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Cloning and expression in *Escherichia coli* K-12 of the structural gene for outer membrane PhoE protein from *Enterobacter cloacae*

(Recombinant DNA; pore protein; phosphate limitation; *pho* regulon; RP4::mini-Mu; $\gamma\delta$ insertion element; vaccines)

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SUMMARY

In *Escherichia coli* K-12, the *phoE* gene encodes an outer membrane pore protein, which is induced by phosphate starvation. The corresponding gene of *Enterobacter cloacae* was transferred to *E. coli* K-12 by using RP4::mini Mu plasmid pULB113 and selecting for R-prime plasmids that carry the genes *proA* and *proB*, which are closely linked to *phoE* in *E. coli* K-12. The *phoE* gene was subcloned into the multicopy vector pACYC184, and the location of the gene was determined by analysis of in vitro constructed deletion plasmids and mutant plasmids generated by $\gamma\delta$ insertions. The *E. cloacae phoE* gene is normally expressed in *E. coli* K-12, and the regulation of the expression is similar to that of the *E. coli phoE* gene. Functionally, the products of the *phoE* genes of *E. coli* K-12 and *E. cloacae* behave very similarly since they form pores in the outer membrane with a recognition site for negatively charged compounds and they serve as (part of) the receptor for phage TC45.

INTRODUCTION

The outer membrane of Enterobacteriaceae functions as a barrier for harmful compounds. The membrane contains a number of abundant proteins, designated as porins or pore proteins, which allow the influx of nutrients. These pore proteins function as nonspecific diffusion channels through which small hydrophilic solutes with M_rs up to about 700 can pass (for a review, see Nikaido, 1979).

Under standard laboratory conditions, *E. coli* K-12 produces two distinct pore proteins, namely OmpC and OmpF (Van Alphen et al., 1978). The synthesis of another pore protein, designated as PhoE protein, is induced when cells are grown under phosphate limitation (Overbeeke and Lugtenberg, 1980a). The primary structure of these porins, OmpF, OmpC and PhoE is very similar (Mizuno et al., 1983). However, in contrast to the OmpF and

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Abbreviations: Ap, ampicillin; bp, base pairs; Cm, chloramphenicol; EtBr, ethidium bromide; kb, 1000 bp; Kn, kanamycin; LPS, lipopolysaccharide; ^{R}(superscript), resistant; Rf, rifampicin; SDS, sodium dodecyl sulfate; Sm, streptomycin; Tc, tetracycline;::, novel joint; [ ], indicates plasmid-carrier state.

OmpC proteins, PhoE protein forms pores with a preference for negatively charged solutes (Korteland et al., 1982; Nikaido et al., 1983; Overbeeke and Lugtenberg, 1982).

The synthesis of PhoE protein is subject to a complex regulatory system in which three regulatory genes are involved, namely *phoB*, *phoR* and *phoM* (Tommassen and Lugtenberg, 1980; Tommassen et al., 1982a, 1984; Wanner and Latterell, 1980). Also, mutations in the genes *phoS*, *phoT*, *phoU* and *pst*, which are primarily involved in phosphate transport, affect the expression of PhoE protein, in that the protein is produced constitutively in strains carrying any of these mutations (Tommassen and Lugtenberg, 1980).

For two reasons we are interested in studying the expression of *phoE* genes from other Enterobacteriaceae in *E. coli* K-12: (i) cloning, sequence analysis and studies on the expression and functioning of the "foreign" *phoE* genes and their products are expected to contribute significant information to our studies on the regulation, biogenesis and structure–function relationship of PhoE protein, and (ii) since outer membrane proteins are potential constituents of vaccines against pathogenic Gram-negative bacteria, their expression in *E. coli* K-12 would be very useful for isolating large quantities of these proteins. Thus, it would be interesting to determine whether the structural genes for outer membrane proteins of other Gram-negative bacteria are expressed in *E. coli* K-12. The cloning of the *Enterobacter cloacae phoE* gene in *E. coli* K-12 is described in this paper. It appears to be normally expressed.

MATERIALS AND METHODS

(a) Bacterial strains, phages and growth conditions

The bacterial strains and their characteristics are listed in Table I. Spontaneous Rf-resistant mutants were obtained by plating 3×10^8 cells of an overnight culture on plates containing 40 μg/ml Rf. Mutants with a heptose-deficient LPS were isolated with the aid of the LPS-specific phages T3, T4 and T7 as described earlier (Havekes et al., 1976). Phage TC45 uses PhoE protein as part of its receptor (Chai and Foulds, 1978). Phage GU5 is specific for strains containing IncP plasmids (Van Gysegem and Toussaint, 1982).

Except where noted, cells were grown overnight at 37°C under aeration in L-broth, which contains 1% tryptone, 0.5% yeast extract, 0.5% NaCl, 0.002% thymine, pH 7.0. Minimal medium used for the selection of auxotrophic markers (Lugtenberg et al.,

TABLE I

Bacterial strains and their characteristics [a]

Strain	Characteristics	Reference/Source
***E. coli* K-12**		
AB1157	F⁻, *thr leu Δ(proA-proB-phoE-gpt) his thi argE lacY galK xyl rpsL*	Adelberg
CE1194	*phoS*21 *bgl* transductant of AB1157	Tommassen et al., 1982b
CE1195	*pro*⁺ *phoE*⁺ transductant of CE1194	Tommassen et al., 1982b
CE1224	*ompR* derivative of AB1157	Tommassen et al., 1983
CE1223	*recA*56 *his*⁺ derivative of CE1194	Tommassen et al., 1983
CE1226	*thr*⁺ *hsdR hsdM his*⁺ *recA*56 derivative of CE1194	This study
MXR	*Δ(lac-pro) galE thi recA*1	Van Gysegem and Toussaint, 1982
CE1227	F⁻, *thr leu thi pyrF thy ilvA lacY argG tonA tsx rpsL cod deoC vtr glpR phoS*200 *phoB*201 *recA*56	This study
CE1225	*thi rpsL phoR*68 *phoM*453 *lac recA*56	Tommassen et al., 1984
CE1238	F⁻, *thi pyrF thy ilvA his argG tonA rpsL deoC vtr glpR ompR*471 *phoR*69 *phoA*8 *Δ(proA,B-phoE)*	Korteland et al., 1982
CE1304	F′ *lac*⁺, *thr thi recA*171 *lacZ*22 *lacI rpsL rpoB supE*	De Geus et al., 1983
Enterobacter cloacae		
H478	*his*	De Graaf

[a] Nomenclature according to Bachmann (1983).

1976) and low- and high-phosphate containing minimal media (Tommassen and Lugtenberg, 1980) have been described previously. To select for antibiotic resistance, the following concentrations were used: Ap 50 μg/ml, Cm 25 μg/ml, Kn 50 μg/ml, Sm 100 μg/ml and Tc 10 μg/ml. For plating, media were solidified with Difco agar at a final concentration of 1.5%.

(b) Genetic techniques

Matings between donor strains carrying RP4-derived plasmids and recipient strains were performed as described by Van Gysegem and Toussaint (1982). Transformation was carried out as described by Brown et al. (1979). Sensitivity to phages was determined by cross-streaking.

(c) Cell envelope preparation

Cell envelopes were isolated by differential centrifugation after ultrasonic disruption of cells (Lugtenberg et al., 1975). Protein patterns of cell envelopes were analyzed by SDS-polyacrylamide gel electrophoresis as described by Lugtenberg et al. (1975), except that 4 M urea was added to the running gel where indicated.

(d) DNA preparation

Crude plasmid DNA was prepared as described by Birnboim and Doly (1979). DNA, needed for in vitro recombination experiments, was further purified by CsCl-EtBr isopycnic centrifugation. Ligation with T4 DNA ligase was performed as described by Tanaka and Weisblum (1975). Analyses of plasmid DNA and DNA fragments were performed by electrophoresis in a horizontal 0.6% agarose slab gel (Van den Hondel et al., 1979).

(e) Isolation of plasmids carrying an inserted γδ-transposable element

Insertions of the transposable element γδ in plasmid pEC17 were obtained as described by Guyer (1978). Plasmid pEC17 was transformed into the F′ *lac*$^+$-containing strain CE1304, selecting for Cm^r colonies. One transformant was used as a donor in a mating with strain CE1238 as the recipient, selecting for Cm^R, *leu*$^+$-colonies. Transconjugants with a γδ insertion on pEC17 inside or outside of *phoE* were identified as resistant or sensitive to phage TC45, respectively.

(f) Rate of permeation of β-lactam antibiotics

The rate of permeation of β-lactam antibiotics through the outer membrane and the inhibition of this permeation by polyphosphate were measured using a method originally described by Zimmerman and Rosselet (1977) and modified by Overbeeke and Lugtenberg (1982), except that the final concentrations of the antibiotics cefsulodin and cephaloridine in the suspensions were 0.8 mM instead of 0.7 and 0.9 mM, respectively.

RESULTS

(a) Transfer of the *proA-proB* region of the *E. cloacae* genome to *E. coli* K-12

For cloning of the *phoE* gene of *E. cloacae* the RP4::mini Mu plasmid pULB113 was used. This plasmid is a derivative of the wide host-range conjugative plasmid RP4 and carries a deleted Mu prophage which allows the plasmid to pick up any chromosomal DNA segment to form R-prime plasmids (Van Gysegem and Toussaint, 1982). The plasmid renders cells resistant to the antibiotics Ap, Kn and Tc. Whereas there is no direct selection for expression of a cloned *phoE* gene available, we could take advantage of the observation that *phoE* in *E. coli* K-12 is located very close to the selectable markers *proA* and *proB* (Tommassen and Lugtenberg, 1981; Tommassen et al., 1982b).

Plasmid pULB113 was transferred to an Rf^R derivative of *E. cloacae* strain H478 by conjugation with donor strain MXR[pULB113], selecting for Kn^R and Rf^R transconjugants. One transconjugant was subsequently used as a donor strain in a mating with *phoS recA* strain CE1226 as the recipient. The latter host contains a *proA-proB-phoE-gpt* deletion. All ten Sm^R *pro*$^+$ transconjugants tested contained a RP4::mini Mu plasmid, since they were Ap^R, Kn^R, sensitive to the Inc-P plasmid-specific phage GU5, and immune to Mu. Plasmid preparations

from these transconjugants all exhibited a band which migrated more slowly on agarose gels than the original pULB113 (results not shown), suggesting that R′ plasmids were obtained. One of these plasmids, designated as pEC10, was further analyzed.

(b) Presence of *E. cloacae phoE* gene on pEC10 and its expression in *E. coli* K-12

Evidence for the presence of an *E. cloacae phoE* gene on pEC10 is obtained if a protein, similar to the *E. coli* PhoE protein, is expressed in a pEC10-containing derivative of *phoS phoE* strain CE1226. In contrast to the parental strain CE1226, the pEC10-containing transconjugant turned out to be sensitive to the PhoE protein-specific phage TC45, showing that a PhoE-like protein is expressed in the latter strain.

Analysis of the cell envelope protein patterns of these strains on SDS-polyacrylamide gels did not reveal a band in the position of the *E. coli* PhoE protein in any of these strains (Fig. 1A, lanes c and d). However, the band in the position of OmpF protein, which migrates slightly faster in the gel than PhoE protein, was heavier stained in case of the cell envelopes of the pEC10-containing strain, suggesting that this band might contain the *E. cloacae* PhoE protein as well as the OmpF protein. Therefore, the cell envelopes were also analyzed in a running gel, which was supplemented with 4 M urea, since this is known to result in another resolution of the proteins (Overbeeke and Lugtenberg, 1980b). As shown in Fig. 1B, an additional band was now found in the cell envelopes of the pEC10-containing strain (lane d), which migrates slightly faster in the gel than *E. coli* PhoE protein and which was not observed in the cell envelopes of a plasmidless strain (lane c) or in the cell envelopes of a TC45-resistant derivative, isolated from CE1226-containing pEC10 (lane e). Therefore, this band represents the *E. cloacae* PhoE protein.

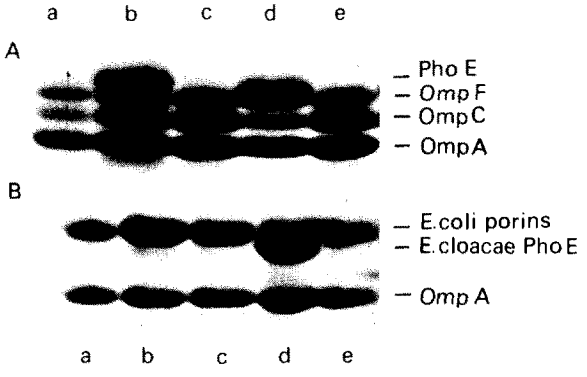

Fig. 1. Cell envelope protein patterns as analyzed by SDS-polyacrylamide gels, either according to Lugtenberg et al. (1975) (panel A), or on gels supplemented with 4 M urea (panel B). The lanes contain cell envelope preparations of *phoS phoE* strain CE1194 (a), *phoS phoE*$^+$ strain CE1195 (b), *phoS phoE* strain CE1226 (c), CE1226 [pEC10] (d), and of a TC45-resistant derivative of the latter strain (e). Only the relevant part of the gels, containing the proteins with apparent M_rs between 35000 and 40000, is shown.

(c) Subcloning of the *E. cloacae phoE* gene

For a physical analysis of the *E. cloacae phoE* gene, this gene was subcloned from pEC10 into the multicopy vector pACYC184. The latter plasmid renders cells resistant to Cm and Tc and contains unique sites for *Sal*I and *Bam*HI in the Tc^R gene (Chang and Cohen, 1978). For cloning of the *phoE* gene, pACYC184 was digested with both enzymes to prevent re-circularization of the vector DNA during subsequent ligation. Purified pEC10 was also digested with *Sal*I and *Bam*HI and, after inactivation of the restriction enzymes, the DNA preparations were mixed, ligated with T4 DNA ligase and transformed into strain CE1226, selecting for Cm^R colonies. Plasmid DNA was isolated from one transformant that was sensitive to TC45, showing that the *E. cloacae phoE* gene had been cloned. This 19.7-kb plasmid, designated as pEC11, contains one *Sal*I site, two *Bam*HI sites and five *Pst*I sites (not shown). *Pst*I does not cleave the vector DNA. For further subcloning of *phoE*, pEC11 was partially digested with *Pst*I. After inactivation of the enzyme and ligation with T4 DNA ligase, the DNA preparation was transformed into strain CE1226. Plasmid DNA was isolated from one Cm^R, TC45-sensitive transformant. The plasmid, pEC12, is 10.7 kb long (not shown). To adapt the plasmid DNA to the restriction-modification system of *E. coli* K-12, the DNA was passed through *hsdR*$^+$ *hsdM*$^+$ strain CE1223. Plasmid DNA from one Cm^R, TC45-sensitive transformant was further studied and is designated as pEC13.

To prepare a restriction map of pEC13 (Fig. 2), fragments of pEC13 generated by single and double digestions with several restriction enzymes, were

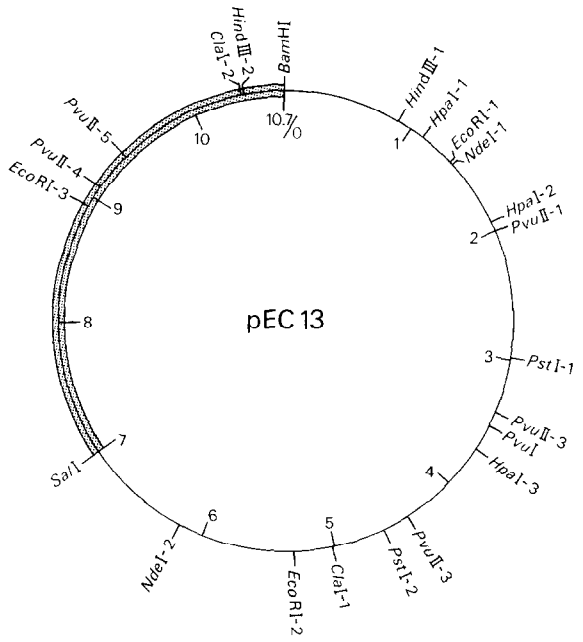

Fig. 2. Restriction map of plasmid pEC13. The pACYC184 vector is indicated by the shaded segment. Map units are in kb. The plasmid contains no *Bgl*II, *Mlu*I and *Sma*I sites.

analyzed on agarose gels. The position of the pACYC184 vector was deduced from its known restriction map (Chang and Cohen, 1978).

(d) Physical localization of the *E. cloacae phoE* gene on pEC13

To localize the *phoE* gene, deletion mutant plasmids of pEC13 were constructed in vitro by digesting purified pEC13 DNA with *Hin*dIII, *Cla*I, *Nde*I, *Hpa*I or *Pst*I. After subsequent ligation, the DNA preparations were transformed into strain CE1223, selecting Cm^R colonies. Plasmid DNA was isolated from the transformants and plasmids pEC14, 15, 16, 17 and 18 were selected for further characterization. The presence of a functional *phoE* gene on the plasmids was determined by testing the transformants for sensitivity to TC45. The results of the analysis of the deletion plasmids are shown in Fig. 3. Since pEC17, in contrast to pEC18, contains a functional *phoE* gene, this gene must be (partially) located on the

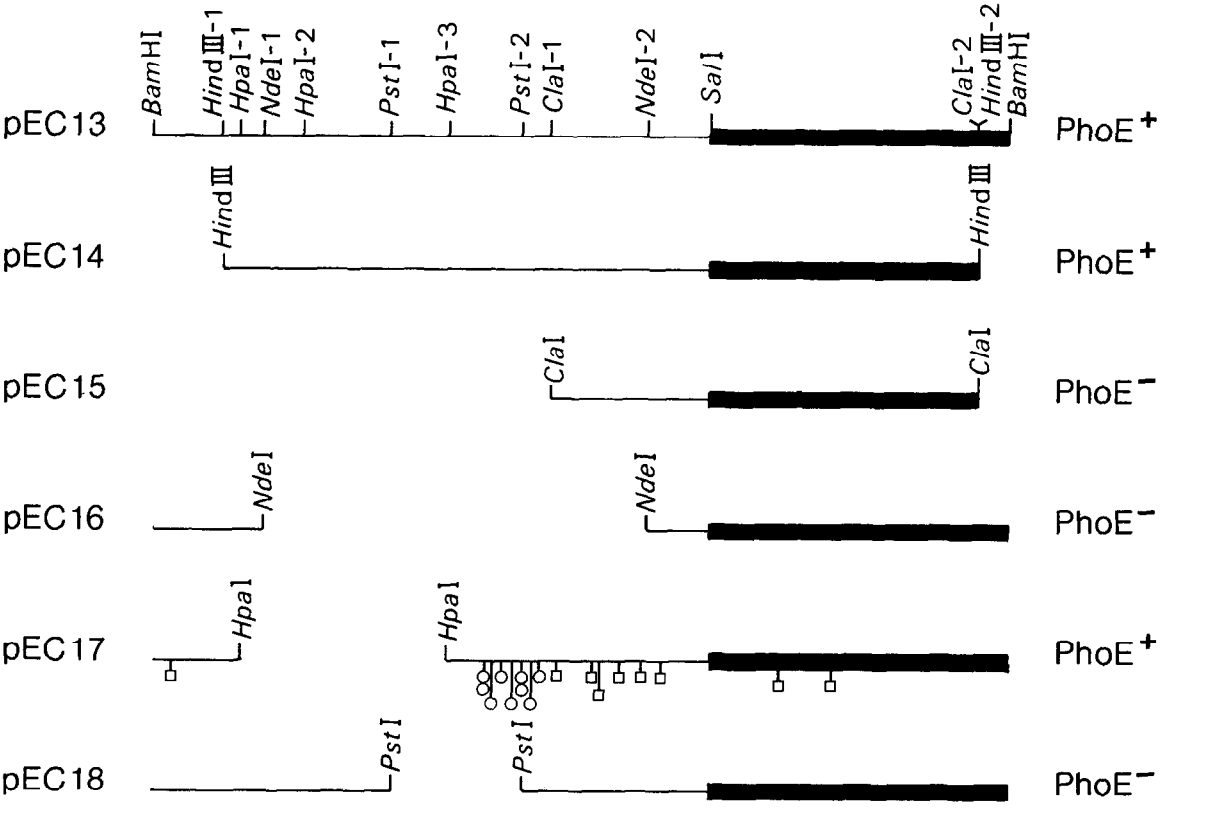

Fig. 3. Localization of *E. cloacae phoE* gene on pEC13 and on derivative plasmids. The presence of a functional *phoE* gene on the plasmids was determined by testing plasmid-containing strains for sensitivity to TC45. The locations of the $\gamma\delta$ insertions in pEC17 are also indicated: insertions that inactivate the *phoE* gene are indicated by circles (o) and insertions outside *phoE* by squares (□). Shaded segments represent pACYC184 vector (see Fig. 2).

0.9-kb DNA fragment between the *Hpa*I-3 and *Pst*I-2 sites of pEC13, whereas the rest of the gene must be located on the *Pst*I-2 to *Sal*I fragment.

This result was confirmed by the analysis of mutant plasmids of pEC17, generated with the aid of the transposable element $\gamma\delta$ of the F plasmid. The $\gamma\delta$ insertions in pEC17 were obtained as described in MATERIALS AND METHODS, section **e**, and the locations of the insertions were mapped using *Cla*I-, *Sal*I-, and *Bam*HI-generated restriction fragments. *Cla*I and *Sal*I cleave the 5.6-kb $\gamma\delta$ element approximately in the middle, whereas *Bam*HI cleaves at 0.2 kb from one end. The results (Fig. 3) show that the insertions in *phoE* are clustered in a region of approx. 0.7 kb, which corresponds with the region predicted from the analysis of the deletion mutant plasmids.

(e) Regulation of the expression of the *E. cloacae phoE* gene in *E. coli* K-12

To determine whether the expression of the *E. cloacae phoE* gene in *E. coli* K-12 is subject to the same regulatory system as the expression of the *E. coli phoE* gene, pEC13 was transformed into strain CE1224, which is wild type for all *pho* regulatory genes, *phoS* strain CE1194, *phoS phoB* strain CE1227, *phoR* strain CE1238 and *phoR phoM* strain CE1225. Cell envelope protein patterns of the transformants were analyzed on SDS-polyacrylamide gels containing 4 M urea (Fig. 4). The *E. cloacae* PhoE protein is produced in CE1224 only when cells are grown under phosphate limitation (lanes a and b). The protein is produced constitutively in *phoS* strain CE1194 (lane c) but the synthesis of the protein is dependent on the *phoB* product (lane d). The protein is also produced constitutively in *phoR* strain CE1238 (lane e), and the constitutive synthesis in a *phoR* strain is dependent on the *phoM* product (lane f). In all these respects, the expression of the *E. cloacae* PhoE protein is exactly the same as that of the *E. coli* K-12 PhoE protein (Tommassen and Lugtenberg, 1980; Tommassen et al., 1984).

The expression of *E. coli* PhoE protein is dependent on a wild-type *E. coli* K-12 LPS, in that mutants with a heptose-deficient LPS produce the protein in severely decreased amounts (Tommassen and Lugtenberg, 1981). Probably, during the synthesis of PhoE protein an interaction with the LPS is required at some post-translational event (Tommassen, J., Overduin, P. and Lugtenberg, B., in preparation). Also in this respect, expression of *E. cloacae* PhoE protein in *E. coli* K-12 is similar to that of *E. coli* PhoE protein, since the cell envelope protein pattern of a heptose-deficient mutant of CE1238 containing pEC13 contains decreased amounts of PhoE protein (Fig. 4, compare lanes e and g).

(f) Pore properties of the *E. cloacae* PhoE protein

It has been reported that PhoE protein pores of *E. coli* K-12 have a preference for anionic solutes, in contrast to OmpF and OmpC protein pores (Korteland et al., 1982; Nikaido et al., 1983; Overbeeke and Lugtenberg, 1982). This preference for negatively charged solutes has been explained in terms of a recognition site on the pore for negatively charged compounds (Overbeeke and Lugtenberg, 1982). Proof for this assumption was obtained in experi-

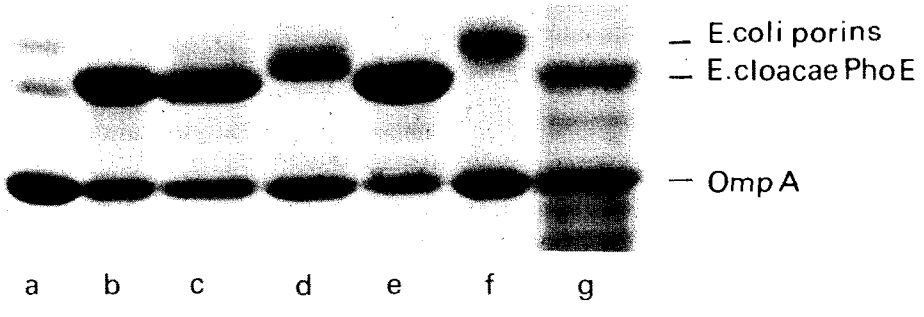

Fig. 4. Expression of the *E. cloacae phoE* gene in *E. coli* K-12 strains. Cell envelope protein patterns of pEC13-containing derivatives of: *phoE* strain CE1224, grown in high (a) and low (b) phosphate minimal medium; *phoSphoE* strain CE1194 (c); *phoSphoB* strain CE1227 (d); *phoR phoE* strain CE1238 (e); *phoR phoM* strain CE1225 (f) and a mutant strain of CE1238 with heptose-deficient LPS (g) are shown on an SDS-polyacrylamide gel containing 4 M urea.

ments showing competition for this site. Thus, permeation of β-lactam antibiotics through PhoE protein pores, but not through OmpF protein pores, is inhibited by negatively charged compounds, e.g., polyphosphates (Overbeeke and Lugtenberg, 1982). To determine whether this recognition site is also present on the *E. cloacae* PhoE protein pores, the influence of polyphosphate on the rate of permeation of the β-lactam antibiotics cephaloridine and cefsulodin was measured (Table II). The results show that the permeation of the antibiotics through the *E. cloacae* PhoE protein pores is inhibited even slightly more strongly than that through the *E. coli*-K-12 PhoE protein pores.

DISCUSSION

Growth of *E. coli* K-12 under phosphate limitation leads to the induction of pore protein PhoE, which is particularly efficient in the uptake of organic and inorganic phosphate (Overbeeke and Lugtenberg, 1980; Korteland et al., 1982). Its structural gene is located at min 6 on the chromosome, close to the genes *proA* and *proB* (Tommassen and Lugtenberg, 1981). We were able to demonstrate the presence of a similar gene in *E. cloacae* by transferring the corresponding region of the *E. cloacae* chromosome to *E. coli* K-12.

Previous studies on the intergeneric exchange of major outer membrane protein genes gave varying results with respect to the expression of these genes. Whereas the cloned *ompA* genes of *Shigella dysenteriae*, *Enterobacter aerogenes* and *Serratia marcescens* are normally expressed in *E. coli* K-12 (Cole et al., 1982), it has also been reported that the *ompA* genes of two clinical *E. coli* isolates are only very poorly expressed when moved to *E. coli* K-12 by transduction (Beher et al., 1980a). Similarly, the *ompF* gene of *E. coli* K-12 is very poorly expressed in *E. coli* B (Pugsley and Rosenbusch, 1983). The poor expression of these genes was supposed to be either due to a defect in transport of the proteins to the outer membrane, for which an interaction with LPS molecules is presumed to be required (Beher et al., 1980a), or due to the presence of a heterologous regulatory gene (Pugsley and Rosenbusch, 1983). We showed that the *E. cloacae phoE* gene is normally expressed in *E. coli* K-12 and the regulation of the expression is similar to that of the *E. coli* K-12 *phoE* gene. Thus, apparently those features of PhoE protein that are recognized by the protein export system are highly conserved, whereas the operator region of the *phoE* gene is also conserved.

Nucleotide sequence analysis of the cloned *ompA* genes from various Enterobacteriaceae (Beck and Bremer, 1980; Braun and Cole, 1983; Movva et al., 1980) suggested that OmpA protein is composed of "variable" and "constant" regions, with the variable regions probably all exposed on the cell surface. Similarly, comparison of the sequences of the *E. coli* K-12 pore protein genes, *phoE*, *ompF* and *ompC* reveals "variable" and "constant" regions (Mizuno et al., 1983). However, comparison of the PhoE proteins of *E. coli* K-12 and *E. cloacae* shows that those

TABLE II

Influence of polyphosphate on the rate of permeation of β-lactam antibiotics through PhoE protein pores in the outer membrane

β-Lactam antibiotic (0.8 mM)	Polyphosphate[b]	Rate of uptake by intact cells[a]	
		CE1238 [pJP12, pBR322] (*E. coli* PhoE)	CE1238 [pEC10] (*E. cloacae* PhoE)
Cefsulodin	−	11.2	7.3
	+	3.4 (69%)	1.2 (82%)
Cephaloridine	−	9.3	12.8
	+	2.8 (70%)	1.9 (85%)

[a] Rate of uptake in the presence or absence of polyphosphate is expressed as nmol/min/μg pore protein. In parentheses: percentages of inhibition.

[b] The presence or absence of 0.2 mM linear polyphosphate with average chain length of 15 phosphate residues during the uptake experiments is indicated by + or −, respectively.

functions, which are related to the cell surface exposed part of the protein, i.e., a recognition site for negatively charged compounds and the phage TC45 receptor function, are conserved. In addition, it has been shown (Van der Ley, P., Amesz, H., Tommassen, J. and Lugtenberg, B., submitted for publication) that five out of six monoclonal antibodies directed against the cell surface-exposed part of PhoE protein also recognize *E. cloacae* PhoE protein. These results suggest that, in addition to the transmembrane stretches, the cell surface-exposed parts of PhoE protein are also highly conserved in *E. coli* K-12 and *E. cloacae*. However, a strong homology between these *phoE* genes is not apparent from the restriction maps of the genes. Whereas the *E. coli* K-12 *phoE* gene contains unique *Pst*I, *Mlu*I, *Cla*I, *Nde*I and *Bgl*II sites (Tommassen et al., 1982b; Overbeeke et al., 1983), in the *E. cloacae phoE* gene only the *Pst*I site (*Pst*I-2 in Fig. 2) is found in a position which might correspond to the *Pst*I site in the *E. coli phoE* gene. The *Pvu*II site in the *E. cloacae phoE* gene (Fig. 3) is not found in the *E. coli phoE* gene.

Whereas the *E. cloacae* PhoE protein is able to act as the receptor for TC45 when produced in *E. coli* K-12, the phage is not irreversibly absorbed by *E. cloacae* cells, induced for PhoE protein by growth under phosphate limitation (unpublished observation). Similarly, it has been reported that OmpA protein of *Salmonella typhimurium* functions as the receptor for phage Ox2 when produced by *E. coli* K-12 but not when produced by *S. typhimurium* (Freudl and Cole, 1983). However, rough mutant strains of *S. typhimurium* are sensitive to Ox2 (Beher et al., 1980b), suggesting that the O-antigen part of the LPS masks the receptor for the phage. Analogously, a permeability barrier created by the O-antigen or a capsule might prevent the adsorption of TC45 to PhoE protein producing *E. cloacae* cells.

It is obvious that nucleotide sequence analysis of the *E. cloacae phoE* gene and comparison to the known sequence of the *E. coli phoE* gene (Overbeeke et al., 1983) will contribute to our understanding of the structure-function relationship of PhoE proteins. In addition, the sequence might be very useful for the analysis of the operator sequence of the *phoE* gene, which is subject to the complex *pho* regulatory system. Therefore, we are in the process of sequencing this gene.

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