MS5-P30 A novel p-nitrophenyl butyrate-specific esterase from Photobacterium sp. 6M7-44 : gene cloning and characterization

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A bacterial strain that produced an esterase was isolated from the whole body of arkshell, *Scapharca broughtonii* and identified as *Photobacterium* sp. 6M7-44. In the present study, the corresponding gene was cloned using the shotgun method. The amino acid sequence deduced from the nucleotide sequence (918bp) corresponded to a protein of 305 amino acid residues with a calculated molecular weight of 34,299 Da. The esterase showed 43~45% identities with the putative esterases of *Photobacterium halotolerans*, *Photobacterium angustum* and *Vibrio* sp. N418, respectively. The esterase contained a putative leader sequence, as well as the conserved catalytic triad (Ser, His, Asp), consensus pentapeptide GXSXG, and oxyanion hole sequence (HG). The protein 6M7-44 was produced in both soluble and insoluble forms when *Escherichia coli* cells harboring the gene were cultured at 18°C. The enzyme showed specificity for C4 (butyrate) as a substrate, with little activity toward the other *p*-nitrophenyl esters tested. The optimum pH and temperature for enzyme activity were pH 9.0 and 30°C, respectively. Relative activity remained up to 90% even at 5°C with an activation energy of 6.29 kcal/mol, which indicated that it was a cold-adapted enzyme. Enzyme activity was inhibited by Cd^{2+}, Cu^{2+}, and Hg^{2+} ions. As expected for a serine-esterase, activity was inhibited by phenylmethylsulfonyl fluoride. It was remarkably active and very stable in the presence of commercial detergents and organic solvents. This cold adapted esterase has potentials for use as a biocatalyst and detergent additive for use at low temperature.

Keywords: Photobacterium sp., cold-adapted esterase, gene expression, substrate specificity

MS5-P31 Structural studies of a novel type of chitinase from archaea

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Themococcus chitonophagus is a hyperthermophilic archaeon, isolated from media containing only chitin as carbon source. It is the first hyperthemophile which can grow in such media. To elucidate its ability to degrade and assimilate chitin, we performed genome analysis and found "*Tc*-ChiX", a putative novel chitinase. *Tc*-ChiX has a secretion signal peptide and two regions having high sequence identity with chitin binding domain of chitinase from a hyperthermophilic archaeon *T. kodakarensis* in the N-terminal domain. On the other hand, the C-terminal domain contains no DXDXE motif conserved in the GH18-type chitinase family and even shares no sequence similarity. We have performed X-ray structure analysis of *Tc*-ChiX to clarify the reaction mechanism of this chitinase.

As the overexpression of *Tc*-ChiX in full-length was not straightforward, we constructed *Tc*-ChiX(ΔBD), only consisting of a putative chitinase domain. This truncated mutant could be sufficiently overexpressed and retained its enzymatic activity. We initially crystallized the sample of this mutant at 20°C, and obtained crystals of the substrate complex diffracting X-rays to 2.55 Å resolution. Moreover, when the crystallization temperature was changed to 35°C, crystals with a different shape were obtained. Diffraction experiments revealed that the maximum resolution of new crystals was improved to 1.95 Å and the space group was also changed from $P2_12_12_1$ to $P6_3$. Finally, we determined both crystal structures. These structures are different in crystal packing, and substrate-binding modes are also remarkably different. We will discuss the reaction mechanism and the substrate selectivity of *Tc*-ChiX based on obtained structural and biochemical information.

Keywords: archaea, chitinase, crystal structure