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Outer Membrane and Porin Characteristics of *Serratia marcescens* Grown In Vitro and in Rat Intraperitoneal Diffusion Chambers

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The composition and antibiotic permeability barrier of the outer membrane of *Serratia marcescens* were assessed in cells grown in vivo and in vitro. Intraperitoneal diffusion chambers implanted in rats were used for the in vivo cultivation of bacteria. Outer membranes isolated from log-phase bacterial cells recovered from these chambers were compared with membranes isolated from cells grown in vitro. Analysis revealed that the suspected 41-kilodalton porin and the OmpA protein were recovered on sodium dodecyl sulfate-polyacrylamide gels in equal quantities. Several high-molecular-weight proteins, thought to be iron starvation induced, appeared in the diffusion chamber-grown cells. The outer membrane permeability barriers to cephaloridine were similar in in vivo- and in vitro-grown cells based on permeability coefficient calculations. The permeability coefficient of cephaloridine in *S. marcescens* cells (30.3×10^{-5} to 38.9×10^{-5} cm s^{-1}) was greater than that obtained for an *Escherichia coli* strain expressing only porin OmpC but smaller than those obtained for the *E. coli* wild type and a strain expressing only porin OmpF. Functional characterization of the suspected porin was performed by using the planar lipid bilayer technology. The sodium dodecyl sulfate–0.4 M NaCl-soluble porin from both in vitro- and in vivo-grown cells showed an average single-channel conductance in 1 M KCl of 1.6. A partial amino acid sequence (19 residues) was obtained for the *S. marcescens* porin. The sequence showed a very high homology to the *E. coli* OmpC porin. These data identified the *S. marcescens* outer membrane 41-kilodalton protein as a porin by both functional and amino acid analyses. Also, the methodology used allowed for efficient growth and recovery of diffusion chamber-grown bacterial cells and permitted identification of specific in vivo-induced changes in bacterial cell membrane composition.

Serratia marcescens produces a wide range of opportunistic infections and is an important pathogen in nosocomial outbreaks (36, 38, 44, 45). Consistent resistance to many antimicrobial agents has been documented in several reports on clinical isolates of *S. marcescens*. The mechanisms by which multiresistance occurs in such clinical isolates is often plasmid determined, and in combination with the species-specific intrinsic resistance of *S. marcescens*, it makes this organism difficult to control (13, 29, 41).

The outer membrane of gram-negative bacteria interacts with the environment and plays a major role in limiting the access of antibiotics to most inner cell targets (33, 35). Porins are outer membrane proteins that form transmembrane channels through which many hydrophilic solutes, including some antibiotics, may diffuse. The exclusion limit and ion selectivity of these pores restrict the permeability of solutes (2, 19). In recent reports (17, 18), major *S. marcescens* outer membrane proteins with apparent molecular masses ranging from 40 to 41 kilodaltons (kDa) were tentatively identified as porins, although specific experiments to determine the pore-forming activity of these proteins were not undertaken. Nevertheless, decreased quantities of these proteins, visualized on sodium dodecyl sulfate (SDS)-polyacrylamide gels, have been associated with nonenzymatic resistance to β-lactam antibiotics (17), as well as to nalidixic acid, trimethoprim, and chloramphenicol (18).

In this study, we report the first systematic functional characterization of the *S. marcescens* outer membrane. Experiments were undertaken to discover the role of outer membrane permeability in the natural resistance of *S. marcescens* to antibiotics and more specifically the role of a suspected *S. marcescens* porin in the penetration of antibiotics and other hydrophilic molecules. In addition, because the outer membrane of gram-negative bacteria interacts with the environment and because knowledge of the outer membrane is usually acquired from the study of cells grown in vitro, we designed the study to compare the outer membrane structures and functions of in vivo- and in vitro-grown *S. marcescens* cells. Rat intraperitoneal diffusion chambers (14, 23) were used to achieve in vivo growth.

Our data suggest that *S. marcescens* possesses a greater permeability barrier than *Escherichia coli* cells because of the expression of a single pore-forming protein with a pore size diameter estimated at 1.06 nm. This 41-kDa protein corresponded to the previously suspected porin candidate mentioned in other studies (17, 18), and its expression was unchanged in cells cultivated in vivo or in vitro.

MATERIALS AND METHODS

Strains and cultivation media. *S. marcescens* UOC-69 is a clinical isolate from a septic episode and was obtained from K. Williams (Seattle Harbor View Hospital, Seattle, Wash.). Strain UOC-69 produces the TEM-2 β-lactamase from plasmid RP4 (30).

A variety of *E. coli* strains (34) were used as controls in planar lipid bilayer experiments and in whole-cell β-lactam permeability assays: JF568, parent strain with all porins, including OmpF and OmpC; JF694, porin PhoE only; JF701, OmpF only; JF703, OmpC only. *E. coli* cells were transformed with the pBR325 plasmid DNA by a calcium chloride procedure (28).

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The media used for in vitro cultivation of bacterial strains were minimal broth Davis (Difco Laboratories, Detroit, Mich.) and Trypticase soy agar or broth (BBL Microbiology Systems, Cockeysville, Md.). Nutrient broth no. 2 (Oxoid Ltd., Basingstoke, England) diluted at 8% (wt/vol) in water was also used to create iron-restricted conditions by a modification of the method of Brown et al. (10), as follows. The medium was supplemented with 0.5% NaCl (wt/vol) before treatment with the Chelex 100 ion-exchange resin (Bio-Rad Laboratories, Richmond, Calif.), and after sterilization by filtration, the medium was then supplemented with 0.4 mM magnesium sulfate and iron-free human transferrin at 100 μg/ml (Sigma Chemical Co., St. Louis, Mo.). Iron sulfate at 60 μM was added to the iron-sufficient control broth.

Diffusion chambers and in vivo growth conditions. Diffusion chambers were constructed from 3-ml polypropylene syringe barrels by modifications of the method of Day et al. (14). Millipore MF 0.22-μm-pore-size filters (Millipore Products Division, Bedford, Mass.) were cut to the diameter of the syringe barrels and attached by melting the barrels on a hot plate to affix the filter. The chambers were autoclaved. Overnight cultures diluted to 10^4 to 10^7 CFU/ml in physiological saline were injected through a needle hole melted through the side of the barrels. The hole was subsequently closed either by heating with a glass rod or with UHU brand glue (Lingner and Fisher GmbH [14]). Cells remained viable with no diminished counts for at least 6 h at 20°C. Chamber volume was approximately 500 μl. Sprague-Dawley rats, weighing approximately 250 g, were anesthetized with ketamine hydrochloride (88 mg/kg) and xylazine (4 mg/kg) (Aveco Co., Inc., Fort Dodge, Iowa) intramuscularly. Three chambers were placed in the peritoneal cavity through a small longitudinal incision in the abdomen. The abdomen wall was sutured, and the skin was clipped. Animals were euthanized with carbon dioxide before the chambers were removed. The rats had free access to food and water.

Bacterial cells were removed from the chambers, collected by centrifugation, washed twice with saline, and suspended in either saline for CFU determination and outer membrane and lipopolysaccharide (LPS) analyses or in 20 mM HEPES (*N*-2-hydroxyethylpiperazine-*N'*-2-ethanesulfonic acid) buffer (pH 7.0) for whole-cell permeability assays.

Outer membrane preparation. Membranes were isolated by the method of Hancock and Nikaido (20) as modified by Godfrey et al. (16). Log-phase cells from 1-liter cultures were harvested by centrifugation. The cells were suspended in 15 ml of 20% sucrose–50 mM Tris hydrochloride (pH 7.9)–0.2 mM dithiothreitol and were disrupted by three passages through a French pressure cell (18,000 lb/in^2). Debris were removed by low-speed centrifugation (500 × *g* for 10 min). The supernatant was loaded onto a discontinuous sucrose gradient (52, 58, 64, and 70%) and was centrifuged at 100,000 × *g* for 16 h. Outer membranes were collected, suspended in 50 mM Tris hydrochloride (pH 7.9), and stored frozen (−20°C). Protein concentration was determined by the method of Lowry et al. (26). The outer membranes (25 μg) were suspended in a electrophoresis sample buffer containing 1% SDS and 5% 2-mercaptoethanol. The samples were solubilized at either 20°C or 100°C for 10 min before being loaded for electrophoresis in discontinuous SDS-polyacrylamide (12%) gels (24). Gels were stained with 0.1% Coomassie brilliant blue.

LPS preparation. Whole-cell lysis to obtain LPS was performed as described by Hitchcock and Brown (21). LPS preparations were applied on SDS-polyacrylamide (15%) gels containing 4 M urea, and visualization was achieved by silver staining (42).

Porin isolation. The porins were isolated from membranes collected from 1 ml of sonicated cells (10^8 to 10^9 CFU) or from 25 to 50 μg of outer membrane proteins by previously published methods (39, 40). The membranes were suspended in 0.5 ml of a solution containing 50 mM HEPES buffer (pH 7.2), 1% SDS, and 5 mM EDTA, and the insoluble material was collected by centrifugation at 100,000 × *g* for 1 h in a Beckman TL-100 ultracentrifuge. The porins were solubilized from the pellet with 0.4 M NaCl in 100 μl of the buffer mentioned above at pH 7.7. Solubilization was allowed for 1 h at room temperature, and insoluble debris were removed by centrifugation as described above. Sodium azide (0.05%) was added to the porin-containing supernatant.

Planar lipid bilayer experiments. Functional characterization of porin activity was done by using the black lipid bilayer technology and equipment as previously detailed (3–5). By using dilutions of about 100,000-fold, the porin preparations were used at concentrations of 0.2 to 2 ng/ml in the Teflon chamber. The artificial lipid membrane used was composed of 2% oxidized cholesterol in *n*-decane and was generously provided by L. C. Blaszczak (Lilly Research Laboratories, Eli Lilly and Co., Indianapolis, Ind.). Experiments were done at 22°C.

Whole-cell permeability assays. The rate of diffusion of cephaloridine (Sigma) across the outer membrane of whole bacterial cells was calculated by the method of Zimmermann and Rosselet (47). Assays were performed in 20 mM HEPES buffer (pH 7.0) at 37°C. Permeability coefficients were calculated as described by Nikaido et al. (34).

Amino acid sequencing. The partial amino acid sequence of the 41-kDa porin isolated from *S. marcescens* was determined by using automated Edman chemistry on an Applied Biosystems 477A protein sequencer (Foster City, Calif.). First, the soluble porin from the SDS-NaCl extract was precipitated with an equal volume of acetone at 0°C for 10 min. The precipitate was spun at −10°C for 30 min at 20,000 × *g*, suspended in 2 ml of 20 mM HEPES (pH 7.0), spun at 25°C through a Centricon-10 microconcentrator unit (Amicon Div., W. R. Grace and Co., Danvers, Mass.) for 15 min at 3,000 × *g*, acetone precipitated, suspended in 75 μl of 12.5% trimethylamine in water, and applied to the sequencer. The amino acid sequence was compared with the protein sequence data base of the Protein Identification Resource by using the FASTP program (25).

Rat peritoneal fluid. Analyses of diffusion chamber fluids were performed with the SMAC system at the John L. McClellan Memorial Veterans Hospital (Little Rock, Ark.). In these analyses, the pH and the concentrations of the following were determined: glucose, protein, carbon dioxide, chloride, potassium, sodium, calcium, phosphate, urea, creatine, and cholesterol.

RESULTS

Growth of *S. marcescens* in diffusion chambers. Growth of *S. marcescens* UOC-69 was monitored after implantation of diffusion chambers in rats (Fig. 1). Diffusion chambers allowed for reproducible efficient growth and recovery of bacterial cells. The calculated doubling time was 1.6 h. The analysis of rat peritoneal fluids recovered from diffusion chambers after a 6-h period of incubation showed a decrease in pH and glucose content from 7.80 to 7.25 and 2.70 to 0.09 mg/ml in control and *S. marcescens*-infected fluids, respec-

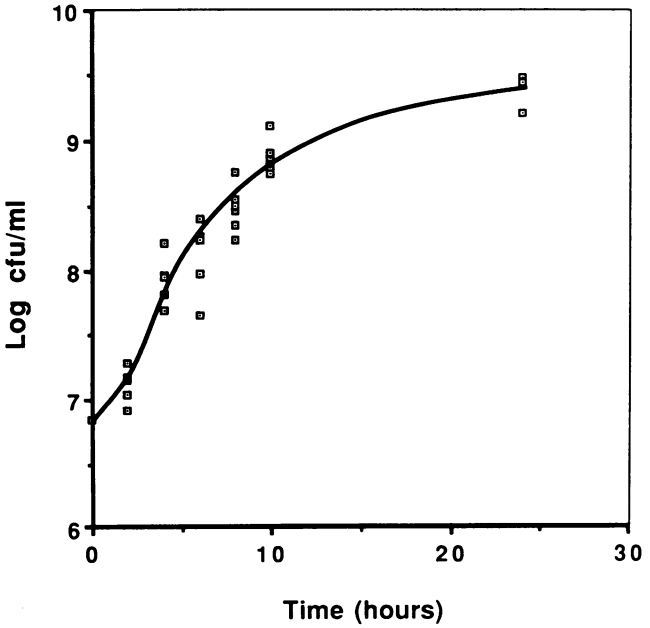

FIG. 1. Growth of *S. marcescens*. Growth was monitored by viable-cell plate counting at intervals after implantation of diffusion chambers in rats. The bacteria were grown overnight in vitro and were diluted in saline before placement in chambers. Data shown represent three to six chambers per time point.

tively. Other physical and chemical parameters measured were similar in both infected and sterile chambers.

Whole-cell permeability to β-lactams. Permeability coefficients were used to compare the outer membrane penetration of cephaloridine in cells grown in diffusion chambers and in vitro (Table 1). There was no change in the permeability of *S. marcescens* cells grown in diffusion chambers or in vitro. *S. marcescens* cells showed a considerable permeability barrier to the β-lactam tested, compared with *E. coli* JF568 ($OmpF^+$ $OmpC^+$) cells. The permeability of *S. marcescens* to cephaloridine was intermediate to that observed for *E. coli* JF701 (expressing OmpF) and JF703 (expressing OmpC).

Examination of cell envelopes. LPS extracted from *S. marcescens* cells grown in diffusion chambers showed an SDS-polyacrylamide gel banding pattern very similar to LPS patterns obtained from in vitro-grown cells (Fig. 2). From

TABLE 1. Permeability coefficients (*P*) of cephaloridine across the outer membrane of bacteria

Species and strain or growth conditions	Porin expressed	*P* (10^{-5} cm s^{-1})
E. coli[a]		
JF568	OmpF and OmpC	150.0
JF701	OmpF	59.0
JF703	OmpC	3.9
JF694	PhoE	3.0
S. marcescens		
In vitro[a]	41 kDa	30.3
In vivo[b]	41 kDa	38.9

[a] Cells grown in Trypticase soy broth.
[b] Cells grown in diffusion chambers.

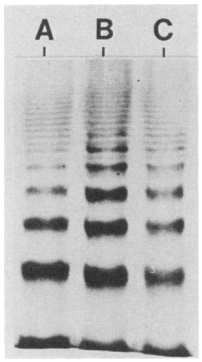

FIG. 2. Analysis of isolated LPS. LPS preparations from whole bacterial cells were electrophoresed on an SDS-polyacrylamide gel (15%) containing 4 M urea and were silver stained. Lanes: A, diffusion chamber-grown *S. marcescens*; B, *S. marcescens* grown in minimal broth Davis; C, *S. marcescens* grown in trypticase soy broth.

cells cultivated in vivo or in vitro, the two major outer membrane proteins (the suspected porin and OmpA, 41 and 38 kDa, respectively) showed identical electrophoretic characteristics (Fig. 3). However, additional high-molecular-weight proteins were uniquely seen in the outer membrane of cells cultivated in diffusion chambers (Fig. 3, arrow). (Figure 3 shows the upper part of the gel, in which the only change in protein expression was observed.) Examination of outer membrane proteins from *S. marcescens* grown under iron-restricted conditions created in vitro (see Materials and Methods) showed the induction of an identical group of high-molecular-weight proteins that were absent in the same cultivation media supplemented with iron sulfate (data not shown).

Porin isolation and functional characterization. The *S. marcescens* suspected porin was isolated by differential solubilization as described in Materials and Methods. Various samples from this isolation procedure were analyzed by SDS-PAGE (Fig. 4). Typically, the OmpA protein showed an increased electrophoretic mobility when samples were not heated to 100°C before electrophoresis (Fig. 4, lane 4a), while the suspected porin showed an oligomeric form under that condition (Fig. 4, lane 5a). The suspected porin was a

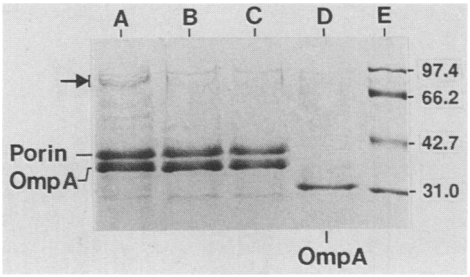

FIG. 3. Analysis of the outer membrane proteins of *S. marcescens* by SDS-polyacrylamide gel (12%) electrophoresis. Lanes: A, diffusion chamber-grown cells; B, cells grown in minimal broth Davis; C and D, cells grown in Trypticase soy broth; E, molecular weight markers (weights [10^3] indicated on the right). Samples were solubilized under reducing conditions at either 100°C (lanes A to C and E) or 20°C (lane D) for 10 min before electrophoresis. OmpA is the major protein band seen in lane D. The arrow indicates the series of high-molecular-weight proteins specifically seen in in vivo-grown cells.

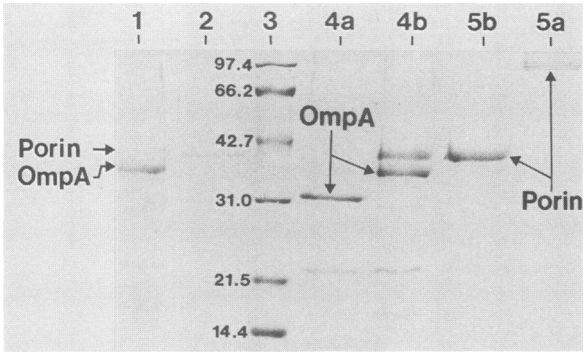

FIG. 4. Analysis of the isolated *S. marcescens* 41-kDa porin by SDS-polyacrylamide gel (12%) electrophoresis. Lanes: 1 and 2, 1% SDS-soluble and 1% SDS–0.4 M NaCl-insoluble outer membrane proteins, respectively; 3, molecular weight (10^3) markers; 4, total outer membrane proteins; 5, 1% SDS–0.4 M NaCl-soluble protein fraction showing the isolated porin. Samples were solubilized at 100°C or at 20°C (lanes 4a and 5a) for 10 min under reducing conditions before electrophoresis.

major outer membrane protein soluble only in the SDS-NaCl fraction during the isolation procedure (Fig. 4, lanes 5a and b).

Functional characterization of the isolated porin was carried out using the planar lipid membrane system as described in Materials and Methods. A stepwise increase in conductance was observed with the addition of 1 ng of porin per ml (Fig. 5). The histogram of conductance steps obtained in 1.0 M KCl showed that the magnitudes of these increments were clustered around a mean (Fig. 6). Considering

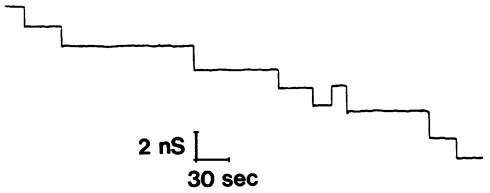

FIG. 5. Planar lipid bilayer experiment. Stepwise increase in conductance recorded in planar lipid bilayer experiments using 1 ng of the *S. marcescens* 41-kDa porin per ml of a 1.0 M KCl solution.

the average single-channel conductance, there was no difference in the pore-forming activity of porins isolated from diffusion chamber- or in vitro (trypticase soy broth)-grown cells. This was also the case for the average conductance increments obtained in other KCl concentrations or in 1.0 M NaCl (Table 2). Table 2 shows that the average single-channel conductance of *S. marcescens* porin (Λ) was a linear function of the bulk aqueous conductivity (σ) of the salt solution with, as a consequence, relatively constant Λ/σ ratios. This is consistent with the pore being water filled. The increase in conductance over time was dependent on the amount of the 41-kDa porin added in the planar lipid bilayer experiments. In bulk conductance experiments, the current measured was directly proportional to the voltage applied, indicating that the pore-forming activity was not influenced by voltage and that the channels formed were water filled and opened (data not shown). These data are consistent with those for most other bacterial porins studied to date. Our planar lipid bilayer system was calibrated with the *E. coli* OmpC purified porin, which showed an average single-channel conductance of 1.5 nS (n = 319).

Amino acid sequence. The amino acid sequence of the *S.*

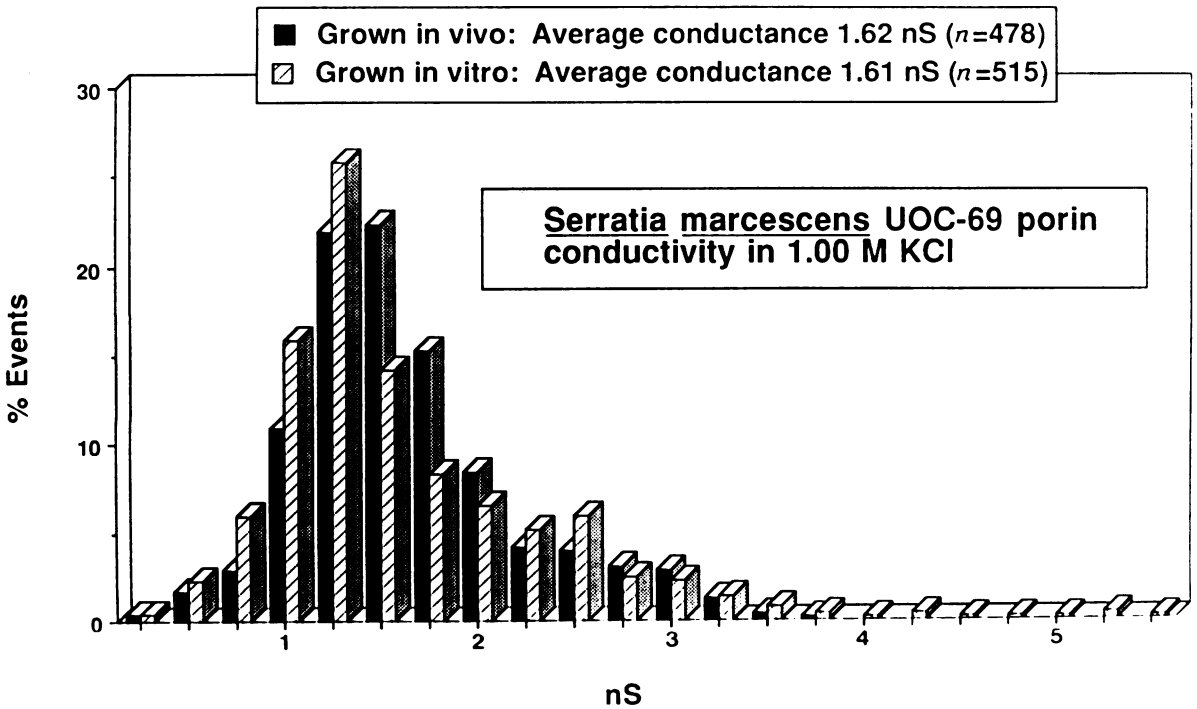

FIG. 6. Histogram of the single-channel conductance steps for the 41-kDa porin (1 ng/ml) of *S. marcescens* grown in vivo (rat diffusion chambers) or in vitro (Trypticase soy broth). Planar lipid bilayer experiments were done in 1.0 M KCl. The number of events (n) and the mean conductance are indicated.

TABLE 2. Average conductance increments[a] for the *S. marcescens* 41-kDa porin

Porin origin[b]	Salt	Concn (M)	λ (nS)[c]	σ (mS/cm)[d]	Λ/σ (10^8 cm^{-1})	*n*
In vitro	KCl	1.0	1.6	110	1.5	515
	KCl	0.5	0.8	56	1.4	261
	KCl	0.2	0.3	22	1.5	236
	NaCl	1.0	1.0	84	1.2	244
In vivo	KCl	1.0	1.6	110	1.5	478
	KCl	0.5	0.8	56	1.4	323
	KCl	0.2	0.3	22	1.6	176
	NaCl	1.0	0.9	84	1.1	271

[a] Increments were measured on membranes made from 2.0% oxidized cholesterol in *n*-decane in the presence of the solubilized *S. marcescens* outer membrane 41-kDa porin.

[b] The 41-kDa porin was extracted from *S. marcescens* cells grown in Trypticase soy broth (in vitro) or in diffusion chambers (in vivo).

[c] Λ, Average single-channel conductance from the number (*n*) of measured single-channel events.

[d] σ, Bulk conductivity of the given salt solution in the absence of a membrane.

marcescens porin was determined for the first 19 residues (Fig. 7). Very strong homology (>84%) was found with published *E. coli* porin sequences (22, 31, 37), including that of the *E. coli* outer membrane Lc porin coded by the bacteriophage PA-2 (8). The strongest homology was seen with the *E. coli* OmpC porin: only Val-3 was replaced by Ile in the *S. marcescens* porin.

DISCUSSION

This is the first report of the functional characteristics of the *S. marcescens* 41-kDa porin. This study was also designed to address the relevance of in vitro growth of bacteria in the investigation of whole-cell permeability, outer membrane components, and pore-forming proteins.

In our electrophoretic systems (Fig. 3 and 4), the 41-kDa porin was distinguished from OmpA (38 kDa) by its molecular mass, its oligomeric form (Fig. 4, lane 5a), and its solubility in a SDS-NaCl solution, as shown with many other peptidoglycan-associated porins from members of the family *Enterobacteriaceae* (2, 19, 39). In addition, the characteristic heat modifiability of the OmpA protein of *S. marcescens* (9, 11) also helped to distinguish this protein from the porin (Fig. 4, lanes 4a and 4b).

The data collected from planar lipid bilayer experiments demonstrated the poreforming activity of the *S. marcescens* 41-kDa porin. The average single-channel conductance in 1.0 M KCl was 1.6 nS for the *S. marcescens* porin extracted from both in vitro- and diffusion chamber-grown cells (Table 1). These values are intermediate to those reported for *E. coli* K-12 OmpC (1.5 nS) and OmpF (1.9 nS) porins (6). The

		+1																	19
S. marcescens	41-kDa	A	E	I	Y	N	K	D	G	N	K	L	D	L	Y	G	K	V	D	G
E. coli	OmpC	·	·	V	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·
E. coli	OmpF	·	·	·	·	·	·	·	·	·	V	·	·	·	·	·	A	V	·	·
E. coli	PhoE	·	·	·	·	·	·	·	·	·	V	·	·	·	·	·	K	A		
PA-2	Lc	·	·	·	·	·	·	·	S	·	·	·	·	·	·	·	N	A		

FIG. 7. Partial amino acid sequence of the *S. marcescens* porin. The homology between the *S. marcescens* porin and the published sequences of the *E. coli* OmpC (31), OmpF (22), and PhoE (37) porins and the bacteriophage PA-2 *lc* gene-encoded porin (8) is indicated by the dots.

pore diameter of the *S. marcescens* porin was estimated to be 1.1 nm by using the following equation: $\Lambda = \sigma\pi r^2/l$ where Λ is the average conductance, σ is the bulk aqueous conductivity, *r* is the radius of the pore, and *l* is the length of the pore, approximated as 6.0 nm (2). In this calculation it was assumed that the pore was a water-filled cylinder with a spherical cross section. The calculated pore size of 1.1 mm is in agreement with our β-lactam whole-cell permeability assay (Table 1) that showed *S. marcescens* cells to be less permeable than *E. coli* JF701 (expressing OmpF) but more permeable than strain JF703 (expressing OmpC). The permeability coefficients of cephaloridine across the outer membrane of the *E. coli* strains were very similar to those previously reported (34).

The amino acid homology of the 19 amino-terminal residues of the *S. marcescens* porin with the same region of all *E. coli* porins was striking (Fig. 7). Greatest homology (95%) was found with *E. coli* OmpC, but a high homology (84%) was also seen with *E. coli* OmpF, *E. coli* PhoE, and bacteriophage PA-2 porins. A tentative model for the topological arrangement of *E. coli* PhoE in the outer membrane of *E. coli* K-12 has been proposed (2). In this model, the amino-terminal 9 amino acids extend into the periplasm and the next 10 residues form the first of 16 membrane-spanning segments. The sequence that we report here for the *S. marcescens* 41-kDa porin is homologous with this N-terminal periplasmic extension and with the first membrane-spanning region. It has been suggested that Lys-18 of PhoE, which is a part of this first membrane-spanning segment, is involved in the anion selectivity of the PhoE pore (43). The *S. marcescens* 41-kDa porin may not be anion selective because it does not contain a Lys at this position and also because it forms a relatively large pore size, as determined by the single-channel experiments.

In the present study, the use of diffusion chambers to grow *S. marcescens* cells allowed us to collect information on the function of the outer membrane and examine its composition after exposure to a physiological environment. Under these conditions, the outer membrane permeability barrier to cephaloridine was unchanged compared with that observed with cells grown in vitro (Table 1). This was consistent with the unchanged LPS banding patterns (Fig. 2) and the unmodified expression of OmpA and the 41-kDa porin in the outer membrane of *S. marcescens* grown under various conditions (Fig. 3). In another study, the growth of *Pseudomonas aeruginosa* in diffusion chambers implanted in rats yielded different results (23). In that study (23), *P. aeruginosa* showed faster growth rates, although a lower net yield of bacteria was obtained, LPS changes were observed, and iron-regulated outer membrane proteins were poorly induced. In our opinion, this divergence of results further supports the need for reassessing the structural and physiological properties of pathogenic bacteria placed in an in vivo environment.

At this point, it would not be reasonable to assume that *S. marcescens* possesses a single pore-forming protein. There are a number of porins from members of the *Enterobacteriaceae* that exhibit specific osmoregulation (12) or that are induced by specific nutrients (7, 27). However, the 41-kDa *S. marcescens* outer membrane protein is certainly a major porin which had consistent expression in our study and showed identical functional characteristics when extracted from either in vitro- or in vivo-grown cells (Fig. 6 and Table 2).

The presence of a group of high-molecular-weight proteins in the outer membrane of cells grown in diffusion chambers

(Fig. 3) demonstrated the ability of this cultivation system to induce specific bacterial components in response to growth conditions. An important limitation for bacterial growth in mammals is the restriction of iron in this environment (1, 15). As a result of iron-restricted conditions in vitro, *S. marcescens* cells expressed a group of high-molecular-weight outer membrane proteins which were very similar to those expressed in diffusion chamber-grown cells (Fig. 3). Iron starvation-induced outer membrane proteins are also seen in other members of the *Enterobacteriaceae* (32). The function of these low iron-induced proteins and their relation to a novel iron transport system described for *S. marcescens* (46) are currently under investigation in our laboratory.

This paper reports the functional characterization of the *S. marcescens* 41-kDa porin. The sequence of the first 19 amino acids of this protein strongly supports its porin identity because of the very high homology with sequences of *E. coli* porins. Finally, although the expression of this pore-forming protein was unchanged in the outer membranes of in vivo- and in vitro-grown cells, the diffusion chamber methodology will provide more opportunities to assess further other differences and similarities of in vivo and in vitro conditions.

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