A Bacillus subtilis gene cluster similar to the Escherichia coli phosphate-specific transport (pst) operon: evidence for a tandemly arranged pstB gene

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We have determined the complete nucleotide sequence of the Bacillus subtilis homologues of the Escherichia coli phosphate-specific transport (pst) genes in the framework of the international B. subtilis genome sequencing project. The pst genes in E. coli form an operon arranged in the order pstS, pstC, pstA, pstB and phoU. In the case of B. subtilis, there are also five ORFs presumably forming an operon. The deduced amino acid sequences of the products of these ORFs show striking similarities to their E. coli counterparts. Comparison of the organization of the pst operon of B. subtilis with that of E. coli revealed that the gene corresponding to phoU is missing, while there are two genes homologous to pstB in B. subtilis. The pst operon is located at 222° on the B. subtilis chromosome.

Keywords: Bacillus subtilis, genome sequencing project, phosphate-specific transport operon, pstB, tandem genes

The phosphate-specific transport (Pst) system of Escherichia coli comprises four distinct subunits encoded by the pstS, pstA, pstB and pstC genes (Amemura et al., 1985; Surin et al., 1985). These genes, together with the phoU gene, form the pst operon, which maps at about 84 min on the E. coli chromosome (Bachmann, 1990). The nucleotide sequences of all five genes have been determined, and the amino acid sequences of the corresponding proteins have been deduced (Amemura et al., 1985; Surin et al., 1985). Apart from transporting phosphate, the Pst system plays an important role in the regulation of a number of coordinately regulated genes collectively referred to as the phosphate regulon (Wanner, 1987; Wanner & Letterel, 1980).

As part of the international Bacillus subtilis sequencing project, we report here the cloning and sequencing of B. subtilis homologues of the E. coli pst gene products.

A λ phage library of B. subtilis strain JH642 (trpC2 pheA1)

The DDBJ accession number for the nucleotide sequence reported in this paper is D58414.

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Fig. 1. For legend see facing page.
Nucleotide sequence of *B. subtilis* *pst* operon

It was demonstrated that *E. coli* PhoB protein, a response regulator of the bacterial two-component regulatory system, bound to the *pho* box and activated the transcription of *pst* operon *in vitro* (Makino et al., 1988). We believe that the *B. subtilis* *pho* box-binding protein will recognize these sequences and regulate the transcription of this operon.

The deduced amino acid sequence of ORF108 shows 20.7% identity to the PstS protein, which is a phosphate-binding protein located in the periplasmic space (Gerdes & Rosenberg, 1974). The ORF72 product has 26.1% identity to the PstC protein, and the protein encoded by ORF73 has 27.2% identity to the PstA protein. The PstA and PstC proteins are hydrophobic and likely to form the transmembrane portion of the Pst system (Amemura et al., 1985; Surin et al., 1985). The hydropathy plots of the ORF72 and ORF73 products show significant similarities to those of their *E. coli* counterparts (data not shown). Importantly, the ORF74 and ORF75 products show 58.6% identity with each other and 57.3% identity to the PstB protein (Fig. 2). The PstB protein is hydrophilic and is likely to interact on the cytoplasmic side with the PstA and PstC proteins. Two key residues (Gly-48 and Lys-49) have been shown to be required for phosphate transport by the Pst system (Cox et al., 1989) and are located in the conserved sequence associated with a nucleotide-binding site (Higgins et al., 1985).

Comparison of the organization of the *pst* operon of *B. subtilis* and that of *E. coli* is represented schematically in Fig. 3. It is very interesting that the gene corresponding to *phoU*, which is involved in the regulation of the phosphate regulon in *E. coli* (Wanner, 1987), is not present in the *B. subtilis* *pst* operon, while there are two genes homologous to *pstB* in *B. subtilis*. The location of *phoU* gene homologue in *B. subtilis* is not known at present.

**Fig. 1.** Complete nucleotide sequence of the *B. subtilis* *pst* operon. Potential RBSs for the ORFs are underlined. Putative -35 and -10 promoter regions are doubly underlined. A potential rho-independent transcription terminator is indicated by arrows. The deduced amino acid sequences of the ORFs are shown in single-letter code with the respective termination codons indicated by asterisks. Two boxed sequences represent putative *pho* boxes. The consensus sequence for the *pho* box of *E. coli* consists of the 18 bp sequence 5'-CT(G/T)CTATA(A/T)(A/T)GTCA(C/T)-3' (Makino et al., 1986). All sequences were determined using the Taq Dye Primer Cycle Sequencing Kit and the 373A sequencer from Applied Biosystems as described previously (Takemaru et al., 1995).

**Fig. 2.** Alignments of the amino acid sequences of the predicted products of ORFs 74 and 75 with *E. coli* PstB. The two key residues (Gly-48 and Lys-49) of the *E. coli* PstB protein, which have been shown to be important for phosphate transport (Cox et al., 1989), are underlined. Hyphens indicate gaps introduced to improve the alignments. Asterisks and dots represent identical and conserved amino acid residues, respectively. The amino acid sequence of *E. coli* PstB is from Amemura et al. (1985) and Surin et al. (1985).

**Fig. 3.** Comparison of the organization of the *pst* operon of *B. subtilis* with that of *E. coli*. The percentage of identical amino acids between corresponding proteins is shown. Sizes of ORFs are to scale. Numbers below the gene name refer to the number of amino acid residues.
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REFERENCES


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