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Crystallization of N-terminal Strep-tagged Fusion Lipase from Thermostable *Bacillus* sp. Strain 42.

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Lipases have great potential to be used in industries due to their favourable properties such as substrate specific, enantiomerically selective, regioselective and mild reactions conditions. Lipases of microbial origin are generally more stable than lipases from animal or plant and as such they are useful source for industrial enzymes. A 1.2 kb lipase gene (AY 78735) [1], isolated from solvent stable and thermostable *Bacillus* sp. strain 42 was overexpressed using pET51b vector with *E. coli* host strain BL21(DE3) pLysS, in which the fusion lipase contains N-terminal Strep-tag II affinity tag [2]. The purified fusion lipase, at protein concentration of about 4.0 mg/mL, was induced to crystallize in 0.1 M MES buffer at pH 6.5 without the presence of salt, but in the presence of only 12% w/v PEG 20 000 as precipitant. Crystallization reactions were carried out using vapour diffusion methods at 16˚C. Crystals were formed after 12 hours incubation. The crystals with size measuring around 0.04 X 0.12 mm were shown to be heavily stained with protein dyes. Lip 42 lipase is highly homologous to three crystallized lipases from thermophilic *Bacillus sp*., namely T1 lipase [3], P1 lipase [4] and L1 lipase [5]. Lip 42 protein crystals, despite having almost 97% similar homology in amino acid sequence, showed a different shape and crystallization condition. The shape of Lip 42 crystal appeared to be partly attributed to the presence of N-terminal tag.

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Keywords : thermostable lipase; strep-tag fusion; lipase crystal

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Crystallization of Mutated T1 Lipase from Thermostable *Geobacillus Zalihae* Strain T1.

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A novel thermoalkaliphilic lipase producer *Geobacillus zalihae* strain T1 was isolated from palm oil mill effluent in Malaysia [1,]. The mature T1 lipase was overexpressed in *Escherichia coli* harboring pGEX/T1S recombinant plasmids with lipase activity of 42 U/ml [2]. The enzyme can be crystallized up to 60°C [3]. Optimization process revealed that a balance of hydrophobic interaction, packing rate, and some flexibility was needed to obtain a good crystal solved at 1.5Å [4]. Point mutation D311E (inter-loops networking) created an additional one salt-bridge and two hydrogen bonds as compared to K344R (intra-loops networking) with additional two salt-bridges and one hydrogen bond. Denatured protein analysis revealed that mutation D311E gave higher Tm (70.59 °C) as compared to K344R (68.54 °C) and native T1 lipase (68.52 °C). The mutant D311E was able to form preliminary crystal interface with formulation 9, 13 and 21 of Crystal Screen 2 with 2 M NaCl, 30% PEG-MME-2000 and 2 M NaCl as precipitants, respectively.

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Hydrophilic Polymers as Efficient Protein Surface Shielding Agents in Protein Crystallization.

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The paper introduces a new concept of “protein surface shielding agents” into protein crystallization. This concept follows from ***“analytical crystallography”***, i.e. analysis of processes leading to formation of crystals and the analysis of intermolecular contacts observed in the crystalline state “***crystal contact areas” (CCA)*** [1]. It shows that large molecules have many various modes of mutual adhesion but only some of these “***adhesion modes” (AM)*** are suitable for compact stacking of macromolecules into the crystal lattice. It shows also that different adhesion modes lead to crystals of different diffraction quality, and to different space groups, and some adhesion modes are