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High level expression of enantio selective lipase gene from Bacillus pumilus in E.coli

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Soluble overexpression of enantioselective lipase gene from Bacillus pumilus designated as lipA in E.coli using T7 expression system and surface display expression systems were used for optimizing the overexpression of the enzyme that would allow us to produce large amounts of lipase enzyme for crystallization as well as characterization for its commercial exploitation.

Expression plasmids pET-Lip, pETB-Lip and pEst100-Lip were constructed in E.coli using pET21 (a)+, pETBlue2 and pEst100 as expression plasmids. pET-Lip, and pEst100-Lip were transformed into E. coli BL21(DE3) and pETB-Lip was transformed into E. coli BL21(DE3) pLacI.The lipase gene ljpA in all the constructs was placed directly under the control of T7 promoter.Lipase activity was found in the soluble fraction of E.coli bearing pET-Lip and pETB-Lip. However, major amount of lipase activity was only displayed on the surface of E. coli harbouring pEst100-Lip.

The increase in enzyme expression levels corresponded to 12-60 fold improvements as compared to native enzyme containing lipA under the control of its native promoter.

The recombinant enzyme BPE from the E.coli pETB-Lip at its maximum expression was purified from the CFE in a three-step procedure by ammonium sulphate precipitation, hydrophobic interaction chromatography and subsequently followed by anion-exchange chromatography.

SDS-PAGE analysis indicated that purified BPE is a monomer with a molecular mass of ~19.2 kD.

The recombinant clone as such or the cell free extract prepared from the clone exhibited original selectivity for the hydrolytic activity of various racemates.

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