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Gene cloning and expression of the *Pseudomonas aeruginosa* periplasmic phosphate-binding protein

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1. SUMMARY

The *pstS* gene, encoding the *Pseudomonas aeruginosa* phosphate-binding protein, was cloned onto a cosmid vector into *Escherichia coli,* and localized by subcloning, mapping the insertion site of Tn*501* in a *P. aeruginosa pstS*::Tn*501* mutant, and hybridization to an oligonucleotide pool synthesized according to the aminoterminal amino acid sequence of the purified protein. The cloned *pstS* gene was transferred to *P. aeruginosa pstS* mutants and was shown to complement these mutants. The *P. aeruginosa* phosphate-binding protein was also expressed and secreted into the periplasm of *E. coli pstS* mutants.

2. INTRODUCTION

Pseudomonas aeruginosa, like *Escherichia coli* [1,2], possesses an inducible high affinity phosphate specific transport (PST) system [3,4]. A key component of this transport system has been demonstrated to be a periplasmic phosphate-binding protein, since mutants lacking this protein are unable to transport phosphate via the PST system [2,4,5]. In this communication we have cloned the structural gene of the *P. aeruginosa* phosphate-binding protein into *E. coli* and obtained expression in both *E. coli* and *P. aeruginosa* phosphate-binding protein deficient mutants.

3. MATERIALS AND METHODS

3.1. Bacterial strains and media

P. aeruginosa PA01 strains used included the wild type strain H103 [6] and its transposon-induced and diethyl sulphate-induced phosphate-binding protein-deficient derivatives H574 *pstS*::Tn*501* [7] and H585 *pstS* [4], respectively. *E. coli* K-12 strains included strain LE392 F^-, *hsdR*-514 (r_{k^-}, m_{k^-}), *supE*-44, *supF*-58, *lacY*-1 or Δ(*lacIZY*), *galK*-2, *galT*-22, *metB*-1, *trpR*-55, λ^- [8], strain C86 (CGSC5009) HfrC *relA*-1, *tonA*-22, *pit*-10, *spoT*, $T2^r$, *pho*-21, and strain ANCC75 *leu, LacY, purE, trp, hrs, argG, rpsL, metA, thi, pstS*-164 [5]. Media utilized for maintenance of strains, growth under phosphate-sufficient or deficient conditions, and selection of plasmids with antibiotic resistance makers were as described previously [9].

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3.2. *Cloning of the* pstS *gene*

DNA procedures utilized were as described by Maniatis et al. [10] with additional methods as described previously [9]. Chromosomal DNA was isolated from strain *P. aeruginosa* H574 *pstS*::Tn*501* and digested with *Pst*I (since Tn*501* contains no *Pst*I sites [11]). The digest was separated on a 10–40% sucrose gradient and the fraction containing an 11.4 kilobase (kb) *Pst*I fragment that hybridized to a radiolabeled Tn*501* sequence, was collected. This DNA was ligated to *Pst*I-digested plasmid pBR325 and used to transform competent *E. coli* strain LE392 cells. A maximum of 10^6 cells was spread on Luria broth agar plates containing 15 μg/ml $HgCl_2$. Colonies from these plates were screened for ampicillin sensitivity and chloramphenicol resistance. The colonies with the correct phenotype contained a plasmid, pPG-1, with the desired Tn*501*-containing *Pst*I fragment. A 3.4 kb *Eco*RI fragment of *P. aeruginosa* chromosomal DNA flanking the Tn*501* insertion was used as a probe for our gene bank. The gene bank contained *P. aeruginosa* chromosomal DNA partially digested with *Eco*R1 inserted into the cosmid pLAFR1 [15], in *E. coli* strain LE392. Plasmid pPBP1, which hybridized with the 3.4 kb *Eco*R1 fragment, was isolated from this bank.

3.3. *Amino-terminal amino acid specific probe*

The purified phosphate-binding protein [4] was subjected to 20 cycles of automated amino-terminal sequencing by S. Kielland, U. of Victoria, Canada to yield the sequence -I-PALPEYQ-KASGVSTNLL (a dash indicates the identity of the amino acid at this position was unclear). A collection of 32 synthetic 23 mer oligonucleotides (5′ GC$^{G}_{C}$CT$^{C}_{C}$CC$^{G}_{C}$GA$^{C}_{A}$TACGA$^{G}_{A}$AAGGC 3′) was deduced from amino acids 5–12, taking into account codon bias in *P. aeruginosa*. This oligonucleotide pool was radiolabelled as previously described [9].

3.4. *Other assays*

Osmotic shock procedures for *P. aeruginosa* [12] and *E. coli* [13] were utilized to release periplasmic components. ^{32}Orthophosphate (New England Nuclear, Quebec, Canada) transport assays were as described previously [4]. Antisera to purified phosphate binding protein was obtained by the published protocol [4]. SDS polyacrylamide gel electrophoresis [6] and Western immunoblotting [14] were performed as described previously.

4. RESULTS AND DISCUSSION

Plasmid pPBP1 (Fig. 1) was isolated as described above from a gene bank of *P. aeruginosa* chromosomal DNA partially digested with *Eco*RI and inserted into the cosmid pLAFR1 [15] in *E. coli* strain LE392. This plasmid, when transformed into *E. coli* K-12 strain C86, expressed high levels of *P. aeruginosa* phosphate binding protein (Fig. 2). The *pstS* gene encoding this protein was localized as follows. Firstly subcloning experiments demonstrated that subclones pPBP2 and pPBP3 both encoded the *P. aeruginosa* phosphate binding protein, thus demonstrating that the *pstS* gene was present on the 5.6 kb *Eco*R1-*Hind* III fragment of plasmid pPBP3 (Fig. 1). Secondly the site of transposon Tn*501* insertion in the phosphate-binding protein-deficient *P. aeruginosa* mutant

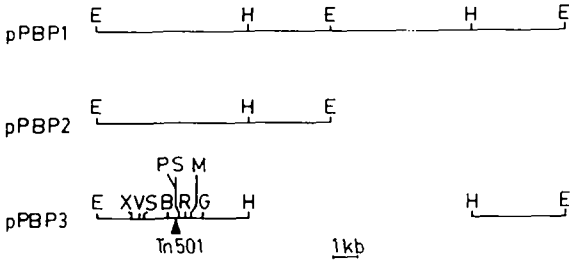

Fig. 1. Restriction maps of *P. aeruginosa* DNA inserts of plasmids containing the cloned *pstS* gene. All inserts were inserted into the *Eco*RI site of the cosmid pLAFR1. A detailed restriction map is shown only for pPBP3. Plasmid pPBP1 was the original clone obtained as described in MATERIALS AND METHODS. pPBP2 was obtained by subcloning one of the two *Eco*RI fragments of pPBP1 whereas pPBP3 was obtained by deleting a 10.2 kb *Hin*dIII fragment from plasmid pPBP1. The site of insertion of Tn*501* in *P. aeruginosa* strain H574 *pstS*::Tn*501* DNA is indicated by a triangle. Enzyme sites are indicated by a one letter designation as follows: E, *Eco*RI; X, *Xho*II; V, *Pvu*II; S, *Sal*I; B, *Bam*HI; R, *Eco*RV; *Sma*I; G, *Bgl*II; H, *Hin*dIII; P, *Pst*I.

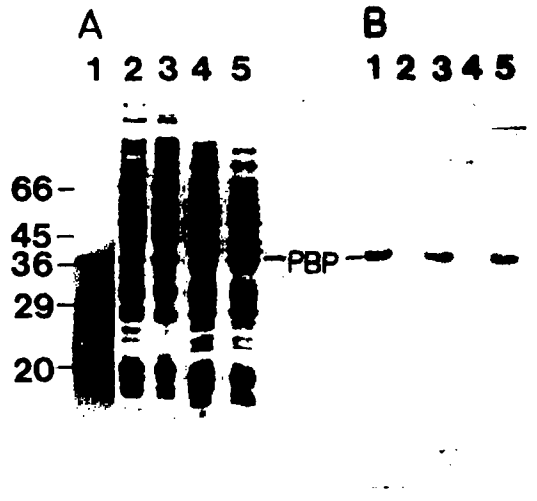

Fig. 2. SDS-polyacrylamide gel electrophoretogram and corresponding Western immunoblot of cell lysates of various bacterial strains containing or lacking plasmid pPBP1. Panel A is the Coomassie blue-stained SDS electrophoretogram. Panel B is the immunoblot of the identical samples seen in panel A probed with phosphate-binding protein specific polyclonal sera at a 1:200 dilution. Lanes 1, purified phosphate-binding protein; lanes 2, *P. aeruginosa* H574; lanes 3, *P. aeruginosa* H574/pPBP1; lanes 4, *E. coli* C86; lanes 5, *E. coli* C86/pPBP1. Molecular weight markers (in thousands) are indicated on the left. The position of *P. aeruginosa* phosphate-binding protein is indicated by PBP. All cell lysates were obtained from cultures grown under phosphate-limiting conditions.

H574, was mapped to a unique site within this 5.6 kb insert (Fig. 1). Finally an oligonucleotide pool synthesized on the basis of the amino terminal sequence of *P. aeruginosa* phosphate-binding protein hybridized uniquely to the 1.5 kb *Bam*HI-*Bgl*II restriction fragment straddling the Tn*501* insertion site in plasmid pPBP3 (Fig. 1). However, some portion of the *pstS* gene lay outside of this fragment since subclones containing only this fragment in the plasmid pUC18 did not express *P. aeruginosa* phosphate-binding protein.

Plasmids bearing the cloned *P. aeruginosa pstS* gene were transformed into the *E. coli* K12 phosphate-binding protein-deficient [5,16] strains ANCC75 *pstS*-164 and C86 *pho*-21. In addition, plasmid pPBP1 was conjugated, with the aid of the helper plasmid pRK2013 [17] into *P. aeruginosa* phosphate-binding protein-deficient mutant strains H574 *pstS*::Tn501 and H585 *pstS*. The resultant plasmid-carrying strains were grown under phosphate-limiting conditions to ensure derepression of the production of phosphate-binding protein, and cell lysates were examined by SDS polyacrylamide gel electrophoresis and on Western immunoblots using phosphate-binding protein-specific sera obtained from immunized rabbits (Fig. 2). Phosphate-binding protein having the same molecular weight and antigenic properties as authentic phosphate-binding protein was observed in all *P. aeruginosa* and *E. coli* plasmid-bearing strains but not in the original mutants (as shown for strains C86 and H574 and their plasmid pPBP1-containing derivatives in Fig. 2). In the case of *P. aeruginosa* strain H574/pPBP1 it was confirmed by phosphate transport assays that the phosphate-binding protein expressed from this plasmid reconstituted phosphate transport to the level observed in the wild type strain H103 *pstS*$^+$.

The phosphate-binding protein of *P. aeruginosa* is normally found in the periplasmic space of this organism [4]. To determine if the phosphate-binding protein encoded by plasmid pPBP1 was also located in the periplasm of both *E. coli* C86/pPBP1 and *P. aeruginosa* H574/pPBP1, these strains were subjected to osmotic shock procedures optimized for the individual organisms. The resulting periplasmic fractions were examined by SDS polyacrylamide gel electrophoresis and Western immunoblot analysis (Fig. 3). In both the *E. coli* and *P. aeruginosa* strains as well as strain H585 (pPBP1 (data not shown), the *P. aeruginosa* phosphate-binding protein expressed from plasmid pPBP1 was found in the osmotic shock fluid, suggesting that in each case, the phosphate-binding protein was properly processed to reach its native location in the periplasm.

In this communication we have described the cloning of the structural gene of the *P. aeruginosa* phosphate-binding protein and the expression of the gene product in the periplasm of both *E. coli* and *P. aeruginosa* phosphate-binding protein deficient mutants. The electrophoretic and immunological characteristics of the protein expressed by the *E. coli* clone were identical to the purified protein from *P. aeruginosa* (Fig. 2 and 3). This indicates that the signals for synthesis, assembly

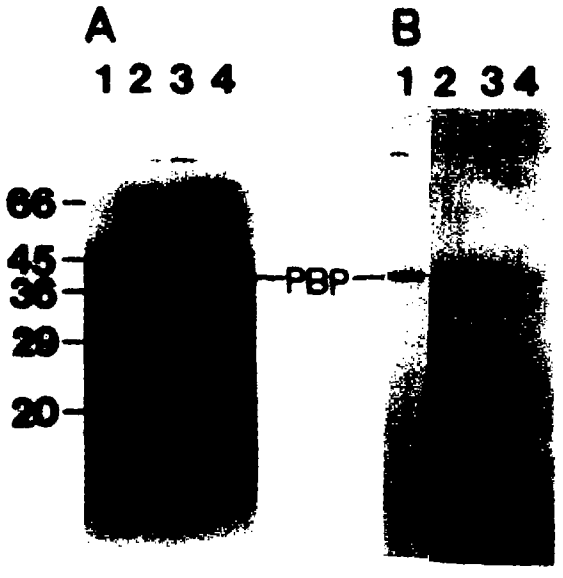

Fig. 3. SDS-polyacrylamide gel electrophoretogram and corresponding Western immunoblot of osmotic shock fluids. Panel A is the Coomassie blue-stained electrophoretogram. Panel B is the immunoblot of the same samples probed with phosphate-binding protein specific polyclonal rabbit sera at a 1:200 dilution. Lanes 1, purified phosphate-binding protein; lanes 2, *P. aeruginosa* H103 osmotic shockate; lanes 3, *P. aeruginosa* H574/pPBP1 osmotic shockate; lanes 4, *E. coli* C86/pPBP1 osmotic shockate. Molecular weight markers (in thousand) are indicated on the left. The position of phosphate-binding protein (PBP) is indicated.

and translocation of this *p. aeruginosa* protein to the periplasmic space must function in *E. coli*.

Based on the aminoterminal amino acid sequences, there appears to be some homology between residues 4–11 (PALPEYQK) of the *P. aeruginosa* phosphate-binding protein and residues 11–17 (PA-PVYAK) of the equivalent *E. coli* protein [5]. Despite this, we have been unable to demonstrate to date that the *P. aeruginosa* phosphate-binding protein actually can complement *E. coli pstS* mutants. Nevertheless, it will be of some interest to obtain the sequence of the *P. aeruginosa pstS* protein to determine the full extent of evolutionary conservation of this gene product.

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