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# Characterization of an *Escherichia coli* K-12 F⁻Con⁻ Mutant

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An *Escherichia coli* K-12 F⁻ mutant defective in conjugation was isolated by means of a zygotic induction enrichment procedure. The recipient ability of the mutant was reduced about 50 times owing to a block in one of the first steps of the conjugation process. In the mutant, cell envelope alterations could not be observed. Sensitivity toward detergents, antibiotics, and phages was unaltered. The mutation appeared to be co-transducible with *pyrD*. The linkage order in the region of the mutation is origin KL 99-*con-pyrD-aroA*.

Bacterial conjugation is commonly considered to occur in a series of stages (6). The first step is the formation of mating pairs between donor and recipient cells by means of the sex pilus (specific pair formation). The next step is thought to be the conversion of a specific pair to an effective pair. A conjugation tube or bridge is then made between donor and recipient that would permit subsequent transfer of donor deoxyribonucleic acid (DNA). The third step is the transfer mobilization of donor DNA. After these steps, transfer of donor DNA occurs. The last step is the integration of transferred chromosomal DNA into the recipient chromosome or the establishment of a replicon containing the transferred DNA in the recipient cell.

The role of the donor cell and its sex pilus during the conjugation process has been extensively investigated (for reviews, see Achtman [1], Brinton [4], and Willetts [29]). Studies on the role of the recipient cell during the conjugation process have been concentrated so far on the transfer (28) and integration or the establishment of the donor DNA (see Clark [5] for review). Recently, studies on the role of the recipient cell during the first stages of the conjugation process have been reported (18, 20, 22, 27).

Cell envelope alterations in *Escherichia coli* K-12 F⁻ mutants may lead to F⁻ mutants unable to conjugate owing to an altered or absent attachment site for the sex pilus. Moreover, it is possible that sex pilus and bacterial viruses use the same or closely related cell envelope components for adsorption and/or injection. It could thus be possible to isolate F⁻ mutants unable to conjugate among F⁻ mutants resistant to bacterial viruses. Mutants of female strains of *E. coli* K-12 have indeed been isolated that are impaired in both mating ability and phage adsorption (20, 22; L. Havekes et al., Mol. Gen. Genet., in press).

The conjugation-defective mutants isolated until now frequently are pleiotrophic cell envelope mutants. To get more specific conjugation-defective mutants, we decided to use a zygotic induction enrichment procedure as a more direct isolation method. A conjugation-defective (Con⁻) mutant isolated in this direct way will be described. We will show that this Con⁻ mutant apparently differs from the Con⁻ mutants described before (20, 22).

## MATERIALS AND METHODS

**Bacterial strains.** All strains were derivatives of *E. coli* K-12. Genotype and source of the strains used are listed in Table 1.

**Phages.** Stocks of wild-type *E. coli* phages T2, T3, T4, T5, T6, T7, P1, and λ were used. Phages K3 (22) and χ (21) were obtained from E. J. J. Lugtenberg. As male-specific phage we used phage μ.

**Media.** Bacterial cells were cultivated in peptone-yeast broth. For plating, peptone-yeast agar (1% peptone, 0.5% NaCl, 0.5% yeast extract, 0.1% $Na_2HPO_4 \cdot 2H_2O$, 1.5% agar [BBL]) or minimal medium agar (31) was used. Required growth factors, amino acids, purines, or pyrimidines were added in final concentrations varying from 20 to 60 μg/ml. Streptomycin (Mycofarm Delft) was added in a final concentration of 100 μg/ml. MacConkey agar was obtained from Oxoid.

**Bacterial growth.** Growth was measured by means of a Klett-Summerson photometer using a red filter (660 nm).

**Isolation of Con⁻ mutants.** For the isolation of a Con⁻ mutant we used a zygotic induction enrichment procedure. Strain KMBL1563, an F′ donor strain carrying an F′ factor with a thermoinducible Mu-1 insertion, was used as donor, and strain AM3000, treated with ethyl methane sulfonate, was used as recipient. Donor cells ($5 \times 10^8$/ml) were mixed with $10^8$ recipient cells per ml. After 3 h of mating at 42 C, streptomycin was added and the mixture was incubated at 42 C with shaking for 1 h. The mixture was then diluted 100-fold in peptone-yeast broth with streptomycin and incubated overnight at 37 C. This culture was used as a recipient

TABLE 1. *Bacterial strains*[a]

| Strain | Mating type[b] | Genotype | Source |
|---|---|---|---|
| KMBL1563 | F′ | Chromosome: *trp*-103 by insertion Mu-1<br>Plasmid: *thy* by insertion Mu-1 cts 62 | P. van de Putte |
| PC1072 | F⁻ | *thr leu proA his thi argE thyA lacY strA* | |
| AM3000 | F⁻ | As PC 1072 but *pon* | This paper |
| AM3001 | F⁻ | As Am3000 but *con* | This paper |
| AM3002 | F⁻ | As AM3000 but colicin I resistant | This paper |
| AM3003 | F⁻ | As AM3001 but colicin I resistant | This paper |
| AM3004 | F⁻ | As AM3001 but *lacZ22 lacI* | This paper |
| AM3005 | F⁻ | As AM3001 but P1 sensitive | This paper |
| AM3006 | F⁻ | As AM3005 but *pyrD* | This paper |
| AM3007 | F⁻ | As AM 3005 but *aroA* | This paper |
| AM3008 | F⁻ | As AM3000 but rifampin resistant | This paper |
| AM3009 | F⁻ | As AM3001 but rifampin resistant | This paper |
| PC0008 | HfrH | | |
| PC0031 | Hfr R4 | | |
| PC0515 | Hfr KL16 | | |
| PC0441 | Hfr B7 | | |
| PC0034 | Hfr G6 | | |
| PC1511 | Hfr KL14 | | |
| PC0617 | Hfr Cav | | |
| PC1837 | Hfr KL99 | | |
| PC1524 | F⁺ | *aroA* | |
| PC0658 | F15 | F′ *thy*⁺/*pyrF strA* | |
| AM1034 | HfrH | *lacZ22 lacI* | J. Bergmans |
| AM1553 | F′ | F′ *lacZ5 lacI* | J. Bergmans |
| I5-3 | R144*drd*3 | *colI*⁺ | E. Meynell |
| χ-1100 | R100-1 | $R_F$ TcCmSuSmSp*drd* | R. Curtiss III |

[a] All PC strains were obtained from the Phabagen Collection, State University Utrecht. All donor strains are streptomycin sensitive unless otherwise indicated.

[b] Marker position, origin, and direction of transfer of donor strains are given in Fig. 1.

culture for a second enrichment procedure. After three enrichment cycles, the mixture was diluted and spread on agar plates (about 100 colony-forming units per plate) containing streptomycin. After 16 to 20 h of incubation at 37 C, the colonies were replicated on: (i) a minimal medium plate plus streptomycin and all required growth factors (plate A) and (ii) a minimal medium plate with streptomycin but without threonine and leucine and covered with a lawn of about $5 \times 10^8$ Hfr R4 donor cells (plate B). After 16 to 20 h of incubation at 37 C, plate B was scored for poorly developed colonies. These colonies were picked from plate A and tested for sensitivity to the male-specific phage $\mu$ and for recipient ability. Phage $\mu$-sensitive colonies were discarded.

**Crosses in liquid medium.** Crosses in liquid medium were performed with log-phase donor and recipient cells grown to a Klett reading of 30 ($2 \times 10^8$ to $3 \times 10^8$ cells/ml). Donor and recipient cells were mixed in a ratio of 1:10 and incubated without shaking in a water bath at 37 C to allow transfer. Transfer was terminated by violent agitation using a Low and Wood shaking machine (14).

**Crosses on filters.** Donor and recipient cells were grown to log phase (Klett reading of 30). Donor and recipient cells were mixed in a ratio of 1:10. The mixture (5 ml) was immediately collected on a membrane filter (Millipore Corp.; diameter, 25 mm; pore size, 0.45 $\mu$m) by suction. The membrane filter was placed on a prewarmed (37 C) peptone-yeast agar plate (collected bacteria on top) and incubated at 37 C to allow transfer. After transfer the filter was added to an appropriate volume of peptone-yeast broth, and cell separation was achieved by violent agitation.

**Gradient of transmission curves.** The method of de Haan et al. (9) was used with the modification that the mating mixture was not shaken during transfer.

**Mating pair formation.** For determination of the number of mating pairs formed in a mixture of donor and recipient cells, we used the method described by Skurray et al. (22). The method is based on the fact that mating pairs from Lac⁺ donor and Lac⁻ recipient cells give colonies with many sectors on lactose-tetrazolium plates.

**Transduction.** Transduction with phage P1 was performed according to Willetts et al. (30).

**Transfer of *colI* factor.** Log-phase donor cells (*colI*⁺, streptomycin sensitive) and recipient cells (colicin resistant, streptomycin resistant) were mixed in a ratio of 1:10, respectively, and incubated for 30 min in a water bath without shaking at 37 C to allow transfer. Transfer was terminated by violent agitation. The mating mixture was diluted and plated on peptone-yeast agar plates (about 100 colony-forming units per plate) containing 100 $\mu$g of streptomycin per ml and grown overnight at 37 C. The resulting colonies were killed by exposing them to chloroform vapor for about 20 min. After the chloroform vapor had disappeared, the plates were overlaid with about $10^7$ cells of a colicin-sensitive

indicator strain suspended in 4 ml of peptone-yeast soft agar. After overnight growth at 37 C, a clear halo could be seen around a *col*$^+$ colony.

**Lethal zygosis in liquid and on solid media and filters.** For lethal zygosis in liquid media, we used the method described by Skurray and Reeves (24), with a donor-to-recipient ratio of 100:1. For lethal zygosis on solid medium, we used the method described by Skurray and Reeves (24) with some modifications. An overnight aerated culture of a streptomycin-resistant F$^-$ strain was diluted 100-fold. A 0.2-ml sample of this suspension was spread on peptone-yeast agar containing streptomycin. As soon as cells were fixed on the plate, 1 drop of a log-phase streptomycin-sensitive Hfr donor culture was placed on the plate. After overnight incubation at 37 C, a zone of growth inhibition is observed if the F$^-$ strain is sensitive to lethal zygosis. Lethal zygosis on membrane filters was performed as were the crosses on filters except that a donor-to-recipient ratio of 100:1 was used.

**Test for sensitivity to detergents, bile salts, and antibiotics.** One-tenth milliliter of a $10^{-5}$ dilution of an overnight culture was spread on the surface of a peptone-yeast agar plate containing one of the detergents or antibiotics in a series of concentrations near the minimal inhibitory concentration for the wild type. To test bile salts sensitivity cells were spread on MacConkey agar. After incubation overnight at 37 C, colony formation was scored.

**Test for sensitivity to bacteriophages.** Various dilutions of phage suspensions were mixed with about $10^7$ bacteria in soft peptone-yeast agar and added as a top layer on peptone-yeast agar plates. After overnight incubation at 37 C, plaque formation was scored.

**Cell envelope preparation and polyacrylamide gel electrophoresis.** For sample preparation and polyacrylamide gel electrophoresis, we used the method described by Lugtenberg et al. (15).

**Isolation of LPS and characterization of the LPS sugar composition.** We isolated lipopolysaccharide (LPS) on a microscale as described by Galanos et al. (8). Hydrolysis of LPS for characterization of the sugar composition of LPS by gas-liquid chromatography was performed according to the method described by Hellerqvist et al. (10).

**Screening test for mating ability.** To localize the mutation by conjugation and transduction, we used an AM3001 derivative with *lacZ22 lacI* mutations (AM3004). Transconjugants or transductants derived from AM3004 were screened for mating ability in the following way. One milliliter of a log-phase culture was added to 1 ml of a log-phase culture of AM1553 (F'*lacZ5 lacI*). After 1 h of mating without shaking, streptomycin and the detergent potassium oleate (0.001% [vol/vol] final concentration) were added to prevent growth of AM1553 and additional mating pair formation, respectively. After 1 h of further incubation at 37 C under aeration, the β-galactosidase activity formed on the basis of interallelic complementation between *lacZ5* and *lacZ22* (2) was measured. One drop of toluene was therefore added, and the suspension was shaken at 37 C for 30 min. A 0.5-ml amount of *o*-nitrophenyl-β-D-galactopyranoside at a concentration of 4 mg/ml was added to the samples. In this test, transconjugants or transductants with wild-type recipient ability developed a yellow color after one night at room temperature. For each cross, at least 25 transconjugants or transductants were tested for recipient ability by this screening test.

## RESULTS

**Isolation and characterization of a conjugation-deficient mutant.** Almost all colonies isolated after the enrichment procedure that gave a reduced growth on mutant identification plate B, as described in Material and Methods, were sensitive to the male-specific phage μ. Presumably, F$^+$ or F' factors lacking an intact prophage had infected recipient cells, resulting in F$^+$- or F'-carrying recipient colonies. The presence of these cells interfered with the isolation of mutants with reduced recipient ability. Experience taught us that three zygotic induction cycles were optimal; after more enrichment cycles the number of F$^+$ or F' derivatives increased such that the presence of conjugation-deficient cells was completely masked. After three cycles of zygotic induction, we isolated five colonies resistant to phage μ and impaired in recipient ability. One of these mutants (AM3001) was used for all experiments described in this paper.

When mutant strain AM3001 was used as a recipient strain in crosses with several donor strains, a significant reduction in transconjugant formation with F' or Hfr donor cells was observed (Table 2). Table 2 shows that the mu-

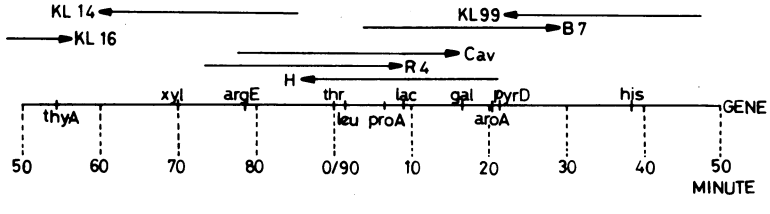


FIG. 1. *Marker position, origin, and direction of Hfr strains. The arrows indicate the position of the origin and the direction of transfer of the Hfr strains used (13).*

tant was a poor recipient for chromosomal DNA as well as for plasmid DNA. The latter observation suggests that the stage at which the conjugant formation process is disturbed precedes the recombination step and justifies the tentative description Con⁻ for the mutant. When transconjugants isolated from crosses of AM3001 with Hfr donor strains were used again, they still were poor recipients. This means that the few transconjugants from AM3001 were not derived from Con⁺ revertants.

**Stage at which the Con⁻ mutant is impaired.** Five separate steps in the conjugation process are thought to occur. The step at which the mutant AM3001 is blocked most likely precedes the recombination step or the establishment of a transferred plasmid (Table 2). In crosses on membrane filters the reduced transconjugant formation frequency was mostly relieved (Table 3). Since mating pairs are more stable in crosses on filters (16, 32), we suggest that the mutant cells form unstable mating pairs that are easily disrupted in liquid medium.

TABLE 2. *Transconjugant formation in crosses with the mutant strain AM3001 and the parental strain AM3000 as recipients*[a]

| Donor | Mating type | Selected marker | Conjugant formation frequency ratio (parent/mutant) |
|---|---|---|---|
| PC0008 | Hfr H | *thr⁺leu⁺strA* | 30 |
| PC0515 | Hfr KL16 | *his⁺strA* | 40 |
| PC1511 | Hfr KL14 | *argE⁺strA* | 60 |
| PC0031 | Hfr R4 | *thr⁺leu⁺strA* | 50 |
| PC0658 | F′ *thy⁺* | *thy⁺pyrF⁺* | 50 |

[a] Crosses were performed in liquid medium as described in the text. Transfer time chosen was equal to the time interval between the origin of the Hfr strain and the selected marker (see Fig. 1) plus 20 min for transfer delay. In the case of the F′ donor, transfer time was 30 min.

TABLE 3. *Transconjugant formation frequency with the mutant strain AM3001 and the parental strain AM3000 in liquid medium and on membrane filter*[a]

| Recipient | Mating conditions | No. of *thr⁺ leu⁺ strA* transconjugants/ml | Conjugant formation frequency ratio (parent/mutant) |
|---|---|---|---|
| AM3000 | Liquid medium | $3.7 \times 10^5$ | |
| AM3001 | Liquid medium | $6.0 \times 10^3$ | 60 |
| AM3000 | Membrane filter | $2.2 \times 10^7$ | |
| AM3001 | Membrane filter | $9.5 \times 10^6$ | 2.5 |

[a] Crosses were performed as described in the text. Strain Hfr R4 was used as donor. Transfer time was 35 min.

Mating of F⁻ cells with an excess of Hfr cells causes a decrease in the number of F⁻ survivors. This phenomenon, called lethal zygosis, requires a specific direct contact between Hfr and F⁻ cells mediated by F pili (23). If mating pair formation in the mutant strain AM3001 is impaired, we would expect the mutant to be less sensitive to lethal zygosis. Figure 2 shows that AM3001 indeed was resistant to lethal zygosis in liquid medium in contrast to the parental strain. Strain AM3001 appeared to be partly sensitive to lethal zygosis on solid medium (not shown) and on membrane filters (Fig. 2). The latter observations could be explained in the same way as the crosses on membrane filters by assuming that on solid medium and on filters mating pairs are more resistant to disruption. To get direct evidence for the supposition that mating pair formation is impaired, we measured the number of mating pairs formed in a mating mixture with mutant AM3001 and the parental strain as recipient strains. To measure

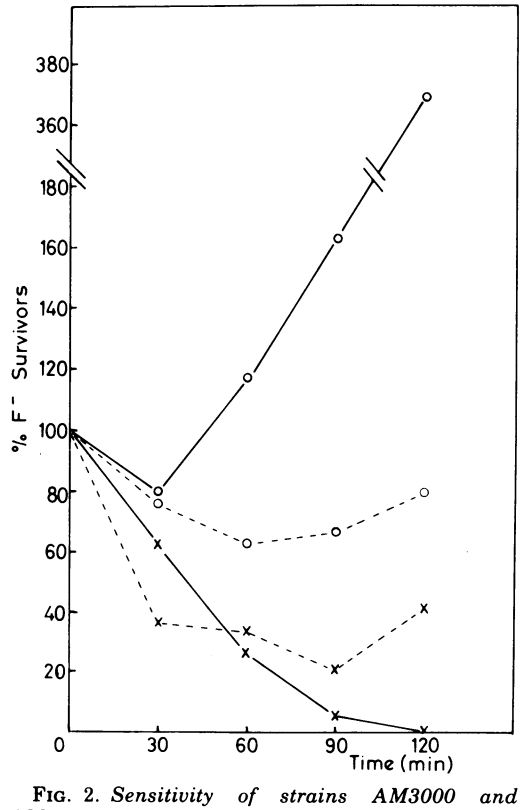


FIG. 2. *Sensitivity of strains AM3000 and AM3001 to lethal zygosis. Recipient cells and donor cells were mixed in a ratio F⁻ to Hfr of 0.01. At intervals samples were taken, diluted, and plated for F⁻ survivors. Symbols: (×) AM3000 survivors; (○) AM3001 survivors; (——) lethal, zygosis in liquid medium; (- - - -) lethal zygosis on membrane filters.*

the number of mating pairs, we used the technique described by Skurray et al. (22). The reduction in transconjugant formation frequency of AM3001 was due to poor specific mating pair formation. The observed poor mating pair formation might have been due to weak mating pairs that were easily disrupted. Results in Tables 2 and 4 show a reduction in transconjugant formation and mating pair formation, respectively, by a factor of about 50.

To study genetic transfer in mating pairs formed with the mutant strain AM3001 as recipient, we compared the gradient of transmission of Hfr donor markers in crosses with the mutant and its parent as recipients. In the mutant strain AM3001, the gradient of transmission of Hfr R4 donor markers was similar to that in the parental strain (Fig. 3). This appeared in crosses performed in liquid medium as well as on membrane filters. The similarity in gradient of transmission suggests that, whereas the preliminary specific mating pairs with the mutant recipient are very weak, the effective mating pairs once formed are as resistant to disrupting forces as mating pairs formed with the parental recipient.

**Specificity of the mutant.** Sex pili are involved in mating pair formation (18). There are at least two kinds of sex pili, F-like and I-like, which differ in their serology and adsorption activities to male-specific phages (12). The mutant strain AM3001 was isolated by selecting for conjugation deficiency with donor cells carrying sex pili specified by an F factor. It is therefore possible that the mutant is impaired specifically in crosses with F-type donor strains. We tested the specificity of the defect in recipient ability by using as a donor strain I5-3, carrying a derepressed *colI* factor (R144*drd*3) and I-like sex pili. Strain I5-3 transferred the *colI* factor to a colicin I-resistant derivative of strain AM3001 (AM3003) as well as it did to a colicin I-resistant derivative of the parental strain (AM3002). The colicin-resistant derivative of AM3001 was still Con⁻ in crosses with F-type donor cells. The mutant strain apparently is still conjugation proficient with a strain carrying I-like sex pili. To test the conjugation ability with a donor strain carrying F-like pili, we used strain χ-1100 carrying the derepressed R factor R100-1 and pili that are serologically different from pili specified by the F factor. R100-1 was transferred equally well to the mutant recipient and to the parental recipient. The Con⁻ mutant AM3001 is thus specifically disturbed in crosses with donor cells carrying an F factor.

TABLE 4. *Mating pair formation in crosses with AM3000 and AM3001 as recipient strains*[a]

| Recipient | No. of colonies | | | Ratio sectored colonies/ Lac⁻ recipient colonies (%) |
|---|---|---|---|---|
| | Lac⁺ donor | Lac⁻ recipient | Colonies with many sectors | |
| AM3000 | 1,638 | 1,528 | 98 | 6.4 |
| AM3001 | 2,606 | 1,556 | 2 | 0.1 |

[a] A Lac⁺ donor strain Hfr R4 was used in all crosses, which were performed as described by Skurray et al. (22).

**Growth.** The mutant grew as well as the parental strain on peptone-yeast agar plates and in peptone-yeast broth at 30, 37, and 42 C.

**Resistance to detergents, bile salts, and antibiotics.** *E. coli* F⁻ mutants impaired in mating pair formation are thought to be cell enve-

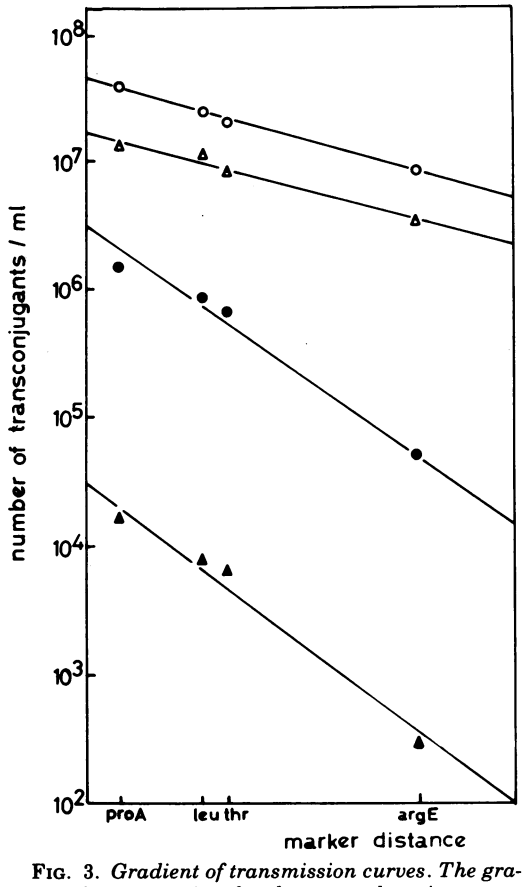


FIG. 3. *Gradient of transmission curves. The gradient of transmission for donor markers in crosses with AM3000 (●, ○) and AM3001 (▲, △) as recipients and Hfr R4 strain PC0031 as donor. Mating was performed in liquid medium (closed symbols) as well as on membrane filter (open symbols). Mating was interrupted by violent agitation after a 45-min transfer.*

lope mutants (16, 18, 23). Most cell envelope mutants showed an altered susceptibility to detergents, bile salts, and/or antibiotics (3, 25, 33). We found that AM3001 was not altered in sensitivity to the detergents sodium dodecyl sulfate (SDS) and deoxycholate, to bile salts in MacConkey medium, or to the antibiotics penicillin G, vancomycin, novobiocin, amoxycillin, ampicillin, and methicillin.

**Phage resistance.** AM3001 was tested for altered phage susceptibility. We tested the sensitivity for a number of phages: T2, T3, T4, T5, T6, T7, λ, C 21, K3, and χ. There was no difference in phage sensitivity in comparison with the parental strain except for phage χ, which presumably uses motile flagella as receptor (21). AM3001 appeared to be less motile than AM3000, as shown by light microscopy. Further investigation showed that Con$^+$ transductants of strain AM3001 were still χ resistant.

**Outer membrane protein and sugar composition of LPS.** Many cell envelope mutants have an altered outer membrane protein or sugar composition of LPS or both (11, 17, 22, 25). The outer membrane protein composition of AM3001 was not different from that of the parental strain, as shown by SDS-gel electrophoresis. The sugar composition of LPS was unchanged as well.

**Genetical characterization.** The mating frequency of AM3001 was sufficient to permit localization of the mutation by conjugation. Recipient ability was tested in a screening method described in Material and Methods. From the linkage data presented in Table 5, we conclude that the lesion is localized in the 17- to 21.5-min region of the *E. coli* map (26). To map the mutation more precisely, the transduction technique was used. Since strain AM3001 is P1 resistant, it was made sensitive for the transducing phage P1 by selecting *argE*$^+$ *strA* transconjugants from a cross of Hfr KL14 and AM3001 and electing a P1-sensitive Con$^-$ recombinant. From the P1-sensitive Con$^-$ strain AM3005, a *pyrD* derivative (AM3006) was isolated after mutagenesis and penicillin enrichment. The Con$^-$ mutation was found to be cotransducible with *pyrD*. Fifteen of 40 *pyrD*$^+$ transductants appeared to be Con$^+$ (Table 6). From this transductional cross, a Con$^-$*pyrD*$^+$ *aroA*$^-$ strain was isolated (AM3007) and used as a recipient in another transductional cross with phage P1 propagated in an *aroA*$^+$ strain (PC0031). One of 36 *aroA*$^+$ transductants was Con$^+$. From these results and from the observation that the *con*$^+$ allele is not introduced by Hfr KL99 (Table 5), we conclude that the mutation is located between *pyrD* and the origin of Hfr KL99, with a distance of about 0.5 min from *pyrD* (26).

TABLE 5. *Genetic analysis of transconjugants from crosses between Hfr donor strains and AM3004*[a]

| Donor | Mating type | Selected transconjugant | Unselected character | Linkage (%) |
|---|---|---|---|---|
| AM1034 | HfrH | *gal*$^+$ *strA* | Con$^+$ | 4 |
| PC0031 | Hfr R4 | *thr*$^+$ *leu*$^+$ *strA* | Con$^+$ | 0 |
| PC0515 | Hfr KL16 | *his*$^+$ *strA* | Con$^+$ | 0 |
| | | *gal*$^+$ *strA* | Con$^+$ | 48 |
| | | *gal*$^+$ *strA* | *his*$^+$ | 36 |
| PC0441 | Hfr B7 | *gal*$^+$ *strA* | Con$^+$ | 52 |
| PC1837 | Hfr KL99 | *his*$^+$ *strA* | Con$^+$ | 0 |
| PC0617 | Hfr Cav | *thr*$^+$ *leu*$^+$ *strA* | Con$^+$ | 0 |

[a] Twenty-five transconjugants from each cross were tested for recipient ability in the screening test for mating ability as described in the text. The reliability of the screening test was checked by testing a representative number of Con$^+$ scored transconjugants for mating frequency in a conjugation experiment. The screening test appeared to give no false scores.

## DISCUSSION

Until now the procedures for the isolation of conjugation-deficient F$^-$ mutants were based on the assumption that they are cell envelope mutants and as a consequence may be resistant to antibiotics or phages. Con$^-$ mutants were therefore isolated by preselecting phage-resistant or antibiotic-resistant mutants (17, 20, 22; L. Havekes, Mol. Gen. Genet., in press).

We used zygotic induction as an enrichment procedure. This more direct procedure was expected to yield more specific mutants. A zygotic induction procedure has been used by others, but the method failed in that F′ or F$^+$ derivatives of the recipient cells, formed during the isolation procedure, outnumbered conjugation-defective mutants (20). The same problem interfered with isolation procedures by lethal zygosis (24). In our hands the zygotic induction procedure presented the same problem but yielded a limited number of mutants. The procedure can be modified to a slightly more efficient method by introduction of plasmid curing

TABLE 6. *Genetic analysis of transductants obtained from Con$^-$ recipients*[a]

| P1 lysate from: | Recipient | Selected marker | Unselected character | Linkage (%) |
|---|---|---|---|---|
| PC1524 | AM3006 | *pyrD*$^+$ | Con$^+$ | 37 |
| | | *pyrD*$^+$ | *aroA*$^-$ | 10 |
| | | *pyrD*$^+$ *aroA*$^-$ | Con$^+$ | 18 |
| PC0031 | AM3007 | *aroA*$^+$ | Con$^+$ | 3 |

[a] At least 25 transductants from each transductional cross were tested for recipient ability in a screening test as described in the text.

after each enrichment cycle (L. Havekes, unpublished data).

A mutant isolated by this direct method was characterized as conjugation deficient in crosses with donor strains carrying an F factor. The reduction in frequency of transconjugant formation varied from 30 to 60. In crosses with a donor strain that carries a sex factor specifying I-like or F-like pili, the mutant strain was as good as the parental strain in recipient activity. A specificity like that of the Con⁻ mutant AM3001 was also found with other Con⁻ mutants (Havekes et al., Mol. Gen. Genet., in press). Direct and indirect evidence showed that pair formation was defective in strain AM3001.

In contrast to other Con⁻ mutants in which a defect in LPS sugar composition and outer membrane protein composition was shown, we did not find any defect. It should be stressed, however, that outer membrane protein composition was tested by an SDS-gel electrophoresis method (15) with limited resolution power. In fact, the mutant described in this paper does not help to determine the chemical nature of the receptor.

We tested the resistance or sensitivity for a number of *E. coli* phages and antibiotics. With the exception of phage $\chi$ interaction, no differences between mutant and parent were found. The resistance of the mutant toward phage $\chi$ is not linked to the Con⁻ mutation and apparently was introduced by chance.

The fact that phage pattern and antibiotic susceptibility were unchanged in the mutant, in contrast to other Con⁻ mutants (20, 22), suggests that the mutation in strain AM3001 is not as pleiotrophic as the mutation in other Con⁻ mutants. The mutant may therefore be advantageous for studies on the F-pilus attachment site.

The *con* mutation in strain AM3001 was ordered on the *E. coli* map in the order origin KL99-*con-pyrD-aroA*. Another Con⁻ mutant was reported to have a mutation or lesion at 14 min on the *E. coli* chromosome map (7), whereas the Con⁻ mutant described by Reiner (20) was reported to be altered in the region of 70 to 74 min. The heptoseless Con⁻ mutants described by Havekes et al. (Mol. Gen. Genet., in press) are impaired in mating pair formation, and their lesions are localized at the 6 to 9 and at the 71 min region of the *E. coli* chromosome, respectively. The scattering on the chromosome of mutations resulting in a Con⁻ phenotype might, at first glance, imply that the receptor structure is complex or that the receptor is highly dependent on structural components in its environment.

## LITERATURE CITED


1. **Achtman, M.** 1973. Genetics of the F sex factor in *Enterobacteriaceae*. Curr. Top. Microbiol. Immunol. 60:79-123.
2. **Bergmans, H. E. N., W. P. M. Hoekstra, and E. M. Zuidweg.** 1975. Conjugation in *Escherichia coli:* a study of recombination and the fate of donor DNA at the level of the zygote. Mol. Gen. Genet. 137:1-10.
3. **Bernstein, A., B. Rolfe, and K. Onodera.** 1972. Pleiotrophic properties and genetic organization of the *tolA,B* locus of *Escherichia coli* K-12. J. Bacteriol. 112:74-83.
4. **Brinton, C. C., Jr.** 1971. The properties of sex pili, the viral nature of "conjugal" genetic transfer systems, and some possible approaches to the control of bacterial drug resistance. Crit. Rev. Microbiol. 1:105-160.
5. **Clark, A.** 1973. Recombination deficient mutants of *E. coli* and other bacteria. Annu. Rev. Genet. 7:67-86.
6. **Curtiss, R., L. J. Charamella, D. R. Stallion, and J. A. Mays.** 1968. Parental functions during conjugation in *Escherichia coli* K-12. Bacteriol. Rev. 32:320-348.
7. **Davies, J. K., and P. Reeves.** 1975. Colicin tolerance and map location of conjugation-deficient mutants. J. Bacteriol. 123:372-373.
8. **Galanos, C., O. Lüderitz, and O. Westphal.** 1969. A new method for the extraction of R lipopolysaccharides. Eur. J. Biochem. 9:245-249.
9. **de Haan, P. G., W. P. M. Hoekstra, C. Verhoef, and H. S. Felix.** 1969. Recombination in *Escherichia coli*. III. Mapping by the gradient of transmission. Mutat. Res. 8:505-512.
10. **Hellerqvist, C. G., B. Lindberg, S. Svensson, T. Holme, and A. A. Lindberg.** 1968. Structural studies on the O-specific side chains of the cell wall LPS from *Salmonella typhimurium* 395 M.S. Carbohydr. Res. 8:43-55.
11. **Koplow, J., and H. Goldfine.** 1974. Alterations in the outer membrane of the cell envelope of heptose-deficient mutants of *Escherichia coli*. J. Bacteriol. 117:527-543.
12. **Lawn, A. M., and E. Meynell.** 1970. Serotypes of sex pili. J. Hyg. 68:683-694.
13. **Low, K. B.** 1972. *Escherichia coli* K-12 F-prime factors, old and new. Bacteriol. Rev. 36:587-607.
14. **Low, K. B., and T. H. Wood.** 1965. A quick and efficient method for interruption of bacterial conjugation. Genet. Res. 6:300-303.
15. **Lugtenberg, B., J. Meijers, R. Peters, P. van den Hoek, and L. van Alphen.** 1975. Electrophoretic resolution of the "major outer membrane protein" of *Escherichia coli* K-12 into four bands. FEBS Lett. 58:254-258.
16. **Matney, T. S., and N. E. Achenbach.** 1962. New uses for membrane filters. III. Bacterial mating procedure. J. Bacteriol. 84:874-875.
17. **Monner, D. A., S. Jonsson, and H. G. Boman.** 1971. Ampicillin-resistant mutants of *Escherichia coli* K-12 with lipopolysaccharide alterations affecting mating ability and susceptibility to sex-specific bacteriophages. J. Bacteriol. 107:420-432.
18. **Ou, J. T.** 1973. Effect of $Zn^{2+}$ on bacterial conjugation: increase in ability of $F^-$ cells to form mating pairs. J. Bacteriol. 115:648-654.
19. **Ou, J. T., and Th. F. Anderson.** 1970. Role of pili in bacterial conjugation. J. Bacteriol. 102:648-654.
20. **Reiner, A. M.** 1974. *Escherichia coli* females defective in conjugation and in adsorption of a single-stranded deoxyribonucleic acid phage. J. Bacteriol. 119:183-191.
21. **Schade, S. Z., J. Adler, and H. Ris.** 1967. How bacteriophage $\chi$ attacks motile bacteria. J. Virol. 1:599-609.
22. **Skurray, R. A., R. E. W. Hancock, and P. Reeves.** 1974. Con⁻ mutants: class of mutants in *Escherichia*

*coli* K-12 lacking a major cell wall protein and defective in conjugation and adsorption of bacteriophage. J. Bacteriol. 119:726-735.

23. **Skurray, R. A., and P. Reeves.** 1973. Characterization of lethal zygosis associated with conjugation in *Escherichia coli* K-12. J. Bacteriol. 113:58-70.
24. **Skurray, R., and P. Reeves.** 1974. F factor-mediated immunity to lethal zygosis in *Escherichia coli* K-12. J. Bacteriol. 117:100-106.
25. **Tamaka, S., T. Sato, and M. Matsuhashi.** 1971. Role of lipopolysaccharides in antibiotic resistance and bacteriophage adsorption of *Escherichia coli* K-12. J. Bacteriol. 105:968-975.
26. **Taylor, A. L., and C. D. Trotter.** 1972. Linkage map of *Escherichia coli* strain K-12. Bacteriol. Rev. 36:504-524.
27. **Walmsley, R. H.** 1973. Physical assay of competence for specific mating pair formation in *Escherichia coli*. J. Bacteriol. 114:144-151.
28. **Wilkins, B. M., S. E. Hollum, and W. D. Rupp.** 1971. Deoxyribonucleic acid transferred from ultraviolet-irradiated excision-defective Hfr cells of *Escherichia coli* K-12. J. Bacteriol. 107:505-512.
29. **Willetts, N.** 1972. The genetics of transmissible plasmids. Annu. Rev. Genet. 6:257-268.
30. **Willetts, N. S., A. J. Clark, and K. B. Low.** 1969. Genetic location of certain mutations conferring recombination deficiency in *Escherichia coli*. J. Bacteriol. 97:244-249.
31. **Winkler, K. C., and P. G. de Haan.** 1948. On the action of sulfanilamide. XII. A set of noncompetitive sulfanilamide antagonists for *Escherichia coli*. Arch. Biochem. 18:97-107.
32. **Wood, T. H.** 1968. Effect of temperature, agitation, and donor strain on chromosome transfer in *Escherichia coli* K-12. J. Bacteriol. 96:2077-2084.
33. **Wu, H. C.** 1972. Isolation and characterization of an *Escherichia coli* mutant with alteration in the outer membrane proteins of the cell envelope. Biochim. Biophys. Acta 290:274-289.