E321 Characterization of a Poly(3-hydroxybutyrate) Depolymerase from *Penicillium pinophilum*

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Purified extracellular poly(3-hydroxybutyrate)(PHB) depolymerase from *Penicillium pinophilum* ATCC 9644 was characterized. PHB depolymerase secretion was increased by fungus at 25℃ higher than at 30℃ and 37℃ after 8 days, but fungus was grown slowly at 25℃. The isolated enzyme was composed of a single polypeptide chain with a molecular mass of about 35kDa as determined by SDS-PAGE. The optimum temperature for PHB depolymerase activity was 50℃. The enzyme was stable over pH 2.0 but unstable at pH 1.0. 0.5mM Fe^{2+} have little or no effect on enzyme activity, 1mM, 2mM and 4mM Fe^{2+} were inhibited about 50%, 85% and 95%, respectively. Effect of various chemicals on PHB depolymerase was examined. 10% SDS have no effect, and 1M Urea and 10mM EDTA have little effect on enzyme activity. 30% EtOH and 10% 2MSH were inhibited about 40% and 60%, respectively.

E322 *Escherichia coli* Dihydroneopterin Triphosphate 2'-epimerase : Gene Organization, Regulation, and Construction of Deletion mutant

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L-*threo*-monapterin is the major pterin in *E.coli*. Dihydroneopterin triphosphate 2'-epimerase is an enzyme catalyzes that the epimerization of dihydroneopterin triphosphate (H_2NTP) to dihydromonapterin triphosphate (H_2MTP), the precursor of monapterin. The gene was recently cloned by using a simple polymerase chain reaction approch with degenerate oligonucleotide primers designed from the N-terminal amino acid sequence. The ORF encoding H_2NTP 2'-epimerase(*mpsA*) and another ORF(*mpsB*) at 3'-downstream region constitute an operon, and named as MPS operon. The expression of H_2NTP 2'-epiemrase in pMPS is much higher than pEPIFL containing only *mpsA*. 3'-deletion analysis revealed gradual reduction in the synthesis of MpsA protein suggesting that *mpsB* region is involved in the high expression of MpsA. However, the transcomplementation experiment with MpsB indicate that MpsB protein is not trans-activator for MpsA synthesis. Although monapterin is the major pterin in *E.coli*, its *in vivo* role is not known. In *E.coli*, the epimerase may function as a regulator system for folic acid biosynthesis or may play a role in regulating GTP concenetration. In an attempt to test these hypotheses, a null mutant strain was constructed by using gene replacement method. It was constructed by homologous integration and segragation of a ColE1-derived recombinant plasmid containing the kanamycin resistance gene for selection, in a temperature-sensitive *polA* strain. Characterization of these mutants are currently underway.