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# Dissociation and Interaction of Individual Components of a Degradative Plasmid Aggregate in *Pseudomonas*

**(transfer plasmid/plasmid compatibility/chromosome mobilization/mercury resistance)**

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**ABSTRACT** **The transfer of the OCT plasmid from *Pseudomonas oleovorans* to *Pseudomonas putida* strain PpG1 results in the acquisition of three independent replicons: OCT, factor K, and the MER plasmid. OCT is a nontransmissible plasmid harboring genes that code for the enzymes responsible for the degradation of *n*-octane. Factor K is a transfer plasmid capable of mobilizing OCT as well as chromosomal genes but incapable of enhancing transfer frequencies of other transmissible plasmids such as CAM, SAL, or RP-1. MER is a self-transmissible plasmid which can confer resistance to high concentrations of mercury salts. While OCT and MER are incompatible with CAM, factor K is compatible with it. Transmissible plasmids such as SAL, CAM, MER, or RP-1 cannot mobilize OCT to any significant extent, and exert strong repression on factor K-mediated transfer of chromosomal genes.**

On the basis of genetic, physical and molecular characteristics, transmissible plasmids have been divided into two kinds: (*i*) where the transfer genes are an integral part of the other genetic determinants (plasmid co-integrates) and (*ii*) where the transfer genes are part of a separate plasmid so that the rest of the genetic determinants are transmissible because of the transfer plasmid (1, 2). In the latter case, in the absence of the transfer plasmid, the genetic determinants usually replicate as a nontransmissible plasmid, which therefore constitutes a component of the total plasmid aggregate. Very little is known, however, about the specificity of *Pseudomonas* transfer plasmids, their interaction or compatibility with other plasmids, and the ability of nontransmissible plasmid components to be transferred by other plasmids besides the transfer plasmid. We have recently demonstrated that the naturally-occurring transmissible plasmid OCT, which confers ability to degrade *n*-octane, can be dissociated into a nontransmissible OCT plasmid and a transfer plasmid termed factor K (3). In this report, we describe the occurrence of another transmissible plasmid, MER, responsible for conferring resistance to mercury ions, as part of the OCT plasmid aggregate. The compatibility and interaction of these components with a number of other degradative and drug-resistance factor plasmids found in *Pseudomonas* have also been studied.

## MATERIALS AND METHODS

*Strains and Their Growth Conditions.* Most of the methods describing the composition of various media and growth conditions of the strains have already been published (4, 5). The relevant genetic properties of the strains described in this report are given in Table 1. The strain of *P. oleovorans* referred to here has been shown to conform to the properties of *P. putida* biotype A as described previously, and has been designated as *P. putida* strain 244 by Stanier *et al.* (5, 6).

*Curing and Transfer of the Plasmids.* The methods employed for curing and transfer of the plasmids have been enumerated elsewhere (4, 5).

*Determination of Resistance to Mercuric Ions.* For determination of resistance to different concentrations of a mercury salt, about $10^7$ cells were inoculated in 5 ml of L broth containing different concentrations of the salt and grown for nearly 48 hr. at 32° on a shaker. For the selection of $MER^+$ exconjugants, the donor–recipient mixture containing about 1 to 2 $\times$ $10^7$ cells of each type was plated on glucose-minimal plates containing appropriate supplements and 25 $\mu$g/ml of $HgCl_2$. Use of lesser concentrations of $HgCl_2$ would usually allow patches of growth even on the control plates.

## RESULTS

The dissociation of the OCT plasmid into a transfer plasmid (factor K) and a nontransmissible plasmid specifying the degradation of octane has been demonstrated previously (3). It is possible to cure either of these two plasmids independently to obtain $OCT^+K^-$ or $K^+OCT^-$ segregants. The role of K in the transfer of not only chromosomal genes but also the OCT plasmid can be seen from the results in Table 2. The loss of K from AC 75 completely abolishes the ability of these cells to transfer OCT or the chromosomal genes, while the presence of K ensures efficient mobilization of chromosomal genes from the donors harboring K to the recipients lacking it.

*Association of the Mercury Resistance Determinant with the OCT Plasmid Aggregate.* Several kinds of plasmids have been reported to be associated with genetic determinants that confer resistance to different inorganic ions (7, 8). Resistance to mercuric ions is important in this respect because a number of drug-resistance factors, sex factors, and other plasmids have often been found to impart resistance to toxic concentrations of mercury salts (8, 9). In an attempt to see if any of the degradative plasmids might have genes that can confer on the host cells the resistance to any of a number of inorganic salts, we tested the resistance pattern of cells harboring CAM (camphor utilization), SAL (salicylate utilization), and OCT plasmids to a variety of inorganic metal ions such as lead, mercury, bismuth, nickel, cobalt, etc. Among different metal ions tested, only mercuric salts had differential inhibition patterns toward cells harboring the OCT plasmid.

TABLE 1. *List of bacterial strains*

| Strain designation* | Phenotype | Plasmids | Derivation† | Refs. |
|---|---|---|---|---|
| AC 4 | Trp⁻,Oct⁺ | OCT,K, MER | Conj. AC 1 → AC 2 | 3 |
| AC 9 | Met⁻ | — | NG | 3 |
| AC 12 | Ilv⁻,Str$^r$ | — | NG | 3 |
| AC 13 | His⁻,Str$^r$ | — | NG | 3 |
| AC 24 | Trp⁻ | K | Mit C | |
| AC 26 | Trp⁻,Sal⁺,Cam⁺, Neo/Kan$^r$ | SAL,CAM, RP-1 | Conj. | 4 |
| AC 27 | Cam⁺, wild type | CAM | Conj. AC 61 → AC 30 | 3 |
| AC 28 | Oct⁺, wild type | OCT,K, MER | Conj. AC 4 → AC 30 | 4 |
| AC 29 | Sal⁺, wild type | SAL | Conj. AC 26 → AC 30 | 5 |
| AC 30 | wild type | — | | |
| AC 75 | Met⁻,Oct⁺ | OCT,K, MER | Conj. AC 4 → AC 9 | 3 |
| AC 76 | Met⁻,Oct⁺ | OCT,K | Mit C | |
| AC 77 | Met⁻,Oct⁺ | OCT,MER | Mit C | 3 |
| AC 90 | Met⁻,Oct⁺, Neo/Kan$^r$ | OCT,MER, RP-1 | Conj. AC 26 → AC 77 | |
| AC 92 | Met⁻Cam⁺ | CAM,K | Conj. AC 26 → AC 75 | |
| AC 93 | Met⁻,Cam⁺ | CAM | Conj. AC 26 → AC 77 | |
| AC 94 | Met⁻,Sal⁺,Oct⁺ | SAL,OCT, K,MER | Conj. AC 26 → AC 75 | |
| AC 95 | Met⁻,Sal⁺,Oct⁺ | SAL,OCT, MER | Conj. AC 26 → AC 77 | |

* All strains are derived from *P. putida* strain PpG1.

† Abbreviations used: Trp, tryptophan; Met, methionine; Ilv, isoleucine-valine; His, histidine; Str$^r$, streptomycin-resistant; Neo/Kan$^r$, neomycin- and kanamycin-resistant; Conj. conjugation; NG, *N*-methyl-*N'*-nitro-*N*-nitrosoguanidine; Mit C, Mitomycin C. Donors in conjugation are on the left of arrows.

Wild-type cells harboring no known plasmids or cells harboring either CAM, SAL, or OCT plasmids had similar inhibition patterns for all the other metal ions tested.

The presence of the OCT plasmid not only imparts resistance to inorganic salts such as $HgCl_2$ or $Hg(CN)_2$, but also to organic mercuric compounds such as phenyl mercuric acetate (Table 3). This resistance to organo-mercurials is, however, not manifested when tested against methyl mercury derivatives such as monomethyl and dimethyl mercury. It is not apparent from the data presented in Table 3 whether the gene responsible for conferring resistance to mercury salts

TABLE 2. *Role of factor K in the mobilization of chromosomal genes and the OCT plasmid*

| Donor | Recipient | Selected marker | Frequency of transfer |
|---|---|---|---|
| AC 75 (OCT⁺K⁺) | AC 30 | OCT | $1 \times 10^{-5}$ |
| | AC 12 | Ilv⁺ | $1 \times 10^{-2}$ |
| AC 77 (OCT⁺K⁻) | AC 30 | OCT | $<1 \times 10^{-8}$ |
| | AC 12 | Ilv⁺ | $<1 \times 10^{-8}$ |
| AC 24 (OCT⁻K⁺) | AC 30 | OCT | $<1 \times 10^{-8}$ |
| | AC 12 | Ilv⁺ | $2 \times 10^{-2}$ |

TABLE 3. *Resistance of P. putida cells harboring different plasmids to various mercury salts*

| Mercury salts | Concentration, μg/ml | Growth* of AC 30 | AC 28 | AC 27 | AC 29 |
|---|---|---|---|---|---|
| $HgCl_2$ | 0 | + | + | + | + |
| | 2.5 | − | + | − | − |
| | 5 | − | + | − | − |
| | 10 | − | + | − | − |
| | 20 | − | − | − | − |
| $Hg(CN)_2$ | 0 | + | + | + | + |
| | 1 | − | + | − | − |
| | 5 | − | + | − | − |
| | 10 | − | − | − | − |
| $CH_3COOHg \cdot C_6H_5$ | 0 | + | + | + | + |
| | 3 | + | + | + | + |
| | 5 | − | + | − | − |
| | 7.5 | − | + | − | − |
| | 10 | − | − | − | − |

* Growth has been measured in L broth in 48 hr; AC 30 is wild-type *P. putida* strain PpG1 without any plasmid, AC 28 is the strain that harbors the OCT plasmid, AC 27 harbors CAM, and AC 29 harbors the SAL plasmid.

is borne on the OCT plasmid. An attempt was, therefore, made to cure the OCT plasmid and the transfer plasmid independently and then examine phenotypically the mercury-resistant nature of the cured cells, or alternatively, to cure the parent cells of their mercury-resistance characteristics and then check the associated loss of the OCT plasmid or factor K. The results are shown in Table 4. It is clear that all of the cells that have lost the OCT plasmid still retain factor K as well as the mercury-resistant characteristics. Similarly, loss of factor K still allows the cells to grow on octane or with a high concentration of mercury salts. Thus, the gene(s) conferring resistance to mercury (*mer* gene) must be separate from either OCT or factor K. This is also confirmed by the fact that it is possible to cure the mercury-resistance character (MER plasmid) without loss of either factor K or the

TABLE 4. *Curing of the individual components of the OCT plasmid aggregate from AC 75*

| Plasmid cured | Mitomycin C concentration, μg/ml | Frequency of curing, % | Plasmids in cured strain* |
|---|---|---|---|
| OCT | 0 | <0.1 | — |
| | 2 | 0.8 | OCT⁻K⁺MER⁺ |
| | 4 | 1.5 | OCT⁻K⁺MER⁺ |
| K | 0 | <0.1 | — |
| | 2 | 0.4 | K⁻OCT⁺MER⁺ |
| | 4 | 1.5 | K⁻OCT⁺MER⁺ |
| MER | 0 | <0.1 | — |
| | 2 | <0.1 | — |
| | 4 | 1.2 | MER⁻K⁺OCT⁺ |

* Presence of the OCT plasmid has been determined by the ability of the cured cells to grow with octane–decane (1:1) as the sole source of carbon; that of MER has been determined by the ability of the cells to grow with L broth in presence of 10 μg/ml of $HgCl_2$; and the presence of factor K has been determined by the ability of the cells to donate chromosomal genes to suitable auxotrophic recipients.

TABLE 5. *Transmissibility of the MER plasmid*

| Recipient | Selected plasmid* | $10^8 \times$ Frequency of transfer | Phenotype of exconjugants |
|---|---|---|---|
| Donor: AC 75 ($K^+OCT^+MER^+$) | | | |
| AC 9 | OCT | 1000 | $OCT^+MER^+Met^+$ (90%) |
| | | | $OCT^+MER^+Met^-$ (10%) |
| AC 30 | MER | 100–1000 | $MER^+OCT^+$ (70%) |
| | | | $MER^+OCT^-$ (30%) |
| AC 13 | MER | 300 | $MER^+OCT^+His^+$ (75%) |
| | | | $MER^+OCT^+His^-$ (10%) |
| | | | $MER^+OCT^-His^-$ (15%) |
| Donor: AC 77 ($K^-MER^+OCT^+$) | | | |
| AC 30 | MER | 100 | $MER^+OCT^-$ |
| AC 13 | MER | 100 | $MER^+OCT^-His^-$ |
| AC 30 | OCT | <1 | $OCT^+MER^+His^-$ |

* Selection for MER plasmid has been made on glucose-minimal plates with appropriate supplements and containing 25 $\mu$g/ml of $HgCl_2$. To prevent diffused patchy growth of recipient cells on such plates, about 1 to 2 $\times 10^7$ cells were plated on the selection plates for scoring $MER^+$ exconjugants.

OCT plasmid (Table 4). The independent curing of the mercury resistance character, therefore, reinforces the concept that it is borne on a plasmid distinct from OCT and factor K.

*Transmissibility and Compatibility of the MER Plasmid.* Since all of the *P. putida* PpGl strains used in this study are sensitive to mercury salts except the $OCT^+$ exconjugants that received the OCT plasmid from *P. oleovorans* (3), it is clear that the mercury-resistant character must have been transferred from *P. oleovorans* along with OCT. The independent segregation of the mercury-resistant character suggests that it is a distinct plasmid separate from OCT and factor K. It is not clear, however, whether MER is a nontransmissible plasmid like OCT, whose transfer is mediated by factor K, or whether MER might be a transmissible plasmid capable of initiating its own transfer. The transmissibility of the MER plasmid was therefore studied. The majority of the recipients that received OCT from an $OCT^+MER^+K^+$ donor (AC 75) also required MER. Similarly, when selected for $MER^+$, the majority of the exconjugants acquired OCT. The transfer of either OCT or MER is almost always associated with chromosomal transfer mediated by factor K (Table 5). The independent transmissibility of MER is evident when a $K^-$ derivative of AC 75 (AC 77) is used as donor (Table 5). The transfer of MER is accomplished without concomitant transfer of chromosomal genes or the OCT plasmid. The mercury-resistant character could be cured from such exconjugants by treatment with mitomycin C, suggesting that the transferred character is determined by a factor that replicates as an independent plasmid inside the exconjugants.

TABLE 6. *Compatibility of CAM, SAL, and RP-1 in exconjugants with components of the OCT plasmid aggregate*

| Selected plasmid | $10^6 \times$ Transfer frequency | Plasmids in exconjugant |
|---|---|---|
| Recipient: AC 75 ($K^+OCT^+MER^+$) | | |
| CAM | 2 | $CAM^+K^+OCT^-MER^-$ (90%) |
| | | $CAM^+K^+OCT^-MER^+$ (5%) |
| | | $CAM^+K^-OCT^+MER^-$ (5%) |
| SAL | 100 | $SAL^+OCT^+MER^+K^+$ |
| RP-1 | 0.1–0.01 | $RP\text{-}1^+K^+OCT^+MER^+$ (60%) |
| | 0.01 | $RP\text{-}1^+K^+OCT^-MER^-$ (40%) |
| Recipient: AC 77 ($K^-OCT^+MER^+$) | | |
| CAM | 8 | $CAM^+OCT^-MER^-$ (98%) |
| | | $CAM^+OCT^+MER^-$ (2%) |
| SAL | 100 | $SAL^+OCT^+MER^+$ |
| RP-1 | 0.03 | $RP\text{-}1^+OCT^+MER^+$ |
| | | $RP\text{-}1^+OCT^-MER^-$ |

The donor in all crosses was strain AC 26 ($Trp^-SAL^+CAM^+$-$RP\text{-}1^+$).

TABLE 7. *Transfer of OCT by other transmissible plasmids*

| Donor | Recipient | Selected marker | Transfer frequency |
|---|---|---|---|
| AC 75 | AC 30 | OCT | $1 \times 10^{-5}$ |
| ($K^+OCT^+MER^+$) | AC 12 | $Ilv^+$ | $2 \times 10^{-2}$ |
| AC 77 | AC 30 | OCT | $<1 \times 10^{-8}$ |
| ($K^-OCT^+MER^+$) | AC 12 | $Ilv^+$ | $<1 \times 10^{-8}$ |
| AC 76 | AC 30 | OCT | $1 \times 10^{-5}$ |
| ($K^+OCT^+MER^-$) | AC 12 | $Ilv^+$ | $1 \times 10^{-2}$ |
| AC 94 | AC 30 | SAL | $5 \times 10^{-3}$ |
| ($SAL^+K^+OCT^+MER^+$) | AC 30 | OCT | $1 \times 10^{-5}$ |
| | AC 12 | $Ilv^+$ | $1 \times 10^{-4}$ |
| AC 95 | AC 30 | SAL | $7 \times 10^{-3}$ |
| ($SAL^+K^-OCT^+MER^+$) | AC 30 | OCT | $<1 \times 10^{-8}$ |
| | AC 12 | $Ilv^+$ | $<1 \times 10^{-8}$ |
| AC 90 | AC 30 | OCT | $4 \times 10^{-8}$ |
| ($RP\text{-}1^+K^-OCT^+MER^+$) | AC 12 | $Ilv^+$ | $<1 \times 10^{-8}$ |
| AC 91 | AC 30 | OCT | $1 \times 10^{-6}$ |
| ($RP\text{-}1^+K^+OCT^+MER^+$) | AC 12 | $Ilv^+$ | $5 \times 10^{-4}$ |
| AC 92 | AC 30 | CAM | $5 \times 10^{-2}$ |
| ($CAM^+K^+OCT^-MER^-$) | AC 12 | $Ilv^+$ | $1 \times 10^{-4}$ |
| AC 93 | AC 30 | CAM | $2 \times 10^{-2}$ |
| ($CAM^+K^-OCT^-MER^-$) | AC 12 | $Ilv^+$ | $1 \times 10^{-8}$ |

The frequent co-transfer of OCT, factor K, and MER as a group raised the question whether these three plasmids might exist inside the cell in some form of physical association. Although most of the chromosomal recombinants that inherit factor K do not receive either OCT or the MER plasmid, and a number of $MER^+$ exconjugants may not receive OCT, it was of interest to determine if these three plasmids could be grouped as a distinct compatibility group from a study of their coexistence with other plasmids. It is known from a previous study that while OCT seems to be compatible with SAL, it is incompatible with CAM and cannot co-exist in the same cell without undergoing recombination with CAM (10). Interestingly, MER seems to show the same compatibility behavior as OCT, while factor K has different characteristics from either OCT or MER. Thus, presence in the recipient of the three plasmids OCT, K, and MER depresses the transfer frequency of CAM by more than 1000-fold. However, most of the $CAM^+$ exconjugants lose both OCT and MER, but not factor K (Table 6). Introduction of RP-1 also leads to the loss of OCT and MER, but the frequency of transfer is much lower than that of CAM. The $SAL^+$ exconjugants

seemed to retain all the three plasmids. Thus, the transfer plasmid (factor K) shows a distinct compatibility behavior separate from OCT and MER. As is shown by the results in Table 6, it is possible to isolate $CAM^+OCT^+$ unstable exconjugants; these are incapable of transferring either CAM or OCT at an appreciable frequency.

*Transfer of OCT by Other Transmissible Plasmids.* The role of the transfer plasmid (factor K) in the transfer of OCT has already been pointed out. It has been demonstrated that $MER^+OCT^+$ cells that have lost factor K are no longer capable of transferring the OCT plasmid (Table 5). Thus, the MER plasmid, although itself transmissible, is incapable of mobilizing OCT. It was of interest to find out (*i*) if other transmissible plasmids such as CAM, SAL, or RP-1 might increase the transmissibility of OCT, or (*ii*) if transmissible degradative plasmids such as CAM or SAL might affect the frequency of chromosome mobilization by factor K, or (*iii*) if the presence of factor K might lead to an enhanced rate of transfer of plasmids such as CAM or SAL. The ability of K to mobilize chromosomal markers as well as OCT, and the inability of MER to mobilize either chromosomal genes or OCT can be seen from the results in Table 7. In the absence of factor K, SAL seems to be incapable of mobilizing the OCT plasmid, although RP-1 appears to transfer OCT at a very low frequency (3 to 4 $\times$ $10^{-8}$ per donor cell). With $RP\text{-}1^+$-$OCT^+K^-$ donor cells, it is possible to isolate $OCT^+$ exconjugants either by selecting on octane plates, or by selecting for $RP\text{-}1^+$ character on minimal glucose plates containing 50 $\mu$g/ml of neomycin and kanamycin. A small proportion of $RP\text{-}1^+$ exconjugants (1–2%) also inherit OCT under such circumstances. The presence of RP-1, CAM, or SAL appears to repress the frequency of K-induced chromosomal gene transfer by 50- to 100-fold. The presence of factor K does not have any effect on the frequency of transfer of transmissible plasmids like CAM, SAL or RP-1. Although incapable of enhancing the frequency of transfer of such transmissible plasmids as CAM, SAL, or RP-1, factor K has been found to enhance transfer of other, nontransmissible, plasmids in *Pseudomonas*.

## DISCUSSION

The simultaneous transfer and frequent association of the three plasmids OCT, MER, and K signifies that they might have useful genetic, as well as physical, properties that might lead to their aggregation in the *P. putida* cells. Although most of the chromosomal recombinants inherited factor K but not OCT or MER, transfer of OCT is almost always associated with transfer of K and MER and, similarly, transfer of MER is accompanied by chromosome mobilization as well as OCT transfer. The associative transfer of these three plasmids might be a reflection of their occurrence as a single unit in *P. oleovorans*, from which they were derived. Thus, in *P. oleovorans* these three plasmids might occur as a plasmid co-integrate and, when transferred to *P. putida*, this plasmid co-integrate might dissociate into three component plasmids.

All $OCT^-$ segregants of *P. oleovorans* also appear to have lost their resistance to high concentrations of $HgCl_2$, implying close physical association of the genes for these properties. A definitive proof of their composite structure, however, must await the physical isolation of such degradative plasmid DNA from *P. oleovorans* and *P. putida* cells, since it has not been possible to isolate these plasmids separately from the chromosome by cesium chloride-ethidium bromide density gradient centrifugation from either of these strains. The presence of a composite OCT-MER-K plasmid in *P. oleovorans* would be reminiscent of the situation with drug-resistance factors 222/R3W (11), or R1 and R6 (12) in *Escherichia coli*, where the drug-resistance factors exist as composite plasmids that dissociate into smaller plasmid components in *Proteus*. On the other hand, drug-resistance plasmid aggregates have been reported to occur in *Salmonella* and *E. coli*, where transfer factors and the drug-resistance determinants occur as separate plasmids, and in the absence of the transfer plasmid the drug-resistance determinants are rendered nontransmissible (1, 2). It is not clear if the transfer plasmid and the drug-resistance determinant plasmids were part of the same replicon in a different bacterial species, but were transferred to *Salmonella*, where they dissociated into component plasmids. Controlled *in vitro* shearing and enzymatic *in vitro* cleavage of isolated plasmid DNA to smaller components and re-introduction of such DNA components to *E. coli* cells by transformation leads to the replication of the components as biologically functional plasmids (13), suggesting that cellular mechanisms exist that can circularize and replicate fragments of a composite plasmid, provided such fragments retain the genes necessary for their replicative autonomy.

The ability of factor K to initiate chromosomal transfer confers on it the properties of a typical sex-factor plasmid. The fertility factor F in *E. coli* can mobilize not only chromosomal genes, but other nontransmissible plasmids as well (2). The inability of other self-transmissible plasmids such as CAM, SAL, or MER to mobilize OCT or chromosomal genes suggests that the transfer plasmids retain considerable specificity in their capability to initiate transfer of chromosomal markers or other nontransmissible plasmids. In contrast, most nontransmissible drug-resistance plasmids in the enterobacteria are mobilized by a variety of $fi^-$ transfer factors (14, 15). CAM, which is a transmissible plasmid co-integrate, has been shown to mobilize chromosomal markers at a low frequency, but ultraviolet irradiation of the donor cells prior to mating leads to an enhanced frequency of chromosomal marker transfer (16). Whether the specificity to initiate transfer of chromosomal genes or nontransmissible plasmids by a transfer plasmid is determined by regions of mutual genetic homology among them is not clear at present. We have no evidence to suggest that factor K needs to undergo recombination or some stable physical association with some portions of the chromosome or the OCT plasmid to initiate their transfer. Since the recombinant or the exconjugant usually inherits the chromosomal gene or the nontransmissible plasmid and factor K separately, it is likely that, like colicin E1 transfer by Flac $traI^-$ mutant (17), there is no stable physical association between factor K and OCT or the host chromosome to effect their transfer. Physical isolation of factor K, MER, OCT, and the other plasmids, and determination of the extent of genetic homologies among the plasmids, as well as the chromosome, by DNA·DNA hybridization and heteroduplex electron microscopy would throw considerable light on the mechanism of transmissibility of nontransmissible plasmids and chromosomal genes by transfer plasmids.

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