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The Gene Locus *yijP* Contributes to *Escherichia coli* K1 Invasion of Brain Microvascular Endothelial Cells

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Most cases of *Escherichia coli* meningitis develop as a result of hematogenous spread, but it is not clear how circulating *E. coli* crosses the blood-brain barrier. A Tn*phoA* mutant of *E. coli* K1 RS218 was shown to be significantly less invasive than its parent strain in bovine and human brain microvascular endothelial cells (BMEC), which constitute the blood-brain barrier. More importantly, traversal of the blood-brain barrier was significantly less with this mutant than with the parent strain in newborn rats with experimental hematogenous meningitis. A DNA segment containing the Tn*phoA* insertion site was cloned from RS218, and the cloned DNA complemented the Tn*phoA* mutant in invasion of BMEC. Nucleotide sequence revealed a near identity to that of a hypothetical *yijP* gene (also called *f577*) in the *E. coli* K-12 genome. Sequence analysis indicated that the *E. coli* K1 *yijP* gene likely encodes a 66.6-kDa membrane protein. Deletion and complementation experiments indicated that the *yijP* gene was involved in *E. coli* K1 invasion of BMEC, i.e., the invasive ability of *E. coli* K1 was significantly reduced after *yijP* was deleted and was restored by complementation with a plasmid containing the *yijP* open reading frame. This is the first demonstration that the *yijP* gene locus plays a role in the pathogenesis of *E. coli* K1 meningitis.

Escherichia coli is the most common gram-negative microorganism causing meningitis in the neonatal period (19, 26). Most cases of *E. coli* meningitis occur as a result of hematogenous spread (14), but it is unclear how circulating *E. coli* crosses the blood-brain barrier. The entry of circulating *E. coli* into the central nervous system (CNS) is most likely to occur at sites of the blood-brain barrier, which is composed of brain microvascular endothelial cells (BMEC).

To study the mechanism of *E. coli* translocation from blood to the CNS, we have developed both in vitro and in vivo models of the blood-brain barrier. The in vitro model of the blood-brain barrier was established by isolation and cultivation of BMEC (12, 22, 23). The resulting BMEC exhibited transendothelial electrical resistance of 100 to 600 Ω/cm^2 (15, 20), a unique property of the brain microvascular endothelial monolayer compared to systemic vascular endothelium. The in vivo model of *E. coli* meningitis was established by induction of hematogenous meningitis in 5-day-old rats (14). In this experimental meningitis model, bacteria are injected via subcutaneous or intracardiac injection, resulting in bacteremia and subsequent entry into the CNS. Using these models of the blood-brain barrier, we have shown that invasion of BMEC is a requirement for *E. coli* K1 penetration of the blood-brain barrier in vivo (12). In an attempt to identify *E. coli* K1 structures contributing to invasion of BMEC, we initially searched for any homology with the known proteins involved in invasion of eukaryotic cells by meningitis causing bacteria. We identified that *E. coli* outer membrane protein A (OmpA) has a sequence homology with *Neisseria* Opa proteins (18), which have been shown to be involved in invasion of eukaryotic cells (30). We have subsequently shown that *E. coli* OmpA contributes to invasion of BMEC (18). Our other approach to identify *E. coli* structures contributing to invasion of BMEC was by use of the transposon Tn*phoA*. These investigations have identified two *ibe* loci, *ibeA* (previously named *ibe10* [12]) and *ibeB* (11), which are located at different sites in *E. coli* K1 chromosome.

In our previous Tn*phoA* mutagenesis of *E. coli* K1 RS218, which is a cerebrospinal fluid (CSF) isolate from a neonate with meningitis, we identified another mutant named 23A-20, which possessed OmpA, IbeA, and IbeB but exhibited significantly less invasion into BMEC in vitro compared to the parent strain (12). In the present study we identified, by cloning and characterizing the Tn*phoA* insertion site of the mutant 23A-20, that the *yijP* locus contributes to *E. coli* traversal across the blood-brain barrier.

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MATERIALS AND METHODS

Bacterial strains, plasmids, and culture conditions. Bacterial strains, plasmid vectors, and their relevant characteristics are described in Table 1. Strain 23A-20 is a Tn*phoA* insertion mutant of *E. coli* K1 E44 as described previously (12). Plasmids constructed in this study are illustrated in Fig. 1 or described in the text. *E. coli* strains were cultured at 37°C in Luria broth (LB; 1% tryptone, 0.5% yeast extract, 0.5% NaCl), brain heart infusion (BHI; Difco Laboratories, Detroit, Mich.), or a synthetic medium (SM) based on M9 (21) supplemented with thiamine and nicotinamide (10 μg of each per ml) and pyruvate (10 mM) as a carbon source. Columbia agar with 5% sheep blood (Remel, Lenexa, Kans.) was used. When necessary, the medium was supplemented with ampicillin (Ap; 100 μg/ml), kanamycin (Km; 40 μg/ml), chloramphenicol (Cm; 25 μg/ml), or rifampin (Rif; 50 μg/ml).

Tissue cultures and invasion assays. BMEC were prepared from bovine and human brains (22, 23), and invasion assays were performed as previously described (11). The primary bovine BMEC were used, and the human BMEC were immortalized by transfection with simian virus 40 large T antigen (24). In invasion assays, bacteria were added to confluent monolayers of BMECs with a multiplicity of infection of 100. The monolayers were incubated for 1.5 h at 37°C to allow invasion to occur. The number of intracellular bacteria was determined on blood agar after extracellular bacteria were killed by incubation of the monolayers with experimental medium containing gentamicin (100 μg/ml). Results were expressed either as the percent invasion ([number of intracellular bacteria recovered/number of bacteria inoculated] × 100) or as the relative invasion (invasion as a percentage of the parent *E. coli* K1 strain).

Neonatal rat model of hematogenous *E. coli* K1 meningitis. The Tn*phoA* insertion mutant 23A-20 was examined for its ability to enter the CNS by using our newborn rat model of experimental hematogenous *E. coli* meningitis as described previously (14). Briefly, at 5 days of age, all members of each litter were randomly divided into two groups to receive, via intracardiac injection, 6.2×10^6 CFU of the parent strain E44 or 1.1×10^7 CFU of the mutant strain

TABLE 1. Bacterial strains and plasmid vectors used in this study

Strain or plasmid	Relevant characteristic(s)	Source or reference
E. coli		
RS218	O18:K1:H7	1
E44	Rif^r of RS218	11
23A-20	E44(*yijP*::Tn*phoA*)	This study
D12	E44(Δ*yijP*)	This study
DH5α	F′ *recA1 hsdR17 thi-1 gyrA96 supE44 endA1 relA1 recA1 deoR* Δ(*lacZYA-argF*) *U169* (θ *80lacZ*M15)	Gibco-BRL
SM10λ*pir*	*thi thr leu tonA lacY supE recA*::RP4-2-Tc:: Mu (λ*pir*) *pro endA hsdA hsdR supF*	25
Vectors		
pUC13	Ap^r; *lacZ′*	27
pCVD442	Ap^r; *sacB oriV oriT*	10
pCVD433	Tc^r (pACYC184 with a *Mlu*I linker)	9
pIB307	Cm^r (temperature-sensitive pSC101 derivative)	6
pK194	Km^r; *lacZ′* (pACYC184 derivative)	13

23A-20. Our pilot experiments revealed that these bacterial inocula for strains E44 and 23A-20 produced bacteremia of 10^5 to 10^8 CFU/ml of blood in almost 100% of the infected animals within 2 h of inoculation. This level of bacteremia has been shown to be sufficient for allowing circulating *E. coli* to enter the CNS (13). At 1 to 2 h after bacterial inoculation, blood and CSF specimens were collected for quantitative cultures. Blood and CSF specimens obtained from animals infected with 23A-20 were cultured in BHI with Km.

Molecular cloning. Standard molecular techniques (21) were used unless otherwise specified. *Mlu*I-digested genomic DNA from the mutant 23A-20 was cloned in the same site of pCVD433 as described (9). Km^r transformants were selected, and a plasmid containing an extra 2-kb insert was purified and partially sequenced by using the 5′ primer Tnp5 and 3′ primer Tnp3, which are complementary to the ends of Tn*phoA* (12). Plasmid DNA was prepared by using the Miniprep Spin Column (Qiagen, Valencia, Calif.). Nucleotide sequencing was carried out with the Applied Biosystems automatic sequencer (Foster City, Calif.).

The genomic library of *E. coli* K1 RS218 was constructed by using the LambdaGEM-12 system (Promega, Madison, Wis.) according to the manufacturer's instructions. The library was screened with ^{32}P-labeled PCR DNA containing the Tn*phoA* insertion site. The recombinant λ DNAs were isolated from the positive plaques and analyzed by restriction digestion. The cloned DNA was subcloned in pUC13 as illustrated in Fig. 1, except for pK23P, which contains only the *yijP* open reading frame (ORF) in the vector pK194. This plasmid was constructed as follows: a 325-bp PCR fragment was generated between two *Mlu*I sites at the 3′ end of *yijP* (Fig. 1), in which the second *Mlu*I was converted to *Pst*I through primer design. This *Mlu*I-*Pst*I PCR DNA was used to replace the 1.2-kb *Mlu*I-*Pst*I fragments in pUC-BS3 to produce the pUC23P. A sequencing reaction was performed to verify the 3′ end of *yijP* in pUC23P. The 2-kb *Bam*HI-*Pst*I fragment containing *yijP* ORF was then transferred to pK194 at the same sites to produce pK23P.

Complementation analysis. The recombinant plasmids were introduced into E44 or its *yijP* mutant derivatives by electroporation as previously described (29). Transformants were tested for their invasion in BMEC. The assays were repeated at least three times, each time in triplicate wells. Results were expressed as relative invasion by comparing to the invasion of the parent strain E44 containing the vector.

Construction of *yijP* deletion mutant in *E. coli*. The *Eco*RV-to-*Mlu*I fragments of 1.85 kb containing the entire *yijP* ORF was deleted from pUC-BS3 (termed pBS-Δibe) (Fig. 1). The 1.1-kb *Bam*HI-*Hin*dIII fragment containing the *yijP*-deleted DNA was cleaved from pBS-Δibe and inserted into the *Hin*dIII-*Bam*HI sites in pEB1.4 (Fig. 1). This recombinant 2.55-kb *yijP*-deleted DNA was cleaved with *Hin*dIII-*Eco*RI and cloned in the suicide plasmid pCVD442 at the *Sma*I site (termed pCVD-Δibe). The *yijP* gene was then deleted from E44 chromosome by the method described earlier (10). Briefly, pCVD-Δibe was transferred into E44 by conjugation with SM10λpir as the host strain. Ap^r Rif^r transconjugants were selected and grown in LB without antibiotics at 32°C for 6 h and then diluted and plated on LB containing no NaCl and 5% sucrose at 30°C. The sucrose-resistant Ap^s Rif^r colonies were selected and tested for the deletion of *yijP* by PCR as described previously (29) by using the TaqPlus Long PCR System (Stratagene, La Jolla, Calif.). E44 DNA, pCVD-Δibe, and an Ap^r Rif^r transconjugant colony were used as control templates in such PCR reactions. The primers used were: PPC-C (5′-CAGAGTCTATTCAGCTAC) located at the end of the *ppc* gene, F283-R (5′-AGATAGCTGACGTCGTGA) located at the beginning of *f283*, and 23A3R (5′-CAGGCTGAAATACTGGCT) and 23A-5 (5′-GCCAGCCTG ATTAAAGAC), both of which were located in the middle of *yijP*.

Construction of antibiotic cassette insertion mutants. To interrupt the *f283* gene, the 1.2-kb *Acc*I-*Bst*31 fragment containing the Cm^r gene was cleaved from pACYC184 and inserted at the *Sal*I site in pUC-B6. The DNA was subcloned in pCVD442 and then transferred to E44 by conjugation, selecting for Cm^r Rif^r and Ap^s transconjugants. To completely delete the ORF *o113*, a 1-kb DNA fragment was amplified from upstream of *o113*, and another from downstream of *o113* by using the *Pfu* DNA polymerase (Stratagene). These two fragments were cloned in pBluescript II KS(+) (Stratagene) and then transferred to the temperature-sensitive plasmid pIB307. A 1.2-kb Km^r cassette was isolated from pUC-4K (Pharmacia) and inserted in the middle of the *o113*-deleted DNA to obtain pIB-o113K. The plasmid was transformed into E44 by electroporation, selecting for Km^r Cm^s colonies at 40°C. To inactivate the ORF *o765*, the 6-kb *Eco*RI fragment from pUC-S10 containing *o765* was subcloned in pACYC184 at the same site selecting for Tc^r Cm^s. The 1.2-kb Km^r cassette from pUC-4K was inserted at the *Bgl*II site in the *o765* ORF. This 7.2-kb recombinant DNA was subcloned in pIB307 and transformed into E44 as described above. All these insertion and/or deletion mutants were verified by PCR by using primers flanking the antibiotic-cassette-inserted sites.

Characterization of *E. coli* mutants. *E. coli* RS218 (and E44) possessed both S and type 1 fimbriae, and the expression of S and type 1 fimbriae was tested by hemagglutination as described previously (18). K1 capsule was detected on an antiserum agar plate as described previously (14). *E. coli* mutant strains were examined by the Vitek Gram-Negative Identification+ Card and the automatic Vitek system for antimicrobial susceptibilities (bioMérieux Vitek, Inc., Hazelwood, Mo.). *E. coli* proteins were fractionated by differential centrifugation and examined on sodium dodecyl sulfate (SDS)–10% polyacrylamide gels as previously described (18, 29).

Nucleotide sequence accession number. The sequence of *yijP* from *E. coli* K1 RS218 has been submitted to the GenBank database under accession number AF112861.

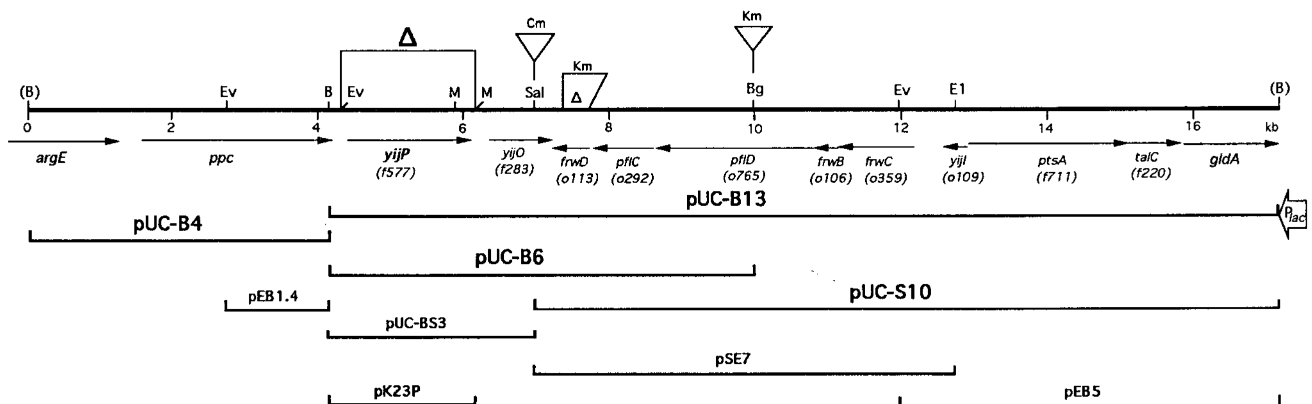

FIG. 1. Gene organization of the cloned DNA and the physical maps of plasmid derivatives. All of these DNA fragments were cloned on pUC13, except pK23P, which was cloned on pK194. Restriction sites: B, *Bam*HI; (B), *Bam*HI from the vector; Bg, *Bgl*II; E1, *Eco*RI; Ev, *Eco*RV; M, *Mlu*I; Sal, *Sal*I. Only the sites involved in plasmid construction are shown. The orientation of the *lacZ* promoter in the vector is indicated. Deletion (Δ) and antibiotic cassette insertion sites in the E44 chromosome are also depicted. The functions of the ORFs with alternative names are unknown.

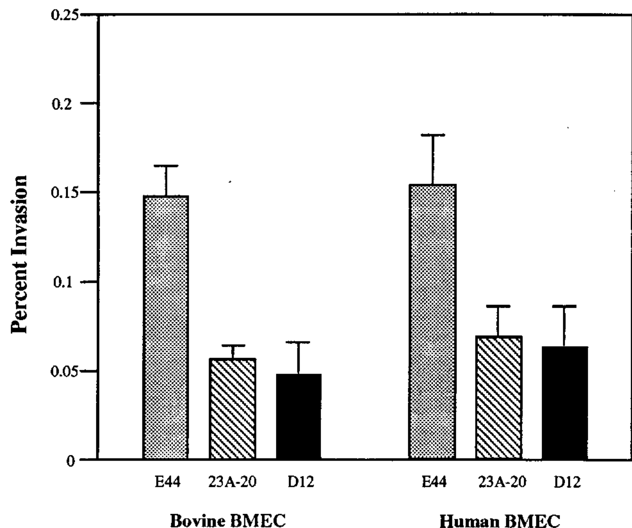

FIG. 2. Invasion frequencies of the *E. coli* K1 parent strain E44 and two *yijP* mutants in BMEC. Values are means of at least three independent assays; bars indicate the standard error of mean.

RESULTS

Isolation and characterization of the mutant strain 23A-20 and its invasion in BMEC. We have identified a Tn*phoA* insertion mutant strain 23A-20 which exhibited significantly less invasive ability in bovine BMEC compared to the parent strain E44 (12). Southern hybridization with *Eco*RV- or *Mlu*I-cleaved genomic DNA of 23A-20 indicated that there was only one copy of Tn*phoA* inserted in the chromosome (not shown). The mutant 23A-20 was found to be positive in the K1 capsule, S fimbriae, and type 1 fimbriae. Repeated in vitro invasion assays in both bovine and human BMECs showed that the mutant 23A-20 was significantly less invasive than E44 in both BMEC cell lines (Fig. 2).

When E44 and 23A-20 were mixed 1:1 for invasion assays, the ratio of intracellular bacteria recovered were about 2.4:1 as determined by the percentage of Km^r colonies in the mixture of recovered intracellular 23A-20 (Km^r Rif^r) and E44 (Rif^r). The result was consistent with that of individual invasion assays (Fig. 2).

To test whether 23A-20 was indeed less invasive in vivo, the mutant 23A-20 and the parent strain E44 were administered via intracardiac injection to 5-day-old rats. Table 2 showed that 23A-20 was able to induce bacteremia in rats to a degree similar to that of E44. The rate of meningitis, defined as positive CSF cultures, induced by 23A-20 was, however, significantly less ($P = 0.019$) than that by E44. These data showed that the mutant strain 23A-20 was significantly less invasive in BMEC both in vitro and in vivo, indicating that the Tn*phoA* inserted region may be important for *E. coli* K1 invasion of BMEC.

TABLE 2. Comparison of bacterial counts in blood and number of animals with positive CSF culture between two groups of animals receiving *E. coli* K1 E44 or its Tn*phoA* mutant 23A-20

Strain (n = 24)	Bacteremia (mean log CFU/ml of blood ± SD)	No. of animals with positive CSF (%)
E44	7.53 ± 0.40	18 (75)[a]
23A-20	7.80 ± 0.67	10 (42)[a]

[a] $P = 0.019$ by Fisher's exact test.

TABLE 3. Relative invasion of *E. coli* K1 containing various plasmid constructs

Strain	Plasmid	% Relative invasion (mean ± SD)
E44	pUC13	100
	pUC-B13	355 ± 86
	pUC-B6	273 ± 73
23A-20	pUC13	23 ± 6.2
	pUC-B13	156 ± 37
	pUC-B6	135 ± 33
	pUC-S10	109 ± 49
	pSE7	6.5 ± 4.9
	pEB5	30 ± 16

Cloning of the Tn*phoA* insertion region. The Tn*phoA* fragment in 23A-20 was cloned in pCVD433 in *E. coli* DH5α by selecting for the Km^r phenotype. A selected plasmid clone was purified, and DNA flanking the Tn*phoA* insertion site was sequenced. A sequence of ca. 800 nucleotides was obtained and was used to search against databases by using the BLAST program (2). The sequence was found to be almost identical to an ORF named *yijP* (also called *f577* [4, 5]), which is located at 90 min of the *E. coli* K-12 chromosome and encodes a hypothetical protein. The Tn*phoA* insertion site was identified to be in the middle of *yijP*.

A 700-bp PCR DNA containing the Tn*phoA* insertion site was synthesized and used to probe the genomic library of RS218. Several positive plaques were obtained, and a recombinant λ clone containing a 17-kb insert was selected for further study (Fig. 1). The cloned DNA was mapped with enzymes *Bam*HI, *Bgl*II, *Eco*RI, *Hin*dIII, *Kpn*I, *Sal*I, and *Sma*I. Most of these restriction sites appeared to be identical to those in *E. coli* K-12 DNA, except that a *Sal*I site in *yijP* and a *Kpn*I site in *o765* were missing in *E. coli* K1 RS218 compared with the *E. coli* K-12 genome (Fig. 1) (5). These results indicated that the cloned 17-kb segment from *E. coli* K1 RS218 was very similar to that in *E. coli* K-12. A sequencing reaction was performed from either end of this 17-kb segment, respectively. Database searching confirmed the presence of the *argE* gene and the *gldA* gene at either end of the cloned DNA (Fig. 1).

Complementation of 23A-20 for invasion of BMEC. Two *Bam*HI fragments from the recombinant λ DNA were first subcloned in pUC13 at the same site and named pUC-B4 and pUC-B13 (Fig. 1). All other deletions were later generated from these two plasmids. The direction of the *yijP* gene was opposite the *lac* promoter on the vector in pUC-B13; the plasmid containing the insert on the other direction was unstable in *E. coli*.

Most of the plasmids shown in Fig. 1 were transferred into the mutant 23A-20 or E44. Transformants were tested for invasion in BMEC together with E44(pUC13) and 23A-20(pUC13) as controls. As shown in Table 3, pUC-B6 and pUC-B13 were able to complement the mutant 23A-20 in our invasion assays to the level observed with the parent strain E44(pUC13). Of interest, pUC-B6 and pUC-B13 increased the BMEC invasion of the parent strain containing the vector by approximately 300%. All other constructs did not complement 23A-20, except that pUC-S10 appeared to increase the invasion of the mutant 23A-20 compared to 23A-20(pUC13), but the degree of complementation varied with relative invasion, ranging from 50 to 175%. These results indicated that the ORF *f577* played a role in *E. coli* K1 invasion of BMEC, but the possible involvement of *f283*, *o113*, and *o292* in *E. coli* invasion of BMEC was not excluded. Plasmid pUC-BS3 affected the growth of its host *E. coli* strain more than other plasmids,

and complementation of 23A-20 with pUC-BS3 was not successful.

Nucleotide sequence of *yijP*. The nucleotide sequence of *yijP* in pUC-B6 was determined. Compared to its counterpart in *E. coli* K-12 (GenBank accession number U00006), 40 of 1,731 nucleotides differed between these two ORFs (97.7% identities). However, only 5 of 577 deduced amino acid residues were different between them. These differences were located near the middle of the deduced YijP protein (Asn377 [K-12 strain] versus Lys [K1 strain], Asn403 versus Asp, Asp412 versus Glu, Ala450 versus Thr, and Asn451 versus Asp).

The sequence downstream of *E. coli* K1 *yijP* was not determined. According to the *E. coli* K-12 sequence, there are several genes of unknown function present downstream of *yijP* (Fig. 1). The deduced *f283* product belonged to the AraC/XylS family of transcriptional regulators (5). The five-gene cluster, *o113-o359*, appeared to form an operon. Their predicted products were 23 to 47% similar to *E. coli* pyruvate formate catabolic enzymes (5).

Deletion and insertion mutagenesis in the E44 chromosome. To further clarify the function of *yijP* and its downstream genes, an isogenic E44(Δ*yijP*) mutant was generated, and one of the colonies, named D12, was chosen for further study. PCR reactions with three pairs of primers indicated that *yijP* was as expected deleted in the D12 strain (not shown).

The growth characteristics of E44 and the Δ*yijP* mutant were the same in all of the media used (see Materials and Methods). Mutation in the *ppc* gene, which is upstream of *yijP*, was reported to render *E. coli* cells unable to grow on several carbon sources such as pyruvate and glutamate (3). However, the Δ*yijP* mutant was found to be able to grow on these carbon sources. The doubling time of the Δ*yijP* mutant in SM supplemented with pyruvate (10 mM) as the sole carbon source was about 70 min, identical to that observed with E44. Like E44, the Δ*yijP* mutant expressed both S fimbriae and type 1 fimbriae and possessed the K1 capsule. The fractionated protein profiles were identical between E44 and the Δ*yijP* mutant in the Coomassie blue-stained SDS-polyacrylamide gel (not shown), suggesting that YijP was a minor protein. The biochemical reactions were identical between E44 and the Δ*yijP* mutant on the Vitek Gram-Negative Identification+ Card. The antimicrobial susceptibility profile of these strains was also identical in the automatic Vitek system. Viability of D12 and 23A-20 cells was unchanged in water containing 0.5% Triton X-100 for 2 h, which was much longer than the exposure time to Triton X-100 (~10 min) used in the in vitro BMEC invasion assay.

Results of invasion assays showed that deletion of *yijP* in E44 reduced the BMEC invasion frequency to the level of the Tn*phoA* mutant 23A-20 (Fig. 2; $P < 0.05$ compared to the parent strain in both bovine and human BMECs). Plasmid pUC-B6 was able to complement D12 in invasion of BMEC compared to E44(pUC13) (Fig. 3). Furthermore, a low-copy-number plasmid pK194 containing only the *yijP* ORF was constructed (pK23P; Fig. 1), and this plasmid fully complemented the Δ*yijP* mutant in invasion of BMEC (Fig. 3).

To study the possible involvement of the genes downstream of *yijP* in *E. coli* invasion of BMEC, *f283*, *o113*, and *o765* were selectively interrupted by antibiotic cassette insertion (Fig. 1). The invasion frequency of these mutants in BMEC was found to be identical to that of E44, indicating that the downstream genes of *yijP* were probably not involved in *E. coli* K1 invasion of BMEC.

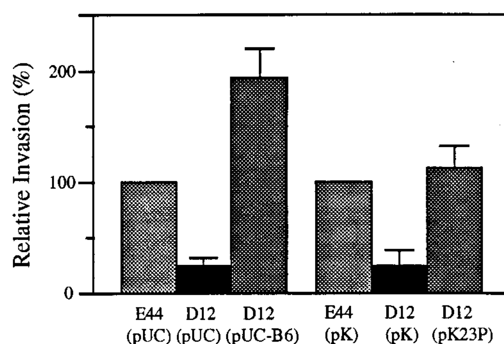

FIG. 3. Complementation of the Δ*yijP* mutant with the plasmids pUC-B6 and pK23P in the invasion of BMEC. Values are means of four separated experiments; bars indicate the standard error of the mean.

DISCUSSION

The most distressing aspect of bacterial meningitis is the limited improvement in the mortality and morbidity attributable to advances in antimicrobial chemotherapy and supportive care. A major contributing factor is our incomplete understanding of the pathogenesis associated with this disease. For example, at present it is unclear how circulating bacteria cross the blood-brain barrier. Our investigations of this issue with *E. coli* as a paradigm have shown that successful traversal of *E. coli* across the blood-brain barrier requires two independent steps of bacterium-BMEC interactions, i.e., *E. coli* binding to BMEC and the invasion of BMEC. We have previously shown that S fimbriae contribute to *E. coli* binding to BMEC (23); however, binding to BMEC via S fimbriae was not accompanied by invasion of BMEC. In contrast, we have shown that OmpA, IbeA, and IbeB contribute to *E. coli* K1 invasion of BMEC (11, 12, 18). The *ibeA* gene was found to be unique in CSF isolates of *E. coli* K1, while *ompA* and *ibeB* have homologues present in the *E. coli* K-12 genome. OmpA is functionally similar between *E. coli* K1 and K-12 strains, as shown by successful complementation of the noninvasive *ompA* deletion mutant of *E. coli* K1 to invade BMEC by the *E. coli* K-12 *ompA* gene (18). In addition, we have identified specific receptors for both OmpA (17) and IbeA (16) present only on BMEC, suggesting that OmpA and IbeA contribute to BMEC invasion via ligand-receptor interactions. IbeB displays the characteristics of outer membrane proteins (11), suggesting that the IbeB-mediated invasion of BMEC may also occur via ligand-receptor interactions. In addition, we have previously shown in the experimental hematogenous *E. coli* meningitis model that the K1 capsule is a critical determinant for *E. coli* to cross the blood-brain barrier as live bacteria (14).

In the present study, we identified a Tn*phoA* mutant 23A-20 that is less capable of invasion into BMEC in vitro. More importantly, this mutant was significantly less invasive into the CNS in vivo than the parent strain in the newborn rat model of hematogenous meningitis. These data suggested that a genetic locus in the mutant 23A-20 affected by Tn*phoA* insertion was most likely to confer the ability of *E. coli* K1 to invade BMEC both in vitro and in vivo. Both the parent strain R218 (or E44) and its mutant 23A-20 possess the K1 capsule, OmpA, IbeA, and IbeB, as well as type 1 and S fimbriae. These findings suggested that a less-invasive property of 23A-20 was unlikely resulted from a polar effect of Tn*phoA* on other known invasion genes. This concept was supported by our demonstration that (i) the *yijP*-deleted mutant of RS218 (strain D12) was less invasive in BMEC and that (ii) plasmids pUC13 and pK194

containing the *yijP* gene were able to complement the *yijP* mutants in the invasion of BMEC to the level of the parent strain (e.g., pUC-B6 and pK23P; Table 2 and Fig. 3). In contrast, other plasmids without the *yijP* gene were not able to restore the ability of 23A-20 to invade BMEC. In addition, selective interruption of other genes downstream of *yijP* by antibiotic cassette insertion did not reduce the invasion frequency of the parent strain. These findings indicated that *yijP* is an important component for *E. coli* K1 invasion of BMEC.

The nucleotide sequence and the deduced amino acid sequence of *yijP* were found to be almost identical to those of *yijP* in the *E. coli* K-12 genome, whose function is currently unknown. Analysis of the deduced amino acid sequence of YijP showed that it has some features of outer membrane protein, including a signal peptide-like sequence and five or six transmembrane segments at its N terminus (28). Comparison of the outer membrane protein profiles revealed that the parent strain and the Δ*yijP* mutant had identical patterns, suggesting that YijP is a minor protein. It is unclear how YijP contributes to *E. coli* K1 invasion of BMEC. We have previously shown that *E. coli* OmpA, a highly conserved outer membrane protein, contributes to *E. coli* K1 invasion of BMEC, and its N-terminal portion is involved in invasion (18). We have recently shown that IbeA interacts with a novel BMEC protein for *E. coli* K1 invasion of BMEC (16). Studies are in progress to examine whether the invasion phenotype is a result of direct interaction of YijP with BMEC and also to determine which domain(s) of YijP is involved in BMEC invasion.

During the preparation of the manuscript, a YijP homologue, termed *L7028* (8) or *ecf3* (7), was reported in the 92-kb virulence plasmid pO157 in *E. coli* O157:H7. The product of this *E. coli* O157 gene shares 82% identities with YijP, and it is predicted to have a similar secondary structure, i.e., a signal peptide-like sequence and five or six transmembrane segments in its N-terminal domain (28). pO157 is known to carry a number of virulence genes, including genes for hemolytic activity and adherence to intestinal cells (8). It would be interesting to find out whether this YijP homologue of *E. coli* O157 and *E. coli* K-12 YijP are able to complement the invasion phenotype in K1 *E. coli* (Δ*yijP*).

In summary, we identified the gene locus *yijP* which contributes to *E. coli* K1 RS218 to invade into the CNS. This is the first demonstration that YijP plays a role in *E. coli* meningitis. Taken together, our findings indicate that several *E. coli* determinants, including OmpA, IbeA, IbeB, and YijP, contribute to *E. coli* K1 crossing of the blood-brain barrier. Studies are in progress to determine how these different *E. coli* structures contribute to invasion of BMEC.

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