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REVIEW ARTICLE

Retrotransfer or gene capture: a feature of conjugative plasmids, with ecological and evolutionary significance

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Overview and general definition

The traditional view on bacterial conjugative gene exchange is a gene flow from the plasmid-containing donor strain into the plasmid-free recipient strain. When mobilization of non-conjugative plasmids is described, it is either a biparental mating with a donor containing both conjugative and a non-conjugative but mobilizable (Mob^+) plasmids, and a plasmid-free recipient, or a triparental mating with a donor, containing the Mob^+ plasmid, a helper strain harbouring a conjugative helper plasmid, and again a plasmid-free recipient strain. In these scenarios, mobilization was always considered as a gene flow from the original donor to the recipient strain. However, the first report of gene flow at high frequencies in two directions was published by Mergeay *et al.* in 1987. By means of an IncP conjugative plasmid, chromosomal genes were not only mobilized from the original donor of the IncP plasmid towards the plasmid-free recipient, but also from the original recipient back into the donor. The terms 'retrotransfer', 'shuttle transfer', plasmid-mediated 'gene capture' or even a kind of bacterial 'hermaphroditism', were used to describe a conjugational biparental event that led to the inheritance (capture) by the original host of a conjugative plasmid, of genetic traits (either chromosomal markers or plasmids) from the mating partner, free of conjugative plasmids. The frequency of this retrotransfer can be very high, being in some cases of the same order of magnitude as the frequencies observed for the transfer of genetic traits promoted in the canonical donor–recipient direction (Mergeay *et al.*, 1987). Although the phenomenon was first observed and analysed in most detail for broad-host-range (BHR) IncP1 plasmids (Mergeay *et al.*, 1987), it was also reported for other plasmids such as some IncN (pULG14) and IncM plasmids (R69.2) (Thiry *et al.*, 1984; Mergeay *et al.*, 1985), the IncF plasmid pDE-FL54 and the IncW plasmid pSa322 (Heinemann & Ankenbauer, 1993a), and for the catabolic IncP9 plasmid pWW0 (Ramos-Gonzalez *et al.*, 1994). This capture of new genetic traits could thus be of great significance for the evolution of microbial communities, especially where stress is selecting rare events, yielding individual populations with new combinations of genes, which may allow better survival or more rapid growth.

Capture of chromosomal markers

The capture of chromosomal markers was first observed during conjugation mediated by derivatives of IncP plasmids carrying a transposable element (Mergeay *et al.*, 1984, 1987) such as R68.45 and RP4::Mu3A (also called pULB113; Van Gijsegem & Toussaint, 1982). The matings involved a donor with multiple markers spread over the chromosome and a recipient carrying one marker. In homologous matings between two *Erwinia chrysanthemi* partners (Schoonejans & Toussaint, 1983) or two *Pseudomonas fluorescens* 6.2 partners (Lejeune *et al.*, 1983; Mergeay *et al.*, 1984), transconjugants were selected for having inherited one marker from each mating partner (Fig. 1). Surprisingly, the transconjugants that carried all the unselected markers of the donor of the conjugative plasmid were often more frequent than those with only one or a few of the unselected donor markers. In other words, a class of recombinants that seemed to have received the whole donor chromosome, and hence expected to be the least

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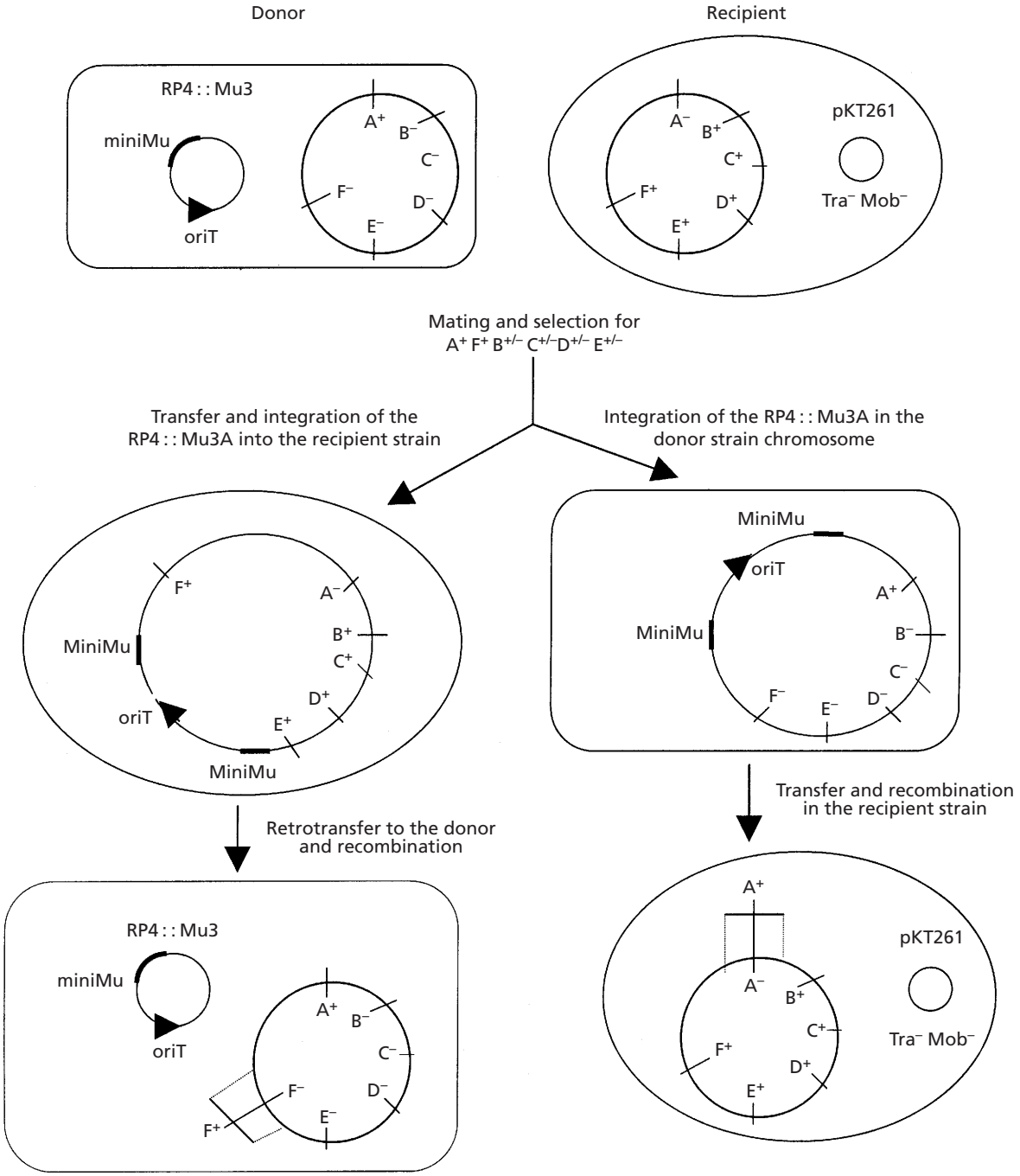

Fig. 1. Schematic of an RP4::Mu3A-mediated cross designed to distinguish retrotransconjugants from direct transconjugants [inspired from data published in Mergeay *et al.* (1987)].The reported cross involves an A^+ B^- C^- D^- E^- F^- donor carrying the conjugative plasmid RP4::Mu3A and a recipient A^- B^+ C^+ D^+ E^+ F^+ strain carrying the Tra^- Mob^- plasmid pKT261, used here exclusively to mark the recipients. A, B, C, D, E and F represent biosynthetic (amino acids) or carbon source chromosomal markers. A^+ F^+ $B^{+/-}$ $C^{+/-}$ $D^{+/-}$ $E^{+/-}$ transconjugants were selected and further analysed by replica plating. As expected, the A^+ B^+ C^+ D^+ E^+ F^+ class is very well represented but the most frequent class is often A^+ B^- C^- D^- E^- F^+, oddly suggesting that transfer of five markers (A^+, B^-, C^-, D^- and E^-), scattered on the chromosome, is more frequent than transfer of one or two. Yet only the class A^+ B^+ C^+ D^+ E^+ F^+ contained the marker plasmid pKT261. This shows that the recombinant class A^+ B^- C^- D^- E^- F^+ (still containing RP4::Mu3A) has in fact inherited only the F^+ marker from the recipient and is a class of retrotransconjugants. The putative miniMu-mediated integration sites of the conjugative plasmid in the chromosomes of both partners are indicated. From these integration sites, the plasmid has transferred the A^+ (direct transfer) and F^+ (retrotransfer) markers to the mating partners.

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frequent, was often the most abundant. This made it impossible to interpret conjugation data in terms of linkage and construction of chromosomal maps. There was, however, an alternative interpretation regarding the origin of this odd class of transconjugants: they might result from the transfer of a single chromosomal marker from the recipient to the donor of the conjugative plasmid, an hypothesis that was easy to test experimentally. A choice of appropriately marked mating partners (e.g. with genetically 'inert' plasmids, i.e. non-self-transferable, non-mobilizable plasmids such as pKT261; Bagdasarian *et al*., 1981) made it possible to demonstrate that this was indeed the case (Fig. 1).

This unconventional direction of gene flow, a plasmid-mediated capture of chromosomal traits, was later also observed in several homologous matings involving a wide variety of bacterial species, including *Ralstonia eutropha*, *Salmonella typhimurium*, *Methylobacillus flagellatus* (Kletsova & Tsygankov, 1990), *Azotobacter vinelandii* (Blanco *et al*., 1991) and *Thiobacillus versutus* (Wlodarczyk & Piechucka, 1995). In all cases, the conjugative plasmids were derivatives of RP4/R68 (IncP/IncP1 plasmids). The same phenomenon was also observed in heterologous matings between *P. fluorescens* and *R. eutropha*, where differences in colour and morphology between the mating partners made it easy to distinguish which partner generated the R-prime-bearing transconjugants. Clearly both parents were involved, even forming sectored colonies (Mergeay *et al*., 1987). Other observations of chromosomal gene capture were reported for the 115 kb *Pseudomonas putida* TOL plasmid pWW0. This plasmid belongs to the IncP9 group; its hosts range mainly among the γ-*Proteobacteria* (*P. putida*, *P. fluorescens*, *Erw. chrysanthemi*, *Escherichia coli*, etc.). In matings between two *P. putida* partners, pWW0 could mobilize antibiotic-resistance markers located at distinct positions within the chromosome of one partner, both in the usual and in the retrotransfer direction. The antibiotic marker was provided by a miniTn5 (a 'one hopper' mini-transposon, which can transpose only once; Simon *et al*., 1983). The frequencies of direct and retromobilization of the chromosomal marker varied by more than five orders of magnitude, depending on the marker's location. However, for each position these frequencies of direct and retromobilization were similar (Ramos-Gonzalez *et al*., 1994). In all the matings considered so far, whether mediated by IncP-derived plasmids or by pWW0, it is important to note that a transposon carried by the plasmid was responsible for mobilization of the chromosomal markers.

The data summarized above suggest the following.

(1) Retromobilization is not a rare event. Its frequency can be as high as the frequency of direct mobilization, suggesting that both types of transfer occur at about the same time after contact between the mating partners.

(2) Recovery of recombinant R-primes carrying chromosomal genes from either partner and the identical linkage values observed in both transfer directions indicates that the conjugative plasmid should enter the recipient, express at least part of the information it carries and interact with the recipient chromosome to promote retrotransfer.

(3) Mobilization of chromosomal genes requires transposon-mediated integration of the conjugative plasmid into the chromosome. The similarity of mobilization frequencies in both directions suggests that the conjugative plasmid integrates at about the same frequency into either partner and that it quickly reaches an equilibrium between episomes (integrated replicons) and autonomous plasmids. Whether mating can stimulate mini-Mu-mediated integration of the conjugative plasmid into the recipient chromosome remains an open question deserving further investigation.

Capture of plasmids

As they mobilize and retrotransfer chromosomal genes, conjugative plasmids can also mobilize and retrotransfer non-conjugative plasmids. Retromobilization of plasmids soon became the most common assay for retrotransfer. 'Promiscuous' IncQ plasmids (Tra^- Mob^+) were most often used in these assays. However, many other plasmids can be retromobilized (Top *et al*., 1991), as can genes located on plasmids containing no transfer gene or transfer origin (Tra^- Mob^-). Examples are the pBR322-derived plasmids (Top *et al*., 1990). Here, the *sine qua non* condition for retromobilization is the presence, either on the mobilized or the conjugative plasmid, of an efficient transposable element. Although plasmid RP4::Mu3A efficiently retromobilizes a pBR325 derivative from *Esc. coli* to *R. eutropha*, RP4 does not. It is therefore assumed that mobilization is possible because of the formation of co-integrates between RP4::Mu3A and the mobilized plasmid, as a result of Mu3A transposition. Formation of co-integrates is also postulated in RP4-mediated retromobilization of the large plasmids from *R. eutropha* CH34, pMOL28 and pMOL30, which encode metal resistance and are either non-self-transferable or self-transferable only at very low frequencies. In this case, formation and resolution of co-integrates would be mediated by mercury transposons carried by these large plasmids (Diels *et al*., 1985). The data in Table 1 show that retrotransfer and direct mobilization of the megaplasmid pMOL30 by RP4 occur at similar frequencies (Mergeay *et al*., 1990). They also highlight a striking phenotypic difference between RP4 (IncP plasmid) and R388 (IncW) as far as retrotransfer is concerned: the frequency of retrotransfer of pMOL30 is 10^5 times lower with R388 than with RP4. In addition to RP4, the catabolic IncP plasmids pJP4 and pSS50 are also able to retromobilize the megaplasmids from *R. eutropha* at rather high frequencies.

pRK2013 provides a particularly good illustration of plasmid 'capture'. In this derivative of RP4, *oriV* has been replaced with the replication origin of the ColE1 plasmid (Figurski & Helinski, 1979). Hence pRK2013 can replicate only in *Esc. coli* and related enterics, and is therefore extremely useful for introducing Tra^- Mob^+

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Table 1. (Retro)mobilization of non-conjugative plasmids from 'donor' strains by conjugative plasmids in the same 'donor' strain or in the final 'recipient' strain

The 'donors' are here the bacteria containing the plasmid to be mobilized: if the recipient contains a conjugative plasmid, the mobilization is considered a retromobilization. Control rows show that no mobilization occurred in the absence of effective conjugative plasmids. The rows containing R388 (IncW) show that it is inefficient as a retromobilizing plasmid. RP4 is IncP; Kn^R, Tc^R, Ap^R; R388 is IncW; Su^R, Tp^R. The data with AE81 and CH34 as donors are from Mergeay *et al.* (1990) (with permission of the publisher).

'Donor'	Final 'recipient'	Plasmid in donor to be (retro)mobilized	Selected marker	Direction of transfer	Transfer frequency
R. eutropha AE81 (RP4)	*R. eutropha* AE104	pMOL30	Zn^R	Direct	10^{-3}
R. eutropha AE81*	*R. eutropha* AE104†(RP4)	pMOL30	Zn^R	Retro	10^{-3}
R. eutropha AE81*	*R. eutropha* AE104†	pMOL30	Zn^R	Control	10^{-8}
R. eutropha AE81*(R388)	*R. eutropha* AE104†	pMOL30	Zn^R	Direct	10^{-4}
R. eutropha AE81*	*R. eutropha* AE104†(R388)	pMOL30	Zn^R	Retro	10^{-8}
R. eutropha AE81*	*R. eutropha* JMP134‡ (contains pJP4)	pMOL30	Zn^R	Retro	10^{-4}
R. eutropha AE81*	*R. eutropha* JMP222§	pMOL30	Zn^R	Control	$<10^{-9}$
R. eutropha CH34‖	*R. eutropha* A5¶ (contains pSS50)	pMOL28	Ni^R	Retro	10^{-5}
R. eutropha CH34‖	*R. eutropha* AE104†	pMOL28	Ni^R	Control	10^{-8}
P. fluorescens PF118**	*R. eutropha* A5¶ (contains pSS50)	pKT212	Tc^R	Retro	10^{-4}
P. fluorescens PF118**	*R. eutropha* AE104*	pKT212	Tc^R	Control	$<10^{-9}$
P. fluorescens PF118**	*R. eutropha* AE707†† (contains pSS50)::Tn*4371*	pKT212	Tc^R	Retro	10^{-3}
P. fluorescens PF118**	*R. eutropha* CH34‖	pKT212	Tc^R	Control	$<10^{-9}$
P. fluorescens PF118**	*Esc. coli* J53 (RP4)	pKT212	Tc^R	Retro	10^{-4}
P. fluorescens PF118**	*Esc. coli* (pRK2013)	pKT212	Tc^R	Retro	10^{-3}
Esc. coli CM1308‡‡	*R. eutropha* AE593 (R388) §§	pMOL187	Zn^R	Retro	$<10^{-8}$
Esc. coli CM1308‡‡	*R. eutropha* AE593 (RP4)§§	pMOL187	Zn^R	Retro	10^{-4}

* CH34 polyauxotroph containing pMOL30 and pMOL28.

† Plasmid-free CH34.

‡ Wild-type strain containing pJP4 (IncP, 90 kb; *clc*: ability to degrade chlorobenzoate; *tfd*: ability to degrade 2,4-dichlorophenoxyacetic acid).

§ Plasmid-free derivative of JMP134.

‖ Wild-type strain containing pMOL30 (240 kb; *czc*: Zn^R, resistance to zinc) and pMOL28 (180 kb; *cnr*: Ni^R, resistance to nickel).

¶ Wild-type strain containing pSS50 (IncP).

** *Pseudomonas fluorescens* 6.2 containing pKT212 (IncQ, Tc^R).

†† CH34 containing pSS50::Tn*4371 bph* (Bph^+, ability to grow on biphenyl as the sole carbon source).

‡‡ DH10B containing pMOL187 [IncQ, Ap^R, czc^+ (Collard *et al.*, 1994)].

§§ Leu^- derivative of AE104.

plasmids into a variety of non-enteric hosts where it is not stably inherited. As shown in Table 1, pRK2013 can promote retromobilization of the IncQ plasmid pKT212 into *Esc. coli* from a *P. fluorescens* strain, in which it cannot replicate. Retrotransfer of pKT212 has also been observed from *Desulfovibrio desulfuricans* (Powell *et al.*, 1989) and *R. eutropha* (Top, 1993; M. Mergeay, unpublished). This indicates that replication of the conjugative plasmid in the recipient partner is not a prerequisite for retrotransfer.

The mechanism of retrotransfer

The observation that retrotransfer and direct transfer occur at about the same time and that retrotransfer does not require replication of the retromobilizing plasmid in the recipient led to a detailed analysis of the retrotransfer mechanism. First, two opposite models were proposed, a unidirectional and a bidirectional model. The unidirectional model involves two transfer events that are indistinguishable from standard conjugation, i.e. transfer of the Tra^+ plasmid from the donor to the recipient, expression of all the *tra* genes in the recipient, and mobilization (retrotransfer) of the Tra^- Mob^+ plasmid from the recipient to the original donor. In the bidirectional model, it is assumed that the bridge made in the first conjugation event (from donor to recipient) can be used for, or at least help, the movement of DNA in the opposite direction (from the recipient to the donor). Mathematical equations were designed for both models, assuming that they could be distinguished. The bidirectional mechanism was associated with the 'one-

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step' mathematical model and the unidirectional mechanism with the 'two-step' mathematical model. The data reported for the RP4 and TOL plasmids fitted the 'one-step' model (Top *et al*., 1992; Ramos-Gonzales *et al*., 1994), thus confirming that retrotransfer is an early event. Yet the data of Perkins *et al*. (1994), obtained with exogenously isolated BHR plasmids (Bale *et al*., 1988), did not completely fit with either model. These authors thus proposed a mathematical description based on the one-step model but allowing for a longer delay before retromobilization is observed. The assertion that retrotransfer might differ from canonical conjugation led to a passionate controversy, thoroughly and subtly recounted by Ankenbauer (1997). It prompted Heinemann and Figurski, and their respective teams, to confront the two models in a set of rigorous experiments (Heinemann & Ankenbauer, 1993a; Heinemann *et al*., 1996; Sia *et al*., 1996). These experiments established that at least one *tra* gene for IncP1 plasmids and multiple *tra* genes for IncF plasmids must be expressed in the recipient for retrotransfer to occur. The investigators concluded that DNA transfer is unidirectional and that retrotransfer involves two transfers that do not differ from the canonical 'Hayes' conjugation mechanism (unidirectional model). To avoid confusion in terminology, it is important to note that although the unidirectional model of DNA transfer is most likely correct, the combination of direct and retrotransfer promotes a bidirectional flux of genetic information between bacterial populations. Heinemann & Ankenbauer (1993b) even showed that expression of chromosomal genes in the recipient of the conjugative plasmid is not required for retrotransfer of a Mob^+ vector to occur, and thus that non-viable cells, receiving a conjugative plasmid, can be transformed into vectors for DNA transmission back to viable cells. In further experiments, to determine the flexibility of gene fluxes promoted by BHR plasmids, it would be interesting to test whether all the *tra* genes of these plasmids are required for retrotransfer and whether retrotransfer can be promoted by repolarization of the bridge with the help of some Tra proteins expressed in the recipient.

Retrotransfer mediated by BHR plasmids

Since retrotransfer is thought to have special bearing on natural gene dissemination in the environment and to horizontal gene transfer, it is of interest here to focus on BHR plasmids in relation to retrotransfer.

BHR conjugative plasmids, or plasmids that can efficiently cross taxonomic barriers, belong to very few families: IncP, IncW and, to a lesser extent, IncN. In practice, plasmids able to cross the barrier between *Esc. coli* and *Pseudomonas* have been placed *ipso facto* in this category. Yet the phylogenetic tree based on 16S rRNA sequences shows that enterics and pseudomonads are quite close to each other within the group of the *γ-Proteobacteria* (Woese, 1987).

Therefore, to maintain the relevance of the term 'BHR plasmids' in the perspective of gene dissemination and capture, we propose to limit its definition, applying it only to those (conjugative) plasmids that can cross at least the barrier between bacteria belonging to two different branches of the *Proteobacteria*. IncP and IncW plasmids surely match this definition. For IncP, the transfer range even spans as many as five branches within the domain of *Bacteria* (*Proteobacteria*, *Firmicutes* (Gram-positive), *Cyanobacteria*, green sulfur bacteria and *Bacteroides/Cytophaga*), extending even to the *Eucarya* such as yeast (Bates *et al*., 1998). We also see that most of the known Inc groups have in fact a narrow host range. An assay (triparental exogenous isolation) has been developed to specifically isolate plasmids that can cross the barrier between *γ*- and *β-Proteobacteria* (Top *et al*., 1994; Smit *et al*., 1993). This procedure has revealed a series of new plasmids that were shown to have a BHR, some of which appear quite different from the familiar IncP or IncW plasmids. These BHR plasmids were tested and compared with RP4 and R388 for the efficiency of different types of biparental transfer: direct mobilization of an IncQ plasmid from *Esc. coli* to *R. eutropha* was compared to retromobilization (also called retrotransfer), and inter-'male' mobilization; the latter represents a mating in which both parental strains contain the same conjugative plasmid from the beginning of the mating (Table 2). The transfer functions seem to be adequately expressed in both hosts (except for pEMT1k and pIPO2k): the plasmids can self-transfer from *R. eutropha* into *Esc. coli* (at a frequency of 10^{-3} to 1 per donor, depending on the plasmid) and conversely from *Esc. coli* into *R. eutropha* (at a frequency of 10^{-4} to 10^{-1}, except pEMT1k and pIPO2k). However, the efficiency of retromobilization of the mobilizable IncQ plasmid pMOL187 differs strongly between the BHR plasmids, ranging through six orders of magnitude (Table 2). For two of the six new BHR plasmids, pIPO2k and pMOL96, the frequency of retrotransfer of the IncQ vector from *Esc. coli* to *R. eutropha* is respectively six and three orders of magnitude higher than the frequency of self-transfer and establishment of the conjugative BHR plasmid from *R. eutropha* to *Esc. coli* during the same mating. This suggests that the low self-transfer frequency of these BHR plasmids is not due to inefficient transfer but to poor replication in the new *Esc. coli* host after transfer. These results seem similar to the observations with pRK2013, which is unable to replicate outside *Esc. coli* and related enterics but can capture plasmids from various other hosts (see 'Capture of plasmids' section and Table 1). The recorded frequencies for inter-'male' mobilization of pMOL187 are identical or very similar to the retrotransfer frequencies, with the exception of the much lower frequency of pIPO2k. This strongly suggests that retrotransfer is an inter-'male' mobilization process. This interpretation is in agreement with the results of Sia *et al*. (1996) and Heinemann & Ankenbauer (1993a, b), which indicate that retrotransfer requires transfer of *tra* genes to the recipient and expression in the recipient of at least one function involved in the formation, maintenance, or functioning of the conjugation bridge. In this mechanism, however,

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Table 2. Transfer phenotypes of 'new' BHR plasmids: comparison of frequencies of retrotransfer and inter-'male' mobilization of the IncQ plasmid pMOL187 from the donor *Esc. coli* CM1308 to the 'recipient' *R. eutropha* AE815, containing one of the BHR plasmids

All the frequencies were determined by dividing the number of transconjugants by the number of donor cells quantified after an 18 h mating. The 'donor' *Esc. coli* CM1308 contains the IncQ plasmid pMOL187, which was selected by its ampicillin resistance (in *Esc. coli*) or its zinc resistance from the czc cassette (in *R. eutropha*) (Collard *et al.*, 1994). The final recipient, *R. eutropha* AE815 is a rifampicin-resistant derivative of the plasmid-free strain AE104. The frequency of self-transfer (R388, pSS50, pJP4, RP4, pA13, pIPO2k, pMOL96, pEMT1k, pEMT8, pSL2T) varies from 1 to 5×10^{-3}. See Table 1 for descriptions of RP4 and R388.

Conjugative plasmid in 'recipient'	Inc group selected marker	Transfer frequency 1*	Transfer frequency 2†	Retrotransfer frequency	Inter-'male' mobilization frequency
pMOL96‡	Km^R	2×10^{-3}	5×10^{-4}	1	4×10^{-1}
pIPO2k§	Km^R	2×10^{-3}	1×10^{-7}	2×10^{-1}	5×10^{-5}
pEMT8k‖	Km^R	4×10^{-1}	2×10^{-2}	1×10^{-6}	1×10^{-6}
pA13¶	IncP, Tc^R	1	7×10^{-2}	8×10^{-5}	4×10^{-5}
pSL2T**	IncP, Tc^R	8×10^{-1}	2×10^{-1}	3×10^{-4}	2×10^{-4}
pEMT1k‖	IncP, Km^R	6×10^{-1}	2×10^{-6}	$<10^{-8}$	$<10^{-8}$
RP4	IncP, Tc^R	1	8×10^{-1}	5×10^{-5}	2×10^{-5}
R388	IncW, Tp^R	1	4×10^{-4}	3×10^{-8}	2×10^{-8}

* Frequency of transfer of conjugative plasmid from AE815 into CM1308.

† Frequency of transfer of conjugative plasmid from CM1308 into AE815.

‡ Isolated from soil by triparental exogenous isolation (Top *et al.*, 1994) and tagged with miniTn5Km1.

§ Isolated by triparental mating and tagged with miniTn5Km1 (van Elsas *et al.*, 1998). This plasmid replicates poorly in *Esc. coli* or integrates in the chromosome.

‖ Isolated from 2,4-dichlorophenoxyacetic acid-treated soil by biparental exogenous isolation and tagged with miniTn5Km1 (Top *et al.*, 1995b, 1996, 1998).

¶ Isolated from soil by triparental exogenous isolation and tagged with miniTn5-Tc (H. De Rore and E. Top, personal communication).

** Isolated from soil by triparental exogenous isolation and tagged with miniTn5-Tc (C. Szpirer, unpublished).

replication of the conjugative plasmid in the host of the Mob^+ plasmid is not necessary to allow retrotransfer, as observed with pRK2013, and now also pIPO2k. During a first transfer, the single-stranded DNA of the conjugative plasmid is transferred to the recipient. The complementary strand of this transferred strand is synthesized by the host's replication machinery. This synthesis is, in some conjugation systems, facilitated by specific Tra proteins (primase for example) which are transported, attached to the transferred strand, from the donor to the recipient bacterium. After DNA synthesis, a double-stranded plasmid is reconstituted in the recipient (Wilkins & Lanka, 1993), which thus contains one complete circular plasmid. The plasmid *tra* genes can be expressed and promote (retro)transfer of a mobilizable plasmid present in the recipient of the conjugative plasmid. In this mobilization, both donor and recipient contain at least one copy of the conjugative plasmid (as in inter-'male' mobilization). In this case, the surface exclusion encoded by the conjugative plasmid could play an important role in retrotransfer. The surface exclusion genes of RP4 are considered quite inefficient compared to those of R388. Table 1 shows a strong phenotypic difference between RP4 (IncP) and R388 (IncW) for retromobilization of the IncQ plasmid pMOL187 and of large plasmids carrying metal-resistance markers. The striking phenotypic differences in retrotransfer capability between the various conjugative BHR plasmids tested, including IncP and IncW, may thus be attributable to differences in the expression of the genes involved in surface exclusion.

Yet another aspect deserves attention: the retrotransfer capability of plasmids is expressed in many matings between unrelated bacteria and in a variety of hosts. This should imply a kind of protection of the captured DNA or even an anti-restriction mechanism. Recently, Althorpe *et al.* (1999) proposed that some leading-region genes helping the immigrant plasmid to establish itself in the new host are transcribed from the incoming strand before synthesis of the complementary DNA, i.e. from promoters active in single-stranded DNA. This region includes an anti-restriction gene such as *ardA* (Delver *et al.*, 1991; Read *et al.*, 1992; Belogurov *et al.*, 1993;

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Chilley & Wilkins, 1995) and an inhibitor of the bacterial SOS response such as *psiB* (Bailone *et al*., 1988; Golub *et al*., 1988; Bagdasarian *et al*., 1992). Some properties of the ArdA system are identified from the cross-protection of the IncPβ R751 by a co-transferring ColIb. Genetic evidence suggests that expression of *ardA* and *psiB* of the IncI-1 plasmid ColIb-P9 is activated when the genes enter the recipient cell on the transferring plasmid strand. In the IncP transfer system, the *tra*C gene product (primase) is transferred from the donor to the mating partner and may protect the entering single-stranded DNA against various aggressions or damage in the recipient (Wilkins & Lanka, 1993).

At first glance, optimal retrotransfer efficiency should rely on four conditions: an efficient conjugation system, a conjugational broad host range, minimized surface exclusion and appropriate protection of the captured DNA.

Retrotransfer and gene dissemination: ecological and evolutionary aspects

Retrotransfer of chromosomal genes or plasmids may have important ecological implications. From the viewpoint of the bacterial cell, it offers the host of the retromobilizing plasmid a kind of gene-capture device, allowing it to pick up new genes from other bacteria, without depending on a third strain with a helper plasmid, as is the case in a triparental mobilization. Since in a natural environment the probability of three different populations (donor, recipient and helper) coming into close contact and exchanging DNA by conjugation is lower than the probability of two populations (donor and recipient) meeting, strains with a retromobilizing plasmid would have easier access to new genetic information than the same strains without such a plasmid. By acquiring new genes in this way, individual bacteria could adapt more easily to changing environmental conditions, such as contamination by xenobiotics (Mergeay *et al*., 1990). Conjugative exchange of catabolic or heavy-metal-resistance genes is reported to occur in habitats such as soils (De Rore *et al*., 1994a, b; Top *et al*., 1990, 1995a, b, 1998), water (Fulthorpe & Wyndham, 1992) and activated sludge (McClure *et al*., 1990; Ravatn *et al*., 1998). These exchanges play an important role in the acquisition of new genetic information enabling bacteria to cope with and/or degrade recently introduced xenobiotics. If such genes are located on chromosomes or on non-conjugative plasmids, a bacterium should have easier access to these genes through retromobilization. It should be stressed that bacteria indigenous to an ecosystem and harbouring retromobilizing plasmids can capture genes, including recombinant DNA, through biparental mating. Although plasmids lacking *mob* (mobilization) and *tra* (transfer) genes have a low probability of dissemination, transfer of genes located on these plasmids is possible (after recombination with, or transposition into, Tra^+ plasmids) and has been demonstrated in soil microcosms (Top *et al*., 1990, 1995a). It has also been shown that a rather rare event, such as retromobilization of heavy-metal-resistance genes located on a Mob^- vector in sterile soil, can become very important when a selective pressure (presence of the corresponding heavy metal) is present. Retrotransconjugants that are undetectable in unpolluted soil because of their low numbers [less than 10 c.f.u. (g soil)$^{-1}$] can increase to a very high level [up to 10^6 c.f.u. (g soil)$^{-1}$] in heavy-metal-polluted soil (Top *et al*., 1995a). Retrotransfer thus occurs in soil and can have a great impact on the microbial community. In addition to this, several groups have reported that both broad- and narrow-host-range plasmids are readily found in several environmental habitats (soils, waste-water treatment plants, etc.) and that some of them can mobilize non-conjugative (Tra^-) plasmids into a wide range of Gram-negative bacteria by triparental mobilization and/or retromobilization (Bauda *et al*., 1995; Fujita *et al*., 1993; McPherson & Gealt, 1986; Hill *et al*., 1992, 1995; Smit *et al*., 1993; Drønen *et al*., 1998; Top *et al*., 1994).

Conclusions

In nature, gene dissemination through horizontal gene transfer involves many different actors (plasmids, phages, transposons and integrons) and mechanisms (homologous and site-specific recombination, transposition, conjugation, transformation and transduction). The ability of a plasmid to mediate retrotransfer (to capture genes that could bring an advantage to its host) is shared by many conjugative plasmids, which possess this ability to a greater or lesser extent. Retrotransfer has mainly been studied with IncP plasmids, but it is more striking in the case of some new, recently described BHR plasmids (Table 1). Retrotransfer may have great evolutionary significance as a force promoting horizontal gene transfer. Once its mechanism has been unravelled, the next step should be to study retrotransfer in detail from an ecological perspective, especially for plasmids with a conjugation range broader than their replication range. Such plasmids could 'pick up' genes (from a plasmid or from the chromosome of a plasmid-free bacterium) from a taxonomic domain much larger than that within which they can replicate. This would have major consequences for horizontal gene fluxes, whether between natural bacterial species or involving genetically modified organisms. We should be aware that our knowledge of BHR plasmids is still limited, even with regard to IncP plasmids, known to self-transfer among Gram-positive bacteria, *Cyanobacteria*, and other major branches of *Bacteria*. Data are scant regarding their capture range and the kinds of genes that can be captured. Biparental, triparental and retrotransfer matings have led to the isolation of new BHR plasmids, but only a few recipient hosts, mainly β- and γ-*Proteobacteria* (Bale *et al*., 1988; Drønen *et al*., 1998, 1999; Top *et al*., 1995; van Elsas *et al*., 1998), and recently one species from the α-*Proteobacteria* (Drønen *et al*., 1999) have been used to capture these plasmids. Other hosts and techniques should be tested to increase the potential catalogue of BHR plasmids and their capture range throughout the various branches of

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Bacteria and even *Archaea* and among some *Eukarya*. This has special bearing on the release of GMOs in the environment. An exciting approach is to analyse DNA exchange between bacterial populations that are usually separated by barriers, caused by physical, metabolic and physiological constraints (pressure, temperature, oxygen, salinity). What a challenge for the microbial ecologist and bacterial geneticist to hunt for overlapping temperature and metabolic domains where two very different bacteria can survive and grow together, to see if those bacteria can capture or exchange genes!

A resolute effort should be made to encompass, in our picture of plasmids as mediators of gene transfer, exchange and capture, at least all the prokaryotic domains. A growing panoply of molecular and physiological tools is available to reach this goal.

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References

Althorpe, N., Chilley, P., Thomas, T., Brammar, W. & Wilkins, B. (1999). Transient transcriptional activation of the IncI1 plasmid anti-restriction gene (*ardA*) and SOS inhibition gene (*psiB*) early in conjugating recipient bacteria. *Mol Microbiol* **31**, 133–142.

Ankenbauer, R. G. (1997). Reassessing forty years of genetic doctrine: retrotransfer and conjugation. *Genetics* **145**, 543–549.

Bagdasarian, M., Lurz, R., Ruckert, B., Franklin, F. C., Bagdasarian, M. M., Frey, J. & Timmis, K. N. (1981). Specific-purpose plasmid cloning vectors. II. Broad host range, high copy number, RSF1010-derived vectors, and a host vector system for gene cloning in *Pseudomonas*. *Gene* **16**, 237–247.

Bagdasarian, M., Bailone, A., Angulo, J. F., Scholz, P., Bagdasarian, M. M. & Devoret, R. (1992). PsiB, an anti-SOS protein, is transiently expressed by the F sex factor during its transmission to an *Escherichia coli* K-12 recipient. *Mol Microbiol* **6**, 885–893.

Bailone, A., Bäckman, A., Sommer, S., Célérier, J., Bagdasarian, M. M., Bagdasarian, M. & Devoret, R. (1988). PsiB polypeptide prevents activation of RecA protein in *Escherichia coli*. *Mol Gen Genet* **214**, 389–395.

Bale, M. J., Day, M. J. & Fry, J. C. (1988). Novel method for studying plasmid transfer in undisturbed river epilithon. *Appl Environ Microbiol* **54**, 2756–2458.

Bates, S., Cashmore, A. M. & Wilkins, B. (1998). IncP plasmids are unusually effective in mediating conjugation of *Escherichia coli* and *Saccharomyces cerevisiae*: involvement of the Tra2 mating system. *J Bacteriol* **180**, 6538–6543.

Bauda, P., Lallement, C. & Manem, J. (1995). Plasmid content evaluation of activated sludge. *Water Res* **29**, 371–374.

Belogurov, A. A., Delver, E. P. & Rodzevich, O. V. (1993). Plasmid pKM101 encodes two nonhomologous antirestriction proteins (ArdA and ArdB) whose expression is controlled by homologous regulatory sequences. *J Bacteriol* **175**, 4843–4850.

Blanco, G., Ramos, F., Medina, J. R., Guttierez, J. C. & Tortolero, M. (1991). Conjugal retrotransfer of chromosomal markers in *Azotobacter vinelandii*. *Curr Microbiol* **22**, 241–246.

Chilley, P. & Wilkins, B. (1995). Distribution of the *ardA* family antirestriction genes on conjugative plasmids. *Microbiology* **141**, 2157–2164.

Collard, J. M., Corbisier, P, Diels, L. & 7 other authors (1994). Plasmids for heavy metal resistance in *Alcaligenes eutrophus* CH34: mechanisms and applications. *FEMS Microbiol Rev* **14**, 405–414.

Delver, E., Kotova, V., Zavilgelsky, G. & Belogurov, A. (1991). Nucleotide sequence of the gene (*ard*) encoding the antirestriction protein of plasmid ColIb-P9. *J Bacteriol* **173**, 5887–5892.

De Rore, H., Demolder, K., De Wilde, K., Top, E., Houwen, F. & Verstraete, W. (1994a). Transfer of the catabolic plasmid RP4::Tn*4371* to indigenous soil bacteria and its effect on respiration and biphenyl breakdown. *FEMS Microbiol Ecol* **15**, 71–81.

De Rore, H., Top, E., Houwen, F., Mergeay, M. & Verstraete, W. (1994b). Evolution of heavy metal resistant transconjugants in a soil environment with a concomitant selective pressure. *FEMS Microbiol Ecol* **14**, 263–274.

Diels, L., Faelen, M., Mergeay, M. & Nies, D. (1985). Mercury transposons from plasmids governing multiple resistance to heavy metals in *Alcaligenes eutrophus* CH34. *Arch Intern Physiol Bioch* **93**, B27–B28.

Drønen, A. K., Torsvik, V., Goksøyr, J. & Top, E. (1998). Effect of mercury addition on plasmid incidence and gene mobilizing capacity in soils. *FEMS Microbiol Ecol* **27**, 381–394.

Drønen, A. K., Torsvik, V. & Top, E. (1999). Comparison of the plasmid types obtained by two distantly related recipients in biparental exogenous plasmid isolations from soil. *FEMS Microbiol Lett* **176**, 105–110.

van Elsas, J. D., Gardener, B. B., Wolters, A. C. & Smit, E. (1998). Isolation, characterization, and transfer of cryptic gene-mobilizing plasmids in the wheat rhizosphere. *Appl Environ Microbiol* **64**, 880–889.

Figurski, D. & Helinski, D. (1979). Replication of an origin-containing derivative of plasmid RK2 dependent on a plasmid function provided in *trans*. *Proc Natl Acad Sci USA* **76**, 1648–1652.

Fujita, M., Ike, M. & Suzuki, H. (1993). Screening of plasmids from wastewater bacteria. *Water Res* **27**, 949–953.

Fulthorpe, R. R. & Wyndham, R. C. (1992). Involvement of chlorobenzoate-catabolic transposon, Tn*5271*, in community adaptation to chlorobiphenyl, chloroaniline and 2,4-dichlorophenoxyacetic acid in a fresh water ecosystem. *Appl Environ Microbiol* **59**, 843–850.

Golub, E., Bailone, A. & Devoret, R. (1988). A gene encoding an SOS inhibitor is present in different conjugative plasmids. *J Bacteriol* **170**, 4392–4394.

Heinemann, J. A. & Ankenbauer, R. G. (1993a). Retrotransfer in *Escherichia coli* conjugation: bidirectional exchange or de novo mating? *J Bacteriol* **175**, 583–588.

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Heinemann, J. A. & Ankenbauer, R. G. (1993b). Retrotransfer of IncP plasmid R751 from *Escherichia coli* maxicells: evidence for the genetic sufficiency of self-transferable plasmids for bacterial conjugation. *Mol Microbiol* **10**, 57–62.

Heinemann, J. A., Scott, H. E. & Williams, M. (1996). Doing the conjugative two-step: evidence of recipient autonomy in retrotransfer. *Genetics* **143**, 1425–1435.

Hill, K. E., Weightman, A. J. & Fry, J. C. (1992). Isolation and screening of plasmids from the epilithon which mobilize recombinant plasmid pD10. *Appl Environ Microbiol* **58**, 1292–1300.

Hill, K. E., Fry, J. C., Weightman, A. J., Day, M. J., Bradley, D. J. & Cousland, B. (1995). Retrotransfer of IncP1-like plasmids from aquatic bacteria. *Lett Appl Microbiol* **20**, 317–322.

Kletsova, L. V. & Tsygankov, Y. D. (1990). Mapping of *pgi* and *gpd* genes involved in C-1 assimilation in the obligate methylotroph *Methylobacillus flagellatum*. *Arch Microbiol* **153**, 139–145.

Lejeune, P., Mergeay, M., Van Gijsegem, F., Faelen, M., Gerits, J. & Toussaint, A. (1983). Chromosome transfer and R-prime plasmid formation mediated by plasmid pULB113 (RP4 mini-Mu) in *Alcaligenes eutrophus* CH34 and *Pseudomonas fluorescens* 6.2. *J Bacteriol* **155**, 1015–1026.

McClure, N. C., Fry, J. C. & Weightman, A. J. (1990). Gene transfer in activated sludge. In *Bacterial Genetics in Natural Environments*, pp. 111–132. Edited by J. C. Fry & M. J. Day. New York: Chapman & Hall.

McPherson, P. & Gealt, M. A. (1986). Isolation of indigenous wastewater bacterial strains capable of mobilizing plasmid pBR325. *Appl Environ Microbiol* **51**, 904–909.

Mergeay, M., Lejeune, P., Gerits, J. & Fabry, L. (1984). RP4::miniMu (pULB113) a genetic tool to study bacterial genomes: application to *Pseudomonas fluorescens* 6.2. *Antonie Leeuwenhoek* **50**, 297–298.

Mergeay, M., Lejeune, P., Thiry, G. & Faelen, M. (1985). Back transfer: a property of some broad host range plasmids. In *Plasmids in Bacteria*, p. 942. Edited by D. Helinski & others. New York: Plenum.

Mergeay, M., Lejeune, P., Sadouk, A., Gerits, J. & Fabry, L. (1987). Shuttle transfer (or retrotransfer) of chromosomal markers mediated by plasmid pULB113. *Mol Gen Genet* **209**, 61–70.

Mergeay, M., Springael, D. & Top, E. (1990). Gene transfer in polluted soils. In *Bacterial Genetics in Natural Environments*, pp. 152–171. Edited by J. C. Fry & M. J. Day. New York: Chapman & Hall.

Perkins, C. D., Davidson, A. M., Day, M. J. & Fry, J. C. (1994). Retrotransfer kinetics of R300B by pQKH6, a conjugative plasmid from river epilithon. *FEMS Microbiol Ecol* **15**, 33–44.

Powell, B., Mergeay, M. & Christofi, N. (1989). Transfer of broad host range plasmids to sulphate-reducing bacteria. *FEMS Microbiol Lett* **59**, 269–274.

Ramos-Gonzalez, M. I., Ramos-Diaz, M. A. & Ramos, J. L. (1994). Chromosomal gene capture mediated by the *Pseudomonas putida* TOL catabolic plasmid. *J Bacteriol* **176**, 4635–4641.

Ravatn, R., Zehnder, A. J. B. & van der Meer, J. R. (1998). Low-frequency horizontal transfer of an element containing the chlorocatechol degradation genes from *Pseudomonas putida* F1 and to indigenous bacteria in laboratory-scale activated-sludge microcosms. *Appl Environ Microbiol* **64**, 2126–2132.

Read, T., Thomas, A. & Wilkins, B. (1992). Evasion of type I and type II restriction systems by IncI1 plasmid ColIb-P9 during transfer by bacterial conjugation. *Mol Microbiol* **6**, 1933–1941.

Schoonejans, E. & Toussaint, A. (1983). Utilization of plasmid pULB113 (RP4::miniMu) to construct a linkage map of *Erwinia carotovora* subsp. *chrysanthemi*. *J Bacteriol* **154**, 1489–1492.

Sia, E. A., Kuehner, D. M. & Figurski, D. H. (1996). Mechanism of retrotransfer in conjugation: prior transfer of the conjugative plasmid is required. *J Bacteriol* **178**, 1457–1464.

Simon, R., Priefer, U. & Pühler, A. (1983). A broad host range mobilisation system for *in vivo* genetic engineering: transposon mutagenesis in gram-negative bacteria. *Bio/Technology* **1**, 784–791.

Smit, E. D., Venne, D. & Van Elsas, J. D. (1993). Mobilization of a recombinant IncQ plasmid between bacteria on agar and in soil via cotransfer or retrotransfer. *Appl Environ Microbiol* **59**, 2257–2263.

Thiry, G., Mergeay, M. & Faelen, M. (1984). Back mobilization of Tra⁻ Mob⁻ plasmids mediated by various IncM, IncN and IncP1 plasmids. *Arch Int Physiol Biochim* **92**, 6465.

Top, E. (1993). *Horizontal Gene Transfer in the Environment and the Significance of Broad Host Range Plasmids*. PhD thesis, University of Gent, Belgium.

Top, E., Mergeay, M., Springael, D. & Verstraete, W. (1990). Gene escape model: transfer of heavy metal resistance genes from *Escherichia coli* to *Alcaligenes eutrophus* on agar plates and in soil samples. *Appl Environ Microbiol* **56**, 2471–2479.

Top, E., Mergeay, M. & Springael, D. (1991). Retrotransfer: a specific trait of certain broad-host-range plasmids (which allows the host to pick up new genes from other species and genera). *Plasmid* **25**, 238.

Top, E., Vanrolleghem, P., Mergeay, M. & Verstraete, W. (1992). Determination of the mechanism of retrotransfer by mechanistic mathematical modelling. *J Bacteriol* **174**, 5953–5960.

Top, E., De Smet, I., Mergeay, M. & Verstraete, W. (1994). Exogenous isolation of mobilizing plasmids from polluted soils and sludge. *Appl Environ Microbiol* **60**, 831–839.

Top, E., De Rore, H., Collard, J. M., Gellens, V., Slobodkina, G., Verstraete, W. & Mergeay, M. (1995a). Retromobilization of heavy metal resistance genes in unpolluted and heavy metal polluted soil. *FEMS Microbiol Ecol* **18**, 191–203.

Top, E., Holben, W. E. & Forney, L. J. (1995b). Characterization of diverse 2,4-dichlorophenoxyacetic acid-degradative plasmids isolated from soil by complementation. *Appl Environ Microbiol* **61**, 1691–1698.

Top, E., Maltseva, O. V. & Forney, L. J. (1996). Capture of a catabolic plasmid that encodes only 2,4-dichlorophenoxyacetic acid:α-ketoglutaric acid dioxygenase (TfdA) by genetic complementation. *Appl Environ Microbiol* **62**, 2470–2476.

Top, E., Van Daele, P., De Saeyer, N. & Forney, L. J. (1998). Enhancement of 2,4-dichlorophenoxyacetic acid (2,4-D) degradation in soil by dissemination of catabolic plasmids. *Antonie Leeuwenhoek* **73**, 87–94.

Van Gijsegem, F. & Toussaint, A. (1982). Chromosome transfer and R-prime formation by an RP4 mini-Mu derivative in *Escherichia coli*, *Salmonella typhimurium*, *Klebsiella pneumoniae* and *Proteus mirabilis*. *Plasmid* **7**, 30–44.

Wilkins, B. & Lanka, E. (1993). DNA processing and replication during plasmid transfer between Gram-negative bacteria. In *Bacterial Conjugation*, pp. 105–137. Edited by D. B. Clewell. New York: Plenum.

Wlodarczyk, M. & Piechucka, E. (1995). Conjugal transfer of plasmid and chromosomal markers between strains of *Thiobacillus versutus*. *Curr Microbiol* **30**, 185–191.

Woese, C. R. (1987). Bacterial evolution. *Microbiol Rev* **51**, 221–271.

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