Thesis Tittle Production and Characterization of a Thermostable Lipase from a Thermophilic Thermus

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Abstract

Thermophilic bacteria Thermus T20 isolated from Tepanom hot spring in Chiang Mai could produce significant extracellular lipase activity and low protease activity in a medium containing 10% (v/v) base mixture, 10% (v/v) 0.2 M phosphate buffer pH 7.2, 0.25% (w/v) nutrient broth and 0.5% (v/v) olive oil in 1l shake flask. The bacterium was precultured for 24 h and 1% (v/v) was inoculated and cultured at 65°C, 200 rpm. The maximum lipase activity (0.45 U/ml) was produced after cultivation for 55 h. Production of lipase (0.65 U/ml) was slightly higher when the bacterium was cultured in 1l medium using 1.5 l fermentor at the same condition including aeration at the flow

rate of 150 ml/min. This might be due to the effective aeration and agitation system in the fermentor.

Comparison study on cell immobilization of Thermus T20 for production of lipase by adsorption on ceramic beads and sponge and entrapment in calcium alginate was made. The porosity of 52.9 g ceramic beads that contained in 2.0 x 25.0 cm column was 0.096 cm^3/g and could adsorb 0.185 g of wet cells but 3.57g of sponge contained in same column has porosity 27.08 cm^3/g and could adsorb 0.406 g of wet cells. Studied on production of lipase from immobilized cells on the three supports in continuous system found that cell immobilized on sponge produced highest lipase activity (0.62 U/ml), and lower lipase activity was produced from immobilized cell in ceramic beads (0.34 U/ml) because of the lower amount of wet cells adsorbed, however cell entrapped in calcium alginate beads did not produce lipase in continuous system. The cultivation of cells entrapped in calcium alginate beads in batch system produced lipase activity (1.22 U/ml) higher than free cells (0.54 U/ml) dued to the higher amount of cells were entrapped and lipase activity in culture medium obtained both from the immobilized cells and the leaked cells which grow in the medium as free cells. Cells entrapped in calcium alginate beads cultivated in batch system seemed to produce higher lipase activity than cells adsorption on ceramic beads and sponge dued to the higher amount of cells and the cultivation in batch system decreased the separation of oil to flow to the upper part of the column which easily occurred in the continuous system.

Some properties of the lipase produced from Thermus T20 were studied. It was found that the optimum temperature and pH are 65°C and 7.2 respectively. The enzyme could be stable up to 65°C when incubated for 1 h and 50% of the activity remained. Thirty two percents of the activity remained when concentrated the enzyme by ultrafiltration and no activity left by lyophilization. Partial purification of the enzyme by alcohol precipitation, dialysis, pH precipitation and gel filtration gave 3 lipase activity peaks with high purification folds 53, 171.2 and 48.48 and specific activity 17.48, 56.5 and 16.0 U/mg protein respectively. Determination for molecular weight of lipases by gel filtration and SDS-PAGE found 3 types of lipases with molecular weight of 232,000, 46,500 and 16,000, respectively. Detail study on other properties of lipase including position specificity, substrate specificity and catalization of the enzyme in organic solvents are needed for proper industrial application.