Nucleotide Sequence of the Surface Exclusion Genes *traS* and *traT* from the IncF₀ lac Plasmid pED208

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pED208 is a 90-kilobase conjugative plasmid belonging to the incompatibility group IncF₀ lac. The surface exclusion system from this plasmid was cloned and sequenced, and two genes demonstrated exclusion ability. *traS* encoded a 186-amino-acid hydrophobic protein which, when transcribed from a vector promoter, caused exclusion of pED208. The product of *traT* (TraTp) was a 245-residue protein which was highly expressed independently of a vector promoter in *Escherichia coli* minicells. The TraTp from pED208 was homologous with *traT* products from the IncF plasmids R-100 and F (80% homology), but recombinants containing the pED208 surface exclusion system excluded F poorly.

**MATERIALS AND METHODS**

**Bacterial strains and plasmids.** Plasmids pED208 and F were carried in the host strain JC6256 (E. coli K-12 F⁻ trp lac) and were kindly donated by N. S. Willetts (Biotechnology Australia, New South Wales, Australia). E. coli P67854 (3) was the strain used for minicell experiments. Recombinants were transformed into JM83 (31) or AA125 (his trp bio Str'), and JM103 was used for M13 cloning and sequencing (34). pED851 is a chimera consisting of the large BamHII fragment from F (containing the entire F *tra* operon) cloned into pBR322 (17). The chimeras that were constructed are described in Table 1.

**Isolation of minicells.** [³⁵S]methionine-labeled minicells were prepared as described previously (10, 13).

**Surface exclusion assay and index.** Surface exclusion-negative recipient JM83 (pBF106) and test strains were used to measure the surface exclusion index. The surface exclusion (sfx) index was calculated by the following formula:

\[
sfx \text{ index} = \frac{[\text{transconjugants}_{pBF106}(\text{transconjugants}_{pBF106} + \text{recipient}_{pBF106})]}{[(\text{transconjugants}_{test} + \text{recipient}_{test})]} ，
\]

where *transconjugants* is the number of colonies containing the marker plasmid, and *recipient* is the number of colonies containing only the marker plasmid. The *transconjugants* and *recipient* are the number of colonies containing the transconjugant and recipient, respectively. A value of 1 indicates no surface exclusion.

Surface (or entry) exclusion (sfx) can be defined as the activity that reduces the ability of plasmid-containing cells to participate as recipients in conjugal matings with other cells containing a closely related or identical plasmid (19). This property is unrelated to plasmid incompatibility which prevents the coexistence of two plasmids within a cell (28). The F plasmid encodes two surface exclusion genes, *traS* and *traT*, which provide exclusion activity by two independent mechanisms (1). These genes are located within the major F transfer (*traYZ*) operon between *traG* and *traD*, immediately following the region required for F pilus biosynthesis (21). The *traS* gene from F encodes an 18,000-dalton inner membrane protein (1) which may affect triggering of conjugal DNA metabolism (20) because *traS* is capable of inhibiting DNA transfer even when stable mating aggregates form. The product of *traT*, TraTp, is a 25,000-dalton protein found in large amounts (greater than 20,000 copies per cell) in the outer membrane of F plasmid-containing cells (1). This protein provides surface exclusion by reducing stable mating aggregate formation (1). It has recently been shown that TraTp from F is a lipoprotein (25).

The nucleotide sequence of *traT* from R-100 has been published previously (24), and the predicted TraTp is highly homologous to TraTp from F (22). It has been demonstrated that the *traT* gene products from R6-5 (the conjugal system of which is closely related to R-100) and R-100 mediate serum resistance (23), and, at least in the case of R6-5, TraTp enhances resistance of *Escherichia coli* to phagocytosis (4).

pED208 (formerly EDP208) is a derepressed derivative of a naturally occurring lac plasmid, Folac, which was originally identified in *Salmonella typhi* (8). F and pED208 plasmids share no serological similarity, and it has been suggested that pED208 does not contain an F-like transfer operon (N. S. Willetts and J. Maule, Genet. Res., in press). Cells carrying pED208 or F-like plasmids are sensitive to the pili-specific phage F₁, while only cells containing F-like plasmids are sensitive to the RNA phages F₂ and R17 (5, 6). In this study we describe the cloning and sequencing of the surface exclusion system from the pED208 plasmid.
TABLE 1. Chimeras constructed from the pED208 plasmid

| Chimera (reference) | Vector/restriction site(s) | Fragment inserted (kb) | Figure no. |
|---------------------|---------------------------|------------------------|------------|
| pBF101 (1)          | pACYC184/HindIII          | HindIII (30)           | 1          |
| pBF106 (11)         | pBR322/HindIII, Sall      | HindIII-XhoI (14)      | 1          |
| pBF111 (11)         | pBR322/Sall              | XhoI (7.51)            | 1          |
| pBF120              | pBR322/Sall, BamHII      | Sall-XhoI (3.7)        | 1, 2       |
| pBF121              | pBR322/Sall, BamHII      | Sall-PstI (0.9)        | 2          |
| pBF122              | pBR322/Sall, BamHII      | PstI-XhoI (1.5)        | 2          |
| pBF124              | pBR322/PstI              | PstI (1.0)             | 2          |
| pBF125              | pBR322/PstI              | PstI (1.3)             | 2          |
| pBF126              | pBR322/PstI              | PstI (1.3)             | 2          |
| pBF129              | pKO1/SalI               | PvuI (0.7)             | 2          |
| pBF130              | pKO1/SalI               | PvuI-PstI (0.19)       | 2          |
| pBF134              | pUC8/Sall, BamHII        | Sall-XhoI (3.7)        | 2          |
| pBF135              | pUC8/Sall, HindIII       | Sall-XhoI (3.7)        | 2          |
| pBF136              | pUC8/PstI               | PstI (1.3)             | 2          |
| pBF137              | pUC8/PstI               | PstI (1.3)             | 2          |
| pBF138              | pUC8/SalI               | Smal-AhaIII (1.1)      | 2          |
| pBF139              | pUC8/SalI               | AhaIII-SalI (1.4)      | 2          |
| pBF140              | pUC8/SalI               | AhaIII (1.5)           | 2          |
| pBF141              | pUC8/SalI               | AhaIII (1.5)           | 2          |
| pBF142              | pUC8/SalI, BamHII        | HpaI-XhoI (1.4)        | 2          |
| pBF148              | pBR328/Sall, EcoRI      | Sall-AhaIII (1.4)      | 2          |
| pBF149              | pBR328/EcoRI, BamHII    | Smal-AhaIII (1.1)      | 2          |

* The BamHII site from the vector of pBF111 was included at one end.
* The HindIII site from the vector of pBF311 was included at one end.
* The EcoRI site from the vector of pBF139 was included at one end.
* The EcoRI and BamHII site from the vector of pBF138 were included at the ends.

activity, while numbers greater than one indicate the presence of a surface exclusion function.

RESULTS

Cloning of the pED208 surface exclusion system. We have previously described the cloning of the four large HindIII fragments from the pED208 plasmid into pACYC184 (pBF101 to pBF104 (11)) (Fig. 1). When these four fragments (which contain 93% of the pED208 plasmid DNA) were tested for surface exclusion activity toward pED208, only pBF101 exhibited detectable activity. pBF101 is the largest of these HindIII clones and encodes the genes required for pilus biosynthesis (11), and presumably, this surface exclusion activity is part of the pED208 transfer operon, as it is in F. Subcloning of pBF101 localized surface exclusion activity to a 7.5-kilobase (kb) XhoI fragment (pBF111) and then to a 3.7-kb SalI-XhoI fragment (pBF120), which was adjacent to the area required for pilus production (Fig. 1 and Table 2).

The Sall-XhoI fragment from pBF120 was extensively subcloned (Fig. 2). Of all the recombinants that were constructed, only chimeras containing three restriction fragments demonstrated surface exclusion (Table 2 and Fig. 2). pBF126 (which contains a 1.3-kb PstI fragment inserted into pBR322) exhibited exclusion of pED208, while the same fragment ligated in the opposite orientation (pBF125) did not exclude pED208. When this PstI fragment was introduced into pUC8, chimeras containing one orientation excluded pED208 (pBF128), while chimeras with the opposite orientation (pBF137) were exclusion negative. From this informa-

FIG. 1. HindIII-XhoI map of the pED208 plasmid, modified from Finlay et al. (11), showing regions that contain various pED208 plasmid-encoded functions, including surface exclusion (sfx).
When the large ORFs, traS, and traT are illustrated above the map, with transcription proceeding to the right. Restriction fragments from pBF120 that were subcloned are shown below the restriction map, with the arrows indicating the direction of vector-promoted transcription. The capability (or lack thereof) of excluding pED208 is indicated to the right of the figure.

**DNA sequencing studies.** To define the components of the pED208 surface exclusion system, the complete nucleotide sequence of the 3,725-nucleotide SalI-XhoI restriction fragment from pBF120 was determined by the M13 dye-sequencing method (27) (Fig. 3). Because the PstI 1.3-kb fragment encodes surface exclusion activity when expressed by a vector promoter (pBF126 and pBF137), but does not do so when oriented in the opposite direction (pBF125 and pBF136), the direction of transcription should proceed from the SaII site toward the XhoI site (to the right in Fig. 2). If the genes of the transfer operon of pED208 are organized in a similar manner to those of F, the surface exclusion genes should be found downstream of the area required for pilus biosynthesis (33). This is precisely the location of the pED208 exclusion loci (Fig. 1), suggesting that the direction of transcription and translation proceeds from the SaII site toward the XhoI site.

When the sequence from Fig. 3 was translated in this direction, four major open reading frames (ORFs) were found. Beginning at the SaII site, there was an ORF of 414 amino acids which stopped at nucleotide 1244 (Fig. 3). After this was a 193-residue ORF (nucleotides 1297 to 1875) which contained the gene we called traS (for reasons to be explained later). After traS was a 179-base-pair intercistronic region and the gene traT, which encoded a 245-amino-acid exclusion-positive phenotype was observed; but the cells grew poorly, and an accurate exclusion index was not obtained. The other two subclones of pBF120 that expressed surface exclusion were pBF140 and pBF141. Both of these chimeras contained the 1.5-kb AhalIII fragment in opposite orientations in pUC vectors. Because pBF140 was not placed under a vector promoter, the exclusion activity encoded by this chimera was expressed independently of a vector promoter and presumably contained its own promoter.

**TABLE 2. Surface exclusion index of pED208 recombinants**

| Donor           | Recipient   | Surface exclusion index |
|-----------------|-------------|-------------------------|
| JC625(pED208)   | JM83(pBF101)| 15.0                    |
| JC625(pED208)   | JM83(pBF106)| 1.0                     |
| JC625(pED208)   | JM83(pBF111)| 7.8                     |
| JC625(pED208)   | JM83(pBF120)| 50.6                    |
| JC625(pED208)   | JM83(pBF125)| 2.2                     |
| JC625(pED208)   | JM83(pBF126)| 31.8                    |
| JC625(pED208)   | JM83(pBF134)| 170                     |
| JC625(pED208)   | JM83(pBF136)| 1.1                     |
| JC625(pED208)   | JM83(pBF137)| 93.2                    |
| JC625(pED208)   | JM83(pBF139)| 1.6*                    |
| JC625(pED208)   | JM83(pBF140)| 9.1                     |
| JC625(pED208)   | JM83(pBF141)| 13.9*                   |
| JC625(pED208)   | JM83(pRS31) | 6.0                     |
| JC625(pED208)   | JM83(pED851)| 3.8*                    |
| JC625(F lac)    | JM83(pRS31) | 297                     |
| JC625(F lac)    | JM83(pED851)| 17.5*                   |
| JC625(F lac)    | JM83(pBF137)| 1.7                     |
| JC625(F lac)    | JM83(pBR134)| 3.8                     |

* Growth rates of bacteria containing these chimeras were two- to three-fold less than other JM83 strains.

* pED851 can also function as a donor resulting in lowered exclusion indices.
FIG. 3. Nucleotide sequence of the 3,725-nucleotide Salt•XhoI fragment, with numbering beginning at the Salt site. \textit{traS}, \textit{traT}, and the two large ORFs are translated below their respective sequences. Putative ribosome-binding sites for \textit{traS} and \textit{traT} are underlined, and the putative \textit{traT} promoter is indicated.
The 414-residue ORF. Several attempts were made to visualize a protein encoded by the first 1,300 nucleotides, but cloning of this region appeared to be deleterious to cells. Although it was possible to clone the Sall-AhaIII 1.4-kb fragment (positions 1 to 1366) into pUC8 (pBF139), bacteria harboring this recombinant grew very slowly. Attempts to reverse the orientation of this insert such that it would be placed under the vector lac promoter were unsuccessful, although we were able to place it into pBR328 under the chloramphenicol promoter (pBF148). When we examined minicells containing pBF139 and pBF148, there were no apparent additional proteins produced (data not shown).

When the SmaI-AhaIII 1.1-kb fragment (nucleotides 244 to 1366) was inserted into pUC8 (pBF138), the large ORF was connected in phase to the beginning of the lacZ gene, creating a fusion polypeptide of 338 amino acids. Again, cells containing this chimera grew slowly. Minicells containing pBF138 produced a polypeptide of 37,000 daltons. When the insert was placed into pBR328 under the chloramphenicol promoter, this protein was removed, suggesting that the polypeptide seen with pBF138 is a fusion protein.

It is not clear why cells harboring recombinants containing this region grow poorly, even when there is no vector promoter transcribing this region (for example, pBF139). There is a possible translational start site at nucleotide 306, preceded by two possible ribosome binding sites (AGAG and CAGGA [29]), beginning at nucleotides 295 and 300, respectively. A translational start site at this position would create a 312-residue polypeptide, assuming that there is an active promoter upstream. As mentioned above, no polypeptides of this size were visible in minicells containing pBF139.

If the tra operons of pED208 and F were ordered similarly, traG should be located immediately before traS. Because traG is large (encoding a 100- to 116-kilodalton protein [32]), it is possible that the 414-amino-acid ORF is the carboxy-terminal region of an pED208 equivalent of traG.

traS. Bacteria containing the recombinants pBF126 or pBF137 both exhibited surface exclusion activity (Table 2). Examination of the nucleotide sequence spanning this PstI fragment (nucleotides 966 to 2262) revealed only one major ORF encompassing nucleotides 1297 to 1875. A probable translational start site for this ORF is the ATG located at position 1318. Finishing five nucleotides upstream of this methionine is a sequence (CAGGAGG) which is highly homologous to the consensus ribosome binding site (AG GAGGT [29]). Translation of a protein beginning with this methionine would yield a 186-residue polypeptide (21,230 daltons; Fig. 3). No polypeptide corresponding to this ORF was detectable in minicells containing pBF125, pBF126, pBF136, and pBF137.

This predicted protein is highly hydrophobic, with 17% of its residues being charged and 50% being aromatic residues and isoleucine, leucine, methionine, or valine. When predictions of the secondary structure of this protein are examined (Fig. 4), these predictions resemble those found for an integral membrane protein. This protein contains stretches of 20 to 25 amino acids which are hydrophobic, with small intervening hydrophilic regions. Furthermore, these hydrophilic regions are all predicted to be in the beta-turn conformation (Fig. 4), while the hydrophobic sequences are predicted to be either in beta-sheet or alpha-helix conformations.

The traS product from F is reported to be an 18,000-dalton inner membrane protein that is produced in small quantities

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**FIG. 4.** Predictive analysis of the secondary structure (Chou and Fasman [7]) and local average hydrophilicity (Hopp and Woods [16]) of the traS gene product. The beta-sheet, alpha-helix, beta-turn, and hydrophilicity values were derived from hexapeptide averages along the length of the sequence. Only predictive values greater than 1 are plotted for beta-sheet, alpha-helix, and beta-turn assignments. Hydrophilic regions appear as positive peaks above zero.
of *traT* from pED208 begins at nucleotide 2055 and proceeds to nucleotide 2789 (25,981 daltons; Fig. 3). Preceding the putative start of this protein is a weak ribosome-binding site (GAGA) eight nucleotides upstream.

The homology of *traT* gene products from pED208, R-100, and F is striking (Fig. 6). TraTps from pED208 and R-100 are 80% homologous, with 46 mismatches and 2 unmatched amino acids. The proposed signal sequences differed significantly, with 7 mismatches and 2 unmatched residues out of 22 amino acids. However, the proposed signal cleavage site (Gly22-Cys23; E. G. Minkley, Jr., personal communication) was conserved, as was the location of glycerol and fatty acid modification in TraT (Cys23 [25; E. Minkley, personal communication]). The remaining differences were interspersed throughout the proteins.

The production of high levels of TraTp independent of the vector promoter (i.e., pBF120 and pBF140) suggests that *traT* encodes its own promoter. Contained within the 179 nucleotides between *traS* and *traT* is a possible promoter centered around position 1950 (Fig. 3) which resembles the consensus proacryotic promoter (15). The -35 region contained four of the six consensus sequence nucleotides (TTGATG), as did the -10 region (TACATT). There were 18 nucleotides found between the -10 and -35 regions.

The 244-amino-acid ORF. Beyond *traT*, beginning at nucleotide 2883, was an ORF which continued into the *XhoI* site at position 3720. There was a potential translational start at nucleotide 2991, preceded by a ribosome-binding sequence (CAGG) eight nucleotides upstream. A translational start at this position would produce an ORF of at least 244 amino acids within the pED208 plasmid. Attempts to detect the production of a polypeptide of this size in minicells by using pBF142 were unsuccessful (data not shown). In the F plasmid, *traD* follows *traT*, producing a 77- to 85-kilodalton protein (18). Thus, if the genetic organization of the pED208 *tra* operon is similar to that of F, this 244-residue ORF may be the amino-terminal region of the pED208 *traD* gene product.

Cross-reactivity between pED208 and F surface exclusion systems. Besides the homology found between the TraTp’s from F and pED208, these plasmids encode conjugative pili that are similar in size, contain an acetylated amino terminus (12, 14), and can be infected by the pilus-specific F1 phage (5). Because surface exclusion may involve, at least partly, blockade of pilus attachment (1, 13), we decided to test for any cross-reactivity between surface exclusion systems from F and pED208. To determine this we utilized recombinants of pED208 and F that encode part or all of their *tra* operons. The results of these assays are shown in Table 2.

When recombinants containing the pED208 surface exclusion system (pBF134 and pBF137) were tested for exclusion of the pED208 plasmid, high levels of surface exclusion were seen. However, when the recipient contained chimeras encoding the entire F *tra* operon (pED851 [17]) or the F surface exclusion loci (pRS31 [30]), levels of exclusion only slightly greater than that of the background were obtained. When the F *lac* plasmid was used as the donor, recipient cells containing the F surface exclusion genes (pED851 and pRS31) produced high surface exclusion indices, but low levels of exclusion were seen with recipient cells containing recombinants of the pED208 surface exclusion system (pBF134 and pBF137). These results suggest that there may be a small amount of surface exclusion cross-reactivity between F and pED208, but it is significantly smaller than the homologous exclusion systems and may be insignificant in vivo.

![FIG. 5. Autoradiograph of a 15% acrylamide gel containing 35S-labeled minicells of pBF140 (lane 1), pBF141 (lane 2), pBF120 (lane 3), and pUC8 (lane 4). The unlabeled arrowheads represent the mobility of size standards of 43, 25.7, 18.4, 14.3, 12.3, 6.2, and 3 kilodaltons from top to bottom, respectively.](http://jb.asm.org/ on May 1, 2019 by guest)

(1). Because this 186-residue polypeptide is similar in size and probably in cellular location, we called this protein the *traS* gene product from pED208. As in the F system, the pED208 *traS* gene immediately precedes *traT* (see below) and follows the region required for pilus biosynthesis (11).

Because bacteria harboring chimeras containing the *PstI* 1.3-kb fragment in opposite orientation to the vector promoter (pBF125 and pBF136) were unable to exclude pED208 (Table 2), this suggests that there is no promoter upstream of *traS*, at least up to the *PstI* site at position 966. *traT*. When the *AhaIII* 1.5-kb fragment (positions 1366 to 2824) was cloned in both orientations into the pUC vectors (pBF140 and pBF141), bacteria containing these chimeras were capable of excluding pED208 (Table 2). Because this *AhaIII* fragment does not encode the entire *traS* gene, these observations suggest that pBF140 and pBF141 contain another surface exclusion gene with its own promoter. Minicells of pBF120, pBF140, and pBF141 all produced a 25,000-dalton protein in large quantities (Fig. 5). When minicells were fractionated, this 25,000-dalton protein was found mainly in the membrane fraction (data not shown). The *traT* gene products of F and R-100 plasmids encode a 25,000-dalton protein which is located in the outer membrane in high copy numbers (21), suggesting that this protein from pED208 may be a TraTp equivalent.

On examination of the nucleotide sequence of this *AhaIII* fragment, a large ORF was found (in addition to the truncated *traS* reading frame) encompassing nucleotides 2001 to 2789. When this translated sequence is compared with the *traT* gene product from R-100 (24), a highly homologous protein from pED208 (which we called TraTp) can be aligned with TraTp from R-100 (Fig. 6). The predicted gene product
DISCUSSION

The regulation of _traT_ in F-like transfer systems is not completely understood. The TraTp of R-100 has been reported to be produced in large quantities, regardless of the transcriptional state of the _tra_ operon (i.e., repressed or derepressed [9]). By sequencing _traT_ from R-100, Ogata et al. (24) found a promoter sequence upstream of R-100 TraTp. Rashtchian et al. (26) reported that TraTp production in F plasmid-containing cells is independent of the _traJ_ product, which is a positive regulator of the _traYZ_ operon, although recent sequence information from the F plasmid suggests that there is no promoter for _traT_ between _traS_ and _traT_ (E. G. Minkley, Jr., personal communication). The high levels of production of TraTp from clones containing pED208 _traT_, in the absence of a vector promoter, suggest that the pED208 system contains a _traT_ promoter, analogous to the R-100 system. Both systems contain two nucleotides in the −35 region of their putative promoters which do not match the procaryotic promoter consensus sequence. R-100 has only one mismatch in the −10 region when compared with the consensus sequence, while pED208 has two mismatches. _traS_ from pED208 does not appear to have a promoter immediately upstream, and presumably, it is under _traYZ_ operon control.

The ribosome-binding site of _traT_ (GAGA) demonstrates poor homology with the consensus sequence (AGGAGG) described by Shin and Dalgarno (25). The synthesis of high levels of TraTp is probably due to a strong promoter which produces large amounts of _traT_ mRNA. In contrast, _traS_ has a ribosome-binding site which is highly homologous to the consensus sequence, but the _traS_ gene product was not detectable in a minicell system. The role of _traS_ in surface exclusion is believed to be involved in the inhibition of DNA transfer (20).

Placement of the surface exclusion genes under different vector promoters greatly varied exclusion capabilities (Table 2). The difference in exclusion levels between pBF126 and pBF137 may be attributed to different vector promoter strengths. _traS_ in pBF126 is promoted by the ampicillin promoter in pBR322, while in pBF137 it is transcribed by the _lac_ promoter. Placement of _traT_ under the _lac_ promoter (pBF141) resulted in slow-growing bacteria, probably because of a higher expression of TraTp. The highest level of surface exclusion involving recombinants of the pED208 surface exclusion system was produced by pBF134. This chimera contains both _traS_ and _traT_ in a pUC8 vector, but they are not transcribed by the vector promoter. When this restriction fragment was placed in the opposite orientation (pBF135), it provided exclusion, but the host cells grew so
poorly that it was not possible to obtain an exclusion index. This lethality may be due to high levels of the gene products of traS and traT, or it may also be due to the ORF found upstream of traS, which appears to be lethal when placed under a vector promoter (see above).

Minkley and Willets (22) reported the production of a 57,000-dalton polypeptide when only the carboxy-terminus fragment of traG from F was present. They attributed it to be a fusion product with the vector or a translational restart product. Although no polypeptides appeared to be produced by the equivalent fragment from pED208 (pBF139 and pBF148), a translational restart within the 414-amino-acid ORF might explain why this region of DNA is difficult to clone and lethal if transcribed with the aid of a strong vector promoter, such as the lac promoter (pBF148 was transcribed by the chloramphenicol promoter in pBR328). In F, traG appears to have several functions, including pilus biosynthesis, stabilization of mating pairs, and DNA transfer (32). If this gene is indeed the traG equivalent in pED208, a translational restart within this gene may define two functional domains of this protein.

The homology between TraTp's from R-100 and pED208 was unexpected, because pED208 was thought to contain a completely unrelated transfer operon. The fact that they have conserved TraTp's and that the order and location of the surface exclusion loci is maintained suggests a common evolutionary origin. The difference between processed TraTp's from F and R-100 is one amino acid (Fig. 5), yet this difference is sufficient to provide plasmid specificity of these traT gene products. The numerous changes in the pED208 TraTp sequence could easily account for a new traT specificity, as seen by the lack of exclusion cross-reactivity between F and pED208 (Table 2). However, with the large homologies in TraTp sequences from pED208 and F, these conjugative plasmids are more related than first believed.

It has been suggested that a possible receptor for TraTp is the pili of a donor cell (13, 22, 33). In IncF plasmid systems it has been shown that pilus type matches traT alleles (13; Willets and Maule, in press). The differences in pili type are due mainly to changes that occur at the carboxy and amino terminal of processed pilin (13). Because the amino-terminal region of pED208 pilin is completely different from that of F-like pilins (12), an altered traT sequence was expected. As more traT sequences are accumulated, the differences and similarities should lead to a better understanding of this important conjugal protein.

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