

Role of Porin Proteins OmpF and OmpC in the Permeation of β -Lactams

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Mutants of *Escherichia coli* K-12 lacking major outer membrane proteins were obtained by selecting for resistance to the β -lactam cefoxitin. Three classes of resistant strains were found: mutants in *ompB*, a regulatory locus for proteins OmpF and OmpC; mutants in *ompF*; and one mutant in *tpo*. The OmpF and OmpC proteins facilitate penetration of β -lactams through the outer membrane.

Mechanisms of resistance to β -lactams in *Escherichia coli* can be divided into three categories: enzymatic inactivation of the antibiotic, alteration of the target, and reduced penetration of the antibiotic. This work concerns the last mechanism, involving alterations in outer membrane proteins leading to decreased penetration of β -lactams through the outer membrane of *E. coli* K-12. The outer membrane, whose function remains unclear, must play a specific role in permeability since it contains several proteins involved in transport (for review, see 18, 21). Permeability of the outer membrane to low-molecular-weight hydrophilic molecules is due to the presence of porin protein molecules such as OmpF and OmpC, which form pores in the outer membrane that allow small molecules to diffuse rapidly into the periplasmic space (18). OmpF and OmpC are two major outer membrane proteins produced in large amounts in *E. coli* K-12 and regulated by the *ompB* locus. Mutants lacking the OmpF protein have increased resistance to the antibiotics chloramphenicol and tetracycline (22) and to copper (15). Permeability of the outer membrane to cephaloridine, 6-aminopenicillanic acid, and ampicillin was shown to be greatly decreased in porin-deficient mutants of *Salmonella typhimurium* (19) and *E. coli* (2, 26). While this work was in progress, Harder et al. (10) isolated carbenicillin-resistant mutants of *Escherichia coli* strains K-12 and B/r which produce diminished levels of the OmpF porin. It was recently proposed by Taylor et al. (25) that the *ompB* locus includes two genes, *ompR* and *envZ* (*tpo* [28] *perA* [30]). The *ompR* gene product is required for expression of OmpF and OmpC proteins. The *envZ* gene decreases the transcription of OmpF and LamB proteins and increases the production of the OmpC protein. In this work, *ompB* and *tpo* mutations will refer to *ompR* and *envZ*, respectively.

In this paper, we analyze three different classes of spontaneous β -lactam-resistant mutants selected in the presence of cefoxitin and demonstrate that they are affected in the structural gene *ompF* and in the regulatory genes *ompB* and *tpo*. We show that permeation of β -lactam compounds through the outer membrane of *E. coli* K-12 involves the action of OmpF and OmpC porins.

MATERIALS AND METHODS

Bacterial strains and phages. The bacterial strains used are listed in Table 1. Bacteriophages Tula, 434, and TP1 were obtained from C. Wandersman, Institut Pasteur, Paris. P1_{vir} was from our laboratory collection.

Strains B1449, B1478, B1467, B1475, B1443, B1485, and B1488, carrying the insertion mutation element Tn5, were constructed by transduction with stocks of P1 phage grown on strains MH450 and MH150 (obtained from C. Wanderman) and selected on ML plates containing 25 μ g of kanamycin per ml and 0.05 M sodium citrate. Strains MH450 (7) and MH150 are insertion mutants defective in the production of OmpF (*ompF*::Tn5) and OmpC (*ompC*::Tn5), respectively.

The episome KLF41::Tn10 was obtained by conjugation (pop1665 \times B1440) and selection on ML plates containing 50 μ g of nalidixic acid and 10 μ g of tetracycline per ml.

Media. Rich medium (ML) was 1% tryptone (Difco Laboratories)—0.5% yeast extract—0.5% NaCl adjusted to pH 7 with NaOH. Mueller-Hinton agar medium (Institut Pasteur Production) was utilized for determination of inhibitory concentrations.

Antibiotics. Antibiotics were obtained from the following sources: ampicillin, Bristol Laboratories; cephaloridine, Eli Lilly & Co.; cefazolin, Allar, S.A.; cefoxitin, Merck Sharp & Dohme-Chibret; tetracycline, Specia, Rhone-Poulenc; and nalidixic acid, Winthrop Laboratories. The following susceptibility testing disks were obtained from Institut Pasteur Production: ampicillin, cephaloridine, cephalothin, cefamandole, cefazolin, cefoxitin, cefuroxime, cefapirin, and ticarcillin. The following additional testing

TABLE 1. Designation, genotype, and origin of strains

Strain	Genotype ^a	Origin
B1343	HfrH <i>thi rpoB</i>	Laboratory collection
pop1010	Hfr <i>his metA aroB rpoB</i>	Wandersman et al. (28)
B1421	Hfr P4X <i>metB1</i>	Laboratory collection
B1350	HfrH <i>thi rpoB ompB1350</i>	B1343/cefotaxim 37°C
B1422	Hfr P4X <i>metB1 ompB1422</i>	B1421/cefotaxim 37°C
B1437	Hfr <i>his metA rpoB ompB1350</i>	pop1010 × P1vir · B1350
B1436	Hfr <i>his metA rpoB ompB1422</i>	pop1010 × P1vir · B1422
B1466	Hfr <i>his metA aroB rpoB ompF1466</i>	pop1010/cefotaxim 30°C
B1449	Hfr <i>his metA aroB rpoB ompF::Tn5</i>	pop1010 × P1 · MH450
B1478	Hfr <i>his metA aroB rpoB ompC::Tn5</i>	pop1010 × P1 · MH150
B1454	HfrH <i>thi rpoB tpo-1454</i>	B1343/cefotaxim 37°C
B1477	Hfr <i>his metA rpoB tpo-1454</i>	pop1010 × P1vir · B1454
B1467	Hfr <i>his metA aroB rpoB ompF1466 ompC::Tn5</i>	B1466 × P1 · MH150
B1475	Hfr <i>his metA rpoB ompB1350 ompF::Tn5</i>	B1437 × P1 · MH450
B1443	Hfr <i>his metA rpoB ompB1422 ompF::Tn5</i>	B1436 × P1 · MH450
B1485	Hfr <i>his metA rpoB tpo-1454 ompC::Tn5</i>	B1477 × P1 · MH150
pop1389	Hfr <i>his metA rpoB ompB101 lac</i>	Wandersman et al. (28)
pop1387	Hfr <i>his metA rpoB top-11 lac</i>	Wandersman et al. (28)
pop1665 (KLF41)	F' <i>argG⁺ rpsL⁺ malT⁺/lhi thr leu argH metA tonA rpsL aroB glpD recA srl::Tn10 ompB101</i>	Wandersman et al. (28)
B1440	<i>malB rpsL thi lac try recA56 nalA</i>	Laboratory collection
B1450	KLF41::Tn10/B1440	pop 1665 × B1440
B1459	KLF41::Tn10/B1437	B1437 × B1450
B1458	KLF41::Tn10/B1436	B1436 × B1450
pop1376	Hfr <i>proC pyrD rpsE</i>	Institut Pasteur collection
DCO	<i>met nalR</i>	Curtis et al. (4)
DC2	<i>met nalR</i>	Richmond et al. (23)
B1488	DC2 <i>ompF::Tn5</i>	DC2 × P1 · MH450

^a Genetic nomenclature is from Bachmann and Low (1), except for *tpo*, which was described previously (28).

disks were prepared in our laboratory (content per disk): benzyl penicillin (Specia; 150 µg), cefoperazone (Pfizer Inc.; 30 µg), cefotaxime (Roussel-Uclaf; 0.125 µg), cefotiam (Cassenne-Takeda; 30 µg), moxalactam (Eli Lilly; 0.25 µg), *N*-formimidoyl thienamycin (Merck Sharp & Dohme; 4 µg).

Antibiotic susceptibility tests. (i) **Determination of antibiotic susceptibility.** Disk susceptibility tests were performed with defined disks and inocula and were recorded after overnight incubation at 37°C. The diameter of the clear zone of inhibition was estimated within an accuracy of 0.5 mm. Mutant strains were considered resistant if the zone of inhibition was reduced by more than 3 mm as compared with the inhibition zones of parental strains measured under the same conditions.

(ii) **Inhibitory concentration.** Serial twofold dilutions of antibiotics were made in Mueller-Hinton agar from 128 to 0.25 µg/ml. Inocula were prepared from 18- to 24-h cultures. Agar plates were inoculated with a Steers replicator, using dilutions to obtain 1,000 to 3,000 bacteria per spot. The endpoint reading was the lowest concentration of antibiotic giving 10 to 30 isolated colonies per spot. These endpoints correspond to 99% inhibition (3). The standard deviation observed with this method is usually 0.3 to 0.5 log₂. A difference in 99% inhibitory concentration of more than twofold was considered significant.

Determination of penicillin-binding proteins. Penicillin-binding proteins of *E. coli* K-12 were investigated

by the method of Spratt (24), using benzyl-[¹⁴C]penicillin (Amersham) and [¹²⁵I]ampicillin (generously given by E. Schinner, Hoechst AG).

Analysis of cell extracts. Samples of cell extracts were prepared as described by Hall and Silhavy (7). Polyacrylamide gel electrophoresis was performed as described previously (13).

β-Lactamase activity. β-Lactamase activity was determined on sonicated bacteria by spectrophotometric assay. Nitrocefin (Glaxo) was used as substrate (20), and the rate of increase in optical density of the compound was measured at a wavelength of 482 nm.

Genetic techniques. Growth of bacteriophage, transduction, and conjugation were carried out as described by Miller (16).

RESULTS

Selection of cefotaxim-resistant mutants. Cefotaxim was used as a selective agent because it has a broader spectrum of antibacterial activity than previously tested cephalosporins, possibly owing to its resistance to a variety of β-lactamases, better penetration into the cell, or tighter attachment to target proteins (5). Spontaneous cefotaxim-resistant mutants were isolated from strains B1343, pop1010, and B1421 at a frequency of 10⁻⁶ to 10⁻⁷. The mutants were selected by plating 10 independently grown overnight sam-

TABLE 2. Plating efficiencies of phages

Strain	Relevant genotype	Plating efficiency of phage:		
		Tu1a	434	TP1
pop1010 ^a		1	1	1
B1466	<i>ompF1466</i>	3×10^{-7}	1	0.5
B1449	<i>ompF::Tn5</i>	$<10^{-7}$	1	1
B1478	<i>ompC::Tn5</i>	1	4×10^{-6}	1
B1436	<i>ompB1422</i>	10^{-6}	5×10^{-8}	1
B1437	<i>ompB1350</i>	1	10^{-8}	1
pop1389	<i>ompB101</i>	$<10^{-7}$	10^{-6}	1
B1477	<i>tpo-1454</i>	6×10^{-8}	1	2×10^{-5}
pop1387	<i>tpo-11</i>	$<10^{-7}$	1	10^{-3}
B1467	<i>ompF1466 ompC::Tn5</i>	$<10^{-7}$	2×10^{-8}	1
B1475	<i>ompB1350 ompF::Tn5</i>	$<10^{-7}$	10^{-7}	1
B1485	<i>tpo-1454 ompC::Tn5</i>	$<10^{-7}$	2×10^{-8}	$<10^{-7}$

^a The plating efficiency on this strain was taken as reference.

ples of each strain on ML agar plates containing 10 µg of cefoxitin per ml and incubating at 30 or 37°C. One colony was picked per sample and purified by repeated streaking on ML agar. The mutants fell into two classes according to their susceptibility spectrum as measured by disk tests. Mutants of the first class (24 of 30) were resistant in increasing order to penicillin, ticarcillin, ampicillin, cefuroxime, cefamandole, cefotaxime, cefoperazone, cephaloridine, cefapirin, cephalothin, cefotiam, cefazolin, moxalactam, and cefoxitin; mutants of the second class (6 of 30) were resistant to the same β-lactams except cephaloridine and cefazolin, to which they were completely susceptible. All of the mutants tested were susceptible to *N*-formimidoyl thienamycin.

The presence of altered penicillin-binding proteins was investigated by fluorography; no differences in the pattern of penicillin-binding proteins could be detected between the mutant and the parental strains. Similarly, the β-lactamase activity of these mutants was unchanged as determined by spectrophotometric assays when the chromogenic nitrocefin β-lactam was used as substrate.

Location and characterization of mutations. Conjugation and transduction tests demonstrated that strains of the first class carried mutations located in the *aroB* region (74 min on the genetic map). When the *aroB* strain pop1010 was transduced to *aro*⁺ with P1vir phage grown on strain B1350 or B1422, 80% of the transductants were found to be cefoxitin resistant. The *ompB* locus, which is involved in the regulation of OmpF and OmpC membrane proteins, has been mapped within the same region of the chromosome (74 min). Strains of the second class carried mutations located between *gal* and *trp*. When the *pyrD* strain pop1376 was transduced to *pyrD*⁺ (21 min on the genetic map) with P1vir phage

grown on strain B1466, 63% of the transductants were found to be cefoxitin resistant. The *ompF* gene coding for the porin protein OmpF lies in the same area (21 min on the genetic map).

In general, *ompB* mutants, because of their decreased synthesis of OmpF and OmpC porin proteins, are resistant to phage Tu1a (receptor OmpF protein [6]) and 434 (receptor OmpC protein [9]), but some *ompB* mutants exhibit an OmpF⁺ OmpC⁻ phenotype. *tpo* mutants are resistant to phages Tu1a and TP1 (receptor OmpF and LamB proteins [29]) because of their decreased synthesis of the OmpF and LamB proteins (28). To test the possibility that the resistant strains we isolated were similar to *ompB*, *tpo*, and *ompF* mutants, their phage sensitivity patterns were compared with those of the known mutants *ompB101*, *tpo-11*, and *ompF::Tn5*. Analysis revealed (Table 2) that mutants of the first class were resistant to either 434 alone (e.g., B1437) or both phages Tu1a and 434 (e.g., B1436), like the known *ompB101* strain, whereas mutants of the second class were resistant only to Tu1a (e.g., B1466), like the *ompF::Tn5* strain B1449.

Since it appeared that an OmpF⁻ phenotype might affect the resistance of *E. coli* K-12 towards β-lactams, we looked for cefoxitin-resistant mutants showing the same phenotype as *tpo* mutants (74 min on the genetic map), which result in a defect in the production of proteins OmpF and LamB (28); *tpo* strains are resistant to phage Tu1a and to phage TP1. Among 100 cefoxitin-resistant mutants selected from an overnight culture of strain B1343, one (B1454) was found to be resistant to both Tu1a and TP1 phages, like *tpo* mutants. When the *aroB* strain pop1010 was transduced to *aro*⁺ with P1vir phage grown on strain B1454, the majority (9 of 10) of transductants were found to be cefoxitin resistant, and the antibiotic resistance spectrum

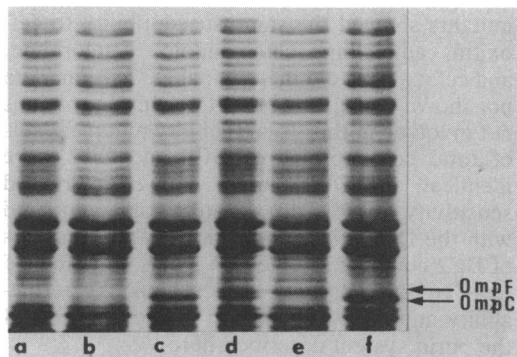


FIG. 1. Analysis of cell extracts on a 12-cm-long 10% polyacrylamide slab gel. The positions of the OmpF and OmpC proteins are shown by arrows. Lane a, strain B1436 (*ompB1422*); lane b, B1437 (*ompB1350*); lane c, B1466 (*ompF1466*); lane d, pop1010 (wild type); lane e, B1478 (*ompC::Tn5*); lane f, B1454 (*tpo-1454*).

of the mutant was similar to those of *ompF* mutants.

Gels of total cell proteins were run (Fig. 1). OmpF and OmpC proteins were not synthesized in mutants B1436 (*ompB1422*) and B1437 (*ompB1350*), the OmpF protein was not synthesized in B1466 (*ompF1466*) and B1454 (*tpo-1454*), and OmpC protein was overproduced in B1454 (*tpo-1454*). Although mutant B1437 was sensitive to phage Tu1a, no OmpF protein could be detected in the gels.

Dominance studies were carried out on *ompB1422* and *ompB1350* after transfer of the *KLF41::Tn10 ompB+* episome into B1436 and B1437. Dominance was tested by analyzing the

antibiotic susceptibility patterns of the merodiploid strains (B1458 and B1459). The *ompB1422* and *ompB1350* mutations were recessive to the wild type (Table 3). The genotype of merodiploid strains was verified by analyzing tetracycline-susceptible segregants which had lost the episome and which were shown to be cefoxitin resistant.

The interpretation of the genetic data is that different spontaneous cephalosporin-resistant mutants isolated in this study are *ompB*, *ompF*, and *tpo* types. Their behavior was identical to that of the previously studied *ompB101*, *ompF::Tn5*, and *tpo-11* strains with regard to their phage susceptibility (Table 2), their pattern of proteins analyzed on cell extracts, and their spectrum of resistance measured by disk susceptibility testing (data not shown) and by inhibition of colony-forming ability (Table 3).

The β -lactams tested can be divided into the following three series according to the behavior of the mutants: *ompB* mutants were quite resistant to the series cefamandole, cefotaxime, cefoperazone, cefapirin, cephalothin, cefotiam, moxalactam, and cefoxitin (represented by cefoxitin in Table 3) and were less resistant to the series penicillin, ticarcillin, ampicillin, and cefuroxime (represented by ampicillin), whereas *ompF* mutants were completely susceptible to cefaloridine and ceftazolin. Table 3 shows the 99% inhibitory concentrations of cefoxitin, ampicillin, cefaloridine, and ceftazolin for the different strains measured at 37°C.

Strain B1466 was originally selected on cefoxitin plates (10 μ g/ml) at 30°C and showed a temperature-dependent phenotype: the 99% inhibitory concentration of cefoxitin for this strain was 25, 8, and 6.25 μ g/ml at 30, 37, and 42°C,

TABLE 3. Susceptibility to β -lactams

Strain	Relevant genotype	99% Inhibitory concn (μ g/ml) ^a at 37°C of:			
		Cefoxitin	Ampicillin	Cefaloridine	Ceftazolin
pop1010		2			
B1466	<i>ompF1466</i>	8	8	2	2
B1449	<i>ompF::Tn5</i>	16	8	2-4	2
B1436	<i>ompB1422</i>	32	8	32	16
B1437	<i>ompB1350</i>	32	8	32	16
pop1389	<i>ompB101</i>	32	8	32	16
B1477	<i>tpo-1454</i>	8	8	2	2
pop1387	<i>tpo-11</i>	8	8	2	2
B1478	<i>ompC::Tn5</i>	2	2	2	2
B1467	<i>ompF1466 ompC::Tn5</i>	128	16	64	64
B1475	<i>ompB1350 ompF::Tn5</i>	32	8	32	16
B1485	<i>tpo-1454 ompC::Tn5</i>	64	16	32	32
B1458	F' <i>ompB+</i> / <i>ompB1422</i>	2	2	2	1
B1459	F' <i>ompB+</i> / <i>ompB1350</i>	2	2	2	1

^a Inhibitory concentration that reduced colony-forming units by 99% when plated onto Mueller-Hinton agar medium.

respectively. Mutants lacking the OmpF protein (B1466, B1449, B1477, and pop1387) had reduced susceptibility to cefoxitin, and a mutant lacking the OmpC protein (B1478) had wild-type susceptibility (Table 3); however, *ompB* mutants lacking both OmpF and OmpC proteins (B1436, B1437, and pop1389) had drastically reduced susceptibility to β -lactams. The case of cefaloridine and cefazolin is remarkable because mutants lacking the OmpF or the OmpC proteins individually were as susceptible to cefaloridine and cefazolin as was the wild type, but mutants lacking both proteins were resistant to these β -lactams. Double mutants combining *ompF ompC* (B1467) or *tpo ompC* (B1485) mutations with defects in both OmpF and OmpC proteins revealed a higher level of resistance to β -lactams than either single mutant. In *ompB ompF* double mutant B1475, no cumulative effects were observed; its susceptibility to β -lactams was identical to that of the corresponding single *ompB* mutant, even for *ompB1350*, which does not abolish sensitivity to phage Tu1a. *ompB ompC::Tn5* double mutants were constructed by transduction and selection on kanamycin but were unstable after removing kanamycin. Strain B1478 (*ompC::Tn5*) was plated on agar plates containing 10 μ g of cefoxitin per ml; the surviving clones (10 of 10) were *ompC::Tn5 ompF* double mutants as tested by transduction, phage sensitivity pattern, and antibiotic susceptibility spectrum. Similarly, when an *ompF::Tn5* strain was plated in the presence of 70 μ g of cefoxitin per ml, surviving clones (five of five) were *ompF::Tn5 ompC* double mutants. Analysis of the susceptibility spectra measured by test disks showed that the *ompF ompC* double mutants, whether obtained by transduction or selection, revealed a higher degree of resistance than *ompF*, *ompB* or *tpo* mutants to all of the penicillin and cephalosporin derivatives tested except *N*-formimidoyl thienamycin.

We could not find any correlation between structure and activity; the only relationship, which may be fortuitous, was between cefaloridine and cefazolin β -lactams. The abnormally highly penicillin-susceptible mutant DC2 isolated by Richmond et al. (23) showed lower minimum inhibitory concentrations of more than 30 penicillin and cephalosporin derivatives than did the parental strain DC0 (5). The only β -lactams tested that were no more active against the mutant DC2 than against the parental strain were cefaloridine and cefazolin. The genotype of the DC2 mutant is not known, but unpublished data (4) indicate that the mutation maps near *purA* (94 min). DC2 *ompF::Tn5* derivatives of DC2 were constructed by transduction, and their susceptibility was compared with that of the parental strains DC0 and DC2. The double

mutants showed the same susceptibility to cefoxitin, ampicillin, cefamandole, cefaloridine, and cefazolin as did the original DC2 strain (data not shown), suggesting that the OmpF protein is not involved in this "permeability mutant"; gels of total cell proteins of DC0 and DC2 were identical, the DC2 mutant showed increased sensitivity to 1% deoxycholate as compared with the DC0 mutant, and no resistant mutants of DC2 could be selected on plates containing 5 μ g of cefoxitin per ml ($<10^{-9}$). Thus, the permeability mutant DC2 seems to create a bypass of the porin system described here.

DISCUSSION

An understanding of outer membrane permeability is important in designing antibiotics and chemotherapeutic agents against gram-negative bacteria. Alterations in outer membrane proteins of moxalactam- and carbenicillin-resistant mutants were previously described by Komatsu et al. (12) and Harder et al. (10), but the genetic characterization of the mutations affecting the permeability to β -lactams was not investigated. In this work, we show that the altered permeability of the outer membrane of *E. coli* K-12 results from three different bacterial mutations localized in the structural gene *ompF* and in the regulatory genes *ompB* and *tpo*. It was unexpected that all of the cefoxitin-resistant mutants isolated in this study should be affected in the permeation of β -lactams, as reflected by mutations in OmpF and OmpC protein expression. The presence of more than a single mutation was unlikely, as the cefoxitin-resistant mutants were isolated without mutagenesis. Increased production of β -lactamase was ruled out for all of the mutants by measuring the hydrolysis of the chromogenic β -lactam nitrocefin, and differences in the patterns of penicillin-binding proteins could not be detected between the mutant and the parental strains.

It was proposed by Nikaido (17) that OmpF and OmpC porins are nonspecific and share similar properties, except OmpF-containing cells are about 10 times more permeable than OmpC-containing cells toward any given solute, and the permeation rate of cephalosporins is related to their ionization.

If the susceptibility of the strains to antibiotics reflects the degree of permeability across the outer membrane, β -lactams can be divided into the following three classes depending on their antimicrobial activity against cefoxitin-resistant mutants: (i) β -lactams such as cefoxitin or ampicillin, which go through OmpF porin much faster than through the OmpC porin, (ii) β -lactams such as cefaloridine and cefazolin, which can penetrate with high enough speed through the less efficient OmpC porin because of their inher-

ently high permeability, and (iii) β -lactams such as *N*-formimidoyl thienamycin, which does not seem to use this porin system.

As predicted by this study, all of the *E. coli* mutants we have selected in the presence of cephaloridine and cefazolin were mutated in the *ompB* gene (manuscript in preparation); similarly, we could not find *N*-formimidoyl thienamycin-resistant mutants altered in the porin OmpF or OmpC proteins.

The diffusion of β -lactams through the outer membrane seems to require a large amount of OmpF protein, since the mutant *ompB1350*, although plating Tu1a phage (receptor OmpF) with an efficiency of 1, showed the same level of antibiotic resistance as *ompB1422*, which does not allow Tu1a phage growth. Previous studies concerning the permeation of other substrates interpreted in a qualitative fashion concluded that peptide f-Met Leu Phe preferred the OmpC pore (11), whereas uptake of AMP is mediated by OmpF (27), and stachyose, raffinose, methionine, and glucose use indifferently the pores formed by protein OmpF or OmpC (11, 15).

The most interesting point is the action of the OmpF and OmpC proteins to facilitate the diffusion of β -lactams, as exemplified by the higher level of resistance of *ompB*, *ompF ompC*, and *tpo ompC* mutants. Evidence has accumulated that the OmpF and OmpC proteins share a number of unique physical and biochemical properties (31). A model of regulation of these proteins has been recently proposed by Hall and Silhavy (8). We do not know whether more than one of these proteins is involved in one type of pore or whether these proteins are involved in separate pores. In wild-type bacteria, the absolute and relative levels of expression of the *ompF* and *ompC* genes are balanced according to internal and external conditions (14). Whether this is the case for the mutants analyzed in the present study is not known. One cannot exclude the possibility of interaction between these proteins, such as the formation of heterotrimer configuration, and the existence of other mechanisms, as in the DC2 mutant, which could also be involved in permeation of cephalosporin derivatives through the bacterial membrane. The study of the mechanisms conferring resistance to β -lactams is important in evaluating the activity of alternative antimicrobial agents and in predictions of resistant mutants that could emerge after therapy.

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