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Alkaline phosphatase/phosphodiesterase from marine bacterium *Cobetia amphilecti* KMM 296

Key words: recombinant alcaline phosphatase, PhoD, phosphodiesterase, marine bacterium, Cobetia amphilecti, properties of phosphodiesterase

Alkaline phosphatases are widely distributed in nature and play a key role in the utilization of soluble phosphorus by hydrolytic cleavage of phosphate monoesters under alkaline conditions, releasing inorganic phosphate from many phosphate-containing compounds [2]. At present, three families of prokaryotic alkaline phosphatases (PhoA, PhoD and PhoX) are known. They differ in structure, substrate specificity, and dependent on different metal ions to exhibit their activity. It has been shown that phosphatase PhoD, belonging to the phosphatase/phosphodiesterase family, is more common in marine bacteria than PhoA and PhoX phosphatases that suggests its important role in marine microorganisms [3].

Using the specific primers based on the gene sequence encoding PhoD-like protein, which was found in the full-length genome of the marine bacterium *Cobetia amphilecti* KMM 296 (GenBank, no. JQJA00000000.1) [1], the recombinant salt-resistant metal-dependent phosphatase/phosphodiesterase CamPhoD with a specific activity 1.6 U/mg (0.025 M tris-HCl, pH 9.0, 2 mM $CoCl_2$, 2 mM $FeCl_3$; 15 mM p-NPP) was produced in the *Escherichia coli* cells Rosetta (DE3), and its physical and chemical properties were studied. The molecular weight of the subunit of the dimeric CamPhoD was 55 kDa. The enzyme was activated by Co^{2+}, Mg^{2+} and Fe^{3+} in a concentration of 2 mM and exhibited its maximal activity at pH 9.2. The Zn^{2+}, Cu^{2+}, Mn^{2+} ions, as well as EDTA and EGTA, did not significantly affect the activity of CamPhoD. The study of CamPhoD specificity revealed that the enzyme catalyze the phosphorus cleavage in the following range: TTP ≥dGMP ≥UTP ≥pNPP ≥CDP ≥TMP ≥bis-pNPP ≥5'-pNP-TMP. The optimal temperature for the exhibition of CamPhoD activity was 45 °C, while it was completely inhibited at 65 °C. The salts NaCl and KCl did not affect the activity of CamPhoD at concentrations up to 1 M, whereas an incubation mixture containing 1.5 M NaCl and KCl reduced the enzyme activity by 50% and 80%, respectively.

Thus, the marine bacterium *Cobetia amphilecti* KMM 296 gene has been confirmed to encode the functionally active extracellular phosphatase/phosphodiesterase of the PhoD family. For the first time the recombinant CamPhoD was obtained to explore its enzymatic properties.

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