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Molecular Analysis of the *Salmonella typhimurium phoN* Gene, Which Encodes Nonspecific Acid Phosphatase

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The *phoN* gene of *Salmonella typhimurium* encodes nonspecific acid phosphatase (EC 3.1.3.2), which is regulated by a two-component regulatory system consisting of the *phoP* and *phoQ* genes. We cloned the *phoN* region into a plasmid vector by complementation of a *phoN* mutant strain and determined the nucleotide sequence of the *phoN* gene and its flanking regions. The *phoN* gene could encode a 26-kDa protein, which was identified by the maxicell method as the product of *phoN*. Results of the enzyme assay and Southern hybridization with chromosomal DNA of *Escherichia coli* K-12 suggests that there is no *phoN* gene in *E. coli*. The regulatory pattern of *phoN* in *E. coli* and Southern hybridization analysis of the *E. coli* chromosome with the *S. typhimurium phoP* gene suggest that *E. coli* K-12 also harbors the *phoP* and *phoQ* genes.

The *phoN* gene in *Salmonella typhimurium* encodes a nonspecific acid phosphatase (EC 3.1.3.2), which is localized in the periplasmic space (10) and maps at 95 min on the *S. typhimurium* linkage map (22). The nonspecific acid phosphatase activity is induced by carbon, phosphate, nitrogen, or sulfur limitation (11). The expression of *phoN* is regulated by the *phoP* and *phoQ* genes (12, 17), which belong to the two-component regulatory systems and regulate the expression of the *Salmonella* virulence genes (6, 17, 19). PhoP apparently functions as a transcriptional activator of *phoN*, and PhoQ apparently functions as a sensor or transducer of the physiological signals (6, 12, 17).

Escherichia coli possesses alkaline phosphatase (EC 3.1.3.1), which is encoded by the *phoA* gene (4) and is localized in the periplasm (3, 8). Alkaline phosphatase is induced only by phosphate limitation and is regulated by the *phoB* and *phoR* genes, which also belong to the two-component systems and regulate expression of a number of genes in the *E. coli pho* regulon (25). While *S. typhimurium* lacks the *phoA* gene, it has the *phoB* and *phoR* genes (24).

We have been interested in the mechanisms of regulation of these phosphate-regulated genes in these organisms. To begin to study the *phoP-phoQ* regulatory system, we cloned the *S. typhimurium phoN* gene and determined the nucleotide sequence of the gene. We also used Southern blot analysis to examine whether the *phoN* gene is present in *E. coli*, and we studied the expression of the *S. typhimurium phoN* gene in *E. coli*.

MATERIALS AND METHODS

Bacterial strains and plasmids. The *S. typhimurium* strains, *E. coli* K-12 strains, and plasmids used in this study are listed in Table 1. Strain CSH26BR was constructed by transducing the *phoB-phoR* deletion marker from ANCH1 (28) into CSH26 by using P1*vir* phage.

Media. Luria-Bertani (LB) broth, LB agar, T broth, and T agar were described by Miller (16). The media used for the routine preparation of M13 phage and for the maxicell method were as previously described (1). Carbon-, nitrogen-, and phosphate-free ($N^-C^-P^-$) medium supplemented with requirements was used for studies of the acid phosphatase levels in cells (11). Ampicillin was added to LB broth or T broth at 100 μg/ml. Kanamycin was added to LB agar at 20 μg/ml. To examine the synthesis of acid phosphatase and β-galactosidase by bacterial colonies, 5-bromo-4-chloro-3-indolyl phosphate and 5-bromo-4-chloro-3-indolyl galactoside were added to agar plates to final concentrations of 40 and 60 μg/ml, respectively.

Recombinant DNA methods. Standard methods for recombinant DNA as described by Sambrook et al. (20) were generally used.

DNA sequencing. The manipulation of M13 phage was as described by Messing et al. (15). A series of phage clones with DNA fragments with one fixed end and the other end formed by successive deletions at the 5′ end was prepared as described by Henikoff (7). The DNA sequences were analyzed by the method of Sanger et al. (23).

Identification of the protein encoded by plasmid. The product of the *phoN* gene carried on a plasmid was identified by the maxicell method (21).

Polyacrylamide gel electrophoresis of acid phosphatase. To prepare acid phosphatase samples for analysis by electrophoresis, cells grown overnight in 1 ml of LB broth were washed, suspended in 0.3 ml of 0.1 M Tris HCl (pH 6.8) containing 1% Triton X, and disrupted with sonication. The cell lysate was centrifuged, and proteins in the supernatant (10 μl) were separated by electrophoresis on a 7.5% polyacrylamide gel at 4°C. After electrophoresis, the gel was stained for acid phosphatase activity by soaking it in a mixture of naphthol-AS-MX-phosphate and fast blue RR salts in 0.1 M acetate buffer (pH 5.5) (16).

Southern hybridization. The presence of the *phoN*- and *phoP*-like sequences in *E. coli* was studied by DNA-DNA hybridization (27) as described by Sambrook et al. (20). The transferred nitrocellulose membrane was prehybridized at 42°C for 4 h without probe and hybridized at 42°C overnight with ^{32}P-labeled probes. The hybridized membrane was washed twice with 1% sodium dodecyl sulfate in 1× SSC (0.15 M NaCl plus 0.015 M sodium citrate) at room temperature and then washed in 0.1% sodium dodecyl sulfate in 0.1× SSC at 60°C for 1.5 h. The DNA fragments of *S. typhimurium* used as *phoN* or *phoP* probes were radiolabeled by the random primer method (5).

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TABLE 1. Bacterial strains and plasmids

Strain or plasmid	Relevant characteristics	Reference or source
S. typhimurium		
LT2	Wild type	K. Kutsukake
SJ10002	*hsdR*	K. Kutsukake
TA2328	*purB12 phoP22*	12
TA2361	*phoN2*	12
TA2362	*phoP12*	12
TA2367	*pho-24*	12
E. coli		
ANCH1	Like ANCK10 except for Δ(*phoB-phoR*)	28
ANCK10	F⁻ *leu lacY trp his argG rpsL ilv metA(orB) thi*	14
CSH26	F⁻ *ara* Δ(*pro-lac*) *met thi*	16
CSH26BR	Like CSH26 except for Δ(*phoB-phoR*)	This study
CSR603	*recA1 uvrA6 phr-1*	21
FE15	F⁻ *thr leu* Δ*phoA thi rpsL*	18
JM103	Δ(*pro-lac*) *supE thi* (F′ *traD36 proAB lacI*^{q}ZM15)	15
Plasmids		
pBR322	High-copy-number vector for cloning; Ap^r Tet^r	2
pMK100	pUC19 derivative carrying *phoN* gene; Ap^r	This study
pMK101	pSCH18 derivative carrying *phoN* gene; Ap^r	This study
pMK110	pRS415 derivative carrying *phoN′-lacZ*; Ap^r	This study
pRS415	Vector for construction of operon fusions with *lacZ*; Ap^r	26
pSCH18	Low-copy-number vector for cloning; Ap^r	9
pUC18	High-copy-number vector for cloning; Ap^r	29
pUC19	High-copy-number vector for cloning; Ap^r	29

Enzyme assay. The nonspecific acid phosphatase assay was performed as described by Kier et al. (10). The β-galactosidase assay was done as described by Miller (16).

Enzymes and radioisotopes. Restriction endonucleases, T4 DNA ligase, T4 DNA polymerase, DNA polymerase (Klenow fragment), exonuclease III, mung bean nuclease, bacterial alkaline phosphatase, an M13 sequencing kit, and a random primer DNA labeling kit were obtained from Takara Shuzo (Kyoto, Japan). [α-^{32}P]dCTP (>400 Ci/mmol) and [^{35}S]methionine were purchased from Amersham Japan, Tokyo.

Nucleotide sequence accession number. The nucleotide sequence data reported in this paper will appear in the DDBJ, EMBL, and GenBank nucleotide sequence data bases under the accession number X59036.

RESULTS AND DISCUSSION

Cloning of the *S. typhimurium phoN* gene. We cloned the *S. typhimurium phoN* gene by complementation of the *phoN* mutant TA2361 with *Eco*RI and *Hin*dIII DNA libraries of strain LT2 cloned into pBR322 that had been propagated in a *S. typhimurium hsdR* strain, SJ10002. Two blue transformants on T plates with 5-bromo-4-chloro-3-indolyl phosphate were examined: one had a 5.5-kb *Hin*dIII insert, and the other had a 2.2-kb *Eco*RI insert. The plasmids had a 1.5-kb *Hin*dIII-*Eco*RI fragment in common; we subcloned this fragment into pUC19. The resultant plasmid (pMK100) and its smaller derivative with the 1-kb *Pst*I-*Eco*RI region (Fig. 1) complemented the *phoN* mutation in TA2361. These results suggest that the 1-kb *Pst*I-*Eco*RI fragment contains the *phoN* gene.

To confirm that the chromosomal DNA cloned on pMK100 contains the *phoN* gene that encodes the nonspecific acid phosphatase described by Kier et al. (12), we assayed acid phosphatase activity in the *phoN* and *phoP* strains carrying the plasmids with or without the chromosomal fragment. First, we measured the activity at pH 5.5 by measuring the release of P_i from 5′-AMP by the method described by Kier et al. (10) (Table 2). The constitutive *S. typhimurium* acid phosphatase mutant *pho-24* (12) produced a higher level of the activity than the wild-type strain, while the *phoN2* and *phoP22* mutants showed very low activity, in agreement with previous studies (12). Introduction of plasmids with the *phoN* region increased the phosphatase activity in the *phoN* and wild-type strains, but these plasmids had very little effect in the *phoP* mutant. The high-copy-number plasmid (pMK100) increased the activity more than the low-copy-number plasmid (pMK101). These results are consistent with *phoP* being a transcriptional activator of *phoN* and the *phoN* region carried on the plasmids containing the promoter region required for activation by PhoP.

To further confirm that the phosphatase encoded by the cloned DNA is the same as the phosphatase encoded by *phoN*, we assayed the phosphatase activity in a native polyacrylamide gel after electrophoresis of proteins in cell extracts (Fig. 2). A thick phosphatase band was observed in samples of the wild-type (lane 1) and *pho-24* (lane 5) strains, while the corresponding band was very lightly stained in

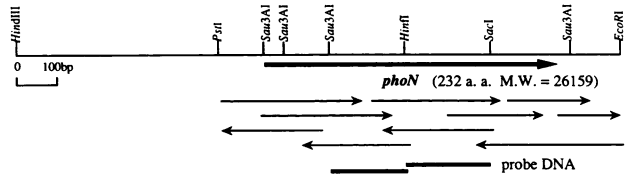

FIG. 1. Physical map of the 1.5-kb *S. typhimurium phoN* region and sequencing strategy. The large arrow indicates the coding region of the *phoN* gene and the direction of transcription. The thin arrows indicate the directions and extents of sequences analyzed. The bars at the bottom indicate the restriction fragments used as probes for Southern blot analysis. a.a., amino acids; M.W., molecular weight.

TABLE 2. Acid phosphatase activities in *S. typhimurium* and *E. coli* strains carrying *phoN*$^+$ plasmids

Host strain	Acid phosphatase activity with plasmid[a]			
	pUC19	pMK100 (*phoN*$^+$)	pSCH18	pMK101 (*phoN*$^+$)
S. typhimurium				
LT2 (wild type)	5.6	42.3	3.9	14.2
TA2361 (*phoN2*)	0.4	42.2	0.6	22.9
TA2328 (*phoP22*)	0.6	1.9	0.5	0.7
TA2367 (*pho-24*)	26.4	NG	20.1	40.5
E. coli FE15 (Δ*phoA*)	0.7	13.2	0.3	4.2

[a] Strains were grown on $N^-C^-P^-$ medium supplemented with 0.4% glucose, 10 mM NH_4Cl, 1 mM sodium phosphate, and synthetic pools (11). Acid phosphatase activity is expressed as units per optical density at 650 nm of the cell culture. One unit of enzyme activity is defined as the amount of enzyme that releases 1 nmol of P_i per min from 5'-AMP. NG, no growth.

samples of the *phoN2* (lane 2) and *phoP22* (lane 4) strains. Introduction of the *phoN*$^+$ plasmid into the *phoN2* strain gave a thick band (lane 3). Therefore, the acid phosphatase present in the wild-type strain and absent in the *phoN2* and *phoP22* strains was recovered by the introduction of the chromosomal fragment carried by pMK101. Essentially the same results were obtained with a plasmid that carried the 1-kb *Pst*I-*Eco*RI fragment (data not shown). From the evidence given above, we concluded that the 1-kb *Pst*I-*Eco*RI chromosomal fragment contains the *phoN* gene.

Nucleotide sequence of the *phoN* gene. The entire nucleotide sequence of the 1-kb *Pst*I-*Eco*RI fragment was determined by the dideoxy chain termination method (23). The sequenced segments are shown in Fig. 1. The sequence of 991 bp containing the *phoN* gene and its flanking regions was determined at least twice on both strands with overlapping junctions. The nucleotide sequence and the amino acid sequence deduced from the nucleotide sequence are shown in Fig. 3. The *phoN* coding region lies between nucleotides 132 and 827, begins with an ATG codon that is downstream of a Shine-Dalgarno sequence (GGAG) at the appropriate position, and ends with a TGA codon. The putative −10 region, cATtAT, and the −35 region, TTGAat, are at nucleotides 82 to 87 and 60 to 65, respectively. A sequence that may form a stem-and-loop structure is located downstream from the TGA translational termination codon. This structure may function as a transcriptional terminator or a regulatory site for the downstream gene. The predicted PhoN

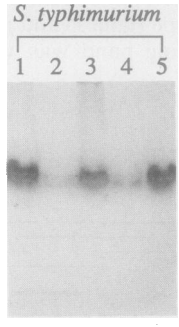

FIG. 2. Activity of acid phosphatase (pH 5.5) stained on polyacrylamide gel after electrophoresis of cell lysates. Lanes: 1, LT2 (*pho*$^+$)/pSCH18; 2, TA2361 (*phoN2*)/pSCH18; 3, TA2361 (*phoN2*)/pMK101 (*phoN*$^+$); 4, TA2328 (*phoP22*)/pSCH18; 5, TA2367 (*pho-24*)/pSCH18.

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          10         20         30         40         50         60
5'CAGTCCGGTATGGACAGACGATAATGCCAGGCGCAGCGTCCTGCTTTTTTACCTGTATGT
          70         80         90        100        110        120
TGAATAACCATTGCAATAAATCATTATAGGATTACATCTGTTTATTATTGCCTGATCCGG
-35                        -10
          130        140        150        160        170        180
AGTGAGTCTTTATGAAAAGTCGTTATTTAGTATTTTTTCTACCACTGATCGTAGCTAAAT
SD          M  K  S  R  Y  L  V  F  F  L  P  L  I  V  A  K  Y
          190        200        210        220        230        240
ATACATCAGCAGAAACAGTGCAACCCTTTCATTCTCCTGAAGAATCAGTGAACAGTCAGT
  T  S  A  E  T  V  Q  P  F  H  S  P  E  E  S  V  N  S  Q  F
          250        260        270        280        290        300
TCTACTTACCACCACCGCCAGGTAATGATGATCCGGCTTACCGCTATGATAAGGAGGCTT
  Y  L  P  P  P  P  G  N  D  D  P  A  Y  R  Y  D  K  E  A  Y
          310        320        330        340        350        360
ATTTTAAGGGCTATGCGATAAAGGGTTCCCCGCGATGGAAACAAGCTGCTGAGGATGCAG
  F  K  G  Y  A  I  K  G  S  P  R  W  K  Q  A  A  E  D  A  D
          370        380        390        400        410        420
ATGTAAGCGTGGAAAATATAGCCAGAATATTCTCGCCAGTAGTGGGTGCTAAAATTAACC
  V  S  V  E  N  I  A  R  I  F  S  P  V  V  G  A  K  I  N  P
          430        440        450        460        470        480
CCAAAGATACGCCAGAAACCTGGAATATGTTAAAGAATCTTCTGACAATGGGCGGCTACT
  K  D  T  P  E  T  W  N  M  L  K  N  L  L  T  M  G  G  Y  Y
          490        500        510        520        530        540
ACGCTACTGCTTCGGCAAAAAAATATTATATGCGTACCCGCCCCTTTGTCTTATTTAATC
  A  T  A  S  A  K  K  Y  Y  M  R  T  R  P  F  V  L  F  N  H
          550        560        570        580        590        600
ATTCCACCTGCCGTCCTGAAGATGAGAATACTTTGCGAAAAAATGGCTCTTACCCTTCCG
  S  T  C  R  P  E  D  E  N  T  L  R  K  N  G  S  Y  P  S  G
          610        620        630        640        650        660
GGCATACTGCTTATGGTACACTTCTGGCATTAGTATTATCCGAGGCCAGACCGGAACGCG
  H  T  A  Y  G  T  L  L  A  L  V  L  S  E  A  R  P  E  R  A
          670        680        690        700        710        720
CGCAGGAGCTCGCCAGACGCGGATGGGAGTTCGGGCAAAGCAGAGTGATATGCGGTGCTC
  Q  E  L  A  R  R  G  W  E  F  G  Q  S  R  V  I  C  G  A  H
          730        740        750        760        770        780
ACTGGCAAAGCGATGTTGATGCTGGCCGTTATGTGGGAGCAGTAGAGTTTGCAAGACTGC
  W  Q  S  D  V  D  A  G  R  Y  V  G  A  V  E  F  A  R  L  Q
          790        800        810        820        830        840
AAACAATCCCGGCTTTTCAGAAGTCACTGGCAAAATCCGTGAGGAGCTGAACGACAAAAA
  T  I  P  A  F  Q  K  S  L  A  K  S  V  R  S  *
          850        860        870        880        890        900
TAATTTATTGAGTAAAGAAGATCACCCCAAACTTAATTACTGAAGGTGAAAGTCTTCCCG
          910        920        930        940        950        960
CAAACTGGCCACAGCAAATGAAAGGAAGTGCAACTGCGTAGGGGCGGCCGGGCGTGGAGA
          970        980        990
ATGCCTTTGGTTTCCCCGATTCGCATGAATT 3'
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FIG. 3. Nucleotide sequence of the *Eco*RI-*Pst*I fragment (991 bp) containing the *phoN* gene and its flanking region. The deduced amino acid sequence is shown by single-letter symbols. The putative −35 and −10 sequences are also indicated. The putative Shine-Dalgarno (SD) sequence is shown by a double line. The putative signal sequence of PhoN protein (amino acids 1 to 15) is underlined. The two convergent arrows indicate inverted repeat sequences.

protein consists of 232 amino acid residues, and its estimated molecular mass is 26,159 Da.

The *phoN* gene product identified by the maxicell method was 26 kDa (Fig. 4), which agrees very well with the predicted value from the DNA sequence and also agrees with the size of the purified acid phosphatase analyzed by SDS-gel electrophoresis by Kier et al. (10). At the N terminus, PhoN protein has a hydrophobic region characteristic of a signal sequence in which positively charged residues at the very N terminus are followed by a stretch of hydrophobic residues that ends prior to Ala at position 15. This indicates that PhoN is secreted across the cytoplasmic membrane by using the signal peptide.

***E. coli* has no PhoN-like acid phosphatase or *phoN*-like gene.** When we attempted to measure acid phosphatase activity at pH 5.5 in *E. coli*, we found that most of the activity was due to the alkaline phosphatase encoded by

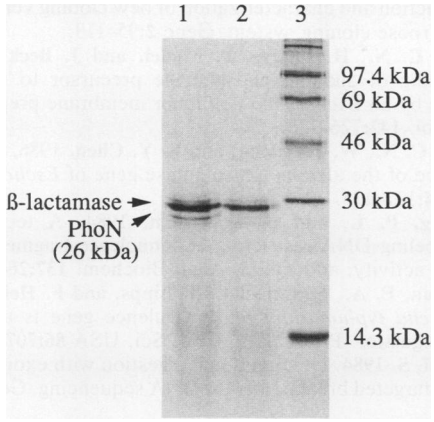

FIG. 4. Autoradiogram of ^{35}S-labeled proteins produced in maxicells with the *phoN* plasmids pMK100 (lane 1) and pUC18 (lane 2). Protein size markers were run on lane 3. The molecular masses of PhoN and the β-lactamase (arrowheads) are estimated to be 26 and 29 kDa, respectively.

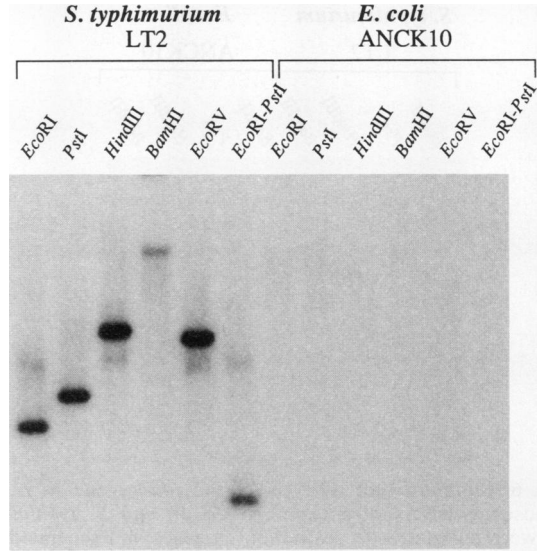

FIG. 5. Southern blot analysis of the *phoN* gene in *E. coli*. Chromosomal DNAs of *E. coli* ANCK10 and *S. typhimurium* LT2 were prepared, digested with restriction enzymes, and separated on a 0.8% agarose gel. The separated DNA fragments were transferred to a nitrocellulose membrane and hybridized with ^{32}P-labeled probe. The 218-bp *Hin*fI-*Sac*I fragment of the *phoN* coding region (Fig. 1) was used as probe. The band shown in the *Eco*RI-*Pst*I lane of LT2 corresponds to the position of 1-kb DNA.

phoA (data not shown). A *phoA* deletion mutant strain, FE15, showed little acid phosphatase activity under the assay conditions (Table 2), indicating that *E. coli* may not possess a PhoN-like phosphatase. However, the *E. coli* strain produced the acid phosphatase when the *S. typhimurium phoN*$^+$ plasmid was introduced. The result suggests that *E. coli* possesses a *phoP*-like gene which is required for *phoN* gene expression.

We investigated whether *E. coli* has a *phoN*-like gene by using Southern hybridization analysis with the 218-bp *Hin*fI-*Sac*I fragment and the 188-bp *Sau*3AI-*Hin*fI fragment internal to the *phoN* coding region of *S. typhimurium* as probes (Fig. 1). The chromosomal DNA of *E. coli* was digested with *Bam*HI, *Eco*RI, *Eco*RV, *Hin*dIII, *Pst*I, and *Eco*RI-*Pst*I and separated by electrophoresis on a 0.8% agarose gel. By the Southern hybridization experiment under the stringent conditions described in Materials and Methods, no DNA sequence hybridizable to the 218-bp *Hin*fI-*Sac*I fragment was detected in *E. coli* (Fig. 5). The same result was obtained with the 188-bp *Sau*3AI-*Hin*fI fragment as probe (data not shown). These results suggest that there is no gene in *E. coli* whose sequence is substantially similar to that of the *S. typhimurium phoN* gene.

Regulation of *S. typhimurium phoN* gene expression in *S. typhimurium* and *E. coli*. To study the regulation of *phoN* expression in *E. coli* and *S. typhimurium*, we constructed a plasmid with a *phoN'-lacZ* operon fusion to measure *phoN* transcription, since accurately measuring the acid phosphatase activity alone in *S. typhimurium* and *E. coli* is difficult because of the presence of other phosphatases (10). β-Galactosidase is absent in *S. typhimurium*, so *E. coli* strains with deleted *lacZ* were used for this study. The protruding ends of the *Hin*dIII-*Sac*I fragment of the *phoN* region (Fig. 1) were converted to blunt ends by T4 DNA polymerase, and the fragment was inserted into the blunt-ended *Bam*HI site upstream of the promoterless *lacZ* gene on pRS415 (26). One of the resultant plasmids (pMK110) that gave a Lac$^+$ phenotype to LT2 was confirmed by restriction enzyme analysis to have the expected structure. pMK110 was introduced into *S. typhimurium* LT2, TA2361 (*phoN2*), TA2328 (*phoP22*), and TA2367 (*pho-24*) and *E. coli* CSH26 (*lacZ*) and CSH26BR [*lacZ*Δ(*phoB-phoR*)]. These strains were grown either in limiting- or excess-phosphate medium (11), and the levels of *phoN* expression in the cells were measured by assaying the levels of β-galactosidase activity (Table 3). The expression of *phoN* was increased threefold by phosphate starvation, and the expression was largely dependent on the function of *phoP* in *S. typhimurium*. This is in agreement with previous work by Kier et al. (12), although the degree of induction by phosphate starvation was lower in the present experiment. Essentially the same results were obtained with a shorter *phoN'-lacZ* operon fusion that contains the *Pst*I-*Sac*I fragment of the *phoN* region (data not shown). These results also show that *phoN* expression is mostly regulated at the transcriptional level, and the upstream regulatory element of *phoN* with which PhoP interacts is included in the sequenced region. The

TABLE 3. Effects of *pho* mutations and phosphate concentration in medium on *phoN'-lacZ* expression of pMK110 in *S. typhimurium* and *E. coli*

Strain/plasmid	β-Galactosidase activity[a]	
	Ex P_i	Li P_i
S. typhimurium		
LT2 (wild type)/pMK110	5,509	13,588
TA2361 (*phoN2*)/pMK110	3,909	11,770
TA2328 (*phoP22*)/pMK110	446	342
TA2367 (*pho-24*)/pMK110	44,867	22,394
E. coli		
CSH26/pMK110	1,199	3,949
CSH26BR [Δ(*phoB-phoR*)]/pMK110	1,447	4,848

[a] Expressed in Miller units. Ex P_i, excess-phosphate medium; Li P_i, limiting-phosphate medium. Strains transformed with vector plasmid pRS415 made <1 to 30 U of the enzyme.

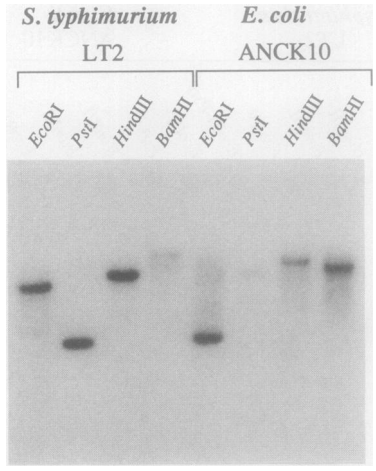

FIG. 6. Southern blot analysis of the *phoP* gene in *E. coli*. Chromosomal DNAs of *E. coli* (ANCK10) and *S. typhimurium* (LT2) were digested with restriction enzymes and separated on a 0.8% agarose gel. The separated DNA fragments were transferred to a nitrocellulose membrane and hybridized with ^{32}P-labeled probe. The 514-bp *Eco*RV fragment of the *phoP* coding region of *S. typhimurium* (6, 17) was used as the probe.

phoN gene was similarly expressed in *E. coli*, and the expression was independent of the *E. coli phoB* gene, which is the positive regulatory gene of the *pho* regulon (13). Since *phoN* is positively regulated by *phoP* and *phoQ* in *S. typhimurium* (17), these results suggest that *E. coli* also has *phoP*- and *phoQ*-like genes whose activities are influenced by phosphate concentrations in the medium, as in *S. typhimurium*.

***E. coli* has a *phoP*-like gene.** To examine the possibility that *E. coli* has a *phoP*-like gene, we cloned the DNA fragment of *S. typhimurium*, which complemented a *phoP* mutation of TA2362, by the same strategy used for cloning the *phoN* gene. The *phoP* region we cloned showed the same restriction patterns as those reported previously (17). We analyzed the *E. coli* chromosome by the Southern blot method under the same conditions as were used for *phoN*, using the 514-bp *Eco*RV fragment internal to the *phoP* gene of *S. typhimurium* as a probe (Fig. 6). The probe hybridized with the *E. coli* DNA fragments as well as with the *S. typhimurium* DNA fragments under stringent conditions, and therefore *E. coli* should contain a sequence very similar to that of the *S. typhimurium phoP* gene. This result and the expression of the *S. typhimurium phoN* gene in *E. coli* strongly suggest that *E. coli* has *phoP* and *phoQ* genes that are very similar to those of *S. typhimurium*.

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