Characterization of Genes Encoding Type 1 Fimbriae of *Klebsiella pneumoniae*, *Salmonella typhimurium*, and *Serratia marcescens*

STEVEN CLEGG,* BRENT K. PURCELL, AND JANET PRUCKLER

Department of Microbiology, University of Iowa, Iowa City, Iowa 52242

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With a minicell system, the organization of genes encoding type 1 fimbriae of *Salmonella typhimurium*, *Klebsiella pneumoniae*, and *Serratia marcescens* was determined. In all cases multiple gene products were necessary for the phenotypic expression of fimbriae; thus fimbrial expression in these strains is similar to that in *Escherichia coli*. The type 1 fimbrial subunit gene was detected by the ability of its product to react with specific antiserum. At least six genes were found to be involved in the expression of type 1 fimbriae by *S. typhimurium*, and at least four genes constituted the fimbrial gene cluster of *K. pneumoniae*. In the case of *S. marcescens*, a minimum of three detectable polypeptides was required for the production of fimbriae. Also, a gene probe consisting in part of nucleotide sequences from the *E. coli* fimbrial subunit gene hybridized to a discrete DNA fragment derived from the plasmid encoding *K. pneumoniae* fimbriae. Such a fragment was assumed to contain a gene encoding the structural component of the type 1 fimbriae. Each of the three cloned systems encoded a number of polypeptides which varied in size; thus, the organization and molecular weight of fimbrial accessory proteins of each genus were not identical.

Type 1 fimbriae are produced by many members of the family *Enterobacteriaceae* (10) and are characterized by their ability to mediate d-mannose-sensitive (MS) agglutination of guinea pig erythrocytes. The role of these fimbrial structures in mediating adherence to eucaryotic cells has led to the conclusion that type 1 fimbriae may potentiate the virulence of enterobacteria. Thus, MS fimbriae of *Escherichia coli* have been implicated in increasing the infectivity of uropathogenic isolates (1, 16). Similarly, these structures have been demonstrated to play a role in the pathogenesis of infections due to *Klebsiella pneumoniae* (11, 20) and *Salmonella typhimurium* (9). Type 1 fimbriae have also been isolated from clinical isolates of *Serratia marcescens*, although in this case their role in the infective process remains to be elucidated (17). In all cases, the adherence of these fimbriae to the appropriate receptors on eucaryotic cell surfaces can be inhibited by d-mannose and related carbohydrates. However, there are serological differences between fimbrial antigens isolated from distinct members of enterobacteria as evidenced by the inability of serum prepared against fimbriae isolated from one species to react with preparations from a second species (18, 24). Thus, these structures demonstrate a functional relatedness while retaining antigenic specificity.

More recently the molecular cloning of the gene cluster encoding the phenotypic expression of MS fimbriae of *E. coli* has facilitated analyses of the proteins required for the control and synthesis of these appendages (15, 25, 26). The construction of a gene probe derived from the fimbrial gene cluster has enabled investigators to determine the degree of relatedness of DNA sequences which encode type 1 fimbriae in many different genera of bacteria (3). These investigations revealed that there was little sequence homology between the *E. coli* fimbrial genes and those from other enterobacteria except *Shigella* species. The construction of recombinant plasmids which encode the expression of type 1 fimbriae belonging to four different genera of the *Enterobacteriaceae* again suggested that the genes involved in the production of fimbriae were not identical (5). Indeed it has been shown that the phenotypic expression of type 1 fimbriae can be determined by gene sequences possessing trans-acting activity provided the genes were derived from the same bacterial species (7). Thus, no complementation to restore fimbrial expression, between genes derived from different species, was observed. These data suggest that the gene clusters encoding type 1 fimbriae have undergone considerable divergence in terms of sequence homology; also, the gene products interact in specific ways to assemble these appendages. Consequently, it would be of interest to compare the genetic organization of fimbrial determinants from distinct bacterial species and to determine what degree of similarity is found among polypeptides encoded by these genes. In this paper we describe the initial studies on the organization of fimbrial genes from *K. pneumoniae*, *S. typhimurium*, and *S. marcescens*.

**MATERIALS AND METHODS**

Media, growth conditions, and bacteria. *E. coli* ORN103 (kindly provided by P. E. Orndorff) is a genotypically nonfimbriate strain derived from the minicell producer *E. coli* P678-54 (27) and was used in all transformation experiments involving recombinant plasmids. The transformants exhibiting MS hemagglutinating activity (MSHA) are shown in Table 1.

Unless otherwise stated, bacterial cultures were grown in Luria broth or in Luria agar (22) for 18 to 24 h at 37°C, and culture media were supplemented with the appropriate antibiotics at the following concentrations: ampicillin, 100 µg/ml; tetracycline, 20 µg/ml; and chloramphenicol, 25 µg/ml (200 µg/ml for plasmid amplification). Spectinomycin (200 µg/ml) was used for the amplification of plasmids conferring chloramphenicol resistance.

**Bacterial plasmids.** Plasmids pISF101, pMH2, and pBP7, encoding the expression of type 1 fimbriae of *S. typhimurium*, *S. marcescens*, and *K. pneumoniae*, respectively, have been described in detail elsewhere (5, 28). Plasmid pBP7 is identical to pBP7, except that the vector pRN2010 (supplied

* Corresponding author.
by W. Dallas, Burroughs Wellcome Foundation) was used to replace the pBR322 cloning vehicle.

Deletion mutants of appropriate plasmids were constructed after digestion with specific restriction endonucleases or the exonuclease Bal 31. Plasmid DNA was purified from amplified cells by sodium dodecyl sulfate (SDS) lysis and ethidium bromide-chromium chloride equilibrium density gradient centrifugation (13, 29). Rapid analysis of transformants for the presence of plasmid DNA was performed by the technique of Holmes and Quigley (14).

The conditions for isolation and ligation of DNA fragments and restriction enzyme digestion and agarose electrophoresis have been described elsewhere (4, 28).

**Preparation and labeling of minicells.** Minicells were prepared as previously described (6), and plasmid-encoded polypeptides were labeled with a mixture of [35S]methionine and [14C]-labeled amino acids (Amersham Corp.). Labeling was allowed to proceed for 2 to 4 h before the removal of unincorporated amino acids by centrifugation. The radiolabeled gene products were detected by autoradiography (2) after electrophoresis through 12.5% SDS-polyacrylamide gels.

For immune precipitation, minicell extracts were prepared after sonication and centrifugation followed by the addition of an equal volume (100 μl) of 0.05 M Tris (pH 7.5)—2 M potassium chloride—2% Triton X-100. Undiluted fimbrial antiserum (50 μl) was added, and the reaction mixture was incubated at 4°C overnight. Antigen-antibody complexes were precipitated by goat anti-rabbit IgG (50 μl) after a second overnight incubation at 4°C. These precipitates were isolated by centrifugation, and the pellet was subsequently washed once with 0.05 M Tris (pH 7.5)—1.2 M KCl—1.2% Triton X-100 followed by 0.05 M Tris—0.1 NaCl. Samples were dissolved in SDS electrophoresis sample buffer and run through 12.5% SDS-polyacrylamide gels.

**Purification of type 1 fimbriae.** A single procedure which could be used for the purification of type 1 fimbriae from all strains of bacteria was not established. Thus, MS fimbriae from a clinical isolate of *S. typhimurium* were prepared by the method of Dodd and Eisenstein (8), whereas the *K. pneumoniae* fimbriae were purified by the scheme of Fader et al. (12). The type 1 fimbriae of *S. typhimurium* were also prepared with deoxycholate and urea as previously described (18); this procedure was also used to prepare the fimbriae of *S. marcescens*. The purity of each preparation was assessed by SDS electrophoresis after boiling of the sample at a pH of 1.8.

Sera raised against fimbrial antigens were prepared after immunization of New Zealand White rabbits. An initial injection of antigen was given subcutaneously, followed by three intramuscular administrations of immunogen each 4 days apart. Antibodies directed against fimbrial antigens were detected by agglutination of fimbriate-phase bacterial suspensions, and hyperimmune sera were used for the immunoprecipitation studies.

**Expression of type 1 fimbriae.** The phenotypic expression of type 1 fimbriae by bacteria was determined by observation of the strains under the electron microscope (4). Hemagglutination tests were conducted using a fresh 3% (vol/vol) suspension of guinea pig erythrocytes in the presence and absence of D-mannose (5). Fimbrial antigens were detected on the bacteria by standard serological agglutination tests or hemagglutination inhibition reactions (5).

**RESULTS**

**Expression of type 1 fimbriae by the minicell-producing strain of E. coli.** It has previously been reported by us that transformants of *E. coli* HB101 were found to express type 1 fimbriae of different serological specificities. Similarly, the genotypically *pil*⁺ strain *E. coli* ORN103 could be transformed by the appropriate plasmids to produce type 1 fimbriae possessing hemagglutinating activity (Table 1). Unlike *E. coli* HB101, the ORN103 strain has previously been shown to lack DNA sequences homologous to the *pil* gene cluster of *E. coli* (25). Transformants appeared fully fimbriate when examined by electron microscopy and exhibited the characteristic MSHA activity associated with type 1 fimbriae. Hemagglutination by these transformants could be inhibited only by homologous antisera; therefore, these results are in agreement with those obtained with *E. coli* HB101 (7).

The type 1 fimbriae were purified from both of the strains containing recombinant plasmids and also from the original wild-type parental isolates. Regardless of the source, the molecular sizes of the fimbrial subunits derived from one species were identical. Thus, the subunit molecular sizes of the *Salmonella*, *Serratia*, and *Klebsiella* fimbriae were 21.5, 16.5, and 19.5 kilodaltons (kDa), respectively (Fig. 1). Preparations of *S. typhimurium* type 1 fimbriae occasionally showed the presence of a smaller 20.0-kDa polypeptide after electrophoresis (Fig. 1). All samples were shown, by electron microscopy, to consist of fimbrial filaments and appeared devoid of any contaminating membrane components. Fimbrial preparations exhibited MSHA when tested in microtiter plates (5), except for preparations of fimbriae which had been exposed to high concentrations of urea (8). In such cases, the purified fimbriae retained the intact fimbrial morphology but did not agglutinate guinea pig erythrocytes.

![FIG. 1. SDS-polyacrylamide gel electrophoresis of purified fimbrial preparations.](http://iai.asm.org/)
**Gene products involved in fimbrial expression.** The parental plasmids pISF101, pMH2, and pBP7 are recombinant molecules which encode type 1 fimbrial expression by *S. typhimurium, S. marcescens*, and *K. pneumoniae*, respectively (5). Deletion mutants were constructed after digestion of these plasmids with either specific restriction enzymes or the exonuclease Bal 31. The deletion mutations along with the hemagglutinating phenotype of corresponding transformants are shown in Fig. 2.

The minicells possessing the parental plasmid pISF101 express at least six polypeptides which appear to be involved in *Salmonella* fimbrial synthesis and assembly (Fig. 3). The molecular sizes of these polypeptides were estimated to be 82.0, 37, 30, 26, 25, and 21.5 kDa, and the locations of the genes encoding these polypeptides (except the 82-kDa protein) are represented in Fig. 2. For example, the 21.5-kDa polypeptide is encoded by only those recombinant plasmids retaining a 3.0-kilobase *Sphl-EcoRI* DNA fragment which is located on pISF101 (Fig. 2). Also, some gene products would consistently appear to be produced in quantitatively different amounts by specific mutants as evidenced by the 37-kDa polypeptide in pISF07. The loss of a 5-kilobase DNA fragment in this recombinant resulted in the accumulation of the 37-kDa polypeptide. Similarly, the 82.0-kDa gene product was routinely seen in the minicell lysates containing the parental plasmid pISF101 but was produced in detectably smaller amounts by the deletion derivatives.

Three detectable polypeptides were associated with type 1 fimbrial production in *S. marcescens*, and the genes encoding these polypeptides were mapped on pMH2 (Fig. 2). The electrophoretic mobilities of these gene products indicated their molecular sizes to be 82, 59, and 16.5 kDa. Mutants which lacked the ability to produce any one of these polypeptides failed to express hemagglutinating fimbriae on their surface. The presence of a 64-kDa polypeptide produced by some of the mutants (pMH14, pMH17, and pMH20; Fig. 4) indicates that a fourth gene product is involved in fimbrial expression. Due to the nature and extent of the deletions in those strains which produce this polypeptide, as well as its absence in lysates of other recombinants, it would appear that this gene product is not a truncated form of the 82-kDa polypeptide. Its absence in extracts derived from the minicells containing the parental plasmid could indicate that such a gene product is produced in relatively small amounts which fimbrial expression is occurring but
that in the absence of expression, under some circumstances, there is an accumulation within the cell of the 64-kDa polypeptide.

The genetic cluster involved in *K. pneumoniae* fimbrial expression is similar in complexity to those described above. Thus, the plasmid pBP9, which encodes the phenotypic expression of fimbriae, was found to possess at least four genes involved in the synthesis of these appendages. The molecular sizes of the plasmid encoded polypeptides encoded by these fimbrial genes were determined to be 80, 24, 22.5, and 19.5 kDa (Fig. 5). Fig. 2 shows the relative positions of the genes encoding these polypeptides. Of particular interest is the gene encoding the 19.5-kDa polypeptide which is found on a 641-base-pair *ClaI* fragment of pBP7 (Fig. 2). The removal of this fragment from pBP7 would appear to be a lethal event, because it proved impossible to attain viable transformants possessing such plasmids. However, much larger deletions within this region (e.g., pBP903, Fig. 2) were able to transform bacteria to a nonhemagglutinating phenotype. Thus, the presence of a 19.5-kDa gene product may be necessary for maintenance of viability of cells transformed with the fimbrial gene cluster.

**Identification of the gene encoding the fimbrial subunits.**

The genes encoding proteins which are integral components of the fimbrial appendages were identified after precipitation of gene products with immune sera raised against the purified fimbrial antigen isolated from the wild-type strains of enteric bacteria (Fig. 6). The major fimbrial subunit gene of *S. typhimurium* was found to encode the 21.5-kDa polypeptide, because this was the only plasmid encoded polypeptide of pISF101 which reacted with the *Salmonella* antifimbrial serum. This gene product possessed an identical electrophoretic mobility to the fimbrial subunit of the biochemically purified antigen. Similarly, the serum raised against the *Serratia* fimbriae precipitated only one gene product of pMH2, and this product exhibited a molecular mobility, equivalent to the 16.5-kDa polypeptide, that was identical to that of the purified subunit.

Serum raised against type 1 fimbriae purified from *K. pneumoniae* reacted with a 19.5-kDa polypeptide, and the electrophoretic mobility of fimbriae purified from the *K. pneumoniae* was identical to that of a 19.5-kDa polypeptide. Due to the similarity of the physical maps of recombinant plasmids encoding type 1 fimbriae of *E. coli* and *K. pneumoniae* (5) and the reported cross-reactivity between these antigens with immune sera (24), the location of the fimbrial subunit gene on pBP7 was determined by using a DNA probe encoding the *E. coli* type 1 fimbriae. Thus a radiolabeled probe derived from pSH2 (3) (kindly provided by S. Hull) which consisted partly of nucleotide sequences comprising the fimbrial subunit gene was constructed by restriction enzyme digestion and nick translation (21). This probe, which was an *EcoRI*-RstI fragment of pSH2, contains DNA sequences encoding the C-terminal portion of the *E. coli* *pilA* structural gene as well as a segment of the *pilB* gene (25). The probe hybridized only to a 1.7-kilobase *PstI*-*EcoRI* fragment of pBP7, indicating a possible homology in the nucleotide sequence of the genes encoding the fimbrial subunits of *E. coli* and *K. pneumoniae*. This region of homology was distinct from the 641-base-pair *ClaI* fragment; thus, pBP7 encodes two different 19.5-kDa polypeptides. One of these is the major fimbrial subunit, whereas the other polypeptide would appear to be involved in the expression of the subunit itself.

**DISCUSSION**

The genetic cluster encoding the type 1 fimbriae of *E. coli* has facilitated the investigation of the control and expression of these organelles in this species (25–27). Recently, we...
demonstrated that the fimbrial genes of distinct enteric bacteria may not be identical because of differences in the restriction enzyme maps of the cloned determinants and also the inability of genes derived from different species to complement each other to restore hemagglutinating activity (5, 7). Certainly, differences in the genes encoding the fimbrial subunit of enteric bacteria may be expected as evidenced by the lack of serological relatedness of the fimbrial antigens (18, 24) which may reflect a difference in the amino acid sequence of the subunit. The results of this study indicate that the phenotypic expression of type 1 fimbriae by species belonging to the genera *Klebsiella*, *Salmonella*, and *Serratia* is due to the presence of a number of gene products. Such results are consistent with those previously reported for the expression of both type 1 (25) and mannose-resistant (6, 19) fimbriae of *E. coli*. Thus, within the family *Enterobacteriaceae*, the biogenesis and expression of fimbrial appendages is a complex process requiring multiple gene products. However, the primary structure of these gene products and the organization of fimbrial gene clusters also differ among genera. As many as six polypeptides are implicated in the expression of type 1 fimbriae by *S. typhimurium*, whereas four polypeptides were found for *K. pneumoniae*. In some cases, the absence of one polypeptide was associated with the concomitant absence of a second accessory gene product or occasionally with the increased production of other polypeptides. For example, in the *Salmonella* system, only the deletion derivative pISF107 was found to encode increased amounts of a 37-kDa polypeptide and, also, a small (<16-kDa) gene product (Fig. 3). Thus, it is possible that the accumulation of the 37-kDa protein is due to the absence of expression of the complete fimbrial gene cluster. Therefore, a complex interrelationship may exist among the elements involved in fimbrial expression. Due to the nature and extent of the deletion in pISF107, the presence of the lower-molecular-weight polypeptide is consistent with this being a truncated form of the 23-kDa protein.

Although the organization of the type 1 fimbrial gene clusters appears to be different with respect to location and sizes of gene products, there are some similarities between these genetic elements and that of the *E. coli* system, especially in the case of *K. pneumoniae*. For example, all systems reported in this study possess a high-molecular-weight polypeptide (Fig. 2) similar to that identified in *E. coli* by Orndorff and Falkow (25) and suggested to be an “anchor” for fimbrial expression. Additionally, the *S. typhimurium* and *K. pneumoniae* gene clusters possess genes encoding both 30- and 26-kDa polypeptides or 35- and 24-kDa polypeptides, respectively. Similarly, the *E. coli* system has 30- and 23-kDa gene products (25). Because of the phylogenetic relatedness of *E. coli* and *K. pneumoniae* it is not surprising that the type 1 fimbrial gene clusters of these two genera appear the most closely related with respect to the organization of individual genes. The fimbria-associated proteins may serve similar functions within their respective systems, but equivalent-sized proteins were not observed in the *Serratia* strain. Previous results by us (1) describing the lack of complementation between species would indicate that each genetic system has evolved to utilize only its own accessory gene products. Currently, we are constructing strains carrying the complete genetic machinery for one fimbrial type along with plasmids carrying only the subunit gene from a different species. In this way it may be possible to determine whether, for example, *Salmonella* fimbriae can be synthesized in the presence of all of the *Klebsiella* accessory genes.

In all three systems the fimbrial subunit could be identified by using immune serum prepared against the purified fimbriae. Those polypeptides encoded by the recombinant plasmids possessed electrophoretic mobilities that were identical to those of the subunits of the biochemically purified fimbriae. Thus, the positions of the subunit genes of *S. typhimurium* and *S. marcescens* are as indicated in Fig. 2. Antiserum to *K. pneumoniae* fimbriae precipitated a 19.5-kDa polypeptide encoded by pBP7. However, a DNA probe carrying the nucleotide sequence encoding the 72 amino acids of the C-terminal region of the *E. coli* fimbrial subunit hybridized to a *PstI-EcoRI* fragment of pBP7. Therefore, we do not believe that the equivalent *K. pneumoniae* gene is located on the 641-base-pair *Clal* fragment of pBP7. Indeed, the preliminary nucleotide sequencing data of the regions immediately adjacent to the *PstI* restriction enzyme site of pBP7 have indicated that these sequences are completely homologous to those reported for the *E. coli* fimbrial gene. Consequently, it is probable that the gene encoding the fimbrial subunit of *K. pneumoniae* is located between the genes encoding the 24- and 22.5-kDa polypeptides of pBP7. Although Buchanan et al. (3) could not demonstrate any sequence homology between nucleotides derived from the cloned *E. coli* fimbrial gene cluster and those of genomic DNA from *Klebsiella* isolates, our results indicate that a portion of the *E. coli* *pIIA* gene does possess sequences homologous to those of the *K. pneumoniae* gene. However, most of the isolates used by Buchanan and her co-workers

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**FIG. 6.** Identification of the major fimbrial subunit encoded by recombinant plasmids. Lanes: A, pBP7-encoded polypeptides; B, immunoprecipitate of this minicell lysate; D and E, pISF101-encoded polypeptides and an immune precipitate, respectively; F and G, pMH2-encoded polypeptides and the corresponding immune precipitate; C, molecular size of the chloramphenicol acetyl transferase encoded by the vector of both pBP7 and pISF101. The chloramphenicol acetyltransferase polypeptide was consistently found to be present in immunoprecipitates. The only other fimbrial gene product which reacted with the appropriate immune serum was identical in molecular size to the corresponding fimbrial subunits.
were nonhemagglutinating and therefore may lack a major portion of the fimbrial subunit gene. Our results would indicate that another gene, mapping on a 641-base-pair ClaI fragment of pBP7 (Fig. 2), encodes a second 19.5-kDa gene product distinct from that of the processed fimbrial protein. This conclusion is supported by the fact that the gene product of the deletion plasmid pBP713, carrying the 641-base-pair ClaI fragment (Fig. 2), does not react with fimbrial antiserum. Deletion of only the small ClaI fragment of pBP7 resulted in the inability of the remainder of the molecule to produce viable transformants. This lethal effect could be overcome by the synthesis of larger deletions extending into the putative fimbrial structural gene and; the possibility of the 19.5-kDa polypeptide suppressing the fimbrial subunit gene is being explored. A polypeptide which suppresses fimbrial expression in the E. coli system has been described; although mutants lacking this gene product were viable, the resulting transformants exhibited a small colony phenotype (26). However, because the deletions described above were DNA sequences residing on a high-copy-number plasmid, it is also possible that the lethal effect of deletions in the small ClaI fragment are due to more indirect effects of gene dosage. Further experiments are currently being performed to determine the physiological role of this gene product.

In most instances the locations of the genes on recombinant plasmids were determined by analysis of appropriate deletion derivatives. However, the precise site of two closely linked genes was sometimes difficult to determine. For example, the relative locations of the genes on pISF101 encoding the 25- and 26-kDa polypeptides must be considered preliminary. In some cases it was not possible to accurately map the position of the genes encoding specific polypeptides encoded by the recombinant plasmids (e.g., the 82-kDa polypeptide of the Salmonella system). However, we believe that these genes are involved in fimbrial expression because they are carried on DNA fragments which are contained within the length of DNA essential for the expression of the Fim" MSHA-positive phenotype as previously determined by transposon mutagenesis (5). Alternatively, these genes are not part of the fimbrial system but are, in some cases, flanked by determinants implicated in fimbrial biosynthesis; we consider this to be unlikely. In the case of S. marcescens only four plasmid encoded polypeptides could be determined by the minicell system. The position of the genes encoding these proteins indicates that other genes may be involved in fimbrial expression. Indeed, we have previously shown that insertions of the transposable element Tn5 into regions of DNA distinct from those encoding the polypeptides result in elimination of the hemagglutinating phenotype (7). Therefore, such fragments of DNA may contain genes encoding proteins which are produced in relatively small quantities.

Recent evidence has suggested that the gene encoding the type 1 fimbriae of E. coli is distinct from that encoding the adhesive function (23). In our studies all strains possessing the parental plasmids pBP7, pMH2, and pISF101 were found to be fimbriate when examined by electron microscopy and exhibited MSHA. However, most of the transformants possessing the deletion derivatives of these recombinant molecules were screened only for hemagglutinating activity. Consequently, some of the hemagglutination-negative strains may in fact be phenotypically fimbriate. Currently, we are using the strains described in this paper to determine the relationships among the fimbrial genes of enteric bacteria and also to determine whether a related MS adhesive component is found among these bacteria.

LITERATURE CITED