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Cloning and expression of genes in vitamin B6 biosynthesis pathways from Geobacillus sp. H6a.

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Vitamin B6, a water soluble vitamin, is found in various forms, i.e., pyridoxine (PN), pyridoxal (PL), pyridoxamine (PM), and their phosphorylated derivatives. It is involved in the metabolism of all three macronutrients, proteins, lipids and carbohydrates. Recently, PM is a promising drug candidate for treatment of diabetic complications by inhibition of glycation reactions and the formation of advanced glycation end products (AGEs). The thermophilic bacterium, Geobacillus sp. H6a can produce extracellular vitamin B6 in PM and pyridoxamine 5- phosphate (PMP) forms. Two genes (*GhpdxS* and *GhpdxT*) encoding pyridoxal 5- phosphate (PLP) synthase in de novo biosynthesis pathway and *GhpdxK* gene encoding PL kinase in salvage pathway were amplified from *Geobacillus* sp. H6a by Touchdown PCR. They were inserted into the expression plasmid pET-28a. The *GhpdxS*, *GhpdxT*, and *GhpdxK* genes consist of 885, 591, and 810 nucleotides, respectively. After induction with 1 mM IPTG at 37°C, the fusion pdxS and pdxT proteins with His-tag were expressed as soluble protein in cytoplasm with molecular weight 32 kDa and 21 kDa respectively. While fusion pdxK protein was expressed in both of soluble protein and inclusion bodies with molecular weight 37 kDa. The soluble pdxS, pdxT and pdxK showed the activities of synthase, glutaminase, and PL kinase, respectively. The properties of these enzymes will be further investigated.

keywords : PLP synthase, PL kinase, Geobacillus, Vitamin B6, Biosynthesis pathways