



Original Research Article

Optimization of process parameters for the extracellular lipase production by newly isolated *Pseudomonas aeruginosa* KDP

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ABSTRACT

Keywords

Ammonium nitrate;
Pseudomonas aeruginosa KDP;
Lipase;
Nickel;
Selenium;
Temperature;
Urea; pH.

Optimization of process parameters for the extracellular lipases production by bacteria is most important because they have a broad range of biotechnological applications. In the present study lipase production, carried out in shake flasks with minimal medium and investigated the influence of different culture conditions including environmental (i.e., pH and Temperature) and nutritional (i.e., Ammonium nitrate, Urea, Selenium and Nickel) factors on the production of lipase by newly isolated *Pseudomonas aeruginosa* KDP. Maximum enzyme production was found to be at pH 7.0 and temperature 45°C. The concentration 0.25mg/l of ammonium nitrate and urea present culture produced maximum lipase is 21.05 U/ml and 15.68 U/ml respectively. The maximum lipase production by *P. aeruginosa* KDP was found to be at 40µm/L Se and 60µm/L Ni supplemented culture. The correlation analyses of all the experiments proved that the physicochemical parameters and the lipase production are interdependent.

Introduction

Lipases (triacylglycerol ester hydrolases, EC 3.1.1.3) are ubiquitous enzymes that catalyze the hydrolysis of fats and oils. Due to this reason, they have high biotechnological potential and currently attracting enormous attention (Reetz, 2002). Lipases occur widely in nature and can be produced by many microorganisms and higher eukaryotes; however, most technologically employed lipases are of microbial origin, because the respective

genes are relatively easy to access and efficiently be expressed. Microbial lipases possess useful features such as high yield and low production cost, diversity in catalytic activities, amenability to genetic manipulation, stability in organic solvents and broad substrate specificity (Shu *et al.*, 2010).

In the last decades, the interest in microbial lipase production has increased, because of its large potential in industrial

applications as additives for foods, fine chemicals production, wastewater treatment, cosmetics, biodiesel production, detergent industry, organic synthesis, oleochemical industry, textile industry, pharmaceuticals, leather, and medical (Hasan *et al.*, 2006; Rajesh *et al.*, 2010; Nadia *et al.*, 2010).

The use of lipases in industry is still limited because it's commercial enzymes. Therefore a considerable interest in reducing the production cost of lipases. The optimization of culture conditions is one way of reducing enzyme production costs. Some of the wastes can be used as a culture medium for lipase production. The lipase production is affected by several extracellular factors such as the carbon sources, nitrogen source, the pH, temperature, the dissolved oxygen level, etc., (Guerzoni *et al.*, 2001). In this study we optimized culture conditions to enhance the lipase production by newly isolated *Pseudomonas aeruginosa* KDP.

Materials and Methods

Microorganism

Pseudomonas aeruginosa KDP was used in this study which was isolated from oil mill soil.

Extracellular lipase assay

Samples of the culture medium were withdrawn and centrifuged for 15 min at 10,000 g. The supernatant was used to estimate the extracellular lipase activity by using an olive oil emulsion as a substrate (olive oil 25%, 0.1 M NaOH 7.5%, polyvinyl alcohol (2%) 67.5%). The enzymatic reaction is initiated by adding 1 mL of supernatant to 4 mL of emulsion

with 5 mL of 0.1 M of phosphate buffer at pH 7. The enzymatic reaction was maintained for 15 min at 37 °C on a rotary shaker (150 rpm) and was subsequently stopped by the addition 20 mL of acetone–ethanol mix [1:1(v/v)]. The free fatty acids released during the reaction were then titrated with 0.05 M NaOH (Destain *et al.*, 2005). One unit of lipase activity is defined as the amount of lipase inducing the release of 1 mmol of fatty acid per minute at 37 °C and pH 7.

Optimization of Fermentation Parameters

Lipase production by strain *Pseudomonas aeruginosa* KDP was conducted in 250 ml Erlenmeyer flasks with 50 ml of the minimal medium containing Glucose 1%, NaCl 0.1%, MgSO₄.7H₂O 0.05%, KH₂PO₄ 0.1%, CaCl₂.2H₂O 0.04%, Olive oil 1.0 (v/v). The remaining nutrients and physical parameters were optimized as follows. Extracellular enzyme from the flask was harvested by centrifugation at 10,000 rpm for 15 minutes and supernatant was used as extracellular enzyme source.

Optimization of different nutrient and physical parameters for lipase production was studied by maintaining all factors constant except the one being studied. Effect of pH on lipase production was studied by cultivating the isolate in different initial pH values (pH 4–8) of the minimal medium. Effect of temperature on enzyme production was studied by incubating the culture at various temperatures ranging from 25 to 60°C. Flasks were kept on shaker at 180 rpm for 48 h.

The effects of different medium components such as metal ions and nitrogen sources were studied. Different

concentration of metal ions such as Selenium (Se) and Nickel (Ni) (10 to 100 µm/L) was used as supplements to study the growth and lipase production. The influence of different concentration of nitrogen sources such as Urea and Ammonium nitrate (0.1 to 1 mg/l) was studied by supplementing in minimal medium along with the 1% olive oil. Minimal medium without selenium, nickel, urea and ammonium nitrate was considered as control and all the experiments were run in triplicates with optimized temperature and pH at 180 rpm for 48h.

Statistical Analysis

All the data was statistically analyzed to find the coefficient of correlations (Pearson) between the variables by Software - MINITAM Release 12.2.

Results and Discussion

The level of enzyme activity produced by an organism from a natural environment is often low and needs to be elevated for industrial application. This can be achieved by microorganisms used for enzyme production is grown in fermenters using an optimized growth medium. Many studies have been undertaken to define the optimal culture and nutritional requirements for lipase production by submerged culture. Lipase production is influenced by the type and concentration of carbon sources, nitrogen sources, culture pH, growth temperature, and dissolved oxygen concentration.

Effect of pH on lipase production

Media pH is one of the important physical parameter which can influence on bacterial growth as well as production. Many

studies have been reported the importance of culture pH. Benattouche and Abbouni (2012) reported that the *Pseudomonas aeruginosa* was able to grow in the pH range from 6 to 8 and produced maximum lipase (38.5 U/ml) at pH 7. Yuzo and Sakaya (2003) also reported that maximum lipase activity from *Pseudomonas fluorescens* HU 380 was detected at pH 7. Similarly, in the present study maximum lipase production (19.32 U/ml) was obtained at pH 7.0.

Effect of temperature on lipase production

Many reports are available in the literature about the optimization of media temperature using different statistical and mathematical models to enhance the production of lipases. In the present investigation the extracellular lipase production by *P. aeruginosa* KDP was optimized and statistically analyzed. The maximum lipase production 21.45U/ml was found to be at 45⁰C (Fig.2). The statistical analyses indicate that the lipase production and initial temperature of culture media are interdependent. The optimal temperature for lipase production by *P. xinjiangensis* CFS14 was 40⁰C (Khemika et al, 2012).

Effect of nitrogen sources on lipase production

Generally, microorganisms produce high amount of lipase when organic nitrogen sources such as peptone and yeast extract used as nitrogen source for lipase production by various *Bacillus* spp (Sharma *et al.*, 2002; Kanwar *et al.*, 2006). Cost of these nitrogen sources are very high than other nitrogen source. In this present study inorganic nitrogen sources such as ammonium nitrate and urea were

Figure.1 Effect of different initial pH on lipase production by *P. aeruginosa* KDP

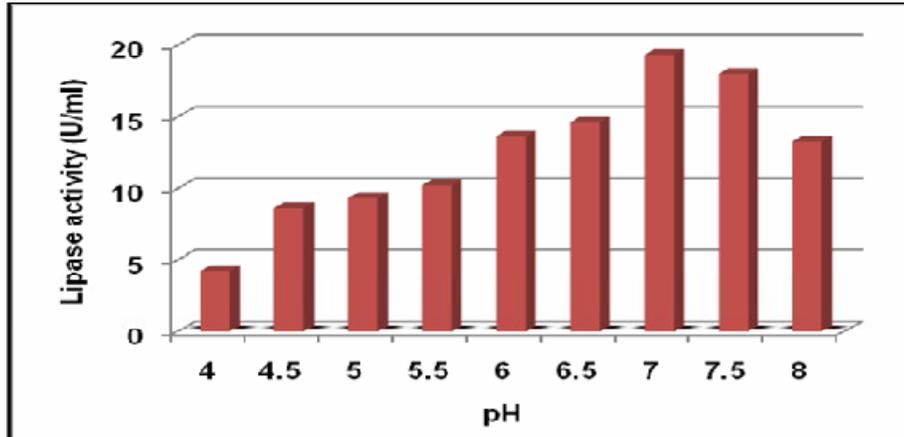


Figure.2 Effect of different initial temperature on lipase production by *P. aeruginosa* KDP

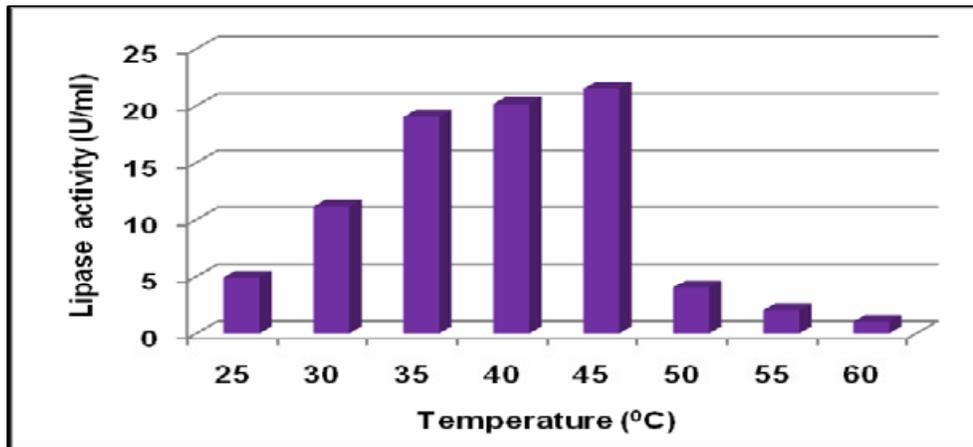
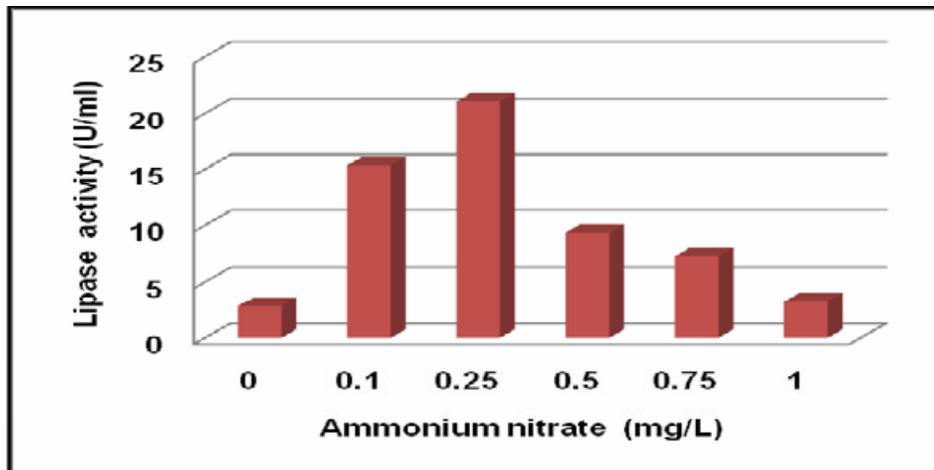


Figure.3 Effect of different concentration of ammonium nitrate on Lipase Production by *P. aeruginosa* KDP



tested individually by supplemented into production media. The maximum lipase production was obtained by *P. aeruginosa* KDP at 0.25 mg/l of ammonium nitrate (21.05 U/ml) and urea (15.68 U/ml) (Figure 3; 4). Among the two inorganic nitrogen sources tested, ammonium nitrate supported lipase production by *P. aeruginosa* KDP because microorganism easily can be utilized than urea. All the concentration of ammonium nitrate grown culture showed significant amount of lipase production when compare to control (without ammonium nitrate). Whereas urea above 0.5mg/l supplement culture showed very poor lipase production because the high concentration of urea inhibited the growth of *P. aeruginosa* KDP.

Effect of metal ion Se and Ni on lipase production

Limited literatures are available on influence of heavy metals on lipase enzyme activity. Reported results concerning the effect of heavy metals on lipase activity are inconsistent. The metal ions have both the positive and negative effects on enzyme activity. In the present study different concentration of Selenium (10 to 100µm/L) was individually supplemented to the culture media to test its effect on lipase production by *P. aeruginosa* KDP. The maximum lipase production (19.86 U/mL) was obtained at 40µm/L of Se present culture (Figure. 5). The optimum Se concentration is 40µm/L because the perfect positive correlation ($\gamma=+1$) was found for this concentration. Metal ions are required for enzyme activity. Selenium is a physiologically essential element to organisms and occurs in them as organic forms at low levels.

The effect of different concentration of Ni on lipase production was tested. The maximum lipase production (21.24U/ml) found to be at 60µm/L Ni supplemented culture grown *P. aeruginosa* KDP (Figure. 6). Saxena and Tanner (2011) reported that the Ni is necessary for growth of *Clostridium ragsdalei* and increasing the concentration of Ni²⁺ from 0.84 to 8.4 µM, increased ethanol production from 35.73 mM to 176.5mM.

Similar experiment was performed in our previous study and found the maximum lipase production by *B. licheniformis* KDP at 60µm/L of Se and 80µm/L of Ni (Sujatha and Dhandayuthapani, 2013).

The present study demonstrated that optimization of culture medium recipe which are play critical role in enhancing the lipase production by newly isolated *P. aeruginosa* KDP. The optimum culture pH and temperature for producing maximum extracellular lipase by *P. aeruginosa* KDP is 7.0 and 45°C respectively. This study indicates that *P. aeruginosa* KDP was a thermophilic organism and it could tolerate higher temperature. The metal ions Se and Ni supported maximum production of lipase at 40µm/L and 60µm/L respectively. As a result, optimizing the culturing conditions and modifying the composition of the medium dramatically improved the lipase production by *P. aeruginosa* KDP. The statistical analysis of present investigation results of all the experiments proved that the physicochemical parameters and the lipase production are interdependent and give useful basic information to achieve the large scale production of lipase by *P. aeruginosa* KDP.

Figure.4 Effect of different concentration of urea on lipase production by *P. aeruginosa* KDP

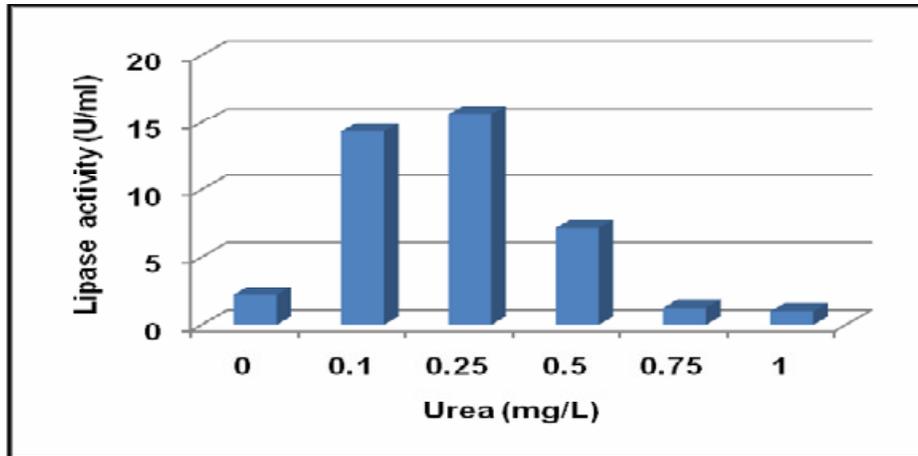


Figure.5 Effect of different concentration of Se on lipase production by *P. aeruginosa* KDP

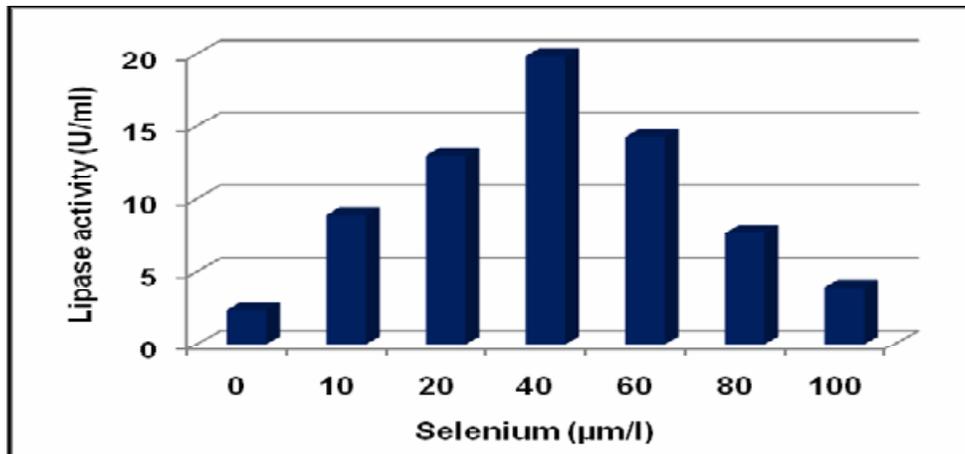
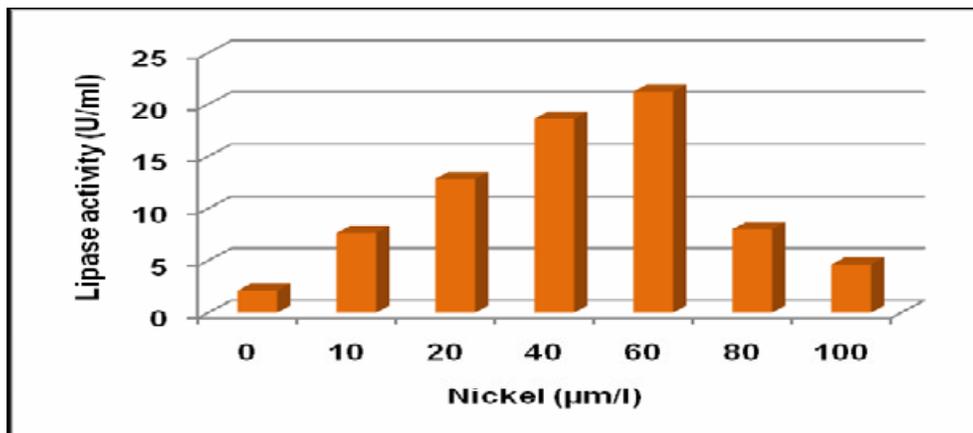


Figure.6 Effect of different concentration of Ni on lipase production by *P. aeruginosa* KDP



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References

- Benattouche, Z., and Abbouni, B. 2012. Production, optimization and characterization of the lipase from *Pseudomonas aeruginosa*. Romanian Biotechbol. Lett. 17(2), 7187 – 7193
- Destain, J., P. Fickers, F. Weekers, B. Moreau and Thonart, P. 2005. Utilization of methyloleate in production of microbial lipase. Appl. Bioche. Biotechnol. 121:269–277
- Guerzoni, M.E., R. Lanciotti, L. Vannini, F. Galgano, F. Favati, F. Gardini and Suzzi, G. 2001. Variability of the lipolytic activity in *Yarrowia lipolytica* and its dependence on environmental conditions. Int. J. Food. Microbiol. 69:79–89.
- Hasan, F., A.A. Shah and Hameed, A. 2006. Industrial applications of microbial lipases. Enzyme. Microb. Technol. 39:235-251.
- Kanwar, S.S., I.A. Ghazi and Chimni, S.S. 2006. Purification and Properties of a Noble Extra-cellular Thermotolerant Metalloproteinase of *Bacillus coagulans* MTCC-6375 Isolate. Protein. Expr. Purif. 46, 421-428
- Khemika, L., B. Angkhameen and Hataichanoke, N. 2012. Investigation of isolated lipase producing bacteria from oil contaminated soil with proteomic analysis of its proteins responsive to lipase inducer. J. Biol. Sci. 12(3): 161-167.
- Nadia, N., Z.A. Nehad, A.E. Elsayed, M.A. Essam and Hanan, M.A. 2010. Optimization of lipase synthesis by *Mucor racemosus* - Production in a triple impeller bioreactor. Malays. J. Microbiol. 6: 7-15
- Rajesh, E.M., R. Arthe, R. Rajendran, C. Balakumar, N. Pradeepa and Anitha, S. 2010. Investigation of lipase production by *Trichoderma reesei* and optimization of production parameters. J. Environ. Agric. Food Chem. 9(7):1177-1189.
- Reetz, M.T., 2002. Lipases as practical biocatalysts. Curr. Opin. Chem. Biol. 6(2):145–150.
- Saxena, J and Tanner, R.S. 2011. Effect of trace metals on ethanol production from synthesis gas by the *Ethanologenic acetogen, Clostridium ragsdalei*, J. Ind. Microbiol. Biotechnol., 38:513–521
- Sharma, R., S.K. Soni, R.M. Vohra, L.K. Gupta and Gupta J.K. 2002. Purification and Characterization of A Thermostable Alkaline Lipase from A New Thermophilic *Bacillus* sp. RSJ-1. Process Biochem. 37:1075-1084.
- Shu, Z.Y., H. Jiang, R.F. Lin, Y.M. Jiang, L. Lin and Huang, J.Z. 2010. Technical methods to improve yield activity and stability in the development of microbial lipases. J. Mol. Catal B: Enzym. 62:1-8.
- Sujatha, K., and Dhandayuthapani, K. 2013. Optimization of lipase production media parameters by newly isolated *Bacillus licheniformis* KDP from oil mill soil, Int. J. Pharm. Bio. Sci. 4(2): (B) 645 - 652
- Yuzo, K., and Sakaya, S. 2003. Purification and characterization of the lipase from *Pseudomonas fluorescens* HU 380. J. Biosci. Bioengineer. 96: (3), 211-226.