Growth characteristics and enzyme production optimization of lipase Producing Strain

Chaocheng Zheng*

Nanjing Vocational Institute of Transport Technology, Nanjing 211188, China

*Corresponding author e-mail: zccnau@126.com

Abstract. 55 samples from different regions were selected and screened by Rhodamine B flat transparent circle method to observe lipase producing effect, among which, LHY-1, identified as *Serratia* sp. has the characteristics of fast growth, high enzyme production and stable ability. The colony of this strain is white, the edge is smooth and tidy, the surface is moist, the cell is straight, rod-shaped, gram negative, 0.1-0.2 μm in diameter and, length 0.3-0.5 μm in length.

1. Introduction

Most of the lipase is extracellular lipase, and the production of lipase, apart from some physicochemical factors (temperature, pH and dissolved oxygen), has a great influence on the production of lipase. The most important nutrient for lipase activity is carbon source, because lipase is an inducible enzyme. Lipases usually produce on oils or other inducers (three acyl glycerol, fatty acids, hydrolyzable esters, Twain, bile salts, glycerol) [1, 2]. To produce lipase, a carbon source of lipids is required. However, the units and essential trace elements should be taken into account in order to optimize the growth and production of bacteria. In general, the expression of lipase can be optimized by optimization of the culture medium, but optimization of the concentration of each medium is a time-consuming process. Classical practice of maintaining one variation without changing other conditions has been proved to be invalid because it does not explain the interplay between individual factors in the fermentation process.

At present, lipase extraction methods often include extraction, chemical synthesis, and microbial fermentation. As resources in extraction method is restricted to climate and soil conditions, complex raw material composition, separation and purification process is tedious. Besides, cost of chemical synthesis is high, which will lead to environmental pollution as well. However, compared to the other two other methods above, fermentation production of microbial lipase, especially in the mature recombinant lipase expression system, production is not affected by the environmental impact of products, products are simple and low cost with shorter enzyme production cycle which is easy to manage, thus indicating a bright prospect [3, 4].

Generally speaking, compared with inorganic carbon source and nitrogen source, organic carbon source and nitrogen source is rich in nutrition which could be utilized by all kinds of bacteria such as *Serratia* sp., *Bacillus*, *Pseudomonas*, Staphylococcus and etc[5,6]. In this paper, fermentation time, carbon source, nitrogen source, temperature, initial pH, salinity and liquid loading were studied to optimize the conditions for growth and enzyme production of the target strain for lipase production, and to provide reference for further study of LYH-1.

Content from this work may be used under the terms of the Creative Commons Attribution 3.0 licence. Any further distribution of this work must maintain attribution to the author(s) and the title of the work, journal citation and DOI.
Published under licence by IOP Publishing Ltd

2. Materials and Methods

2.1. Culture medium and solution

Lipase activity assay medium: 1% Peptone, 0.01% $CaCl_2 \cdot H_2O$, 1% NaCl, 1.5% agar, 1% olive oil emulsions, 0.01% Rhodamine B, pH 7. All compomemts were sterilized at 121℃ for 20 min.

LB liquid medium: 1% Peptone, 1% NaCl, 0.5% Yeast Extract Powder, pH 7. All compomemts were sterilized at 121℃ for 20 min.

The substrate for the reaction of butyric acid with butyric acid was 1.5% Gum Arabric Powder, 2% Triton X-100 and 0.3 mg/mL soluble butyric acid ester dissolved at Tris-HCl (pH 7.0) to constant volume of 100mL.

Lipase activity was assayed by transparent plate ans spectrophotometric method. Enzyme unit was defined as the amount of lipase required for lipase hydrolysis of substrate to 4-Nitrophenyl 1nmol butyrate per minute under certain conditions.

2.2. Growth characteristics and enzyme production optimization

*2.2.1. Effects of culture time on growth characteristics and enzyme activities. S*train was inoculated into LB liquid medium in constant temperature 30 ℃ shaking at 180 rpm for 72 h. The fermentation broth was centrifuged at 10000 rpm every 4 h, diluted with sterile water, and the absorbance of target suspension was determined by UV spectrophotometer at 600nm to draw growth curve of the target strain.

2.2.2. Effects of carbon and nitrogen sources on growth characteristics and enzyme activities. Strain was inoculated into different carbon sources (glucose, sucrose, beef extract, olive oil, Tween-80) and nitrogen source (ammonium sulfate, ammonium nitrate, peptone, yeast powder, urea) under constant temperature at 30℃ for 16 h before centrifuging at 10000 rpm for 10 min and being mixed with steriled water. Ultraviolet spectrophotometer at the wavelength of 600nm was to determine absorbance of the target suspension with sterile water as a blank control.

2.2.3. Effect of culture temperature on growth characteristics and enzyme production. Strain was inoculated into LB liquid medium at 4℃,10℃,15℃,20℃,25℃,30℃,35℃,40℃,45℃ and ℃ for 16 h before centrifuging at 10000 rpm for 10 min and being mixed with steriled water. Ultraviolet spectrophotometer at the wavelength of 600nm was to determine absorbance of the target suspension.

2.2.4. Effects of different initial pH on growth characteristics and enzyme activities. Objective strain was inoculated into LB liquid medium at pH 4.0, 5.0, 6.0, 7.0, 8.0, 9.0, 10.0, 11.0, 12.0 for 16 h before centrifuging at 10000 rpm for 10 min and being mixed with steriled water. Ultraviolet spectrophotometer at the wavelength of 600nm was to determine absorbance of the target suspension.

2.2.5. Effects of salinity on growth characteristics and enzyme production. Objective strain was inoculated into LB liquid medium under different salinities (0%, 1%, 2%, 3%, 4%, 5%, 6%, 7%, 8%, 9%, 10%) for 16 h before centrifuging at 10000 rpm for 10 min and being mixed with steriled water. Ultraviolet spectrophotometer at the wavelength of 600nm was to determine absorbance of the target suspension.

2.2.6. Effect of the fluid volume on the growth of the strain and lipase production. LYH-1 was cultivated in 250 mL LB liquid medium under different loading volumes (10mL, 20mL, 30mL, 40mL, 50mL, 60mL, 70mL, 80mL, 90mL, 100mL) for 16 h before centrifuging at 10000 rpm for 10 min and being mixed with steriled water.Ultraviolet spectrophotometer at the wavelength of 600nm was to determine absorbance of the target suspension. The blank was controlled by sterile water. Lipase

activity of the target strain was determined according to method mentioned above, and the lipase was expressed by OD_{410}. The concentration of the target bacterial suspension was expressed by OD_{600}.

3. Results and dicussion

3.1. Effect of culture time on growth characteristics and enzyme production

Strains were inoculated into LB liquid medium in constant temperature (30℃) , 180 rpm for 72 h. Samples were withdrawn at 4 h at intervals, centrifuged for 10 min at 10000 rpm and mixed with sterilized water for determination of absorbance of the target suspension by spectrophotometer at 600nm, using sterile water as a blank control. The growth curve and enzyme production curve of the target strain were drawn in figure 1. As shown in figure 1, the target strain LHY-1 grew rapidly in the former 12h, and reached a stable stage after 12h. Enzyme production of LHY-1 increased rapidly in the former 12h, and the enzyme production decreased after 20h with the best enzyme production time in 16h.

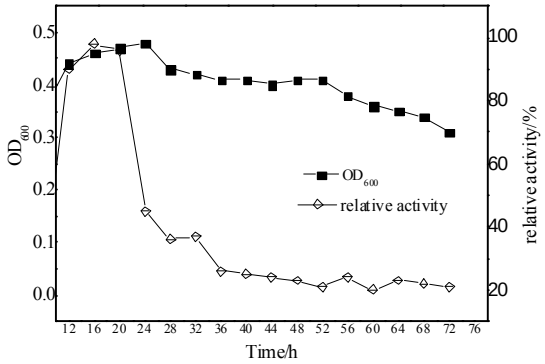

Figure 1. Effect of culture time on the growth of the strain and lipase production.

3.2. Effects of carbon and nitrogen sources on growth characteristics and enzyme activities

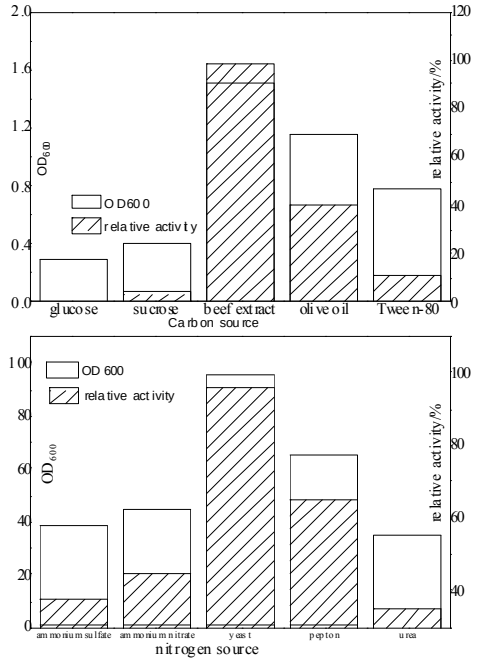

Figure 2. Effect of culture time on the growth of the strain and lipase production.

Effects of different carbon sources and nitrogen sources on the growth and enzyme production of the strain were shown in figure 2. As shown from the diagram, the growth of the target strain LHY-1 is not good with glucose and sucrose as the sole carbon source, and the carbon and nitrogen sources for the growth and production of enzymes are beef extract and yeast powder respectively.

3.3. Effects of different temperatures on growth characteristics and enzyme production

Strain LHY-1 was cultivated under 4℃, 10℃, 15℃, 20℃, 25℃, 30℃, 35℃, 40℃, 45℃, 50℃ in LB liquid medium before being centrifuged at 10000 rpm for 10 min and being diluted mixed with sterilized water for determination of absorbance of the target suspension by spectrophotometer at 600nm, using sterile water as a blank control. Effect of different temperatures on the growth and lipase production of target strain LHY-1 is shown in figure 3. From Fig. 3, the target strain LHY-1 strain has a wide range of temperature adaptability and grows well at a temperature of 10-40℃, but the target strain LHY-1 has the best lipase production at a temperature of 20℃.

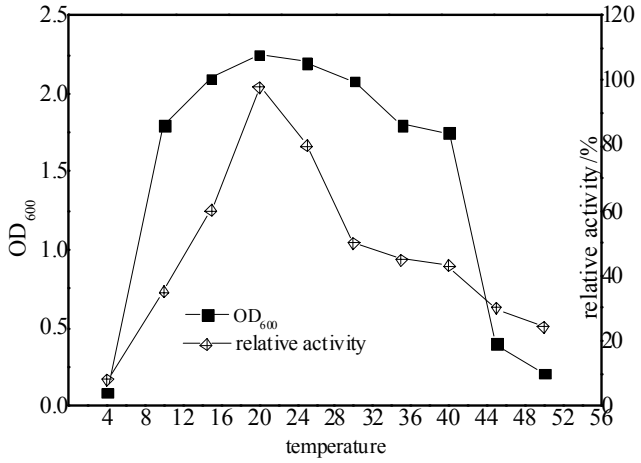

Figure 3. Effect of culture temperature on the growth of the strain and lipase production.

3.4. Effects of different initial pH on growth characteristics and enzyme production

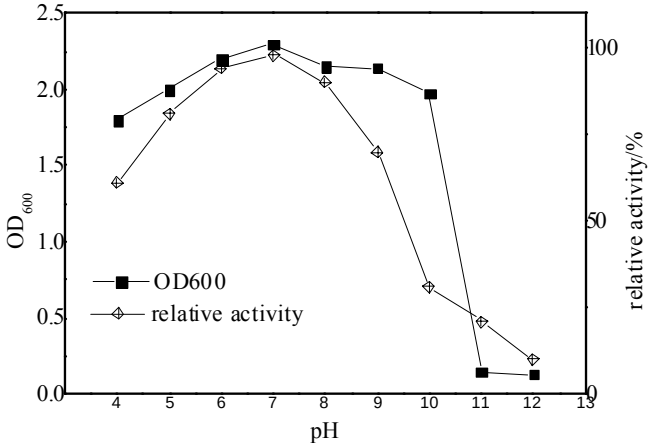

Figure 4. Effect of the initial pH on the growth of the strain and lipase production.

Strain LHY-1 was cultivated under pH 4, 5, 6, 7, 8, 9, 10, 11, 12 in LB liquid medium before being centrifuged at 30 ℃,10000 rpm for 10 min and being diluted mixed with sterilized water for determination of absorbance of the target suspension by spectrophotometer at 600nm, using sterile

water as a blank control. Effect of different initial pH on the growth and lipase production of target strain LHY-1 is shown in figure 4. From Fig. 4, the target strain LHY-1 was found to grow well within the initial pH range of 4.0-10.0, but the target strain LHY-1 was 7 at the initial pH.

3.5. Effects of different salinity on growth characteristics and enzyme activities

LYH-1 was cultivated under different salinity (0%, 1%, 2%, 3%, 4%, 5%, 6%, 7%, 8%, 9%, 10%) in LB liquid medium before being centrifuged at 30 ℃,10000 rpm for 10 min and being diluted mixed with sterilized water for determination of absorbance of the target suspension by spectrophotometer at 600nm, using sterile water as a blank control. Effect of salinity on the growth and lipase production of target strain LHY-1 was shown in figure 5. The target strain LHY-1 has a high salinity tolerance and grows even up to a concentration of 9% (w/v) while the strain grows and produces lipase best with no salinity.

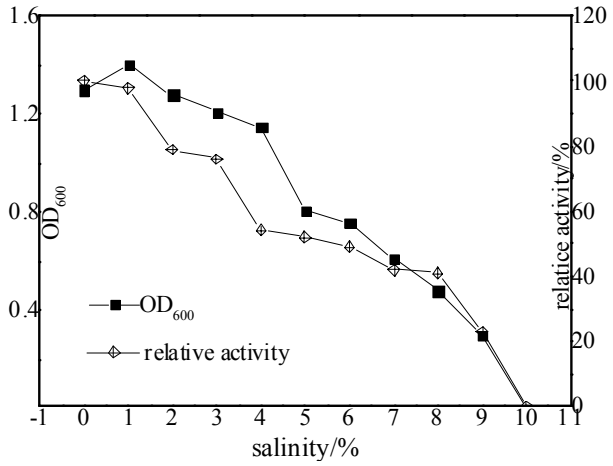

Figure 5. Effect of the salanity on the growth of the strain and lipase production.

3.6. Effects of different loading amount on growth characteristics and enzyme production

The absorbance of the enzyme activity of the target bacteria was determined by ultraviolet spectrophotometer at the wavelength of 410nm with sterile water as blank control. When the liquid loading was 30mL (250 mL triangle bottle), the growth and enzyme production of the strain was the best (Figure 6).

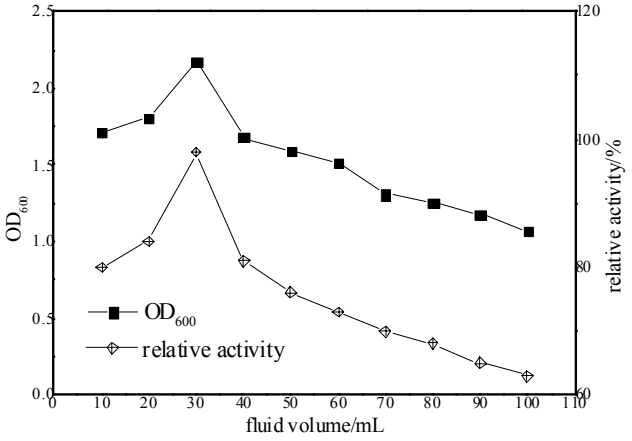

Figure 6. Effect of the fluid volume on the growth of the strain and lipase production.

4. Conclusion

The lipase activity of a strain-LYH-1, screened from petroleum using Rhodamine B flat halo method was relatively high with the characteristics of rapid growth, wide range of adaptation and stable lipase production. In addition, enzyme production conditions of strain LHY-1 were optimized. The strain grew rapidly in the former 10h, and reached a stable stage after 10h. The best enzyme production time was 16h. The optimum growth and the best carbon and nitrogen sources for producing the enzyme are beef extract and yeast powder respectively. The bacteria grew well at 10 -40 C, and the optimum enzyme production temperature was 20 ℃. The strains grew well in the initial pH range of 4.0-10.0, and the optimum enzyme production rate was 7 at the initial pH. The bacterium has a high salinity tolerance and grows at a salinity of 9%, but the salinity of the optimum enzyme production is 0. The optimum growth and optimum enzyme production rate was 30 mL.

Acknowledgments

This work was supported by a grant from High level Scientific Research Foundation for the introduction of talent in Nanjing Vocational Institute of Transport Technology.

References

[1] Vakhlu J, Kour A. Yeast lipases: Enzyme purification, biochemical properties and gene Cloning, Electronic Journal of Biotechnology. 9 (2006)1-17.
[2] Buchanan R E, Gibbens N E. Berger's Bacterial Identification Manual, Beijing: Science Press. 1984, 482-486.
[3] Liu I L, Tsai S W. Improvements in lipase production and recovery from Acinetobacter radioresistens in presence of polypropylene powders filled with carbon sources, Appl. Biochem. Biotech. 104 (2003) 129-140.
[4] Litantra R, Lobionda S, Yim J H et al. Expression and biochemical characterizat- ion of cold-adapted lipases from Antarctic Bacillus pumilus strains, Journal of Microbiology and Biotechnology. 23 (2013) 1221-1228.
[5] Yang X, Wang B, Cui F, Tan, T. Production of lipase by repeated batch fermentation with immobilized Rhizopus arrhizus, Process Biochemistry. 40 (2005) 2095-2103.
[6] Sun S Y, Xu Y. Solid-state for 'whole-cell synthetic lipase' production from Rhizopus chinesis and identification of the functional enzyme, Process Biochemistry. 43 (2008) 219-224.