ABSTRACT

Lipases (triacylglycerol hydrolases, E.C. 3.1.1.3) catalyze the hydrolysis of triglycerides to free fatty acids and glycerol. Extracellular lipase secreted by the gram-negative bacterium, *Pseudomonas aeruginosa* is a promising candidate for biotechnological applications and is extensively used for chiral production of racemic compounds.

In the first part of this thesis, the production of lipase by *Pseudomonas aeruginosa* PGSL 03 (isolated from surfactant gel) utilizing vegetable oil seeds was investigated. Maximum lipase activity was observed at 30°C and pH 7.5 in sesame meal at 2% (w/v) concentration. The amount of lipase correlated well with the relative percentage of calcium and C18: n fatty acid esters present in the media. The study, therefore, identifies vegetable oil seeds as a simple and economical lipid–rich media viable at an industrial scale. Ammonium Sulphate precipitation of culture supernatant resulted in partial purification of the enzyme (4.28 fold) with 90.6% yield. The enzyme was active in the pH range of 6-11 and was stable at temperatures below 55°C.

The effect of various metal stearates on the lipase production by *P. aeruginosa* was studied to understand the role of metals on lipase secretion. Magnesium Stearate and Calcium Stearate enhanced growth as well as lipolytic activity probably due to their role in stability of the active conformation and ribosome binding, respectively. Extracellular lipase secretion in presence of metal stearates could be explained by `membrane
pore hypothesis’, in which the metal stearates might reduce the fluidity of the outer membrane, thereby helping the formation of relatively stable pore-like structures for the transmembrane export of the enzyme molecules.

With the completion of *Pseudomonas aeruginosa* genome project, the database information on the biochemical network responsible for transcription and secretion of this industrially important enzyme was compiled. Two putative $\sigma^{54}$-dependent promoter sequences upstream of *lipA/H* and LipR protein were annotated.

In the final part of the thesis, a series of full-atom molecular dynamics simulation of an analogous enzyme (1EX9) was done to understand the mobility of regions involved in catalysis. Using molecular dynamics simulations, we proposed that in addition to the postulated lid (125-148), there exists a novel second lid (covering residues 211–222) in this lipase, that lies over the binding pocket covering the first lid. Restrained and unrestrained molecular dynamics simulations and molecular dynamics simulations of rational insilico mutants of *P. aeruginosa* lipase in both aqueous environment and octane-water interface showed that the movement of second lid may actually trigger the movement of the first lid, and that this triggering action is driven by hydrophobic contacts (mainly Phe214 and Ala217 residues) between the two lids. This computational study thus, paves a way for experimentalists to study the structure and dynamics of this protein in greater detail and to understand the coupled sub domain movements in a comprehensive fashion.