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*)

chromosomal DNA was constructed as described previously (Takemaru *et al.*, 1995). The library was screened using as a probe plasmid pNEXT27, which is one of the *NotI* linking clones containing a DNA insert located at 222° on the *B. subtilis* chromosome (Itaya & Tanaka, 1991). With this probe, several recombinant λ phage clones covering the entire region of the *B. subtilis* *pst* operon were isolated. Sequencing was performed by a strategy based on the combination of PCR and the random sequencing method, i.e. preparation of a random library, amplification of inserts subcloned in M13 phages by PCR, sequencing of randomly selected inserts, and filling of the gaps by region-specific primers (Ogasawara *et al.*, 1994). The entire DNA sequence was determined on both strands to ensure high accuracy.

The complete nucleotide sequence of *B. subtilis* *pst* operon is shown in Fig. 1 with the deduced amino acid sequences of five ORFs. These ORFs are transcribed in the same direction, and each is preceded by a putative ribosome-binding site (RBS) appropriately spaced from a presumed ATG initiation codon. The canonical vegetative promoter sequence is found upstream of ORF108. A perfect 15 bp inverted repeat that is a candidate for a transcriptional termination site is found downstream of ORF75. In addition, two sequences showing similarities with *E. coli* *pho* boxes are observed in the upstream region of ORF108.

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Abbreviations: Pst, phosphate-specific transport; RBS, ribosome-binding site.

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


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ORF74 1 MSEQVKEKPERAIVIPKQNHVLEVKDLSIYYGNQAVHVNMDIEKNAVTALIGPSGCG
PstB 1 MSNVETAPSKIQVRNLNFIYKGFHALKNINLDIAKNQVTAFIGPSGCG
ORF75 1 MSIAATEAVMKQEVYQVNGMNLWYQGHALKNINLSIYENEVTAIIGPSGCG

ORF74 61 KSTFLRNINRMNDLIPSAEAGEITLLEGNILGNNINVSRLREIGMVFPQKPNFPKSIY
PstB 49 KSTLLRTFNKMPFELYEQRAEGEILLDGNILNLSQDIALLRKAVGMVFPQKPTFPMSIY
ORF75 52 KSTFIKTNLNMIQMTPNVKLAGELWYNGSNILKDKVDIVDLRKNIGMVFPQKGNFPQSIF

ORF74 121 ANITHALKYAGERNKAVLDEIVEESLTKAALWDEVKDRHLSSALSLSGGQQQLCIARTL
PstB 109 DNIAFGVRLFEKLSRADMDERVQWALTKAALWNETKDKLHQSGYSLSGGQQQLCIARGI
ORF75 112 DNVAYGPRVHGTKNKKIKQIEVKSLLKDALWDEVKDRHLTSALSLSGGQQQLCIARAL

ORF74 181 AMKPAVLLLDPEASALDPISNAKIEELITGLKREYSIIIVTHNMQALRVSDRTAFFLNG
PstB 169 AIRPEVLLLDPECSALDPISGRIEELITELKQDYTVVIVTHNMQQAARCSDDHTAFMYLG
ORF75 172 ATNPDIILLMDPEPSALDPISRKIEELILELKDQYTVIVTHNMQQAARVSDQTAFFYMG

ORF74 241 ELVEYQTEQIFTSPPKQKQTEYDINGKFG
PstB 229 ELIEFSNTDDLFTKPAKKQTEYDITGRYG
ORF75 232 ELVECDNTNKMFSNPKDQRTLDYISGRFG

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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).

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 with 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). Interestingly, 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). Importantly, these two residues are also conserved in the ORF74 and ORF75 products (Fig. 2).

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. 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.

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)TCATA(A/T)A(A/T)CTGTCA(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).

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