Microbial Enzyme Technology: Screening, Isolation and Characterization of Lipolytic and Proteolytic Microbes and Enzymes*

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Introduction

Many strains of bacteria, yeast and fungi have been reported to be lipase and protease producing microorganisms. They are drawing more attention compared to the other sources due to their short generation time, and the ability to modify their genetic make up. To day microbial lipases and protease are being used in detergent, food, leather industries and medical purposes. Proteases are increasingly being used in film, silk industry and in the synthesis of biologically actives peptides (Tsuru et al. and Yoshimoto, 1987).

Materials and Methods

Screening and isolation: Screening for lypolitic microbes was done using triolein plate assay. A specific plate assay developed by this laboratory was used (Samad *et.al*, 1989). Samples were taken from soils and palm oil mill effluent. The bacterium protease producer was isolated from dumping ground at Sri Petaling. It is able to grow at temperatures up to 60°C. **Assay of Lipase Activity**: The lipase activity was assayed by method of Razak *et al.*, (1995). The protease activity was assayed according to the method of Keay et al. and Wildi (1970) with slight modifications. **Purification:** Cold acetone (-16°C) was added to the crude enzyme. Chromatographic separation was carried out by applying the sample onto Sephadex G-100 column. **Characterization of enzyme:** Enzyme activity was measured at various temperatures, ranging from 30°C to 70°C. For effect of pH, the enzyme activity was measured at pH range of 4.0 to 10.0. For thermostability, the enzyme was incubated at 40, 50, 60, 70, 80 and 90°C for 30 minutes. After the incubation the enzyme was immediately cooled in the ice for 15 minutes and the residual activity was determined.

Results and Discussion

Over 100 lipolytic microbes and have been isolated,with detail studies done on 3 fungi and 3 bacteria .The optimum growth temperature for Rhizopus oryzae(M) was 37°C, Rhizopus oryzae(T) between 45 -50oC and Rhizopus rhizopodiformis between 45 -50oC. Pseudomonas cepacia has optimun growth at 37°C, Pseudomonas sp. at 45oC and isolate U3 was grown at 45oC.(Razak et al.,1995). Lipases from thermophilic fungi. Rhizopus oryzae and Rhizopus rhizopodiformis have optimum pH and temperature at 6.0 and 45oC, respectively (Razak et al., 1997). High amount of intracellular lipase was produced from Rhizopus oryzae at 30oC for 72h incubation with shaking rate of 100 rpm. (Razak et al.,1991). All the carbon sources generally inhibited the production of extracelullar lipase from Rhizopus oryzae with the exception of sucrose, but enhanced the production of intracellular lipase. Peptone was the best substrate for extracelullar enzyme. For the intracellular lipase, the other substrate gave comparable results. Shaking enhanced the production of both types of enzymes; the optimum temperature was 45oC and 37oC for extra-and intra-cellular lipase, respectively. And pH of 5.0 was optimal for production of both enzymes. (Salleh et al.,1993). Lipase from Rhizopus rhizopodiformis was partially purified by acetone precipitation. The enzyme was purified to about 2.8 fold, with 62.5% recovery and with specific activity of 3.2U/mg.the enzyme was further purified by gel filtration through Sephadex G-100, up to about 9.7 fold and had specific activity of 11.1 U/mg.(Salleh et al.,1996). Extracellular lipase was purified from Pseudomonas sp. by acetone precipitation,, and gel filtration chromatography. The purified enzyme displayed a 19.8 fold purity with the yield of 30%. The purified lipase was found to have three subunits, each with molecular weight of 31500, 33250 and 35250 Dalton on SDS gel electrophoresis. The optimum pH and temperature for the pure enzyme were pH 4.0 and 55oC, respectively. The enzyme was stable at pH range between pH 3 -7 for 30 min at 37oC and it can withstand up to 65oC for 30 min. (Razak et al.,1994a). Glycerol was found to be the best carbon source whereas yeast extract was the best nitrogen source for the production of protease from Bacillus stearothermopilus. The bacteria could grow up to 70oC but optimum protease production was at 60oC. Best initial pH for protease production was pH 5. Alkaline pH inhibited the production. The enzyme was stable at 60oC for 18 h and was inhibited by EDTA, PMSF and HgCl2 (Razak et al., 1994b). The thermophilic *Bacillus stearothermopilus* protease was purified to homogeneity by heat treatment, ultrafiltration and gel filtration chromatography with a 128 fold increase in specific activity and 75% recovery. The protease has a relative molecular mass of 33500 by SDS-PAGE but only 22000 by gel filtration (Rahman et al., 1994). *Bacillus stearothermopilus* strain F1 was found to exhibit the highest protease production in alkaline media. It was able to grow up to 80 oC and within a broad pH range of 5.0 to 11.0.

Conclusions

We believe that somewhere in the soils, water and air of our local environment awaits lypolitic and proteo-

lytic microbes to be discovered. And for that very reason, we will keep on the screening program. In addition, it is hoped that improvement of the strain through mutation or genetic engineering will be done to give the mutant (s) of the required properties.

Benefits from the study

This protease possessed good properties for the purposes of detergent formulary. Some studies of immobilization of lipases were undertaken to improve the enzyme usage.

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