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Cloning and Sequence of the Gene Encoding the Major Structural Component of Mannose-Resistant Fimbriae of *Serratia marcescens*

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***Serratia marcescens* US46, a human urinary tract isolate, exhibits mannose-resistant hemagglutination and agglutinates yeast cells, thereby indicating that it has two types of adhesins. We constructed a cosmid library for the DNA of this organism and isolated DNA clones carrying genes for mannose-sensitive (MS) and mannose-resistant (MR) fimbriae. On introduction of the cloned genes into *Escherichia coli* K-12, MS and MR fimbriae were formed. These fimbriae were functionally and morphologically indistinguishable from those of *S. marcescens*. Subcloning of these gene clusters revealed that the genes encoding MS fimbriae reside on a 9-kilobase (kb) DNA fragment, while those encoding MR fimbriae are present on a 12-kb fragment. Transposon insertion and maxicell analyses revealed that formation of MR fimbriae is controlled by several genes which reside on the 9-kb fragment. The nucleotide sequence of *smfA*, the gene encoding the major structural component of MR fimbriae, revealed that this gene encodes a 174-amino-acid polypeptide with a typical procaryotic signal peptide. The primary structure of the *smfA* product showed significant homology with the primary structure of the *E. coli* fimbrial subunit.**

Serratia marcescens is a bacterial species causing nosocomial infection in the urinary and respiratory tracts (3, 16, 17, 30). Gram-negative bacteria isolated from patients with an urinary tract infection adhere to uroepithelial surfaces by bacterial surface appendages known as pili or fimbriae (13, 15, 43). *S. marcescens* isolated from such patients also possesses fimbriae and adheres to uroepithelial cells (47).

There are at least two classes of adhesins in *S. marcescens* (1, 26, 27). One class, designated MRHA, agglutinates chicken erythrocytes in the presence of D-mannose and is associated with thick rigid fimbriae. The other, named MSHA, exhibits mannose-sensitive hemagglutination of guinea pig and chicken erythrocytes and is associated with thin flexible fimbriae. The latter adhesin is also responsible for agglutinating yeast cells. *S. marcescens* US46, isolated from a patient with a urinary tract infection, exhibits mannose-resistant hemagglutination and agglutinates yeast cells, thereby indicating that this strain possesses two classes of adhesins (26).

To elucidate mechanisms related to the genetic control of formation of mannose-resistant (MR) and mannose-sensitive (MS) fimbriae of *S. marcescens*, we attempted to clone the DNA fragments carrying these genes. We placed fragments of the chromosomal DNA of strain US46 on appropriate vectors and introduced them into the nonfimbriated strain *E. coli* K-12. Thus, we were able to clone the chromosomal determinants for MR and MS fimbriae of *S. marcescens* US46. We determined the nucleotide sequence of the *smfA* (for *S. marcescens* fimbria A) gene, which encodes the major structural component (major fimbrial subunit) of MR fimbriae of *S. marcescens* US46.

MATERIALS AND METHODS

Bacterial strains and growth conditions. The bacterial strains used are listed in Table 1. *E. coli* K-12, strain KD2158, which has type 1 fimbriae, was mutagenized with methyl methanesulfonate, and a nonfimbriated mutant strain, K4, was isolated. Unless otherwise stated, bacteria were grown in L broth (31) and on L broth agar, both without glucose. Culture media were supplemented with appropriate antibiotics at the following concentrations: ampicillin, 50 μg/ml; tetracycline, 10 μg/ml; chloramphenicol, 25 μg/ml (for plasmid amplification, 200 μg/ml).

Vectors. pHC79 (22) was used as a cosmid vector. pBR322 (8), pACYC184 (9), and pUC19 (48) were used as plasmid vectors. We also used M13 phage cloning vectors mWB3225 and mWB3295 (6).

Chemicals and enzymes. Methyl methanesulfonate was purchased from Nakarai Chemicals Co., Ltd. (Kyoto, Japan). Proteinase K and ribonuclease A (type III) were from Sigma Chemical Co., Ltd. Restriction enzymes, T4 DNA ligase, bacterial alkaline phosphatase, and DNA sequencing kits were obtained from Takara Shuzo Co., Ltd. (Kyoto, Japan) and Toyobo Co., Ltd. (Osaka, Japan).

Preparation of DNA. The high-molecular-weight chromosomal DNA of *S. marcescens* was prepared essentially by the method of Berns and Thomas (7). Plasmid DNA was isolated by the alkaline lysis method (31).

Construction of the genomic library. The high-molecular-weight chromosomal DNA of *S. marcescens* was partially digested with *Sau*3A and subjected to 0.5% agarose gel electrophoresis. DNA fragments (35 to 50 kilobases [kb]) were transferred to DE81 paper (Whatman) by electrophoresis. The paper was washed with 800 μl of 0.2 M NaCl, and DNA fragments of appropriate sizes were eluted from the paper with 400 μl of 2 M NaCl. After ligation of these fragments with pHC79, which had been treated with *Bam*HI and bacterial alkaline phosphatase, recombinant molecules were packaged in vitro (23, 34).

Determination of agglutination properties. Hemagglutination properties were determined in phosphate-buffered saline (PBS) with a 2% (vol/vol) suspension of chicken or guinea pig erythrocytes, with or without 1% (wt/vol) D-mannose

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TABLE 1. Bacterial strains

Strain	Type(s) of fimbriae[a]	Relevant genotype	Source or derivation
S. marcescens			
US46	MR, MS		26
US5	MS		27
E. coli			
P678-54	None	F^- *thr leu-6 thi-1 lacY1 malA1 xyl-15 mtl-2 tonA2 gal-6* λ *rpsL minA minB*	2
KD2158	Type 1	*hsdR recA1 leu-6 proA2 his-4 rpsL31 mtl-1 xyl-15 ara-14 galK2 lacY1 tsx-33 supE44*	This paper
K4	None	As KD2158	This paper
JM83	ND	*ara* Δ(*lac-proAB*) *rpsL* (=*strA*) *lacZ M15*	45
DH1	ND	F^- *recA1 endA1 gyrA96 thi-1 hsdR17 supE44*	19
CSR603	ND	F^- *uvrA6 recA1 phr-1 thr leu pro his arg lac gal ara xyl mtl tsx rpsL thi*	41
WB373	ND	MM294, but harboring mini F plasmid, pWB373	6

[a] ND, Not determined.

(10, 14). Yeast cell agglutination was performed as described previously (26).

Antiserum and antibody. MS fimbria-specific serum was prepared from rabbits with purified MS fimbriae of *S. marcescens* US5 as described previously (27). MR fimbria-specific monoclonal antibody was raised against MR fimbriae of *S. marcescens* US46 as reported previously (26).

Electron microscopy. Bacteria were washed twice in 2% ammonium acetate solution, and 1 drop of the suspension was placed on a Formvar-coated grid. These bacteria were stained with 2% sodium phosphotungstate (pH 7.0) and examined under a JEM 100C electron microscope at 80 kV.

Insertion of transposable antibiotic resistance elements (Tn*3*) into the cloned gene. DH1 cells carrying pYM141 encoding MR fimbriae were transformed with pSC301::Tn*3*, which is temperature sensitive in replication, and the cells were grown at 30°C overnight in the presence of ampicillin and chloramphenicol (36). Plasmid DNA was extracted from the culture by the rapid alkaline extraction method (31) and was then introduced into nonfimbriated *E. coli* K4 cells. Transformants resistant to both ampicillin and chloramphenicol at 42°C were selected. Under these conditions, only cells carrying the Tn*3*-inserted pYM141 can survive because pSC301::Tn*3* cannot replicate at 42°C (36). Hemagglutination activity and fimbria formation were then examined. Tn*3*-inserted plasmids were extracted and analyzed by using restriction enzymes to map the insertion sites of Tn*3*.

Labeling of plasmid-encoded proteins. The maxicell method of Sancar et al. (41) was used. Plasmid-encoded proteins were selectively labeled with [^{35}S]methionine and analyzed by sodium dodecyl sulfate (SDS)–15% polyacrylamide or 10 to 20% gradient gel electrophoresis, followed by fluorography.

Immunoprecipitation. Immunoprecipitation of [^{35}S]methionine-labeled, plasmid-encoded proteins was performed essentially by the method of Orndorff and Falkow (37), except that cells radiolabeled by using the maxicell system were used instead of radiolabeled minicells.

Purification of MR fimbriae produced in *E. coli*. *E. coli* P678-54 cells carrying pYM122 encoding MR fimbriae of *S. marcescens* US46 were grown overnight in the presence of ampicillin and harvested by centrifugation. The cells were suspended in 50 ml of 0.1 M Tris hydrochloride (pH 8.0) and homogenized for 5 min in a Waring blender, and the homogenate was centrifuged at 25,000 × *g* for 60 min. The supernatant was concentrated to about 5 ml by dialysis against 20% polyethylene glycol 6000. The concentrated material was subjected to gel filtration (Sepharose 4B). The fractions containing fimbriae were examined by electron microscopy.

Gel electrophoresis of proteins. Polyacrylamide gel electrophoresis in the presence of SDS was done essentially by the method of Laemmli (29).

Determination of the nucleotide sequence. The nucleotide sequence was determined by the chain-termination method (20) using plasmid vector pUC19 and phage vectors mWB3225 and mWB3295.

Amino acid composition and amino-terminal sequence analyses of purified MR fimbriae. The amino acid composition of purified MR fimbriae was determined after hydrolysis of the samples with 5.7 M HCl for 24, 48, and 72 h at 110°C and with 3 M mercaptoethanesulfonic acid containing 0.2% tryptamine for 24 h at 110°C. The amino acid sequence was determined by automated Edman degradation. Details regarding the procedure, including the equipment used, were the same as those described previously (40).

RESULTS

Isolation of recombinant cosmids carrying genes for MR and for MS fimbriae. *S. marcescens* US46, a human urinary tract isolate, seems to possess both MR and MS fimbriae because it agglutinates chicken erythrocytes in the presence of D-mannose and also agglutinates yeast cells. A recombinant cosmid library was constructed by inserting *Sau*3A partially digested DNA from the US46 cells into the *Bam*HI site of pHC79. The recombinant cosmids were packaged in vitro and used to transfect nonfimbriated *E. coli* K4. The resulting ampicillin-resistant transformants were screened for hemagglutination activity, and among the 3×10^3 transformants, 2 MRHA clones and 10 MSHA clones were isolated. The MRHA clones were aggregated with the anti-US46 MR fimbria antibody. The MSHA clones were aggregated with the anti-MS fimbria antiserum and agglutinated yeast cells (Table 2). Two recombinant cosmids, pYM1 (for MSHA) and pYM100 (for MRHA), were used for further analyses.

Subcloning of the MSHA gene cluster. pYM1 carries a 32-kb DNA fragment derived from *S. marcescens* US46. To locate the MSHA gene cluster, various deletion derivatives of pYM1 were constructed by in vitro recombination. It was concluded that the MSHA gene cluster resides on a 9-kb DNA fragment carried by plasmid pYM7 (Fig. 1). The partial restriction map of the 9-kb fragment revealed that it is identical to the map obtained for the MS fimbria gene cluster of *S. marcescens* isolated from sputum (11).

Subcloning of the MRHA gene cluster. Plasmid pYM100 (for MRHA) carries a 45-kb DNA fragment derived from *S. marcescens* US46, and its restriction map differs from that of pYM1 (for MSHA). pYM100 was partially digested with *Eco*RI, and pYM111 was constructed by self-ligation. An 18-kb *Aat*I fragment derived from the plasmid was inserted into the *Eco*RV site of pBR322, and the resulting plasmid, pYM120, showed the MRHA$^+$ phenotype. pYM121 was

TABLE 2. Agglutination properties of *S. marcescens* US46 and of various recombinant *E. coli* derivatives[a]

Strain	Hemagglutination[b] of:		Yeast cell agglutination[c]	Agglutination by antibody[d]:	
	Chicken erythrocytes	Guinea pig erythrocytes		Anti-MS	Anti-MR
US46	MR	MS	+	+	+
K4	–	–	–	–	–
K4(pYM1)	MS	MS	+	+	–
K4(pYM100)	MR	–	–	–	+

[a] Bacteria grown overnight on an LB agar plate at 37°C were suspended in PBS and used for the following tests. For hemagglutination, chicken and guinea pig erythrocytes were suspended to a concentration of 2% in PBS and mixed with the bacterial suspension on a glass slide. To examine MRHA, the erythrocytes were suspended in PBS containing 1% D-mannose. To determine yeast cell agglutination, the yeast cell and bacterial suspensions were mixed on a glass slide. To determine agglutination by antibody, a bacterial suspension was mixed with anti-MS or anti-MR antibody on a glass slide. All agglutination tests were read within a few minutes.

[b] MR, Mannose-resistant hemagglutination; MS, mannose-sensitive hemagglutination.

[c] –, No agglutination; +, agglutination positive.

[d] Anti-MS, Anti-MS fimbria antiserum; anti-MR, anti-MR fimbria monoclonal antibody.

made by self-ligation after partial digestion of pYM120 with *Cla*I, and pYM122 was constructed by partial digestion of pYM121 with *Ava*I. Cells harboring pYM122, which carries a 12-kb DNA fragment from *S. marcescens*, exhibited the MRHA$^+$ phenotype and fimbria formation (Fim$^+$) phenotype. Thus, the genes encoding MRHA appear to reside on the 12-kb DNA fragment. This 12-kb DNA fragment was recloned into the *Hin*dIII site of the pACYC184 vector. The resulting plasmid, pYM141 (Fig. 2), exhibited the MRHA$^+$ phenotype and the Fim$^+$ phenotype.

To define more precisely the region required for both MRHA$^+$ and Fim$^+$ phenotypes, Tn*3* was inserted into the 12-kb DNA fragment from pYM141. Seventy-five insertion mutants were examined. From the locations of the Tn*3* insertions in the mutant plasmids that were MRHA$^-$ or Fim$^-$ (or both), we concluded that the region for MRHA fimbria determinants spans about 8.7 kb (Fig. 3).

Electron microscopic observation of cells carrying the cloned genes. MRHA and MSHA activities expressed in *E. coli* were associated with two types of morphologically distinct fimbriae. The MR fimbriae expressed in *E. coli* harboring pYM122 are 7 nm in diameter and are morphologically indistinguishable from those of *S. marcescens* US46. MS fimbriae of *E. coli* harboring pYM7 are 3 nm in diameter, are flexible, and have essentially the same features as those of *S. marcescens* (Fig. 4).

Polypeptides encoded by pYM141. Polypeptides encoded by plasmid pYM141, by the vector plasmid pACYC184, and

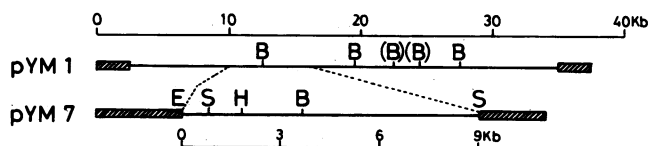

FIG. 1. Physical maps of recombinant cosmids pYM1 and pYM7 carrying an *S. marcescens* chromosome fragment (for mannose-sensitive hemagglutination). Origins and restriction enzyme target sites are indicated as follows: ——, *S. marcescens* DNA; ▨, cosmid pHC79 DNA; H, *Hin*dIII; E, *Eco*RI; B, *Bam*HI; S, *Sal*I. (B) indicates that the restriction site is either of the two.

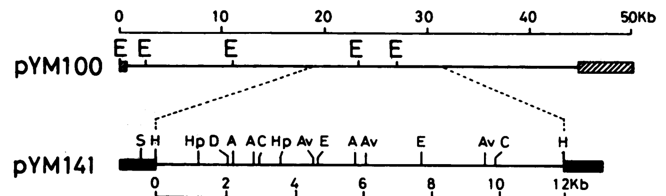

FIG. 2. Physical maps of recombinant cosmid pYM100 carrying a *S. marcescens* chromosome fragment (for MRHA) and of its derivative. Origins and restriction enzyme target sites are indicated as follows: ——, *S. marcescens* DNA; ▨, cosmid pHC79 DNA; ▬, plasmid pACYC184 DNA; E, *Eco*RI; D, *Dra*I; A, *Aat*II; C, *Cla*I; Av, *Ava*I; Hp, *Hpa*I; H, *Hin*dIII; S, *Sal*I.

by various Tn*3* insertion mutants were labeled by the maxicell method (40) and analyzed by SDS-polyacrylamide gel electrophoresis. The positions for Tn*3* insertions are mapped in relation to the left *Hin*dIII site (Fig. 3). Three polypeptides encoded by pYM141 were identified, the molecular weights of which are 84,000 (p84), 28,500 (p28.5), and 17,500 (p17.5). Two Tn*3* insertions, at position 2.4 (pYM1032) and at position 2.6 (pYM1035) within a 700-base-pair *Aat*II fragment, abolished the formation of p17.5, whereas the Tn*3* insertion at position 3.0 (pYM1041) did not (Fig. 5A, lanes c, d, and e). Therefore, the gene *smfA*, encoding p17.5, seems to be located on the 700-base-pair *Aat*II fragment (Fig. 3). Formation of p84 was abolished by Tn*3* insertions at positions 3.9 (pYM1029) and 5.5 (pYM1088), and a truncated protein was formed by pYM1088 (Fig. 5A, lanes i and j). Tn*3* insertion at position 3.6 (pYM1047) did not abolish formation of p84 (data not shown). The size of the region encoding p84 is about 2.3 kb; thus, the gene *smfC*, encoding p84, seems to occupy the region between positions 3.6 and 6 (Fig. 3). Tn*3* insertion at position 6.2 (pYM1018) abolished formation of p28.5, but insertion at position 6.8 (pYM1075) did not (Fig. 5A, lanes f and g). Therefore, the gene *smfD*, encoding p28.5, would reside on the region between positions 6 and 6.8 (Fig. 3). There seem to be several genes in the remaining regions of the cloned DNA, since Tn*3* insertions at these regions affected fimbria formation. However, the products of these genes could not be detected in the present analyses, probably because of low levels of expression of the genes.

Immunoprecipitation of radiolabeled polypeptides encoded by pYM141 revealed that a polypeptide with a molecular weight of 17,500 is specifically precipitated by anti-MR fimbria antibody (Fig. 5B). This polypeptide comigrated with the purified MR fimbriae on SDS-polyacrylamide gel electrophoresis and was not detected in the immunoprecipitated products of pYM1032(*smfA*::Tn*3*). A vector-encoded 26.0-kilodalton (kDa) polypeptide was also detected, probably because of nonspecific precipitation of the overproduced product (Fig. 5B).

Nucleotide sequence of the *smfA* gene encoding the major structural component of MR fimbriae. The polypeptide with a molecular weight of 17,500 (p17.5), encoded by *smfA*, seemed to be the major structural component of MR fimbriae because p17.5 is immunoprecipitated by anti-MR fimbria antibody and the molecular weight of the purified fimbriae from strain P678-54 harboring plasmid pYM122 is 17,500 (Fig. 5C). A 1-kb *Dra*I-*Cla*I fragment carrying *smfA* was then subjected to DNA sequencing (Fig. 6).

The nucleotide sequence of the *smfA* gene and the deduced amino acid sequence are shown in Fig. 7. A 522-nucleotide-long open reading frame was found. A presumed initiation codon (ATG), preceded by a sequence showing

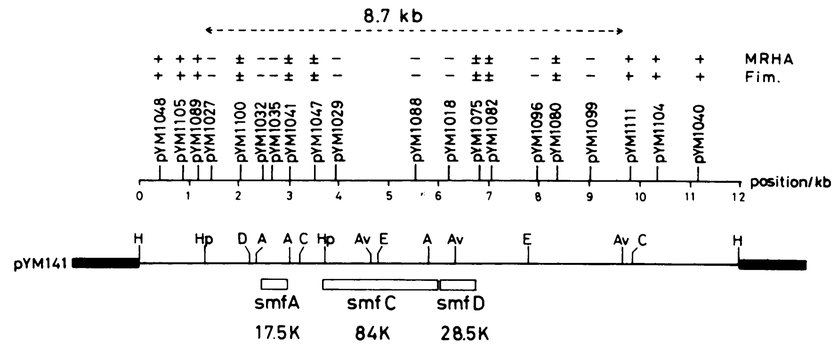

FIG. 3. Locations of Tn*3*-inserted sites and a partial genetic organization of the *smf* operon in pYM141. The upper, dotted line shows the region required for MRHA and for fimbria formation. The positions of various Tn*3* insertions and the mutant phenotypes are given in the upper half of the figure, and the restriction map of pYM141 and the locations of the *smf* genes identified are given in the lower half. The lengths of the open boxes correspond to the lengths of the genes required for encoding each polypeptide. Mutant phenotypes are indicated as follows: +, MRHA or fimbria formation identical to that of the wild-type clone; ±, weak MRHA or reduced fimbria formation; −, abolished MRHA or fimbria formation. ▬, pACYC184; ——, *S. marcescens* DNA; H, *Hin*dIII; HP, *Hpa*I; A, *Aat*II; Av, *Ava*I; D, *Dra*I; C, *Cla*I; E, *Eco*RI.

FIG. 4. Electron micrographs of negatively stained *S. marcescens* strains and *E. coli* P678-54, with or without recombinant plasmids. (A) *S. marcescens* US46, which has both MR and MS fimbriae. (B) *S. marcescens* US5, which has only MS fimbriae. (C) Nonfimbriated *E. coli* P678-54. (D) P678-54 harboring pYM122 encoding MR fimbriae of *S. marcescens*. (E) P678-54 harboring pYM7 encoding MS fimbriae of *S. marcescens*. Bars, 0.1 μm.

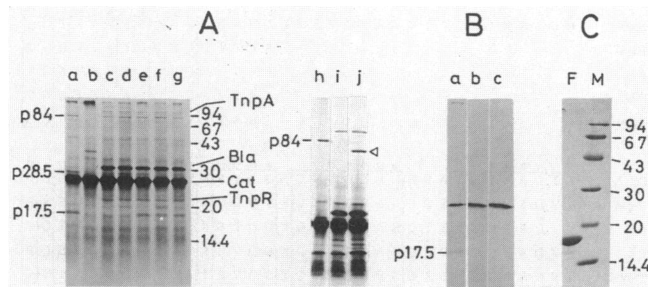

FIG. 5. Analyses of protein products on SDS-polyacrylamide gel electrophoresis. (A) Proteins produced by hybrid plasmids carrying the MR fimbria gene cluster. The polypeptides encoded by plasmids were specifically labeled with [^{35}S]methionine by using the maxicell system and were analyzed by electrophoresis on SDS–15% polyacrylamide (lanes a to g) and on a 10 to 20% gradient SDS-polyacrylamide gel (lanes h to j) followed by fluorography. Each lane represents CSR603 cells harboring the following plasmids. Lanes: a, pYM141; b, pACYC184; c, pYM1032; d, pYM1035; e, pYM1041; f, pYM1018; g, pYM1075; h, pYM141; i, pYM1029; j, pYM1088. ◁, Truncated protein. (B) Immunoprecipitation of the protein products of pYM141, pYM1032, and the vector plasmid pACYC184. ^{35}S-labeled gene products encoded by each plasmid were immunoprecipitated with anti-MR fimbria antibody. The precipitates were then applied to SDS–15% polyacrylamide gel electrophoresis followed by fluorography. Lanes: a, immunoprecipitated products of pYM141; b, immunoprecipitated products of pYM1032 (*smfA*::Tn*3*); c, immunoprecipitated products of pACYC184. (C) Electrophoresis of purified fimbriae from *E. coli* P678-54 carrying plasmid pYM122 encoding MR fimbriae. Purified fimbriae were subjected to SDS–15% polyacrylamide gel electrophoresis and stained with Coomassie brilliant blue. Lane F, Purified fimbriae; lane M, molecular mass markers: phosphorylase *b* (94 kDa), bovine serum albumin (67 kDa), ovalbumin (43 kDa), carbonic anhydrase (30 kDa), soybean trypsin inhibitor (20 kDa), and lactalbumin (14.4 kDa).

homology to the ribosome-binding sites (42), was located at position 250. The sequence initiated here and ending at the TAA triplet at position 772 would encode a polypeptide with 174 amino acid residues. The amino-terminal amino acid sequence of the plausible product has all the features of a procaryotic signal sequence (25).

The amino-terminal sequence analysis of the purified MR fimbriae revealed two types of proteins of almost equal amounts, one showing the amino-terminal sequence Ala-Ala-Asp-Gln-Gly-His-Gly-Lys-Val-Thr-Phe-Thr-Gly-Ser-Ile and the other showing the sequence Ala-Asp-Gln-Gly-His-Gly-Lys-Val-Thr-Phe-Thr-Gly-Ser-Ile-Ile. The former sequence corresponded exactly to the 22nd and following amino acid sequence deduced from the nucleotide sequence, while the latter corresponded to the 23rd and following sequence (Fig. 7). Thus, two types of proteins are apparently produced by the post-translational cleavage, at two adjacent sites. The amino acid composition of the purified MR fimbriae was determined, and the data are shown in Table 3. Since the amino-terminal sequence and the amino acid composition of the purified MR fimbriae match well the amino acid sequence of the hypothetical protein deduced from the DNA sequence of *smfA*, the primary structure of this protein shown in Fig. 7 seems to be correct.

The hydrophilicity profile of the SmfA protein is shown in Fig. 8. It is evident that the 21- or 22-amino-acid-long signal sequence is within a hydrophobic region.

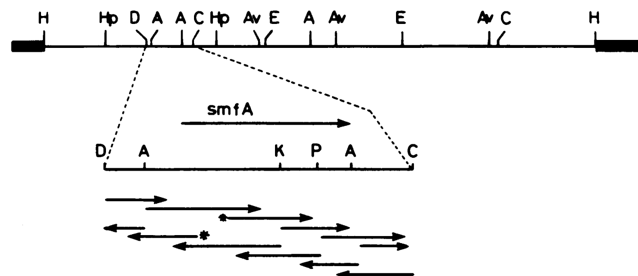

FIG. 6. Organization of a 1.0-kb *Dra*I-*Cla*I fragment and strategy for DNA sequencing. The upper half shows the organization of the *smfA* gene and target sites of restriction enzymes. ▬, Plasmid DNA; ——, cloned *S. marcescens* DNA; H, *Hin*dIII; Hp, *Hpa*I; C, *Cla*I; D, *Dra*I; A, *Aat*II; E, *Eco*RI; Av, *Ava*I; K, *Kpn*I; P, *Pst*I. DNA sequencing was performed by the dideoxy method (20); the strategy is shown in the lower half of the figure. The arrows indicate the direction of DNA sequencing, the region of the *smfA* gene, and the direction of transcription. The asterisks indicate that DNA sequence data were obtained by using a synthetic oligonucleotide primer.

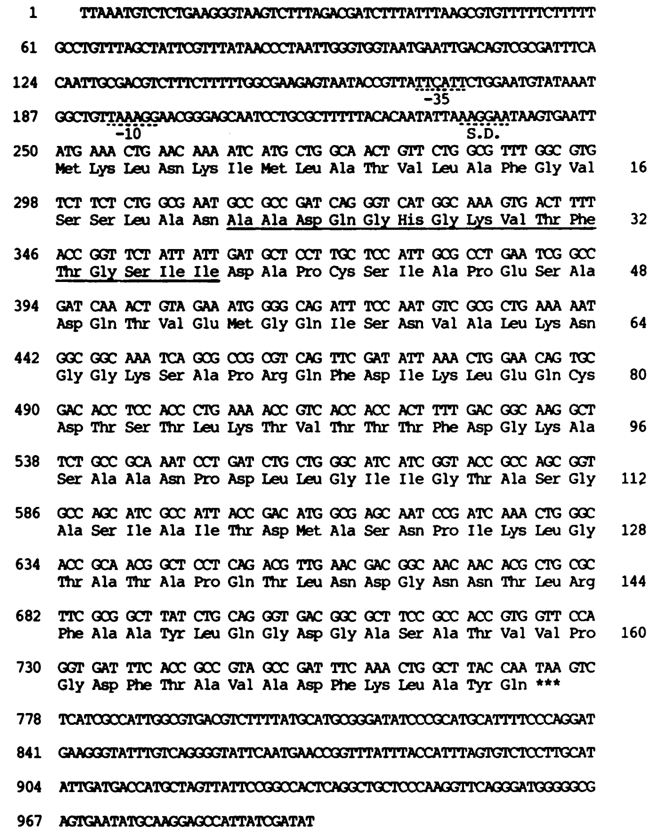

FIG. 7. Nucleotide sequence of the *smfA* gene and primary structure of SmfA protein. The Shine-Dalgarno-like sequence and the consensus sequence for the −35 and the −10 regions of the promoter are indicated by dotted lines. The predicted amino acid sequence of the SmfA protein is also given. The underlined 16-amino-acid sequence was determined by protein analysis. Numbers on the left refer to the nucleotide positions; numbers on the right refer to the amino acid residues.

DISCUSSION

We cloned DNA fragments that encode MR fimbriae and MS fimbriae of *S. marcescens* US46. *E. coli* harboring plasmid pYM7 agglutinated chicken and guinea pig erythrocytes and possessed fimbriae morphologically indistinguish-

TABLE 3. Amino acid composition of SmfA protein

Amino acid	No. of residues/molecule:	
	Determined by analysis[a]	Predicted from nucleotide sequence
Aspartic acid	19.0	12
Asparagine		7
Threonine	17.5[b]	17
Serine	11.0[b]	11
Glutamic acid	11.1	3
Glutamine		8
Proline	6.6	7
Glycine	15.0	15
Alanine	22.6	22/23
Cysteine	1.7[c]	2
Valine	7.1[d]	7
Methionine	1.9	2
Isoleucine	9.4[d]	10
Leucine	10.0	10
Tyrosine	2.0	2
Phenylalanine	6.0	6
Lysine	8.0	8
Histidine	1.0	1
Tryptophan	0.1[e]	0
Arginine	1.9	2

[a] Average values obtained from 24-, 48-, and 72-h hydrolyses with 5.7 M HCl.
[b] Extrapolated values to zero time.
[c] Value determined by using cysteic acid.
[d] Value taken from 72-h-hydrolysis values.
[e] Value for tryptophan that was hydrolyzed with 3 M mercaptoethanesulfonic acid for 24 h.

able from the MS fimbriae of *S. marcescens*. The physical map of the DNA carried by pYM7 was essentially the same as that for the MS pilin genes of *S. marcescens* isolated from sputum (11).

E. coli harboring pYM141 exhibits mannose-resistant hemagglutination (MRHA) to chicken erythrocytes. This adhesive capacity is associated with thick fimbriae present in *E. coli* transformants which are morphologically similar to the thick fimbriae of *S. marcescens* US46. Plasmid pYM141 carries a 12-kb DNA fragment derived from the *S. marcescens* chromosome, and transposon insertion analyses revealed that an 8.7-kb region of the DNA is required for expression of MRHA and for fimbria formation. The physical map of the MR fimbria gene cluster of *S. marcescens* differs from that of uropathogenic and enteropathogenic *E. coli* (4, 12, 18, 35). However, sizes and arrangements of the three genes within the cluster are similar to those for the *E. coli* Pap fimbria gene cluster. The physical maps of the MR and MS fimbria genes of *S. marcescens* (Fig. 1 and 2) are

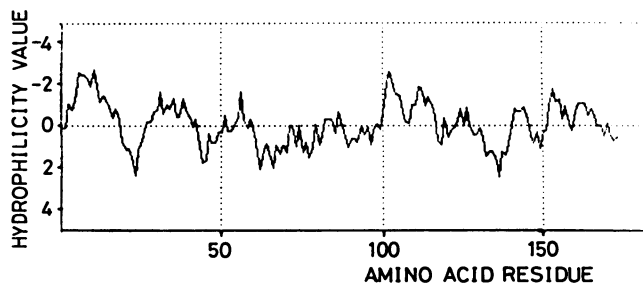

FIG. 8. Hydrophilicity profile of the presumptive SmfA protein. Hydrophilicity was measured by the method of Kyte and Doolittle (28).

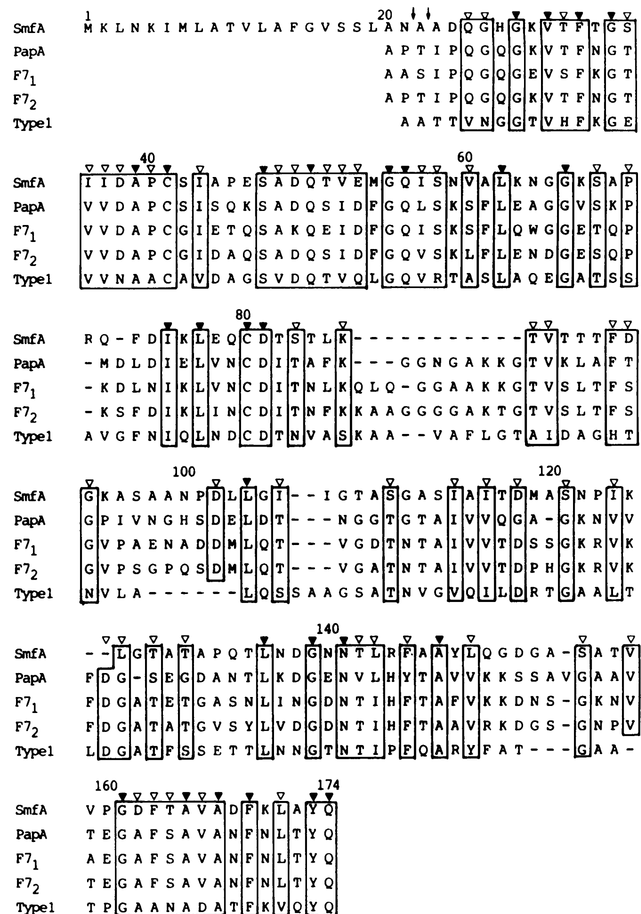

FIG. 9. Comparison of the predicted amino acid sequences of the SmfA protein and of other fimbrial proteins. Sequences of the fimbrial proteins PapA (5), $F7_1$ (43), $F7_2$ (38), and Type 1 (37) are aligned with the SmfA sequence. The amino acids are designated by the one-letter code and are numbered according to those for the SmfA protein. Homologous areas are framed. Symbols: ▼, identical amino acids in all of the sequences; ▽, positions of identical or conservative replacements of amino acids in four of five sequences. Conservative replacements are defined as being within the groups (D,E), (K,R), (S,T), (F,Y,W), and (I,L,V,M). Gaps introduced in order to present a closer homology are indicated by dashes. Vertical arrows indicate signal peptide cleavage sites of the SmfA protein.

entirely different and seem to be derived from different regions of the *S. marcescens* chromosome. *E. coli* J96 has two distinct fimbria gene clusters, one encoding Pap fimbriae and the other encoding type 1 fimbriae (24). *S. marcescens* US46 also seems to have two distinct fimbrial gene clusters.

Morphological and serological surveys of *S. marcescens* US46 showed that this strain has at least two distinct fimbriae (26). This was confirmed genetically in the present study.

We determined the nucleotide sequence of a 1-kb *DraI*-*ClaI* fragment containing the *smfA* gene encoding the major structural component of MR fimbriae. The single open reading frame corresponds to the SmfA protein in respect to size and location. The *smfA* gene encodes a 174-amino-acid polypeptide with a typical procaryotic signal peptide (25). The amino-terminal sequence analysis of the purified MR fimbriae revealed that the peptide is cleaved at two adjacent sites, yielding one signal peptide consisting of 21 amino acids and another with 22 amino acids. This may be due to

ambiguity in recognition by the signal peptidase. There are examples of the presence of multiple cleavage sites in preproteins; the fimbrial subunits of *Neisseria gonorrheae* (21), *Moraxella bovis* (32), *Bacteroides nodosus* (33), bovine growth hormone, and human interferon, cloned in yeast cells (46), are cleaved at more than one site. The signal sequence of the *smfA* gene product is similar in amino acid composition, hydrophilicity, and locations of the charged residues to the signal sequences of *E. coli* fimbrial subunits (5, 38, 39, 44).

The primary structure of the mature SmfA protein is homologous to the structure of *E. coli* Pap, $F7_1$, $F7_2$, and type 1 fimbrial subunits. In particular, sequences of the amino-terminal and carboxyl-terminal regions are highly conserved (Fig. 9). These regions may be involved in membrane transport of fimbrial subunits and in subunit-to-subunit interactions. Other features characteristic of *E. coli* fimbrial subunit molecules, including the two cysteines and the penultimate tyrosine, were also present in the *smfA*-encoded product. Thus, genes encoding the *S. marcescens* fimbriae and the *E. coli* fimbriae may have evolved from a common ancestral gene.

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