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Alkaline active cyanide dihydratase of *Flavobacterium indicum* MTCC 6936: Growth optimization, purification, characterization and *in silico* analysis

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Abstract

The present work explores a rare cyanide dihydratase of *Flavobacterium indicum* MTCC 6936 for its potential of cyanide degradation. The enzyme is purified to 12 fold with a yield of 76%. SDS and native-PAGE analysis revealed that enzyme was monomer of 40 kDa size. The enzyme works well in mesophilic range at wide array of pH. The thermostability profile of cyanide dihydratase revealed that the enzyme is quite stable at 30 °C and 35 °C with half-life of 6 h 30 min and 5 h respectively. K_m and V_{max} for cyanide dihydratase of *F. indicum* was measured to be 4.76 mM and 45 U mg^{-1} with k_{cat} calculated to be 27.3 s^{-1} and specificity constant (k_{cat}/K_m) to be around 5.67 mM^{-1} s^{-1}. MALDI-TOF analysis of purified protein revealed that the amino acid sequence has 50% and 43% sequence identity with putative amino acid sequence of *F. indicum* and earlier reported cyanide dihydratase of *Bacillus pumilus* respectively. Homology modeling studies of cyanide dihydratase of *F. indicum* predicted the catalytic triad of the enzyme indicating Cys at 164, Glu at 46 and Lys at 130th position. The purified enzyme has potential applications in bioremediation and analytical sector.

Keywords: Cyanide dihydratase, *F. indicum*, MALDI-TOF, Homology modeling