CHAPTER VI
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Lipases (Triacylglycerolhydrolase E.C.3.1.1.3) in general, are enzymes that catalyze hydrolysis of triacylglycerol into monoacyl glycerol, diacylglycerol, glycerol and fatty acids. In the recent years lipases have find use in many industries including detergent, chemical, pharmaceutical, leather and food processing. Lipases show different substrate and positional specificities and can catalyze various reactions like hydrolysis, interesterification, esterification, alcoholysis, acidolysis and aminolysis. Alkaline, thermophilic lipases find use in detergent and oleochemical industry. Several plants, animals, fungi and bacteria are known to produce lipases. However focus on bacterial lipases has recently been laid due to their high stability, multifold properties and abundant supply. The present work embodied in the thesis comprises the study made on the occurrence of hot springs of Arunachal Pradesh, India, the isolation of novel lipase from an indigenously isolated microbial strain, its production, characterization, biotechnological application in the field of detergents and surfactants, Immobilization and stability studies.

The objective of the present work was important as hot springs are considered as potential source of thermophilic microorganisms. Based on this assumption, the hot springs of Arunachal Pradesh has been chosen as study area as previously no work has been carried out to exploit the potential of these hot springs.

The hot springs are located in the west Kameng and Tawang districts of Arunachal Pradesh. First hot spring is in the Dirang area of West Kameng district. West Kameng district lies between 26°54' and 28°01' N latitudes and 91°30' and 92°40' E longitudes. It is bounded by Tibet in the North, Bhutan in the west and Tawang district of Arunachal Pradesh in the northwest, East Kameng district in the east and Sonitpur district of Assam in the south. It has an area of 7,422 sq.km. The hot spring of Dirang is located in the bank
of river Dirang-chu at an height of 1495m asl. Dirang hot spring is surrounded by river Dirang-chu on one side and three other sides by subtropical Pine forest. The other hot spring (Kitpi hot spring) is situated in the Greng Khar village of Kitpi area of Tawang district. The Kitpi area is approximately 20-25 Km away from the main Tawang city. The Tawang district lies between 27°22’ and 27°45’ N latitudes and 90°15’ to 90°45’E longitudes. It covers an area of 2085sq km and lies at an altitude of 3,500m asl. It is bounded by Tibet in the north, Bhutan in the south and west; and West Kameng district in the east. It has an area of 2,085 sq.km. Kitpi hot spring is located in the bank of the river Jong at an height of 1850m asl.

A number of lipase producing bacterial strains were isolated from the samples by enrichment culture technique. The zone of clearance surrounding the bacterial colonies was considered as index of extracellular lipase production and based on the zone of clearance an alkaline, thermophilic lipase-producing organism was chosen for further study. The organism was identified taxonomically based on morphological, physiological and biochemical characteristics as Bacillus sp DH4. Production of the lipase from this organism was optimized in shake flasks, using a statistical experimental design. The organism was found to utilize a number of vegetable oils as well as other carbon sources. Groundnut oil was selected as it gave the maximum lipase production among the carbon sources tested and is easily available, inexpensive source of oil in India. Among the sugars sucrose and maltose was found to be good substrates for lipase production. The Bacillus sp grew well in all nitrogen sources with peptone as the suitable nitrogen source. The optimized medium resulted in about 3-fold increase in the enzyme production, as compared to that obtained in the basal medium. The pH 9.0 and temperature 50°C were found to be optimum for lipase production. The lipase production was found to be maximum in the presence of Ca2+ ions and also in the combination of Mg2+ ions and Ca2+ ions.

The extracellular lipase produced by this organism was purified to homogeneity. The culture broth grown cells are harvested to late exponential phase of growth was centrifuged at 10,000xg for 10 min at 4°C followed by filtration through 0.45µm
Millipore membrane filter to remove the cells. The extracellular lipase was purified by ammonium sulphate precipitation followed by gel filtration chromatography. The purification fold of the enzyme is found to be 10.3 and the molecular weight of the protein to be 33KDa. The purified enzyme was characterized in terms of its pH optimum and stability, temperature optimum and stability, effect of metal ions, substrate specificity and stability in the organic solvents. The enzyme was active at alkaline pH. In the present work the optimum pH of the alkaline lipase was found to be 9.0. The enzyme was found to be stable in the pH range of 6-10 with marked activation at alkaline pH of 8-10. More than 145% of the residual activity was observed on incubation of lipase for more than 6 hours at the pH values of 9 at 50°C. Lipase activity was assayed at different temperatures ranging from 10-80°C at pH 9.0. The optimum reaction temperature was 50°C. Thermal stability was investigated by incubating the enzyme at various temperatures (30-80°C) for 1h. Thermo stability profile indicated that the enzyme stability upto 60°C with the retention of 95.8% activity at 60°C and almost 90% of the activity at 70°C.

The enzyme activity is found to be maximum in the presence of Ca²⁺ ions proved the enzyme to be Ca²⁺ dependent metalloenzyme. The best suitable substrate for lipase activity was found to be triolein. Stability in the organic solvents is desirable in synthesis reactions. The stability of the enzyme is tested in various organic solvents and maximum stability was found to be in the presence of acetone followed by methanol.

The compatibility of the lipase in the presence of various surfactants and detergents followed by wash performance analysis to prove the present lipase to be a potential detergent additive. The effects of various surfactants and commercial detergents on the alkaline lipase activity were tested at 10% and 0.1% (w/v). The lipase was found to be stable in both ionic and nonionic surfactants with marked activation in the presence of Triton X-100 Triton X-114 with the retention of 40-68% of activity. The enzyme retains almost 100% of its activity in Tween 20 and Tween 80 while in Tween 40 the drop of activity is quite low. SDS was found to be strong inhibitor and almost leads to total inhibition of lipase activity.
The effect of commercial detergents showed different rates of lipase inhibition. The stability of lipase activity was done in comparison of commercial lipase. Surf and Ariel was found to be strong inhibitor with almost 40% inhibition in relative activity. Whereas wheel, Tide and fena inhibits up to 20%. The commercial alkaline protease inhibits up to 20% of the relative activity. The lipase retained its activity in the presence of commercial detergents in solution and hence such a preparation will be very useful as detergent additive. The wash performance analysis of Bacillus DH4 lipase suggested that large number of fatty acids were released when lipase was used along with the detergent, leading to better stain removal. The compatibility of Bacillus sp DH4 lipase with chlorine was studied as detergents contains chlorine. Chlorine does not affect the stability of lipase. In 400 mg of available chlorine l⁻¹ the enzyme still retained almost 80% of its activity, after exposed for one hour.

The immobilization of lipase was carried out in silica by adsorption method and the stability studies were carried out between free and immobilized lipase. No change in optimum pH for lipase activity was observed in the case of aqueous and immobilized form. Optimum temperature of immobilized enzyme shifted from 50°C to 55°C.