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Molecular Analysis of Phosphate Metabolism-Related Genes in *Serratia marcescens*

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To understand the mechanism of phosphate accumulation, a gene encoding PPK was cloned from the genomic library of *Serratia marcescens* by Southern hybridization using *E. coli ppk* gene as a probe. One positive plasmid was obtained. The recombinant plasmid DNA has 8.5 kb fragment and was designated pDH3. Several recombinant subclones constructed from pDH3. The nucleotide sequence of a 4.0 Kb segment was determined and two open reading frames were detected. The ORF1 and ORF2 encoded the PPK polypeptide and truncated PPX polypeptide, respectively. A gene encoding PPX was found downstream region of the gene for *ppk,* and transcription of the *ppx* gene depends on the *ppk* promoter, indicating a polyphosphate (polyP) operon. Potential CRP binding site and *pho* box sequence were found in the upstream of the putative promoter in the regulatory region. Analysis of the nucleotide sequence showed that homology in their amino acid sequences between PPK proteins was 90%, 93%, and 87% in *E. coli, Yersinia pestis,* and *Klebsiella* sp. respectively. The *ppk* gene product, a polypeptide of 75 kDa, was confirmed by SDS-PAGE. The expression of PPK protein resulted in the formation of inclusion bodies and increased at 23℃. The *E. coli* strain harboring plasmid pSPK5 with *ppk* gene increased enzyme activity of polyphosphate kinase. For cloning of the complete *ppx* gene, remaining *ppx* gene was cloned from the genomic library of *Serratia marcescens by* southern

hybridization using partial *ppx* gene of pDH3 as a probe. The recombinant plasmid DNA has 4.5 kb fragment and was designated pCX1. Analysis of the nucleotide sequence showed that homology in there amino acid sequence between PPX proteins was 89%, 77% and 76% in *Yersina pestis, E. coli and Salmonella typhimurium*, respectively. The *ppx* gene product, a polypeptide of 58KDa was confirned by SDS-PAGE. The *E. coli* strains harboring recombinant plasmids with *ppk* and *ppx* gene were increased to accumulation of polyphosphate.

In a previous study, recombinant plasmid DNA, pDH3 obtained from genomic library of *Serattia marcescens* and several recombinant subclones constructed from pDH3. One of subcloned gene, pPH7, was further analyzed. The nucleotide sequence of a 5,077 bp segment of the pPH7 was determined and three open reading frames was detected. The three ORFs encoded the *pstC, pstA, pstB*, which were *pst* (phosphate specific transport) operon. In the case of *S. marcescens*, there are three ORFs (*pstC, pstA, pstB*) presumably forming an operon and same direction of transcription. Comparison of the *pst* operon of *S. marcescens* with that of other organism revealed that the genes to *pst*S and *pho*U is missing. Potential CRP bonding site and *pho* box sequence were found in the upstream of the putative promoter at the regulatory region. Analysis of the nucleotide sequence showed that homology in amino acid sequence between PstC protein and *Yersnia* sp., *Vibrio* sp. and *Pseudomonas* sp. were 49, 37 and 33%, respectively. PstA protein and *Yersnia* sp., *Vibrio* sp. and *Pseudomonas* sp. were 64, 51, and 47%, respectively. PstB protein and *Methanococcus* sp., *E. coli* and *Mycoplasma* sp. were 60, 50 and 48%, respectively. The *pstC* gene product, a polypeptide of 74 kDa was confirmed by SDS-PAGE. The *E. coli* strain harboring plasmid pPH7 with *pst* genes was increased to transport of phosphate.

A bacterium having high abilities to solubilize inorganic phosphate was isolated from cultivated soils. The strain was identified as *Pseudomonas cepacia* DA23, based on the physiological and biochemical properties. The optimum temperature and initial pH to solubilize insoluble phosphate in sucrose minimal medium were 26℃ and pH 5.0, respectively. In these conditions, phosphate-solubilizing activities of the strain against two types of insoluble phosphate were quantitatively determined. When glucose was used as a carborn source, the strain had a marked mineral phospahte solubilizing activity. Inorganic phospahte solubilization was directly related to the pH drop by the strain. Analysis of the culture medium confirmed the production of gluconic acid as one of the main organic acids released by *Pseudomonas cepacia* DA23. In the previous chapter 1 and 2, the phosphate accumulatiom-related genes were cloned from *S. marcescens* and expressed in *E. coli.* To compare the Pi uptake between *E. coli* and PSM, *P. cepacia* DA23, the recombinant plasmids, pSHK5, pSHX6, pSHKX6, pSPH7 were transferred into *P. cepacia* DA23. Strain *P. cepacia* DA23 harboring pSHK5 and pSHKX6 removed about 90% of phosphate in the medium, sixfold more Pi from the medium than did the control strain. These results indicate the potential for genetic improvement of more useful microorganosms for enhanced Pi removal.