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The *pst* Operon of *Bacillus subtilis* Has a Phosphate-Regulated Promoter and Is Involved in Phosphate Transport but Not in Regulation of the Pho Regulon

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Genes from *Bacillus subtilis* predicted to encode a phosphate-specific transport (Pst) system were shown by mutation to affect high-affinity P_i uptake but not arsenate resistance or phosphate (Pho) regulation. The transcription start of the promoter upstream of the *pstS* gene was defined by primer extension. The promoter contains structural features analogous to the *Escherichia coli pst* promoter but not sequence similarity. Expression from this promoter was induced >5,000-fold upon phosphate starvation and regulated by the PhoP-PhoR two-component regulatory system. These data indicate that the *pst* operon is involved in phosphate transport and is a member of the Pho regulon but is not involved in P_i regulation.

Escherichia coli has two major P_i transport systems (24, 29, 30, 35, 44). The Pst (phosphate-specific transport) system is a high-affinity, low-velocity, free-P_i transport system which is structurally similar to ABC transporters (3). The Pit (phosphate inorganic transport) system is a divalent metal transporter for which P_i (40) or arsenate (48) can serve as the anion (39). Additionally, several organophosphate transport systems result in P_i uptake in *E. coli* (42).

Recently, the genes encoding proteins homologous to those of the *E. coli pst* gene products were cloned and sequenced in *Bacillus subtilis* (36). Five cistrons with homology to genes in the *E. coli pst* operon were named *pstS*, *pstC*, *pstA*, *pstB1*, and *pstB2*. A putative promoter sequence was located 5′ of the *pstS* gene, and a potential rho-independent transcription terminator ($\Delta G = -18.4$ kcal/mol) was observed 3′ of *pstB2*.

The *pst* operon of *E. coli* and the proposed *pst* operon of *B. subtilis* encode proteins similar to ABC transporters (3, 45), which are composed of a binding protein (similar to PstS), two integral inner membrane proteins (similar to PstC and PstA), and an ATP binding protein (similar to PstB for *E. coli* or PstB1 and PstB2 for *B. subtilis*). The *pstB2* gene is at the 3′ end of the operon in the position of *phoU* in *E. coli*. PhoU, which has no similarity to ABC proteins, is not in the *pst* operon of *B. subtilis*.

The first four genes in the *pst* operon of *E. coli*, i.e., *pstS*, *pstC*, *pstA*, and *pstB*, are required for phosphate transport; mutations in any of these genes abolish P_i uptake (6, 33, 46) when the concentration of P_i is low. The *pst* operon also has a role in the regulation of Pho regulon genes (1, 43, 46) in that *phoU* is required for the repression of the Pho regulon but is apparently not required for phosphate transport through the Pst system (25, 34, 50).

E. coli genes induced by phosphate starvation and controlled by *phoB-phoR* constitute the Pho regulon (21, 22, 37, 38, 41). During P_i limitation, PhoR (histidine kinase) turns on the Pho regulon genes by phosphorylating PhoB, a response regulator. The activated *phoB* in turn activates transcription by binding specifically to a DNA sequence of 18 nucleotides (the Pho box) (20), which is part of the promoter of each Pho regulon gene, including the *pst* operon.

In *B. subtilis*, Pho regulon genes are expressed in response to phosphate starvation and regulated by the PhoP-PhoR two-component regulators. Previous data show that PhoP and PhoR are equally required for transcription induction of Pho regulon genes, including *phoA*, *phoB*, *tuaA*, *phoD*, and *phoPR* (12, 13, 16), albeit the *phoPR* operon promoter shows low constitutive *phoPR* transcription in a *phoP* or *phoR* mutant strain (16).

The proposed function for the *pst* operon in *B. subtilis* in phosphate transport (36) has not been tested. It was also of interest to determine whether the *pst* genes in *B. subtilis* are Pho regulon genes and if they play a role in phosphate sensing.

MATERIALS AND METHODS

Bacterial strains and plasmids. The bacterial strains and plasmids used are listed in Table 1. The plasmids were constructed as follows. *pst* genes in plasmids pYQ21, pYQ22, pYQ25, and pYQ26 were amplified by PCR from chromosomal DNA from *B. subtilis* JH642 and cloned into the vector pCRII (Fig. 1A). pYQ21 contains a 131-bp PCR fragment generated with primers FMH219 (^{16}CATGATGTGGGCGTTTTTTTTTATAC^{41}) and FMH220 (GGA^{147}TCCCCCTGCGTATAATGTGTA^{127}). The numbers at the 5′ and 3′ ends of this primer and following primers refer to numbers in the operon sequence of Genbank database accession number D58414. pYQ22 contains the *pst* promoter region and the entire *pstS* gene; the PCR primers used were FMH219 and FMH221 (^{1064}TAAGATCAGCTTTGCTTCCCGTTG^{1041}). pYQ25 contains the *pstC* and *pstA* genes; the PCR primers used were FMH222 (^{1022}TGAAAGTGACACGTGATGCCA^{1042}) and FMH223 (^{3011}GGAACAATAACAGCTCGCTCAGGT^{2988}). pYQ26 contains the *pstB1* and *pstB2* genes; the PCR primers used were FMH224 (^{2898}GATGGCTTGGCACGATGATCTAC^{2920}) and FMH225 (^{4698}TGACTATGTCATTGATGCCGGCT^{4676}). Plasmid pYQ23 contains a *pst-lacZ* promoter fusion constructed by subcloning the *Bam*HI-*Eco*RI fragment from pYQ21 into the *Bam*HI-*Eco*RI site of pDH32 (31). pYQ23 was linearized, transformed (7) into *B. subtilis* JH642, and integrated into its chromosome at the *amyE* locus, creating strain MH5496. MH5496 chromosomal DNA was transformed into the *phoP* mutant strain (MH5117) (15) and the *phoR* mutant strain (MH5124) (15), selecting for chloramphenicol resistance, resulting in strains MH5497 (*phoP pst-lacZ*) and MH5498 (*phoR pst-lacZ*), respectively. To make mutations in the *pst* genes, a chloramphenicol resistance (Cm^r) cassette was used to generate the plasmids designated pYQ27, pYQ29, and pYQ33 (Fig. 1A). pYQ27 contains a Cm^r gene within the *Sma*I fragment (1.5 kb) from pMI1101 (23) which was inserted into the *Sac*II site of the *pstS* gene in pYQ22. pYQ29 was constructed

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TABLE 1. Bacterial strains and plasmids

Strain or plasmid	Genotype or antibiotic resistance	Source or reference
B. subtilis		
JH642	*pheA1 trpC2*	J. A. Hoch
MH5117	*pheA1 trpC2 mdh*::Tet^r *phoP* Δ*Eco*RI	F. M. Hulett (16)
MH5124	*pheA1 trpC2 phoR* Δ*Bal*I::Tet^r	F. M. Hulett (16)
MH5446	*pheA1 trpC2 pstS*::Cm^r	This study
MH5447	*pheA1 trpC2 pstS*ΩpYQ27 Cm^r	This study
MH5504	*pheA1 trpC2 pstC*ΩpYQ29 Cm^r	This study
MH5505	*pheA1 trpC2 pstB1*ΩpYQ33 Cm^r	This study
MH5496	*pheA1 trpC2 amyE*::pYQ23 Cm^r	This study
MH5497	*pheA1 trpC2 amyE*::pYQ23 Cm^r *phoP* Δ*Eco*RI	This study
MH5498	*pheA1 trpC2 amyE*::pYQ23 Cm^r *phoR* Δ*Bal*I::Tet^r	This study
MH5403	*pheA1 trpC2 amyE*::pRC696 Cm^r	This study
MH5502	*pheA1 trpC2 amyE*::pRC696 Spc^r	This study
MH5503	*pheA1 trpC2 amyE*::pRC696 Spc^r *pstS*::Cm^r	This study
MH5506	*pheA1 trpC2 amyE*::pRC696 Spc^r *pstC*ΩpYQ29 Cm^r	This study
MH5507	*pheA1 trpC2 amyE*::pRC696 Spc^r *pstB1*ΩpYQ33 Cm^r	This study
Plasmids		
pCRII	Amp^r Kan^r (3.9)[a]	Invitrogen
pDH32	Amp^r Cm^r (9.9)	31
pJL62	Amp^r Spc^r Tet^r (6.55)	I. Smith
pJM105B	Amp^r Cm^r (3.88)	27
pMI1101	Amp^r Cm^r (9.0)	23
pRC696	Amp^r Cm^r (10.0)	F. M. Hulett (5)
pYQ21	Amp^r Kan^r P_{pst} (4.0)	This study
pYQ22	Amp^r Kan^r *pstS* (4.95)	This study
pYQ25	Amp^r Kan^r *pstC pstA* (5.9)	This study
pYQ26	Amp^r Kan^r *pstB1 pstB2* (5.7)	This study
pYQ23	Amp^r Cm^r P_{pst}::*lacZ* (10.0)	This study
pYQ27	Amp^r Kan^r Cm^r *pstS* (6.5)	This study
pYQ29	Amp^r Kan^r Cm^r *pstC* (6.9)	This study
pYQ33	Amp^r Kan^r Cm^r *pstB1* (6.7)	This study

[a] Values in parentheses are the sizes of the plasmids in kilobases.

from pYQ25 by interrupting its *pstC* gene at the *Cla*I site with a 995-bp *Hinc*II-*Sma*I Cm^r cassette from pJM105B (27) which lacks its transcription terminator, allowing transcription of the genes downstream of *pstC* in the *pst* operon. pYQ33 was derived from pYQ26, in which an 84-bp *Ssp*I fragment was deleted from the *pstB1* gene and replaced by a Cm^r cassette of pJM105B. pYQ27 was linearized and transformed into JH642, selecting for Cm^r, to obtain a *pst* mutant strain (MH5446) in which *pstS* was interrupted by a Cm^r gene (Fig. 1B). The mutation in the *pstS* gene of MH5446 was confirmed by PCR, with chromosomal DNAs used as templates. Plasmid pYQ27 was transformed into the JH642 genome by Campbell integration, creating MH5447 (Fig. 1B). pYQ29 and pYQ33 were transformed into the JH642 strain genome by Campbell integration, resulting in strains MH5504 and MH5505 (Fig. 1B), respectively. To construct *pst* mutants with the Pv-*lacZ* promoter fusion, pJL62 was linearized and transformed into strain MH5403 (*amyE*::pRC696Cm^r) to convert its chloramphenicol resistance to spectinomycin resistance (Spc^r). Chromosomal DNA from the resulting strain, MH5502 (*amyE*::pRC696Spc^r), was transformed into each of the *pst* mutants. The transformants were selected for Spc^r and screened for chloramphenicol sensitivity and loss of α-amylase synthesis (*amyE*).

Medium, growth conditions, and enzyme assays. Low-phosphate defined medium (LPDM), as described by Hulett et al. (14), was used for studies of P_i uptake, alkaline phosphatase (APase) activity, and transcription of the *pst* promoter by phosphate or nitrogen starvation. The concentration of P_i in LPDM is 0.4 mM unless otherwise stated. High-phosphate defined medium (HPDM) is the same as LPDM except that it contains 10 mM phosphate. To analyze phosphate or nitrogen starvation induction of APase and the *pst-lacZ* promoter fusion, cells were grown in LPDM containing 5 mM HK_2HPO_4-KH_2PO_4 overnight. The overnight culture was used to inoculate a fresh culture of LPDM or HPDM (for phosphate induction) or LPDM without ammonium sulfate (for nitrogen starvation). Growth, APase specific activity (SA), and β-galactosidase SA were measured hourly. APase SA was determined as described previously (14). β-Galactosidase SA was determined by the method of Ferrari et al. (9) and expressed in units per milligram of protein. The unit used was equivalent to 0.33 nmol of *ortho*-nitrophenol produced per min. Tryptose blood agar base medium (TBAB) is 33% tryptose blood agar base (Difco) and when supplemented with 0.5% glucose is called TBABG. The concentration of P_i in the medium was assayed by the method of Ames and Dubin (2) after removing the cells by filtration through a 0.45-μm-pore-size filter.

PCR. Amplification reactions were performed with a Gene Amp Kit (Perkin-Elmer Cetus) as described in the manufacturer's protocol. Oligonucleotides were purchased from Integrated DNA Technologies.

General methods. Transformation of *B. subtilis* was by the two-step transformation method of Cutting and Vander Horn (7). Transformants were selected for drug resistance on TBABG plates containing either 5 μg of chloramphenicol per ml or 100 μg of spectinomycin per ml. Plasmid DNA was purified with Qiagen columns as described in the manufacturer's instructions.

Primer extension. Strain MH5496 was grown in LPDM to induce expression of the *pst* operon as monitored by β-galactosidase activity. Total RNA was extracted, and the *pstS* transcription initiation site was determined by primer extension as described previously (5). Primers FMH220 and FMH236 marked in the *pst* sequence shown below (see Fig. 6A) were used. A sequencing ladder was generated by annealing the same primers to pYQ22 and extending them with Sequenase (United States Biochemical Corp.) as described in the manufacturer's instructions.

Phosphate transport assays. P_i uptake assays in *B. subtilis* were done by use of a modification of the procedure developed for *E. coli* (6, 34, 46, 47). Carrier-free $^{32}P_i$ was purchased from ICN Biomedicals, Inc. Cells were grown to the stationary phase in LPDM. The cells were washed twice with P_i-free defined medium, resuspended in the same medium to an optical density at 600 nm of 0.5, and incubated with shaking for an additional 2 h at 37°C to induce maximal phosphate uptake. $^{32}P_i$ (0.2 to 100 μM) was added to 10 ml of the cell culture. At specific intervals (15 or 30 s), 0.5-ml cell samples were transferred to a membrane filter (0.45-μm pore size; Micron Separations Inc.) and washed twice with 5 ml of P_i-free defined medium in an apparatus described elsewhere (28). The membranes were dried, and the radioactivity was measured with a Tri-Carb 1500 liquid scintillation analyzer (Packard Instrument Company, Downers Grove, Ill.). Samples containing known amounts of $^{32}P_i$ were used to calculate the P_i

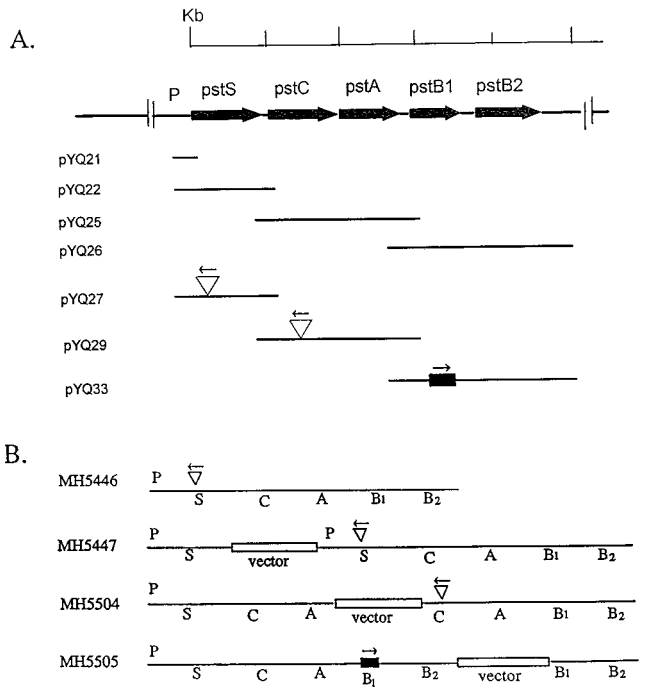

FIG. 1. Organization of the *pst* operon in *B. subtilis* and *pst* mutants. (A) Genes in the *pst* operon and plasmids containing a *pst* mutation. The large arrows indicate the sizes and directions of the genes in the *pst* operon. For details of the plasmid construction, see Materials and Methods. The horizontal lines represent DNA cloned within the plasmids named on the left. The positions of the inserted Cm^r gene (▽) and a *pst* gene deletion and Cm^r gene insertion (■) are indicated. Small arrows indicate the directions of Cm^r gene transcription. (B) Map of the *pst* region in the chromosome of *pst* mutant strains. The horizontal lines represent the chromosomal DNA of the *pst* mutant strains named on the left. The map is not drawn to scale. The letters P, S, C, A, B1, and B2 denote the promoter, *pstS*, *pstC*, *pstA*, *pstB1*, and *pstB2*, respectively.

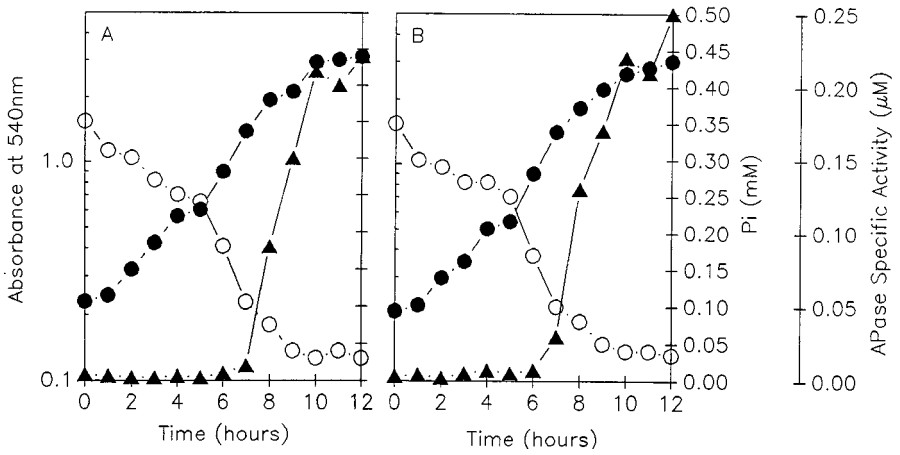

FIG. 2. Growth of parent strain JH642 (A) and *pst* mutant strain MH5446 (B) in LPDM. Symbols: ●, cell growth; ▲, APase SA; ○, concentration of P_i in the medium.

concentration. Concentrations were expressed in terms of nanomoles of P_i per milligram (dry weight), by using an experimentally derived factor (optical density at 600 nm of 1.0 = 0.32 mg of dry weight per ml of culture of *B. subtilis* JH642). For the determination of transport kinetic data, rates of transport performed at various phosphate concentrations were calculated from the linear uptake measurements at 15-s intervals for 2 min.

RESULTS

***pst* mutations affect phosphate transport.** The Pst system of *E. coli* is well defined as a high-affinity phosphate transporter. To test the hypothesis that the *pst* operon in *B. subtilis* may be involved in phosphate transport, four *pst* mutant strains were made (Fig. 1B). Strain MH5446 (*pstS*::Cm^r) contains a polar mutation in the first gene of the *pst* operon since the Cm^r gene has a transcription terminator (23). Strain MH5447 (*pstS*ΩpYQ27Cm^r) contains one good copy of *pstS* followed by the vector and the complete *pst* operon with the Cm^r gene in the *pstS* gene, which is polar on the downstream genes. Strain MH5504 (*pstC*ΩpYQ29Cm^r) has good copies of *pstS*, *pstC*, and *pstA* under control of the *pst* promoter, but the Campbell insertion of pYQ29 is polar on the downstream genes (*pstB1* and *pstB2*). MH5505 (*pstB1*ΩpYQ33Cm^r) has a *pst* operon with a nonpolar mutation in *pstB1*. The organization of the mutated and nonmutated duplicated genes resulting from Campbell integration of the plasmid was detected by PCR (data not shown).

Strain MH5446 harboring a polar mutation in the *pstS* gene grew normally in LPDM when compared to growth of the parent strain, JH642, and the phosphate concentrations in the media containing the two strains dropped in parallel as growth progressed (Fig. 2). APase(s) was not expressed during exponential growth but was induced after 6 h of growth, when the P_i concentrations in the culture media of both strains had dropped below 0.1 mM, signaling induction of the Pho regulon (16). These data suggest that the *pst* null mutation did not markedly affect low-affinity P_i uptake by *B. subtilis* MH5446 and that induction of the Pho regulon was not changed in a *pst* mutant.

In contrast to the results in LPDM containing 0.4 mM P_i, P_i uptake from the same medium containing 10 μM P_i (Fig. 3) or less (data not shown) by phosphate-starved cells was drastically reduced (10- to 25-fold) in all *pst* mutant strains as compared to that of the parent strain. These data indicate that the gene products encoded by the *B. subtilis pst* operon have a role in the high-affinity uptake of P_i. Preliminary kinetic analysis of P_i transport in the parent strain revealed a biphasic Eadie-Hofstee plot, a calculated K_m of 0.51 ± 0.04 μM phosphate, and a V_{max} of 2.20 ± 0.07 nmol of P_i per min per mg (dry weight) for high-affinity P_i transport compared to a calculated K_m of 20.76 ± 0.22 μM phosphate and a V_{max} of 9.4 ± 0.04 nmol of P_i per min per mg (dry weight) for low-affinity P_i transport. The K_m and V_{max} values for the *pst* mutant (MH5446) were similar to the low-affinity data for the parent strain (data not shown). Strains with mutations in a *pst* gene retained the same arsenate sensitivity as the parent strain, JH642 (data not shown), suggesting that the Pst system is not an arsenate transporter.

The *pst* promoter is phosphate starvation inducible and PhoP dependent. To study the regulation of the *pst* operon's expression in *B. subtilis*, a *lacZ* fusion to the *pst* operon promoter was integrated at the *amyE* locus (strain MH5496). This strain retained an intact copy of the *pst* genes. The strain was

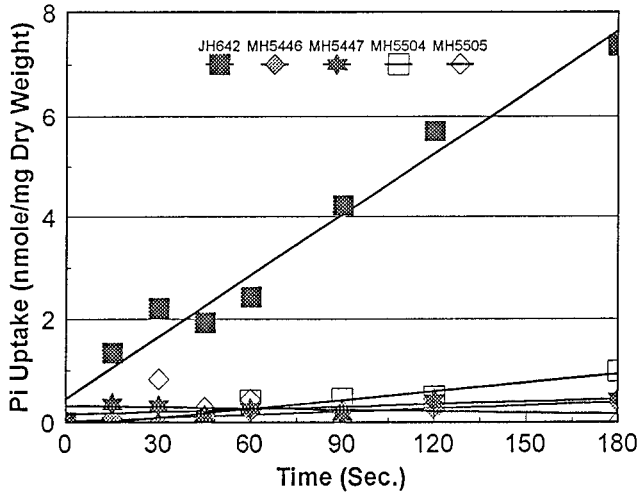

FIG. 3. P_i uptake in the parent and *pst* mutant strains. Cells were grown in LPDM, starved for phosphate, and P_i uptake was measured in the medium containing 10 μM P_i. The procedure is described in Materials and Methods. The strains used were JH642 (parent), MH5446 (polar mutation in *pstS*), MH5447 (Campbell integration of pYQ27), MH5504 (Campbell integration of pYQ29), and MH5505 (Campbell integration of pYQ33).

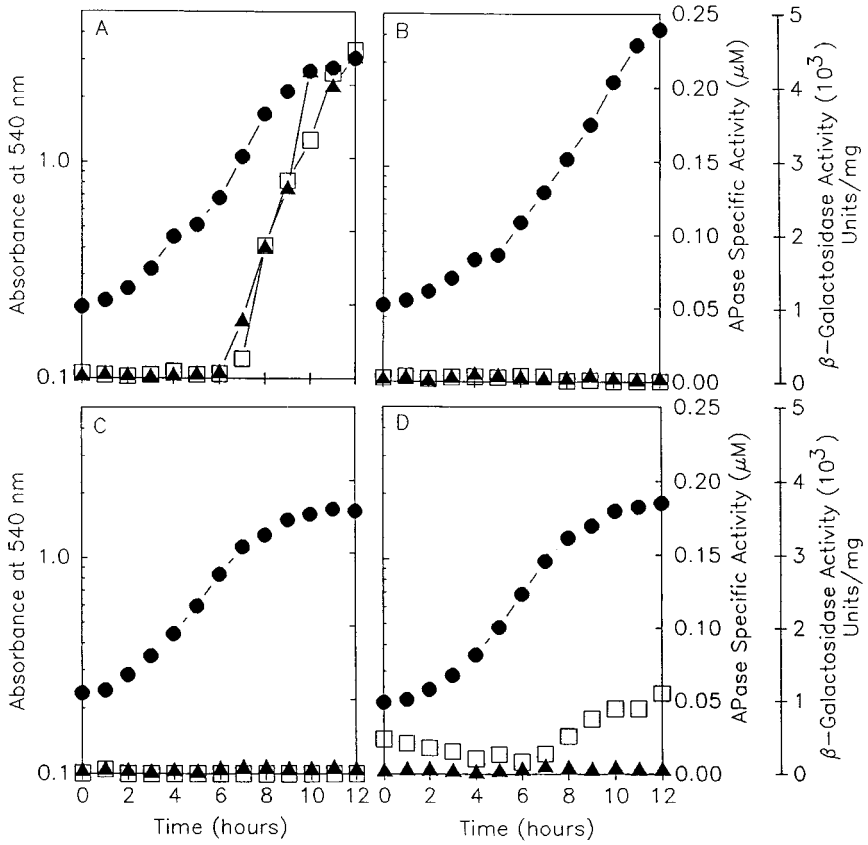

FIG. 4. Induction of APase and β-galactosidase in *pst-lacZ* fusion strains. Graphs show cell growth (●) and total APase (▲) and β-galactosidase (□) SAs in *pst-lacZ* fusion strains. (A) MH5496 (*amyE*::*pst-lacZ*) in LPDM (limited P_i); (B) MH5496 (*amyE*::*pst-lacZ*) in HPDM (excess P_i); (C) MH5497 (*phoP amyE*::*pst-lacZ*) in LPDM; (D) MH5498 (*phoR amyE*::*pst-lacZ*) in LPDM.

grown in LPDM, and the *pst* promoter-driven β-galactosidase SA and APase SA were measured throughout growth and stationary phases (Fig. 4A). Neither enzyme was expressed during log-phase growth. However, as cells entered the stationary phase when phosphate became limiting, the SAs of both enzymes increased. *pst* promoter activation induction was >5,000-fold; total APase induction was >500-fold. There was no *pst* promoter induction or APase induction as the culture entered the stationary phase when cells were grown in HPDM (Fig. 4B) or starved for nitrogen (data not shown).

The induction of APase is *phoP-phoR* dependent (5, 15, 16) and serves as a reporter of Pho regulon expression. To determine the effects of *phoP* or *phoR* mutation on *pst* transcription, strains MH5497 and MH5498 were cultured in LPDM. Neither strain induced APase synthesis in LPDM. There was no *pst* promoter-driven β-galactosidase expression in the *phoP* mutant strain (Fig. 4C), but a low level of β-galactosidase activity was detected in the *phoR* mutant strain (Fig. 4D).

The *pst* operon is transcribed from a single promoter. Primer extension analysis of the *pst* promoter by using RNA extracted from phosphate-starved cells showed that there is one transcription initiation site located 40 bp upstream from the putative translation initiation codon of *pstS* (Fig. 5). The same results were obtained with each of two primers, FMH220 and FMH236 (Fig. 6A). A sequence similar (4 of 6 bp) to the consensus sequence in the −10 region of a sigma A-dependent promoter (11) was centered 12 bp upstream of the transcription start site. A potential −35 sequence (4 of 6 bp) exists in the promoter region; it is 18 bp instead of the usual 17 bp away from the −10 region. The promoter region contains four 6-bp sequences similar to TTAACA, which is a sequence of unknown function positioned upstream of the −10 region of all known Pho-regulated genes in *B. subtilis* (8, 16).

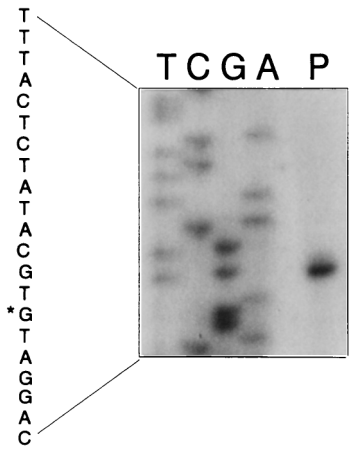

FIG. 5. Primer extension analysis of the *pst* promoter. The end-labeled primer was annealed to RNA from late-exponential-phase cells grown in LPDM and extended with avian myeloblastosis virus reverse transcriptase (lane P). Lanes T, C, G, and A are a sequencing ladder generated by annealing the same end-labeled primer to a plasmid (pYQ22) containing the 5′ end of the *pst* operon and extending it with Sequenase. The asterisk indicates the base to which the primer extension product maps.

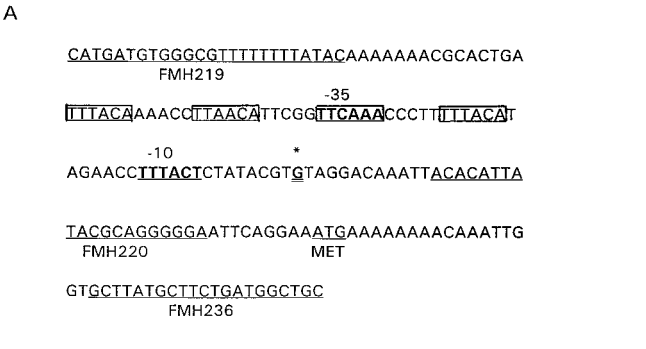

FIG. 6. (A) Nucleotide sequence of the *pst* promoter region. The putative translation start site of *pstS* is indicated (MET). The transcription start site, G, is double underlined, in boldface print, and marked with an asterisk. The putative −10 and −35 regions are underlined and in boldface print. The boxed sequences are 6-bp consensus sequences. The two underlined sequences identified as FMH220 and FMH236 at the 3′ end are the two primers used in primer extension. (B) Alignment comparison of *pst* promoters of *E. coli* and *B. subtilis*. The putative −10 regions are underlined. The transcription start site, G, is double underlined. The double-underlined sequences represent Pho boxes of the *E. coli* promoter and putative Pho boxes of the *B. subtilis* promoter. The consensus sequences are shown in boldface italics.

The *pst* operon does not play a role in Pho regulation. During vegetative growth of *B. subtilis*, when extracellular phosphate levels decrease to below 0.1 mM, a number of previously silent genes are expressed. Those genes depend on PhoP and PhoR for their expression, and they are identified as Pho regulon genes. APase is the traditional enzyme of choice as a reporter of Pho regulon activity during the phosphate deficiency response (13, 16). It was of interest to investigate whether the *pst* operon in *B. subtilis* regulates any other Pho regulon gene expression. The induction of total APase SA and the SA of the Pv promoter, a phosphate starvation-inducible promoter (5), were similar for the *pst* mutants tested (MH5503, MH5506, and MH5507) and the parent strain cultured in either LPDM or in HPDM (data not shown). These data showed that *pst* operon products are not required for normal Pho regulation.

DISCUSSION

The results presented here show that *pst* mutations affect high-affinity P_i uptake, dramatically establishing that the *pst* operon in *B. subtilis* is involved in phosphate transport. Since mutations in different genes in the *pst* operon cause similar decreases in P_i uptake, each *pst* operon gene may play a role in phosphate transport. The kinetic data for P_i uptake and the absence of a change in arsenate sensitivity in the *pst* mutants are analogous to results obtained for *E. coli pst* mutants, supporting the hypothesis that the *B. subtilis pst* operon functions as a high-affinity phosphate transport system. Analysis of the *B. subtilis pst* sequence reveals conservation of three amino acid residues (D-56, R-135, and D-137 for *E. coli*; D-99, R-171, and D-173 for *B. subtilis*) involved in the unusual anion binding site, specific for phosphate but exclusionary for sulfate, found in the PstS-phosphate complex of the *E. coli* protein (19, 44, 49). In addition, modeling and structure analysis of the *B. subtilis* PstS may reveal evolutionary conservation for phosphate binding among phosphate-specific active transport receptors. Cells with a *pst* null mutation utilized extracellular P_i at a rate similar to that of the parent strain in medium containing an initial P_i concentration of 0.4 mM. These data suggest that (i) the *pst* mutants are capable of utilizing P_i as the sole phosphate source and (ii) other low-affinity phosphate transport system(s) must exist. At least one phosphate transport system similar to the Pit system of *E. coli* has been reported (10, 17). P_i plays a key role in the metabolism of all cells; thus, it is not surprising that cells may have multiple systems for P_i transport and accumulation.

The *pst* operon is a member of the Pho regulon. Other Pho regulon genes show a similar dramatic reduction of expression in either *phoP* or *phoR* mutant strains (4, 5, 8, 32), suggesting that PhoR is the sole sensor kinase which phosphorylates PhoP (response regulator) and that the phosphorylated PhoP activates the transcription of Pho regulon genes. The low constitutive activity of the *pst* promoter in a *phoR* mutant strain may result from phosphorylation of PhoP by other kinase proteins or small molecules. This assumes that a lower concentration of PhoP∼P is required for activation of the *pst* promoter than that for the other Pho regulon promoters analyzed. Activation of the *pst* promoter by unphosphorylated PhoP is another explanation, but it seems less likely since the constitutive *phoPR* operon expression in a *phoR* mutant strain (16) fails to promote *pst* expression during high-phosphate culture conditions.

Although the *pst* promoter has a poorly conserved σ^A −10 region (4 of 6 bp) and −35 region (4 of 6 bp), it is a strong promoter compared to other Pho regulon promoters, i.e., *phoA*, *phoB*, and *phoD* (4, 5, 8). There is no consensus Pho box in the *B. subtilis pst* promoter, a common feature of Pho regulon genes in *E. coli* that consists of two 7-bp direct repeats of CTGTCAT separated by 4 bp (20) (Fig. 6B). However, in the *B. subtilis pst* promoter, there are four 6-bp sequences similar to a consensus sequence, TTAACA, positioned upstream of the −10 region in all known Pho-regulated genes (8, 16). These consensus sequences are separated by nonconserved 5-bp sequences. These features are analogous to the *E. coli* Pho box. Perhaps the actual sequence and spacing of the *B. subtilis* Pho box are different from those of the *E. coli* Pho box but the general structure is conserved (Fig. 6B). The *pst* promoter in *E. coli* contains two Pho boxes in tandem which are responsible for a *pst* promoter activity higher than that of other Pho regulon genes (18). Further analysis is required to determine if the consensus sequence in the *B. subtilis pst* promoter (Fig. 6B) represents tandem promoters responsible for the strong *pst* promoter activity.

None of the mutations in the *pst* operon of *B. subtilis* changed the induction pattern of the Pho regulon promoters we analyzed, *phoB* (Pv promoter), *pstS*, or total APases. In both *E. coli* and *Pseudomonas aeruginosa* (26) but not in *B. subtilis*, *PhoU* is a component of the *pst* operon, and in both gram-negative organisms, the Pst system, PhoU, and PhoR are involved in repression of the Pho response during growth under P_i-replete (excess) conditions. Our observations raise the question as to what gene products in addition to PhoR, if any, are involved in sensing the signal which results in induction of the *B. subtilis* Pho regulon.

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