2 (g/L) MgSO₄, 7H₂O, 0.2 (g/L) CaCl₂, at pH of 7.0, and temperature of 30 ºC. The lipase of ABT-206 was characterized by determining its stability at varying range of pH and temperature. The lipase enzyme showed stability in the pH range of 8.0 to 10.0 and a temperature range of 35 ºC to 50 ºC with maximum activity at pH 8.0 and temperature of 45 ºC. The new marine Actinomycete isolate, ABT – 206 could be used as potential strain for commercial lipase production.

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6. References


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