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Complementation Analyses of Recombinant Plasmids Encoding Type 1 Fimbriae of Members of the Family *Enterobacteriaceae*

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Insertion mutants of recombinant plasmids encoding type 1 fimbriae of four genera of enteric bacteria were used to detect genetic complementation. After transformation by pairs of plasmids, double transformants were screened for their ability to express type 1 fimbriae. Complementation was observed between genes derived from the same genus but was absent with chimeric molecules carrying genetic information from two different genera. The results indicate that diffusible gene products of the *fim* cluster are necessary for phenotypic expression of type 1 fimbriae.

Type 1 mannose-sensitive (MS) fimbriae are produced by many strains of enterobacteria (8) in which they are responsible for adherence of the organisms to mannose-containing receptors on eucaryotic cells. The exact role of the type 1 fimbriae in the infective process remains to be elucidated, but initial evidence suggests that these organelles play a role in potentiating the virulence of some species of the family *Enterobacteriaceae* (9, 12). The detection of type 1 fimbriae produced by bacterial cells is facilitated by the observation that fimbriate bacteria agglutinate guinea pig erythrocytes only in the absence of D-mannose (8).

The molecular cloning of the type 1 fimbrial gene cluster of both *Escherichia coli* (13) and *Klebsiella pneumoniae* (14) has previously been described. Also, we recently reported the construction of recombinant plasmids encoding the fimbriae of *Salmonella typhimurium*, *Serratia marcescens*, and *Enterobacteria cloacae* (5). In that study, using a limited number of insertion mutants, it was demonstrated that plasmids encoding the *K. pneumoniae* fimbriae could complement each other to direct expression of fimbriae as evidenced by the MS agglutination of erythrocytes and electron microscopy. However, combinations of mutant plasmids encoding type 1 fimbriae of *K. pneumoniae* and *S. typhimurium*, respectively, did not possess the ability to restore the fimbrial phenotype. The present paper describes experiments using insertion mutants of recombinant plasmids encoding the fimbriae of four different species of enterobacteria and the ability of such mutants to complement each other to produce functional fimbrial adhesins.

The recombinant plasmids encoding antigenically distinct type 1 fimbriae are listed in Table 1 and were used as target molecules for transposon (Tn*5*) mutagenesis. The construction of fimbria-negative, nonhemagglutinating insertion mutants by using Tn*5* has been described in detail elsewhere (14). Hemagglutinating activity was determined by the guinea pig erythrocyte test (7), and representative isolates were examined for the presence of fimbriae by electron microscopy (1). All transformation experiments were performed with a restriction- and modification-minus strain, *E. coli* HB101 (*hsdM hsdR recA*), as the recipient. Transformation by two distinct plasmid species was achieved by using pairs of recombinant molecules which differed with respect to the cloning vector possessed by each recombinant. Thus, the compatible cloning vectors pBR322 (3) and pACYC184 (4) were used in all the double transformations, and such transformants were selected after plating on agar containing kanamycin and the appropriate antibiotics. Confirmation that transformants contained plasmids with two independent insertions of Tn*5* was made by analysis of the restriction enzyme profile of double transformants with subsequent comparison of the restriction fragments of plasmids prepared from transformants possessing only a single plasmid type. In all cases it was determined that the physical maps of plasmids isolated from double transformants were consistent with the presence of two types of recombinant molecules in such tranformants. Also, selection for *E. coli* strains which had spontaneously lost one type of plasmid by plating on media which did not contain a specific antibiotic and subsequent analysis of the remaining plasmid suggested that no illegitimate recombination had occurred between the two plasmid types.

Figure 1 illustrates the result of the complementation analyses with the chimeric molecules. Independent Tn*5* insertion mutants which fail to complement each other to restore the MS hemagglutinating phenotype are defined as belonging to the same complementation group. For example, four distinct Tn*5* insertions comprise complementation group 1 of pBP7 (Fig. 1). Thus, each of these mutants, when present as pairs of plasmids in the bacterial cell, failed to restore hemagglutinating activity. However, when one of these plasmids was used to transform *E. coli* HB101 along with one of the chimeric molecules from complementation group 2 of pBP7, the double transformants were found to agglutinate guinea pig erythrocytes. Consequently, two complementation groups were found on plasmids pBP7, pECH3, and pMH2, and each of these complementation groups was defined by at least two independent Tn*5* insertions. The recombinant plasmid pISF101 was found to possess three distinct clusters of Tn*5* insertions which appeared to constitute three complementation groups. Complementation group 1 consisted of a single Tn*5* insertion, whereas the remaining two groups were delineated by multiple insertion sites.

In all complementation experiments performed, the restoration of the hemagglutinating phenotype was observed only within a single genus. Thus, intact fimbrial genes from one genus could complement each other but were never observed to complement genes from a different genus. For example, fimbrial gene sequences from *K. pneumoniae* did not possess *trans*-acting activity with those from the remaining three genera.

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TABLE 1. Recombinant plasmids encoding type 1 fimbriae of enteric bacteria

Plasmid	Size (kbp)[a]	*fim* genes derived from:
pECH3	19	*E. cloaceae*
pBp7	14.5	*K. pneumoniae*
pISF101	17	*S. typhimurium*
pMH2	15	*S. marcescens*

[a] kbp, Kilobase pairs.

Our results indicate that the fimbrial gene clusters within a single genus of the family *Enterobacteriaceae* encode diffusible gene products which are required for the phenotypic expression of type 1 fimbriae. Thus, genetic complementation could be observed between DNA sequences derived from a single isolate. Also, the results provide initial evidence that the fimbrial genetic apparatus of the four genera examined consists of more than one transcriptional unit since, regardless of the site of the Tn*5* insertion within a gene, mutants of one group were consistently complemented by those of a distinct group. Therefore, the polar effects of transposon insertion would be expected to result in hemagglutination-negative phenotypes if the fimbrial genetic system was found within one operon. Similar results regarding the number of transcriptional units comprising genes involved in type 1 (13) and mannose-resistant (6) fimbrial expression of *E. coli* have previously been reported. However, Tn*5* insertion into a gene cluster has not consistently been found to exert polar effects (2), and therefore, the concise nature of the transcriptional units of the gene cluster in these enteric bacteria must await identification of RNA polymerase binding sites and transcription initiation sequences.

The serological heterogeneity of the type 1 fimbriae produced by different genera within the *Enterobacteriaceae* has been reported elsewhere (7, 11). These differences may simply reflect nucleotide sequence diversity of the genes encoding the fimbrial subunit within each genus. However, our results suggest that the fimbrial gene clusters of distinct genera differ widely because no complementation between fimbrial genes of the four recombinant plasmids was observed regardless of the site of the Tn*5* insertion. Consequently, the genes encoding some of the accessory polypeptides involved in fimbrial expression appear to be significantly different among members of the *Enterobacteriaceae* such that they can only be used in the homologous system. However, from our results we can not conclude that all these accessory proteins are genus specific because of the uncertainty that all relevant genes were the target for Tn*5* insertion. Therefore, a comparison of the organization of the fimbrial gene cluster belonging to distinct members of the enteric group of bacteria may provide information concerning the evolutionary relatedness of this genetic system.

Finally, recent evidence has suggested that the mannose-resistant adhesins of uropathogenic *E. coli* may consist of two gene products encoding a fimbrial subunit and a digalactoside binding moiety (10). All MS hemagglutinating transformants which were observed in this study were also

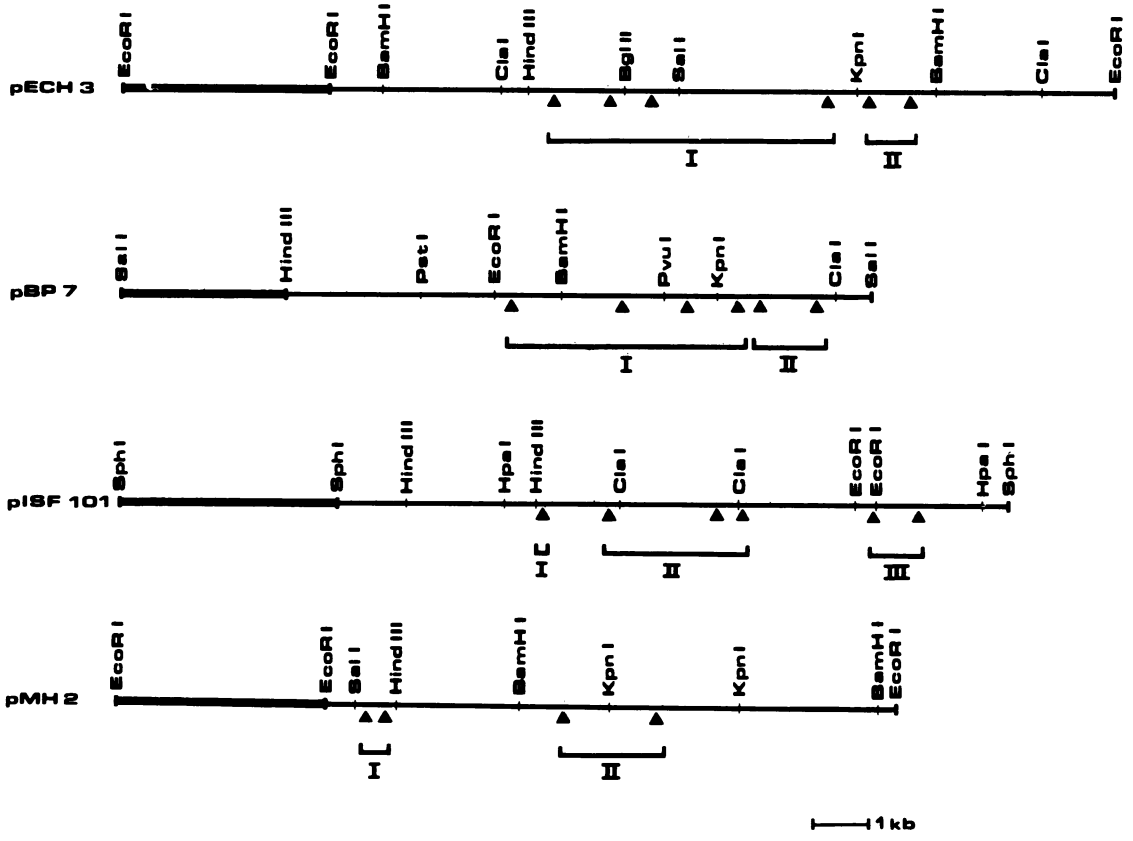

FIG. 1. Complementation groups of recombinant plasmids are designated by Roman numerals, and the approximate sizes are indicated. Closed triangles indicate the site of Tn*5* insertion mutants (MSHA$^-$) which were used in the complementation studies.

found to be fimbriate when examined by electron microscopy. However, we have not, as yet, defined specific genes of the relevant systems and, therefore, from our data it would be premature to speculate on the nature of the MS adhesin organelles in the four genera.

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