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Kinetic and Substrate Selectivity Analysis of Nitroreductase Enzyme of Metagenomic Origin

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Abstract: Nitroreductases are important class of biocatalysts involved in varied processes including biodegradation and detoxification of nitro compounds. We have applied functional metagenomics, a culture independent technique, to isolate a novel nitroreductase. The metagenome derived nitroreductase (NR1) was purified to homogeneity through Ni-NTA purification. To determine the substrate specificity and positional selectivity of NR1, the enzyme was assayed in the presence of various nitro aromatic compounds (as substrate). Michaelis-Menten kinetic constant km,Vmax and catalytic efficiency factor k_{cat}/k_m were determined to find the substrate specificity of NR1. Lowest k_m (2.43 µM) and highest k_{cat}/k_m 374 on 2,4,6 Trinitrotoluene (TNT) among tested substrate specifies that 2,4,6-TNT as the best substrate for NR1. Also highest Vmax values were obtained when 4-nitrophenol and 4-nitrotoluene is used as substrate. Enzyme NR1 did not show any reductase activity against the aromatic compounds with nitro substitution at 2nd position. Very less activity was found for 3-nitrobenzene, also very high km value was observed. By comparing the km and k_{cat}/k_m values it was clear that the enzyme showed lower k_m value for aromatic substrate carrying nitro group at para (4th) position when compared to nitro group at other position. The NR1 was found to be stable in the pH range of 6.0 to 9.0 and showed maximal activity at pH 8.0. Thermal stability studies on NR1 showed that the enzyme was stable at 40 °C for 2 hrs and optimal temperature for the enzymatic activity was found to be 30 °C. The isolated nitroreductase found to be a potential enzyme for application in reduction of nitro aromatic compounds.

Keywords*: Nitroreductase, Kinetics, Substrate Selectivity, Purification*

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