

Abstract:

Lipase (triacylglycerol acyl Hydrolase EC 3.1.1.3) are the most important industrial enzymes are used in various industries including food, detergents, pharmaceutical products, leather, textiles, cosmetics, and paper. Bacterial lipases are member of β/α hydrolase family that hydrolase triacylglycerol at the water-lipid interface. *Bacillus thermocatenulatus* lipase2 (BTL2) is a thermoalkalophilic lipase that show optimal activity at 60–75 °C and pH 8–10. BTL2 is important research target because of its potential industrial applications.

At the present study, the effect of substitution Phe¹⁷ with Ser on enzyme activity in chimeric *Bacillus thermocatenulatus* Lipase (of penta-peptide sequence (¹¹²Ala-His-Ser-Gln-Gly¹¹⁶) *Bacillus thermocatenulatus* Lipase was replaced with correspondence sequences of *Candida rugosa* lipase (²⁰⁷Gly-Glu-Ser-Ala-Gly²¹¹)). At first. For survey the effect of substitution Phe¹⁷ with Ser on structure and function of lipase bioinformatics studies and structure prediction of lipase was investigated. Then this mutation induced by site directed mutagenesis. Then the mutant BTL2 was cloned and expressed in *E. coli* as secretion protein. Finally, Catalytic activity of new chimeric lipases was evaluated at presence of various triglycerides as substrates and compared with activity of old chimeric lipase, also the effects of different parameters such as temperature, pH, detergents, organic solvents and metal ions were evaluated on chimeric enzymes activities using pH-stat assay. In comparison with old chimeric lipase, the new chimeric lipases showed better activity for all substrates except Tributyrin (C₄). In addition, The mutation created improved to most studied, pH stability, solvent and metal ions resistance of new chimeric lipase.

Keywords: lipase, *Bacillus thermocatenulatus*, BTL2, Protein engineering



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Title:

**Investigation the effect of substitution Phe¹⁷ with Ser on
enzyme activity in chimeric *Bacillus thermocatenuatus* Lipase**

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