5.0 SUMMARY

Out of 11 isolates only three isolates viz SLP2, SLP4 and SLP8 have been screened as high lipase producers. These elite isolates were identified SLP2 as *Bacillus brevis*, SLP4 as *Bacillus licheniformis* and SLP8 as *Pseudomonas aeruginosa* based on morphological observation and biochemical tests were carried out according to Bergey’s Manual of Determinative Bacteriology. Then these elite bacterial strains used for more investigation as a result of they were the foremost potent lipase producers.

Highest lipase production was achieved by *B. licheniformis* at pH 7.0 whereas by the *B. brevis* and *P. aeruginosa* was at pH 7.5. The temperature 45°C and agitation speed 200rpm was found to be optimum conditions for same bacterial strains. In the present study found that the 8mL⁻¹ was the optimum inoculum size for fermentation processes. At these optimum fermentation conditions all the 3 new isolates were made two fold lipase productions with in 48h.

In this research finding, highest lipase production by *B. brevis*, *B. licheniformis* and *P. aeruginosa* were obtained with sunflower oil followed by olive oil. The carbon sources 10gL⁻¹ of glucose, 7.5gL⁻¹ of sucrose, 7.5gL⁻¹ of maltose, 7.5gL⁻¹ of lactose and 5% glycerol were found to be an ideal concentration for the maximum lipase production by new bacteria isolates.

The optimum concentration of different nitrogen sources was: yeast extract-10mgL⁻¹, peptone - 10mgL⁻¹, urea - 0.25mgL⁻¹ for the very best production of lipase by *B. brevis*, *B. licheniformis* and *P. aeruginosa*. 1mgL⁻¹ of ammonium sulphate and 0.75mgL⁻¹ ammonium nitrate was the optimum concentration for *B. brevis*. *B. licheniformis* produced highest lipase at 0.75mgL⁻¹ ammonium sulphate and ammonium nitrate. 1mgL⁻¹ ammonium sulphate and ammonium nitrate was found to be a best concentration for maximum lipase production by *P. aeruginosa*. 
All the 3 new isolates showed 9-10 fold lipase production when the medium was supplemented with 40µmL⁻¹; Cu, 60µmL⁻¹; Fe, 60µmL⁻¹; Ni, 60µmL⁻¹; Zn and 60µmL⁻¹ of Se for *B. licheniformis*, 80µmL⁻¹ of Se for *B. brevis*, and *P. aeruginosa*.

A 10% (v/v) of freshly prepared crude extract of seaweed such as *Ulva, Caulerpa, Padina, Sargassum* and *Gracilaria* was used as substrate for the maximum lipase by *B. brevis*, *B. licheniformis*, and *P. aeruginosa*. Among the seaweeds, genus *Ulva* and *Gracilaria* was found to be the best source for the highest lipase production.

Among the six different polymers that the Ca-alginate was found to be the best polymer for immobilization of newly isolated bacterial cells for extracellular lipase production. Ca-alginate immobilized cells showed high stability for repeated use. Six repeated batches were carried out in flasks for 144 h. Bacterial cells immobilized in the remaining materials showed only 3 to 4 repeated batches for lipase production.

Extracellular lipase was purified by the two step processes such as ammonium sulphate precipitation and column chromatography. Then the purified lipases were characterized and found the optimum pH and temperature for the more stability with maximum activity was 8.0 and 40°C respectively. The three bacterial strains lipases showed the maximum activity only in the presence of Ca²⁺ and Co⁺, whereas other metal ions inhibited activity of the lipase more or less. Also found that the lipases from *B. brevis*, *B. licheniformis* and *P. aeruginosa* were showed a marked stability in the hydrophobic solvents such as benzene, p-xylene and n-hexane after 30 min incubation than hydrophilic solvents. The purified lipase was used for environmental application and found as a good oil degrader.