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Cloning, Heterologous Expression, Refolding, and Characterization of a Cold-active Lipase from *Acinetobacter baumannii* B5

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A gene encoding lipase of the *Acinetobacter baumannii* B5 isolated from thermal water of Baek-du mountain was cloned and sequenced. The deduced amino acid sequence of the lipase and lipase chaperone were found to encode mature proteins of 325 aa (35kDa) and 344 aa (37kDa), respectively. The lipase was expressed using pET-32a vector in the *Escherichia coli* BL21 (*trxB*) cells as inclusion body, which were subsequently solubilized by urea. After being purified by Ni-affinity chromatography, the lipase was refolded by incubation at 4℃ in the presence of a 1:30 molar ratio of lipase:chaperone. The lipase has a unique optimum temperature of 35℃, which was found to be lower that of other *Acinetobacter* sp. lipases. The lipase also maintained 28% of optimum activity at 0℃. These results indicate that the lipase from *Acinetobacter baumannii* B5 is a cold-active enzyme. The maximum activity of lipase was exhibited at 35℃ and a pH 8.3 when *p*-NP caprate was used as a substrate. The refolded lipase was activated by Ca^{2+}, Mg^{2+}, and Mn^{2+}, whereas Zn^{2+}, Cu^{2+}, EDTA, DTT, and 2-mercaptoethanol inhibited it. 0.1% of Tween20 increased the activity by 33%, however, Triton X-100 and SDS inhibited it by 34-60%.

Key words: *Acinetobacter baumannii* B5, A cold-active lipase, Ni-affinity chromatography

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Paenibacillus xylanilyticus Strain DZ-8: a New Source to Produce Mannanase from Konjac Field

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To investigate predominant bacteria in rhizosphere of *Amorphophallus konjac* field, we isolated a total of 10 bacterial strains and found onebacteria strain can grow very well in konjac curd medium but no pathogenicity to *A. konjac*. This strain is Gram-negative, rod-shaped, no spore-forming bacterium. Comparative analysis of 16S rDNA gene revealed that the greatest sequence similarity was found with respect to *Paenibacillus xylanilyticus* strain XIL14 (99%). This strain is proposed to be *Paenibacillus xylanilyticus* strain DZ-8. Strain DZ-8 can produce many extracellular enzyme including mannanase, xylanase, carboxymethyl cellulase (CMCase) and chitinase etc. In this paper we focused on strain DZ-8 as new source to produce mannanase. Higher mannanaseactivity (255.33U/ml) can be detected by using konjac glucomannan other than locust bean gum as the only carbon source. When konjac glucomannan is added to1.5% in medium the highest mannanase activity (256.67U/ml) can be detected. The molecular mass of mannanase produced by strain DZ-8 is about 60 KDa by zymogram. Finally TLC was employed to detect oligosaccharide in media with galactomannan or glucomannan as substrate. As culture time going on, mannohexaose, mannopentaose, mannotetraose, mannotriose and mannobiose come into being in succession. While monosaccharide could be detected in fourth day.

Keyword: *Paenibacillus xylanilyticus, Amorphophallus konjac,* mannanase, zymogram, TLC