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Characterization of a Lipolytic Enzyme of Pseudomonas nitroreducens Isolated from Mealworm's Gut

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Abstract : In this study, a symbiotic bacteria from yellow mealworm's (Tenebrio molitor) mid-gut was isolated with characteristics of growth on minimal-tributyrin medium. After a PCR-amplification of its 16s rDNA, the resultant nucleotide sequences were then analyzed by schemes of the phylogeny trees. Accordingly, it was designated as Pseudomonas nitroreducens D-01. Next, by searching the lipolytic enzymes in its protein data bank, one of those potential lipolytic α/β hydrolases was identified, again using PCR-amplification and nucleotide-sequencing methods. To construct an expression of this lipolytic gene in plasmids, the target-gene primers were then designed, carrying the C-terminal his-tag sequences. Using the vector pET21a, a recombinant lipolytic hydrolase D gene with his-tag nucleotides was successfully cloned into it, of which the lipolytic D gene is under a control of the T7 promoter. After transformation of the resultant plasmids into Eescherichia coli BL21 (DE3), an IPTG inducer was used for the induction of the recombinant proteins. The protein products were then purified by metal-ion affinity column, and the purified proteins were found capable of forming a clear zone on tributyrin agar plate. Shortly, its enzyme activities were determined by degradation of p-nitrophenyl ester(s), and the substantial yellow end-product, p-nitrophenol, was measured at O.D.405 nm. Specifically, this lipolytic enzyme efficiently targets p-nitrophenyl butyrate. As well, it shows the most reactive activities at 40°C, pH 8 in potassium phosphate buffer. In thermal stability assays, the activities of this enzyme dramatically drop when the temperature is above 50°C. In metal ion assays, $MgCl_2$ and NH_4Cl induce the enzyme activities while $MnSO_4$, $NiSO_4$, $CaCl_2$, $ZnSO_4$, $CoCl_2$, $CuSO_4$, $FeSO_4$, and $FeCl_3$ reduce its activities. Besides, NaCl has no effects on its enzyme activities. Most organic solvents decrease the activities of this enzyme, such as hexane, methanol, ethanol, acetone, isopropanol, chloroform, and ethyl acetate. However, its enzyme activities increase when DMSO exists. All the surfactants like Triton X-100, Tween 80, Tween 20, and Brij35 decrease its lipolytic activities. Using Lineweaver-Burk double reciprocal methods, the function of the enzyme kinetics were determined such as Km = 0.488 (mM), Vmax = 0.0644 (mM/min), and kcat = $3.01x10^3$ (s^{-1}), as well the total efficiency of kcat/Km is $6.17\ x10^3$ (mM^{-1}/s^{-1}). Afterwards, based on the phylogenetic analyses, this lipolytic protein is classified to type IV lipase by its homologous conserved region in this lipase family.

Keywords : enzyme, esterase, lipotic hydrolase, type IV
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