Kinetic Study for Recombinant Benzaldehyde Dehydrogenase in Rhodococcus Sp

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Background & Objectives: Rhodococcus ruber UKMP-5M was isolated from soils contaminated with crude oil in Malaysia and identified as a petroleum-hydrocarbon degrader.

Methods: The benzaldehyde dehydrogenase gene was amplified by polymerase chain reaction (PCR) and cloned and expressed in *E. coli* BL21(DE3). The expression of recombinant protein was induced by isopropyl β-D-1-thiogalactopyranoside (IPTG) and the product was purified by AKTA prime system. The protein concentration was measured by bicinchoninic acid (BCA) methods and the kinetic characters were determined by spectrophotometer at 340 nm. The metabolite was identified by gas chromatography-mass spectrometry (GC-MS).

Results: Benzaldehyde dehydrogenase (BZDH) was encoded by xylC amplified at 64 ºC. The recombinant BZDH was induced by 1 mM IPTG after 4 hours incubation at 37 ºC. The recombinant protein was purified by ion exchange chromatography and confirmed the purity by SDS-PAGE %12 and western blot. The protein size was 27 kDa and the protein concentration was 1.181 µg/mL. The BZDH activity measured based on NAD-NADH reaction that was 9.4 U/mL. The purified BZDH showed optimum pH and temperature at 8.5 and 25 ºC, respectively. The BZDH lost 90% of activity after 16 hours incubation at 25 ºC. The BZDH activity had a sharp decline at the range of 40-50ºC that had lost around %40 of its activity and after that, the loss of activity is not significant. The steady state kinetics determined Km and Vmax, which were 4.2 mM, and 19.73 U/mL, respectively based on Linewaver Burk plot. The GC-MS analysis shown BZDH was able to convert benzaldehyde to benzene compounds.

Conclusion: The recombinant BZDH is able to utilize benzaldehyde to less toxic compounds, which is essential in biodegradation reactions.

Keywords: Benzaldehyde; Dehydrogenase; Rhodococcus Ruber; Kinetic