Bulletin of Environment, Pharmacology and Life Sciences
Bull. Env. Pharmacol. Life Sci., Vol 6 [8] July 2017: 109-111

Online ISSN 2277-1808
Journal's URL:http://www.bepls.com
CODEN: BEPLAD
Global Impact Factor 0.533
Universal Impact Factor 0.9804

ORIGINAL ARTICLE **OPEN ACCESS**

A Thermostable bacterial alkaline lipase: an ideal choice for application in detergent formulations

Nidhi Tripathi, Anjali Choudhary* Preeti Chandurkar, Tanuja Murab, Nidhi Gurjar and Rohit Rawat
Department of Biotechnology and Biochemistry, Career College, Bhopal.
*Corresponding Author

ABSTRACT
Lipases are amongst the most important biocatalysts carrying out novel reactions in an aqueous medium. This is primarily due to their ability to utilize a wide spectrum of substrates. During the present work extracellular Lipases are extracted from Aeromonas hydrophila which is isolated from Laharpur Water reservoir, Bhopal. Lipases were found to be stable at extreme pH and temperature, because of these properties it can be used as a component of detergent in order to remove stains of oil on the clothes, it can reduce the environmental load of detergent products as the chemicals used in conventional detergents but bacterial lipases are biodegradable, non-toxic and leave no harmful residues.
Keywords: thermostable bacteria, alkaline lipase

Received 10.06.2017 Revised 25.06.2017 Accepted 02.07.2017

INTRODUCTION

Microbial enzymes are used as biological catalysts due to their high specificity and economic advantages [2], this is due to the fact that it is easy to characterize microbial enzymes for their optimal condition. When we compare microbial enzymes with that of enzymes in comparison to plants or animals, microbial enzymes have a variety of catalytic activities, high production capacity within a short period of time ease for genetic manipulation and it is cost effective. However, microbial enzymes can be produced at any time and do not affect by seasonal fluctuations. The other advantage of microorganisms over that of plants and animals is that they can grow rapidly on inexpensive media and microbial enzymes are more stable in terms of activity.

Lipases are hydrolytic enzymes (EC 3.1.1.3) act at fat-water interfaces to catalyze the hydrolysis of triglycerides to fatty acid and glycerol [15, 13, 3]. Lipases are very important because of the role they play in the postmortem quality deterioration of seafood and other foodstuffs during handling, chilled frozen storage and widely used for biotechnological applications in dairy industry, oil processing, production of surfactants and pure pharmaceuticals. Compared with other hydrolytic enzymes (e.g., proteases and carbohydrases), lipases are relatively less well studied and in this regard, lipases from aquatic animals are even less well known versus their counterparts from mammalian, plant and microbial sources [11].

Apart from this Lipases represent an important group of biotechnologically valuable enzymes [5, 6, 9]. They are widely distributed in nature. Although lipases have been found in many species of animals, plants, bacteria, yeast, and fungi, the enzymes from microorganisms are the most interesting because of their potential applications in various industries such as food, dairy, pharmaceutical, detergents, textile, biodiesel, and cosmetic industries and in synthesis of fine chemicals, agrochemicals, and new polymeric materials [1, 10, 4]. Detergent industries are the primary consumers of enzymes, in terms of both volume and value [12]. The use of enzymes in detergents formulations enhances the detergents ability to remove tough stains and making the detergent environmentally safe. Nowadays, many laundry-detergent products contain cocktails of enzymes including proteases, amylases, cellulases, and lipases [8].

From an enzyme point of view, detergents on the international market contain principal ingredients that operate by almost identical detergency mechanisms. Soil and stains are removed by mechanical action assisted by surfactants, builders, and enzymes. 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. However, nitrogen sources and essential micronutrients should also be carefully considered for growth and production optimization [17].

Oils and other oil-based food products, cosmetics, automobile products etc. are a major problem as they form dirty and non-removable spots on clothes, and this is because oil is not soluble in water so it is difficult to remove the stain from clothes. To overcome with this problem, we should use lipases as a component in washing detergent, Lipases present in the detergent will hydrolyses oils and after that it would be easy to remove the stain of oil from clothes. During the present study lipases producing *Aeromonas hydrophila* were isolated from the Laharpur water reservoir, Bhopal. Laharpur is a water reservoir but it is highly polluted by sewage and domestic waste having oils. The aim of the study is characterized lipase production by *Aeromonas hydrophila* in different temperature and pH in order analyze whether lipases are suitable or not as a component in detergent.

Materials and Methods

Isolate used in this experiment was previously isolated from Laharpur Water Reservoir, Bhopal and identified as *Aeromonas hydrophila* [16].

Screening for lipase activity by Tributyrin Clearing Zone. The predominant bacteria in the nutrient agar plate were isolated and screened for lipolytic activity. Lipolysis is observed directly by changes in the appearance of the substrate such as tributyrin and triolein, which are emulsified mechanically in various growth media and poured into a Petri dish. The bacterial isolates were screened for lipolytic activity on agar plates containing tributyrin (1%, w/v), agar (2%, w/v) in Luria–Bertani medium. Lipase production is indicated by the formation of clear halos around the colonies grown on tributyrin containing agar plates [17].

Lipase production

Aeromonas hydrophila was initially enriched by using the medium containing (w/v): beef extract (0.15%), peptone (0.5%), sodium chloride (1.0%) and glucose (0.5%), pH 7, at 32°C for 24 h. Then after 24 hours of incubation, 5% of this was then incubated at 37°C. After incubation of 48 hours it was centrifuged at 10000 rpm and the supernatant was used as lipase enzyme source.

Lipase assay

The activity was assayed with the reaction mixture, in a final volume of 1 mL, containing 40 mM Tris-HCl buffer (pH 8.0), 20mM pNPP, a substrate, and 25µL of the enzyme (5 mg/mL). After10 min of incubation at 40ºC, the reaction was stopped by the addition of 2mL of ethanol 96%, and the p-nitrophenol released was monitored spectrophotometrically at 420 nm, using a standard curve. One lipase unit (U) was defined as the amount of enzyme that released 1 µmol p-nitrophenol per minute [14].

Optimization of lipase production

Characterization of lipase production at different pH

The effect of medium pH on lipase production was determined at different pH varied from 5 to 9 during different time intervals of 24, 48 and 72 h [14].

Characterization of lipase production at different temperature

Effect of medium temperature on lipase production was determined by incubating the production media at different temperatures such as 20^{o}C, 30^{o}C, 37^{o}C, 40^{o}C and 50^{o}C for the time intervals of 24, 48 and 72h [14].

RESULT AND DISCUSSION

Most soils, dirt stains are acidic in nature; therefore most of the detergent is on the alkaline side. Oils that means lipids are made up of fatty acids which also require alkali as a cleaning agent but the only detergent are not sufficient because fatty acids are insoluble in water so during the present study lipases were characterized for the stability at different pH.

Most of the companies are not currently manufacturing the detergents alone; they are producing enzyme-based detergents. More than 50% of the detergents produced in the developed countries presently contain enzymes to improve the detergency by removing tough stains. The role of a detergent lipase is to digest the lipidic molecules from the soiled substrates. Most of the chemical detergent ingredients are hazardous to human beings and cause pollution to the environment. The use of alkaline lipase in detergent formulations can reduce or substitute the use of these harmful ingredients in higher amounts. The detergent lipases active at ambient temperature are now preferred as the quality of the cleaned fabric is maintained and energy saved. Review papers on the production, purification, characterization

and application of lipases in various industries are available, but no specific review on the microbial alkaline lipases or detergent compatible lipases. In the present review, screening, production and properties of detergent compatible lipases are reported with emphasis on the stability and compatibility of alkaline lipases in detergent and detergent constituents and the methods for the examination of oil stain removal [7]. Lipases were found to be most optimum at pH 8.5 but it also showed good activity up to pH 9.5 that means lipases were alkaline pH resistant. Similarly lipases were also characterized by different temperature Lipases was found to be active up to 45^0C but it showed optimum activity at 40^0C. From the present study it is concluded that *Aeromonas hydrophila* isolated from Laharpur water reservoir was found to produce lipases which were pH stable and found to be most active at 40^0C that means if this lipases add as a component in detergent it would be a great tool to remove oil stains from the clothes.

REFERENCES

1. Abdelkafi S, Ogata H, Barouh N, Fouquet B, Lebrun R, Pina M, Scheirlinckx F, Villeneuve P, Carrière F. (2009). Identification and biochemical characterization of a GDSL-motif carboxyl ester hydrolase from *Carica papaya* latex. Biochim Biophys Acta. 1791:1037–1046.
2. Abo, M. (1990). Method of purifying dry-cleaning solvent by decomposing liquid contaminants with a lipase. World Organization Patent 9007606
3. Desnuelle, P., L. Sarda and G. Ailhaud, (1960). Inhibition de la lipase pancréatiquepar le diethyl-p-nitrophényl phosphate en émulsion. Biochem. Biophys. Acta, 37: 570-571.
4. Dharmaraj S. (2010). Marine *Streptomyces* as a novel source of bioactive substances. World J Microbiol Biotechnol. ;26:2123–2139. doi: 10.1007/s11274-010-0415-6.
5. Grant WD, Heaphy S. (2010). Metagenomics and recovery of enzyme genes from alkaline saline environments. Environ Technol.;31:1135–1143. doi: 10.1080/09593331003646661.
6. Guncheva M, Zhiryakova D, Radchenkova N, Kambourova M. Properties of Immobilized lipase from *Bacillus stearothermophilus* MC7. Acidolysis of triolein with caprylic acid. World J Microbiol Biotechnol. 2010;25:727–731.
7. Hans Sejr Olsen and Per Falholt. (1998). The role of enzymes in modern detergency. Journal of Surfactants and Detergents. 1:4 , 555–567.
8. Jeon J.H., J.T. Kim, Y.J. Kim, H.K. Kim, H.S. Lee, S.G. Kang, S.J. Kim and J.H. Lee. (2009). Cloning and characterization of a new cold-active lipase from a deep-sea sediment metagenome. Appl. Microbiol. Biotechnol. 81: 865,74.
9. Jiang Y, Zhou X, Chen Z. (2010). Cloning, expression, and biochemical characterization of a thermostable lipase from *Geobacillus stearothermophilus*. Environ Technol.;31:1107–1114. doi: 10.1080/09593331003677872.
10. Lee HK, Lee JK, Kim MJ, Lee CJ. A newly high alkaline lipase: an ideal choice for application in detergent formulations. Immobilization of lipase on single walled carbon nanotubes in ionic liquid. Bull Korean Chem Soc. 2010;31:650–652. doi: 10.5012/bkcs.2010.31.03.650. lipids Health Dis. 2011; 10: 221.
11. Lopez-Amaya, C., A.G. Marangoni, N.F. Haard and B.K. Simpson, 2001. Lipases: Seafood Enzymes. Utilization and Influence on Postmortem Fish Quality. 1st Edn., Marcel Dekker, New York, pp: 121-146.
12. Mitidieri S, Martinelli AHS, Schrank A, Vainstein MH. Enzymatic detergent formulation containing amylase from *Aspergillus niger*: a comparative study with commercial detergent formulations. Bioresour Technol. 2006; 97:1217–1224. doi: 10.1016/j.biortech.2005.05.022.
13. Norin, M., O. Olsen, A. Svendsen, O. Edholm and K. Hult, 1993. Theoretical studies of *Rhizomucor miehei*lipase activation. Protein Eng., 6: 855-863.
14. Prazeres Janaina Nicanuzia dos; Juliana Aparecida Bortollotti Cruz; Gláucia Maria Pastore. Characterization of alkaline Lipase from *Fusarium oxysporum* and the effect of different surfactants and detergents on the enzyme activity.
15. Sarda, L. and P. Desnuelles, (1958). Action de la lipase pancréatique sur les estersen émulsion. Biochem. Biophys. Acta, 30: 513-521.
16. Tripathi N and Choudhary. (2014). Isolation and identification of enteropathogenic *Aeromonas hydrophila* from laharpur water reservoir, Bhopal. International journal of science, environment and Technology .
17. Veerapagu m, .A. sankara Narayanan, K .Ponmurugan, K.R.Jey . (2013). screening selection identification production and optimization of bacterial lipase from oil spilled soil. Asian Journal of pharmaceutical and clinical research. Vol 6, Suppl 3, 2013, 62-67.

CITATION OF THIS ARTICLE

N Tripathi, A Choudhary, P Chandurkar, T Murab, N Gurjar and R Rawat. A Thermostable bacterial alkaline lipase: an ideal choice for application in detergent formulations. Bull. Env. Pharmacol. Life Sci., Vol 6 [8] July 2017: 109-111