P30

Cloning of Novel Bacterial Lipase Gene and Production of Active Enzyme by Refolding System

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Determination of nucleotide sequence of the gene encoding a lipase from *Pseudomonas sp.* P-S4 revealed that P-S4 lipase (PSL) is composed of 313 amino acid residues with a calculated molecular weight of 33,501. Analysis of the amino acid sequence revealed significant homology (around 63%) to lipases of *Vibrio cholerae*. Open reading frame fragment (939 bp) was inserted a expression vector, pET29a(+). Recombinant P-S4 lipase (rPSL) was overproduced in *Escherichia coli* (BL21 DE3) in an insoluble form, solubillized in the presence of 8 M urea, purified in a urea-denatured form and refolded by removing urea in the presence of the Ca^{2+} ion. Gel filtration chromatography indicated that this refolded protein is monomeric. Analysis by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) revealed an molecular weight of 33 kDa for the purified protein. rPSL showed relatively broad substrate specificities. rPSL was classified into lipase family I.1 based on the amino acid sequence similarities but into lipase family I.2 based on molecular weight (33 kDa) and active-site motif (GHSQG), suggesting that this lipase is a novel type.