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

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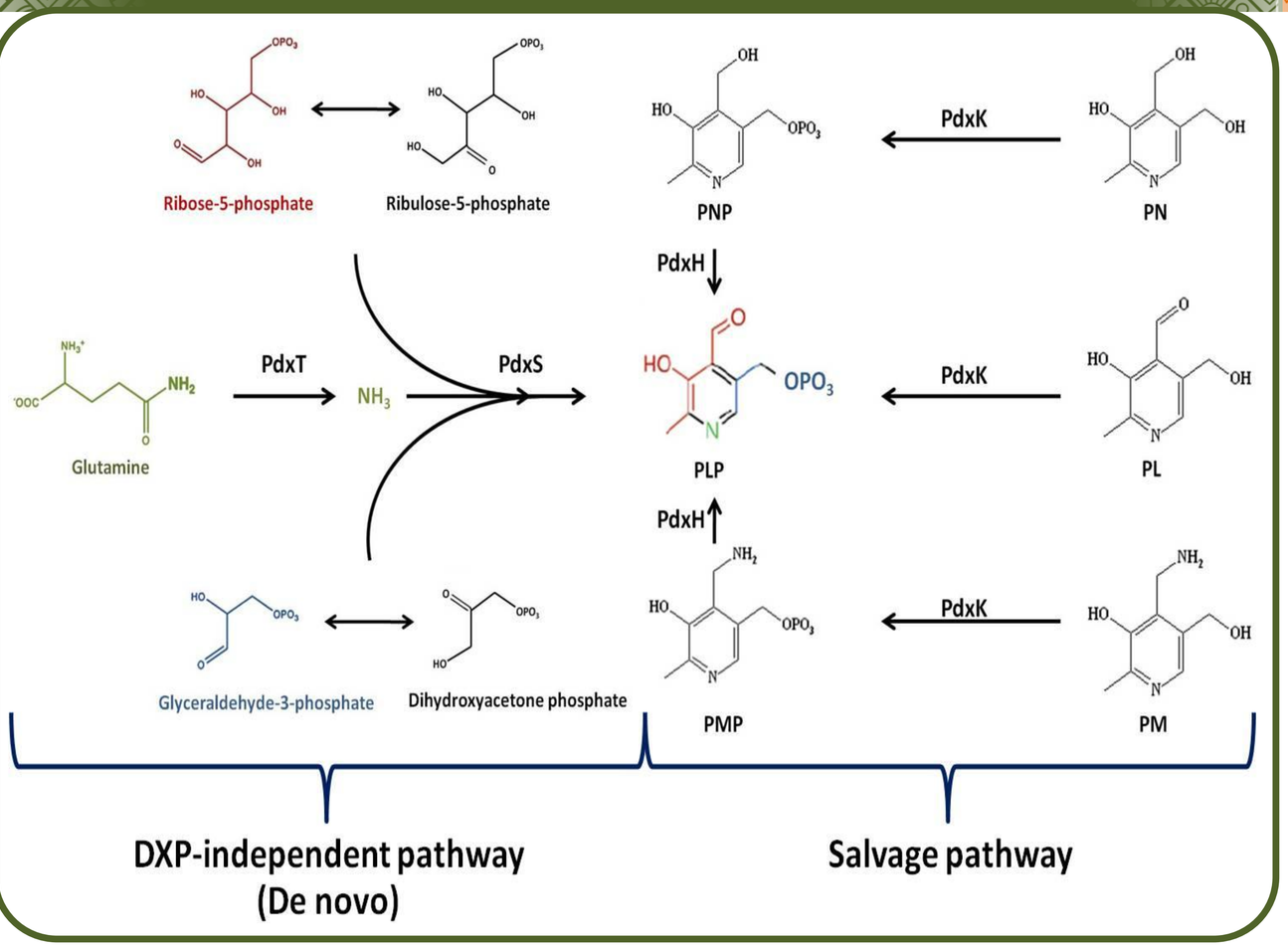
ABSTRACT

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.

INTRODUCTION



RESULTS

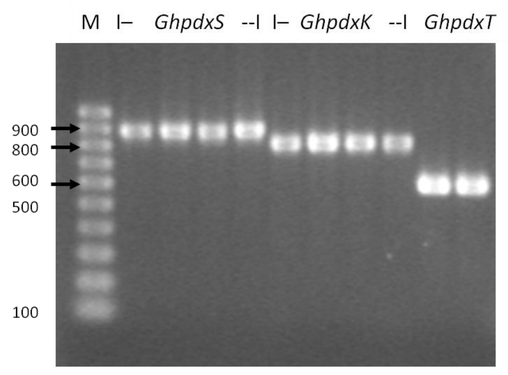


Figure 1. PCR products of *pdxS*, *pdxT* and *pdxK* genes from *Geobacillus* sp.H6a using touchdown PCR.

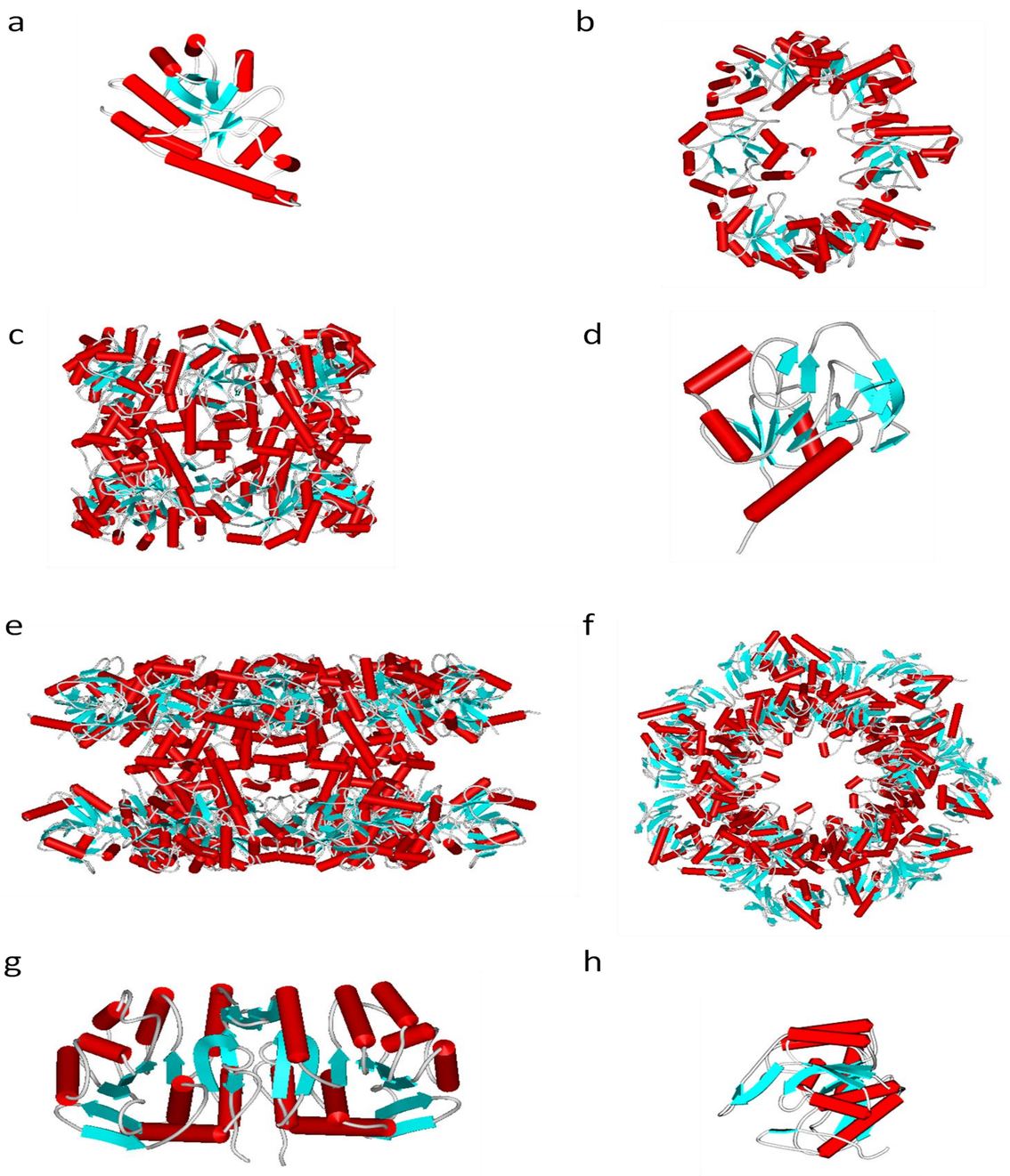


Figure 2. The putative 3D structure of GhPdxS , GhPdxT and GhpdxK established by homology-based modeling. a) The (β/α)8 barrels form of PdxS subunit (PDB 1ZNN). b) The hexameric ring of PdxS . c) Two hexameric rings of PdxS with the active sites positioned on the inside (PDB 1ZNN.pdb1). d) The PdxT subunit (PDB 1Q7R). e) PLP synthase complex contains a dodecamer of PdxS and pdxT ($PdxS_{12}$:$PdxT_{12}$) in lateral view (PDB 2NV2). f) PLP synthase complex in top view. g) Dimeric subunit of PdxK (PDB 2I5B). And h) Monomeric subunit of PdxK.

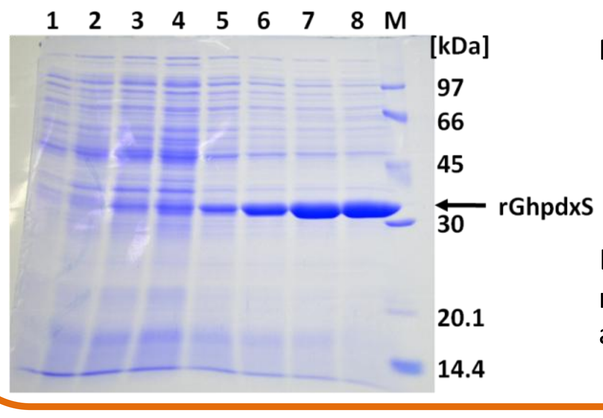


Figure 3. SDS gel electrophoresis in 12% polyacrylamide gel of the crude extract of rGhpdxS obtained from induced and uninduced *E.coli* BL21 (DE3) pET28a(+)-*GhpdxS.*

Lane 1-4: uninduced cultures at 0, 1, 3 and 6 h, respectively. Lane 5-8: induced cultures at 0, 1, 3 and 6 h, respectively. Lane M: protein marker.

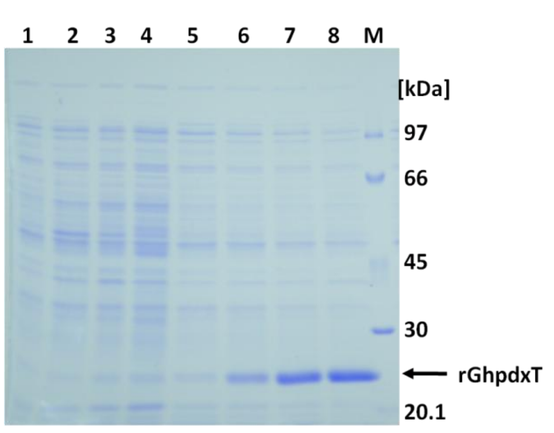


Figure 4. SDS gel electrophoresis in 12% polyacrylamide gel of the crude extract of rGhpdxT obtained from induced and uninduced *E.coli* BL21 (DE3) pET28a(+)-*GhpdxT.*

Lane 1-4: uninduced cultures at 0, 1, 3 and 6 h, respectively. Lane 5-8: induced cultures at 0, 1, 3 and 6 h, respectively. Lane M: protein marker.

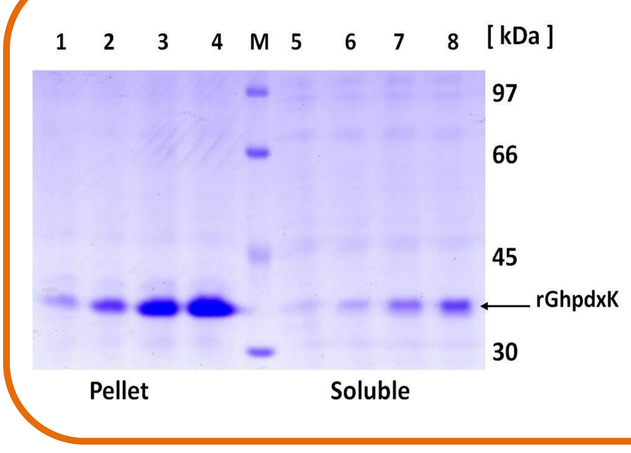


Figure 5. SDS gel electrophoresis in 12% polyacrylamide gel of the crude extract of rGhPL kinase obtained from induced in *E.coli* BL21 (DE3) pET28a(+)-*GhpdxK.*

Lane 1-4: Pellet induce cultures at 0, 1, 3 and 6 h, respectively. Lane M: protein marker. Lane 5-8: Soluble induced cultures at 0, 1, 3 and 6 h, respectively.

References

1. Strohmeier M, Raschle T, Mazurkiewicz J, Rippe K, Sinning I, Fitzpatrick TB, Tews I. (2006) PNAS 103:51:19284-19289.
2. Newman JA, Das SK, Sedelnikova SE, Rice DW. (2006) J.Mol.Biol. 363: 520-530.
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