Cloning, expression and characterization of 3-hydroxyisobutyrate dehydrogenase from *Pseudomonas denitrificans* ATCC13867

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The gene encoding an NAD^+-dependent 3-hydroxyisobutyrate dehydrogenase (3HIBDH-IV) from *Pseudomonas denitrificans* ATCC 13867 was cloned and expressed in *Escherichia coli* BL 21 (DE3) and characterized to understand its physiological relevance in 3-hydroxypropionic acid (3-HP) degradation. The deduced amino acid sequence exhibited a high similarity to other 3-hydroxyisobutyrate dehydrogenase isozymes (3HIBDHs) of *P. denitrificans* ATCC 13867. The comparison of 3HIBDH-IV with its relevant enzymes indicates that Lys171 is important for catalytic function on 3-hydroxyacids. Purified 3HIBDH-IV is specific to (S)-3-hydroxyisobutyrate, but also catalyzes the oxidation of 3-HP to malonate semialdehyde. The half-saturation constant (K_m) for 3-HP was 1.0 mM and the specific activity of 3HIBDH-IV was 17 U/mg protein for 3-HP with NAD^+ as a cofactor at 30 ◦C and pH 9. The specific activity constant (K_{cat}/K_m) for the oxidation of 3-HP was estimated at 10.24×10^3 $M^{-1}S^{-1}$. The heavy metals such as Ag^+ and Hg_2^+ inhibited 3HIBDH-IV activity, whereas dithiothreitol, 2-mercaptoethanol and ethylenediamine- tetraacetic acid increased its activity by 1.5-1.8 fold.