

## SCREENING, ISOLATION AND CHARACTERISATION OF LIPOLYTIC AND PROTEOLYTIC MICROBES AND ENZYMES

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### Introduction

Microbial lipases and proteases constitute important groups of biotechnologically valuable enzymes due to the versatility of their applied properties and ease of mass production. Both enzymes have been the subject of intensive research with considerable emphasis on biotechnological aspects. Lipases are enzymes which catalyse hydrolysis of water-insoluble acyl esters, trans- and interesterification reactions leading to the production of altered lipids. Proteases are enzymes which catalyse the hydrolysis of peptide bond. Industrially, proteases and lipases are widely used in food production, detergent, chemical and pharmaceutical industries. In view of their importance, during the last 7 years, our group have started an intensive screening programme for proteolytic and lipolytic microbes from local environments. New microbial enzymes with new properties may permit better understanding of the enzymes and exploitation of their biotechnological potential.

### Materials and Methods

Primary screening for lipolytic microbes was done using tributyrin and triolein agar. Skim milk agar plates were used for proteolytic microbes. Lipase activity was determined according to Samad et al. (1990). Protease activity was assayed according to method described by Rahman et al. (1993).

### Results and Discussion

Over 100 lipolytic and proteolytic microbial isolates, mainly bacteria and fungi, were isolated and purified from various locations, such as dumping places, palm oil mill effluents (POME), soils and drains. The cultural and nutritional factors affecting the growth of selected microbes and their enzyme production were studied in detail (Razak et al. 1997a). For microbial lipases, detailed studies were done on *Rhizopus rhizopodiformis*, *R. oryzae* (thermophilic) and *R. oryzae* (mesophilic), (Razak et al. 1997b). Both the intracellu-

lar/membrane bound and extracellular lipases from the three fungi were purified and characterised. Two bacterial lipases from *Pseudomonas* sp. were studied in detail. Lipase production by immobilised cells were also investigated, the matrix employed were alginate, polyurethane and poly-HEMA (Salleh et al. 1996). For microbial proteases, focus was on thermostable proteases. Most of the potential protease producers isolated belonged to *Bacillus* sp. A detailed study was done on *B. stearrowthermophilus* strain F1, since it produced remarkable amount of protease under alkaline condition. The protease from these bacteria has been purified and characterised. It showed very high thermostability up to 80°C. Detergency studies on the protease showed that it washed better than Savinase (a commercial enzyme) at temperatures higher than 50°C suggesting its potential as a detergent enzyme. Strains improvement through mutagenesis to improve the enzyme's yield was also studied (Razak et al. 1996). Currently, work is being carried out to clone and sequence the protease gene from *B. stearrowthermophilus* strain F1.

### Conclusions

Three fungal lipases were purified and characterised. Their growth and lipase production parameters were determined. Higher lipase yield was achieved with immobilised cells. Protease from *B. stearrowthermophilus* strain F1 was purified and characterised. The protease is very thermostable, stable in alkaline condition and display remarkable washing ability.

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