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Original Research Article

Optimization and purification of lipase production by aeromonas hydrophila

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Abstract

Lipases are industrially important enzyme. During the present work extracellular Lipases are extracted from non-hemolytic *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, *Aeromonas hydrophila* was found to be non pathogenic so it can be use to extracted extracellular lipases which can be used to reduce the pollution of aquatic system.

Key Words: Alkaloid, non rhizobia, plant growth promotion, 16Sr DNA sequence, root nodule bacteria, *Trigonella foenumgraecum*

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INTRODUCTION

Microbial lipases constitute an important group of biotechnologically valuable enzymes, mainly because of versatility of their applied properties and ease of mass production (Godris et.al, 1998, Macrae 1983, Suzuki et.al, 1988). Besides their industrial applications, novel biotechnological applications have been successfully established using lipases for the synthesis of biopolymers and biodiesel, the production of pharmaceuticals, agrochemicals and flavor compounds (Jaeger 2002). Because of huge variation in applications, the availability of lipases with specific characteristics is still a limiting factor. Thus, to search for new lipases with different characteristics continue to be important research topics (Borkar et.al, 2009). Lipases are the group of enzymes that catalyzes the formation or hydrolysis of fats (Svendsen 2000); they have the ability to hydrolyze Triacylglycerols at an oil water interface to release free fatty acids and glycerol. Microbial lipase may originate from fungi, molds, or bacteria and most of them are formed extracellular (Pandey *et al*1999). Lipases are produced by submerged culture, and release during the late logarithmic or stationary phase (Ghosh *et al*.1996). Some studies have also shown that producing lipase by bacterial strains is more common because they offer higher enzymatic activity compared to fungi, tend to have optimal pH at neutrality or alkalinity, they are often thermo stable and are mostly extracellular, facilitating their extraction from the fermented medium (Hasan *et al*., 2006; Li *et al*., 2010). There are opportunities to extend the use of enzymes in detergents both geographically and numerically. They have not found widespread use in developing countries which are often hot and dusty, making frequent washing of clothes necessary. The recent availability of a suitable lipase may increase the quantities of enzymes employed very significantly. There are, perhaps, opportunities for enzymes such as glucose oxidase, lipoxygenase and glycerol oxidase as means of generating hydrogen peroxide *in situ*. Added peroxidases may aid the bleaching efficacy of this peroxide..(http://www1.lsbu.ac.uk/water/enztech/detergent.html).

Biotechnology based cleaning agents such as enzyme based agents, are widely used in industries. The biotechnology based cleaning agents are cheaper and less harmful to the environment. They have specific leaning action and can also be used at lower temperatures. They produce effluents with lower COD and non-corrosive nature. Enzyme-based cleaners are becoming increasingly popular in the food industry as compared to caustic or acid cleaning regimes (D'Souza and Mawson, 2005).The

enzyme based detergents have better cleaning properties as compared to synthetic detergents. They are active at low washing temperatures and environment friendly also (Kumar *et al*., 1998). The enzymes in the detergents do not lose their activity after removing stain. The enzyme containing detergents also improve the fabric quality and keeping color bright. The enzyme based detergents are used in small quantity as compared synthetic chemicals. They can work at very low temperature, environment friendly and completely biodegradable. Four classes of enzymes are generally used in detergents as given in (Gormsen *et al*., 1991).Removal of proteins by non-enzymatic detergents can result in permanent stains due to oxidation and denaturing caused by bleaching and drying. Proteases hydrolyze proteins and break them down into more soluble polypeptides or free amino acids. As a result of the combined effect of surfactants and enzymes, such hard to remove stains can be removed from fibre (Hasan et.al 2010). During the present investigation extracellular lipases are extracted and characterized which is isolated from non-pathogenic *A.hydrophila.*

MATERIALS AND METHODS

Isolate used in this experiment was isolated from Laharpur Water Reservoir, Bhopal and identified as *Aeromonas hydrophila* (Tripathi and Choudhary, 2015, Tripathi et.al, 2017; 2018).

Screening for lipase activity: It was done 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 (Veerapagu et.al, 2013, Tripathi *et.al,* 2017)

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 hour 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 reaction mixture, in a final volume of 1 mL, containing 40 mM Tris-HCl buffer (pH 8.0), 20mM pNPP, as substrate, and 25 µL of enzyme (5 mg/mL). After10 min of incubation at 40ºC, the reaction was stopped by the addition of 2 mL 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 perminute (Prazeres et.al, 2006).

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 (Prazeres *et.al*, 2006).

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 (Prazeres et.al, 2006)

Effect of inhibitors /activators: Lipase activity was assayed in the presence of various inhibitors and activators (Borkar et.al 2009).

OBSERVATION AND RESULTS

Enzyme activity at pH 5 to 6 was almost negligible but remarkable increase was obtained from pH 7 to 9 lipases showed good activity above 30^{0}C but maximum lipase activity was found at 40^{0}C. Lipases showed excellent activity with Ca^{2+}, Zn^{2+}, Mg^{2+}, Mn^{2+}, $Cu^{2+}$and Fe^{2+} but it showed inhibitory effect with EDTA.

DISCUSSION

In the present study, extracellular lipase isolated from non-hemolytic strains of *A.hydrophila* was found to be more active in pH range of 8 to 9. It shows less activity pH below 7. Optimum temperature for these enzymes was 40^{0}C, it also showed good activity at 37^{0}C. The results showed that enzyme activity was decreased in presence of 5 mM EDTA. On the basis of results, we can conclude that it might be a metalloprotein which is effective at alkaline pH and the bacteria is mesophilic so enzymes were found to be active at 37^{0}C to 40^{0}C temperature.

CONCLUSION

Lipases was found pH sensitive, it cannot work at acidic pH. On the basis of results it can be concluded that this enzyme can be useful in various industrial and biotechnological applications viz detergents and food industries, environmental bioremediations. Also this bacteria was found non hemolytic it can also be used as bio remediating agents in aquatic system polluted with oils and effluents. However, further works relating to improvement in enzyme yield and other kinetic aspects of

enzyme activity are required to understand the catalytic properties of this enzyme.

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