Conjugative transfer of *tet(S)* between strains of *Enterococcus faecalis* is associated with the exchange of large fragments of chromosomal DNA

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The tetracycline resistance determinant *tet(S)* was first detected in antibiotic multiresistant *Listeria monocytogenes* BM4210 and subsequently in strains of *Enterococcus faecalis*. Transfer of *tet(S)* from clinical isolate *E. faecalis* BM4242 to *E. faecalis* strains JH2-2 and OG1RF was found to require the presence in the donor strain of the 55 kb conjugative plasmid pIP825. Comparison of restriction endonuclease generated maps of the donor, the two recipients, and of four transconjugants indicated that transfer of *tet(S)* (i) was from chromosome to chromosome, (ii) resulted in the acquisition of an approximately 40 kb element in the same chromosomal region and (iii) was associated with the exchange of large chromosomal fragments. Similar observations were made following conjugal transfer of *tet(S)* from four other *E. faecalis* clinical isolates.

Keywords: *Enterococcus faecalis*, chromosomal mobilization, conjugation, tetracycline resistance

INTRODUCTION

The first multiresistant *Listeria monocytogenes* was isolated from a patient in 1988 (Poyart-Salmeron *et al.*, 1990). This strain was resistant to tetracycline, chloramphenicol, erythromycin and streptomycin. All the resistance genes were carried by the self-transferable plasmid pIP811. Resistance to tetracycline was mediated by a new determinant, *tet(S)*, encoding a protein closely related to Tet(M), which acts by ribosomal protection (Charpentier *et al.*, 1993). It was speculated that pIP811 could originate in enterococci since it was closely related to the prototype enterococcal plasmid pAM β 1 and since its stability and transfer efficiency were higher in enterococci than in other Gram-positive bacterial genera (Poyart-Salmeron *et al.*, 1990). Indeed, eight *Enterococcus faecalis* clinical isolates were found later to harbour *tet(S)* and were able to transfer this determinant by conjugation to other *E. faecalis* strains (Charpentier *et al.*, 1994). Four of these isolates were studied further and found to harbour plasmids that did not hybridize with the *tet(S)* probe, suggesting that the resistance gene

was located in the chromosome. In Gram-positive bacteria, tetracycline resistance genes are borne by a variety of mobile genetic elements including transposons, both conjugative and non-conjugative, and self-transferable plasmids (Salyers *et al.*, 1990, 1995; Courvalin & Carrier, 1986; Bedzyk *et al.*, 1992).

We have investigated the transfer of *tet(S)* from *E. faecalis* BM4242. The presence of a conjugative plasmid, pIP825, was found to be required for conjugative transfer of *tet(S)* to occur although pIP825 did not harbour *tet(S)*. Analysis of the transferred DNA using rare-cutting enzymes and PFGE confirmed that *tet(S)* was transferred from chromosome to chromosome. Comparison of restriction endonuclease generated maps of the donor, recipients and transconjugants revealed that acquisition by the recipient of an approximately 40 kb *tet(S)* element was associated with the exchange of large pieces of chromosome.

METHODS

Bacterial strains, media and antibiotic susceptibility testing.

The strains used in conjugation experiments are listed in Table 1. Bacteria were grown at 37 °C in brain heart infusion (BHI) broth and agar (Difco), except for *Bacillus subtilis* which was grown on Mueller–Hinton agar (Sanofi Diagnostics

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Abbreviation: ZIFE, zero-integrated-field electrophoresis.

Table 1. Bacterial strains

Antibiotics: Cm, chloramphenicol; Em, erythromycin; Fu, fusidic acid; Rif, rifampicin; Sm, streptomycin; Sp, spectinomycin; Tc, tetracycline. Rec⁻, recombination-deficient.

Strain	Resistance phenotype	Plasmid present	Source (reference)
<i>E. faecalis</i>			
BM4242	Tc	pIP825	Wild strain (Charpentier <i>et al.</i> , 1994)
BM4333	Fu Rif Tc	pIP825	BM4242 × JH2-2
BM4334	Fu Rif Tc	No	Plasmid-cured derivative of BM4333
BM4335	Fu Rif Tc	pIP825	BM4242 × JH2-2
BM4336	Fu Rif Tc	No	BM4242 × OG1RF
BM4337	Fu Rif Tc	No	BM4242 × OG1RF
BM4338	Fu Rif Tc	pIP825	BM4242 × OG1RF
BM4236	Cm Em Sm Tc	Yes	Wild strain (Charpentier <i>et al.</i> , 1994)
BM4239	Cm Sm Tc	Yes	Wild strain (Charpentier <i>et al.</i> , 1994)
BM4240	Tc	Yes	Wild strain (Charpentier <i>et al.</i> , 1994)
BM4241	Tc	Yes	Wild strain (Charpentier <i>et al.</i> , 1994)
JH2-2	Fu Rif	No	Spontaneous mutant of JH2 (Jacob <i>et al.</i> , 1974)
JH2-SmSp	Sm Sp	No	Spontaneous mutant of JH2 (Jacob <i>et al.</i> , 1974)
OG1RF	Fu Rif	No	Spontaneous mutant of OG1 (Murray <i>et al.</i> , 1993)
UV202	Fu Rif	No	Rec ⁻ derivative of JH2-2 (Yagi & Clewell, 1980)
<i>E. faecium</i>			
BM4107	Sm	No	Spontaneous mutant of BM4105 (Leclercq <i>et al.</i> , 1989)
<i>L. monocytogenes</i>			
L017RF	Fu Rif	No	Spontaneous mutant of L017 (Perez-Diaz <i>et al.</i> , 1982)
LSm	Sm	No	Spontaneous mutant of L. EGD (Gaillard <i>et al.</i> , 1986)
<i>B. subtilis</i>			
BM4150	Sm	No	QB166 derivative (Courvalin & Carlier, 1986)

Pasteur). Susceptibility tests were performed on BHI agar for the clinical isolates and on Mueller–Hinton agar for the other strains by the disk-agar diffusion test according to the recommendations of the Comité de l'Antibiogramme de la Société Française de Microbiologie (Courvalin & Soussy, 1996).

Filter mating experiments. Filter mating was performed as described by Poyart-Salmeron *et al.* (1990) and Charpentier *et al.* (1994). The transfer frequencies were expressed as the number of transconjugants per donor after the mating period.

DNA manipulations. Plasmid and total DNA was prepared as described by Trieu-Cuot *et al.* (1988) and Leclercq *et al.* (1992), respectively. Total high-molecular-mass DNA was prepared in agarose blocks (Murray *et al.*, 1990). Forty microlitres of plugs were digested overnight with 40 U *Sfi*I or *Sma*I or 100 U *Not*I. Southern hybridizations were carried out under conditions of high stringency (Sambrook *et al.*, 1989). Probes were labelled with [α -³²P]dCTP by random priming using a commercially available kit (Amersham).

PFGE. The software-assisted zero-integrated-field electrophoresis (ZIFE) apparatus (Auto Base, Q-Life System) was used.

Preparation of a BM4242 genomic library in bacteriophage λ . Total DNA of BM4242 was digested partially with *Sau*3A and ligated into λ FixII according to the manufacturer's recommendations (Stratagene). The ligation mixture was packaged *in vitro* using Gigapack II packaging extracts (Stratagene). The bacteriophages were transduced into *Escherichia coli* XL-1 Blue MRA (P2).

Construction of probes. The probe used to screen for the presence of pIP825 was a 7 kb *Hind*III fragment of a λ clone from the BM4242 genomic library that hybridized with pIP825 DNA. To prepare the F_{OG} probe, total DNA from *E. faecalis* OG1RF digested with *Not*I was resolved by ZIFE in low-melting agarose, the 350 kb F_{OG} fragment was cut from the gel, digested with *Hind*III and DNA was extracted from agarose as described by Murray *et al.* (1993). A similar procedure was used to obtain the *Not*I J_{JH} fragment of JH2-2 and the *Sma*I Sm-1 fragment of OG1RF. To prepare BF1, BF2, and BF3 probes, λ DNA was extracted using a rapid procedure (Grossberger, 1987). The 1443 bp NADH peroxidase (nucleotides 624–2066, Ross & Claiborne, 1991) and 560 bp NADH oxidase fragments (nucleotides 500–1060, Ross & Claiborne, 1992) were generated by PCR.

Plasmid curing. Twenty-five microlitres of an overnight culture of BM4333 were diluted in 10 ml BHI broth containing ethidium bromide at a subinhibitory concentration (8 mg ml⁻¹) and incubated at 37 °C for 24 h. Bacteria were plated on BHI agar and isolated colonies were screened by hybridization with the pIP825 probe.

RESULTS AND DISCUSSION

Intra- and intergeneric conjugal transfer of tet(S) from *E. faecalis* BM4242

The ability of clinical isolate BM4242 to transfer *tet*(S) to other Gram-positive bacteria was studied by filter mating. Two *E. faecalis* recipients devoid of plasmids

Table 2. Transfer frequencies of *tet(S)*

Frequencies of transfer are expressed as the number of transconjugants per donor after a filter mating period of 18 h.

Strain	Donor		Recipient	Transfer frequency
	Origin	Plasmid		
BM4242	Wild isolate	pIP825	OG1RF	1×10^{-9}
BM4338	BM4242 $\times$ OG1RF transconjugant	pIP825	JH2-SmSp	1×10^{-9}
BM4336	BM4242 $\times$ OG1RF transconjugant	None	JH2-SmSp	$< 10^{-10}$
BM4337	BM4242 $\times$ OG1RF transconjugant	None	JH2-SmSp	$< 10^{-10}$
BM4242	Wild isolate	pIP825	JH2-2	5×10^{-7}
BM4333	BM4242 $\times$ JH2-2 transconjugant	pIP825	JH2-SmSp	1×10^{-7}
BM4335	BM4242 $\times$ JH2-2 transconjugant	pIP825	JH2-SmSp	1×10^{-6}
BM4334	Plasmid-cured BM4333 derivative	None	JH2-SmSp	$< 10^{-10}$
BM4242	Wild isolate	pIP825	UV202	1×10^{-9}

Fig. 1. Analysis of *Sfi*I-digested total DNA by ZIFE. Conditions were optimized to separate DNA fragments in the 100–1100 kb size range (ROM card 5). Strains in lanes: 1, BM4242; 2, JH2-2; 3, BM4333; 4, BM4335; 5, OG1RF; 6, BM4336; 7, BM4337. (a) 0.8% agarose gel. (b) Southern hybridization with the *tet(S)* probe.

were used: OG1RF, whose chromosomal physical map has been established (Murray *et al.*, 1993), and JH2-2 (Jacob & Hobbs, 1974) (Table 1). Strain BM4242 transferred *tet(S)* at frequencies of approximately 5×10^{-7} to JH2-2 and 1×10^{-9} to OG1RF (Table 2). This variation in transfer frequency could be related to differences in the genetic background of the two recipients used, although both were *E. faecalis*. Four transconjugants, the two JH2-2 derivatives BM4333 and BM4335 and the two OG1RF derivatives BM4336 and

BM4337, were selected for DNA analysis. The *tet(S)* determinant could not be transferred by conjugation (frequency $< 10^{-10}$) from BM4242 to *L. monocytogenes* L017RF and LSm, *B. subtilis* BM4150, or *Enterococcus faecium* BM4107 (Table 1).

Involvement of plasmid pIP825 in the transfer of *tet(S)*

E. faecalis BM4242 harboured plasmid pIP825 with a size of approximately 55 kb, as determined by agarose gel electrophoresis after *Hind*III or *Eco*RI digestion of purified plasmid DNA (data not shown). A probe internal to *tet(S)* (Charpentier *et al.*, 1993) did not hybridize with pIP825 DNA (data not shown). pIP825 was present in four transconjugants obtained with JH2-2 (including BM4333 and BM4335) but in only two of four OG1RF transconjugants (including BM4338). To assess a putative role for this plasmid in *tet(S)* transfer, re-transfer experiments were carried out using the transconjugants as donors and *E. faecalis* JH2-SmSp as a recipient (Table 2). The *tet(S)* determinant conjugated from JH2-2-derived BM4333 and BM4335 transconjugants that harboured pIP825 at frequencies ranging from 10^{-6} to 10^{-7} , which was similar to that observed for transfer from BM4242 to JH2-2. From the OG1RF-derived BM4338 transconjugant, which also contained pIP825, *tet(S)* transferred at a frequency of 10^{-9} , similar to that for transfer from BM4242 to OG1RF. Based on analysis of plasmid content and dot blot hybridization, the two OG1RF-derived transconjugants BM4336 and BM4337 did not harbour pIP825 and could not re-transfer tetracycline resistance (frequency $< 10^{-10}$) in three independent experiments. Similarly, *tet(S)* could not be re-transferred from BM4334, a plasmid-cured derivative of the JH2-2 transconjugant BM4333 (frequency $< 10^{-10}$) (Table 2). Taken together these data suggest that pIP825 was required for conjugal transfer of *tet(S)*. In transconjugants BM4333, BM4335 and BM4338, the *Eco*RI and *Hind*III restriction profiles of

Fig. 2. Analysis of *SmaI*-digested total DNA by ZIFE. Electrophoresis conditions were optimized to separate DNA fragments in the 8–500 kb size range (ROM card 3). Strains in lanes: 1, BM4242; 2, JH2-2; 3, BM4333; 4, BM4335; 5, OG1RF; 6, BM4336; 7, BM4337. (a) 0.8% agarose gel. (b) and (c) Southern hybridization with the *tet(S)* (b) and the Sm-1 (c) probe.

pip825 DNA remained unchanged compared to that in BM4242 (data not shown), indicating that *tet(S)* was not plasmid-borne in the transconjugants.

***tet(S)* is carried by an approximately 40 kb element and is transferred from chromosome to chromosome**

There are five *SfiI* recognition sites in the chromosome of OG1RF (Murray *et al.*, 1990), six in that of JH2-2 (Quintiliani & Courvalin, 1994) and seven in that of BM4242 (Fig. 1a). The *SfiI* restriction profiles of the four transconjugants were indistinguishable from those of the respective recipient (Fig. 1a). A *tet(S)* probe hybridized with the largest fragment, of approximately 1200 kb, in all transconjugants and in the BM4242 donor (Fig. 1). By contrast, hybridization of a pip825 probe with BM4242, BM4333 and BM4335 DNA was confined to the well, suggesting that there is no *SfiI* site in pip825 DNA. These results indicate that *tet(S)* is present in the chromosome of the donor and of the transconjugants.

The *SmaI* restriction profiles of the OG1RF recipient differed from those of transconjugants BM4336 and

BM4337: a 340 kb fragment, designated Sm-1, was replaced by a 380 kb fragment which hybridized with a probe made from Sm-1 DNA and with the *tet(S)* probe (Fig. 2). This indicates that *tet(S)* was transferred from chromosome to chromosome as part of an approximately 40 kb fragment. Results of hybridization of *SmaI* restricted DNA from JH2-2 and JH2-2-derived transconjugants BM4333 and BM4335 with the Sm-1 probe indicated that the size of the corresponding fragments was 280 kb in the recipient and 320 kb in the transconjugants and that the latter also hybridized with the *tet(S)* probe (Fig. 2). The Sm-1 and *tet(S)* probes also hybridized with a 320 kb *SmaI* fragment in the BM4242 donor (Fig. 2). These results confirm that *tet(S)* was transferred from chromosome to chromosome as part of a 40 kb element and indicate that, in the transconjugants, insertion occurred in the same chromosomal *SmaI* fragment. Since *SmaI* cuts *E. faecalis* DNA in more than 20 fragments that are difficult to resolve, we performed a similar analysis using *NotI* restriction endonuclease.

Analysis of the *NotI*-generated profiles indicated that

Fig. 3. Analysis of *NotI*-digested total DNA by ZIFE. Electrophoresis conditions were optimized to separate DNA fragments in the 8–500 kb size range (ROM card 3). Strains in lanes: 1, BM4242; 2, JH2-2; 3, BM4333; 4, BM4335; 5, OG1RF; 6, BM4336; 7, BM4337. The size marker used is bacteriophage λ DNA concatamers (λ). (a) 0.8% agarose gel. (b) and (c) Southern hybridization with the *tet(S)* (b) and *npr* (c) probes.

the chromosomal rearrangements in the transconjugants were more complex than the mere insertion of a 40 kb fragment (Figs 3a and 4, Tables 3 and 4). The *NotI* restriction map of OG1RF has been established by Murray *et al.* (1993). In OG1RF-derived transconjugants BM4336 and BM4337, insertion of the 40 kb *tet(S)* element was found to occur in the 350 kb *NotI* fragment, designated F_{OG} in OG1RF. This fragment carries the NADH peroxidase gene (*npr*) (Murray *et al.*, 1993). In the OG1RF- and JH2-2-derived transconjugants, an *npr*-specific probe and the *tet(S)* probe co-hybridized, confirming that insertion of *tet(S)* had occurred in the same chromosomal region (Fig. 3).

Comparison of the physical maps of the chromosomal regions flanking the insertion site of the *tet(S)* element in OG1RF, JH2-2 and BM4242

By repeated probing of Southern blots of *NotI*-, *SmaI*- and *NotI*- plus *SmaI*-digested total DNA of OG1RF, JH2-2 and BM4242 resolved by ZIFE, we could establish the physical map of a 700 kb chromosomal region surrounding the insertion site of the *tet(S)* element (Fig. 5). The *NotI* F_{OG} fragment (350 kb) hybridized with a 310 kb *NotI* fragment in JH2-2 (designated F_{JH}) and with two BM4242 *NotI* fragments of 160 ($F1_{BM}$) and 180 kb ($F2_{BM}$). The *npr* probe hybridized with fragments F_{JH} and $F1_{BM}$.

The size of fragment G was 180 kb in OG1RF (G_{OG}), 160 kb in JH2-2 (G_{JH}) and 170 kb (G_{BM}) in BM4242.

The inserts of two recombinant λ bacteriophages BF1 and BF2, part of a genomic library from BM4242, were shown to originate from G_{BM} (data not shown). Both hybridized with the *Sm*-1 fragment in OG1RF, with a 280 kb *SmaI* fragment in JH2-2 and a 320 kb *SmaI* fragment in BM4242 (data not shown). This confirmed the homology between fragments G_{OG} , G_{JH} and G_{BM} . The *nox* probe, which hybridized with fragment J_{OG} of OG1RF, also hybridized with *NotI* fragments of 200 kb from JH2-2 (J_{JH}) and 180 kb (J_{BM}) from BM4242 (data not shown). Similar hybridization patterns were obtained using the J_{JH} fragment as a probe. The insert of λ BF3, containing a *NotI* site, hybridized with *NotI* fragments I_{OG} and J_{OG} from OG1RF, with fragments of 200 kb (J_{JH}), 30 kb (H_{JH}) and 16 kb (I_{JH}) from JH2-2, and of 180 kb (J_{BM}) and 53 kb (I_{BM}) from BM4242 (data not shown). Following *SmaI* restriction, BF3 hybridized with *SmaI* fragments of 105 kb from OG1RF, 100 kb from JH2-2 and 145 kb from BM4242 (data not shown). Taken together, these results indicate a similar organization of this chromosomal region in *E. faecalis* OG1RF, JH2-2 and BM4242 (Fig. 5).

Comparison of the *NotI* maps of the donor, recipients and transconjugants reveals exchange of chromosomal DNA adjacent to the *tet(S)* element

As already discussed, insertion of the *tet(S)* element in the transconjugants occurred in the F_{OG} (BM4336 and BM4337) or F_{JH} (BM4333 and BM4335) *NotI* fragments. However, in transconjugants BM4335 and BM4336, one

Fig. 4. Analysis of *NotI*-digested total DNA by ZIFE on a 0.8% agarose gel. Electrophoresis conditions were optimized to separate DNA fragments in the 8–200 kb size range (ROM card 2). Strains in lanes: 1, BM4242; 2, JH2-2; 3, BM4333; 4, BM4335; 5, OG1RF; 6, BM4336; 7, BM4337. The size markers used are: λ , bacteriophage- λ DNA concatamers, and R, Raoul (Appligene).

in each recipient, the *npr*-hybridizing fragment of the recipient (F_{OG} of 350 kb in OG1RF and F_{JH} of 310 kb in JH2-2) was replaced by two fragments of respectively

220 and 160 kb in BM4336 and of 180 and 160 kb in BM4335 (Fig. 3). The new fragments hybridized with the F_{OG} probe but only the 160 kb fragment hybridized with the *npr* and the *tet(S)* probes, as did the $F1_{BM}$ fragment from the donor (Tables 3, 4 and Fig. 5). This implies that the DNA acquired possesses a *NotI* restriction site or, less likely, that a *NotI* site was generated upon insertion. In the two remaining transconjugants BM4333 and BM4337, modifications occurred in other *NotI* fragments than the one hybridizing with the F_{OG} probe. In BM4333 the 200, 160, 30 and 16 kb fragments of the recipient were replaced by fragments of 180, 170 and 53 kb (Figs 3 and 4). These fragments hybridized with the *nox* and the J_{JH} probes for the fragment of 180 kb, the BF1 and BF2 probes for the fragment of 170 kb and the BF3 probe for the fragment of 53 kb. For all these probes the length of the *NotI* restriction fragments was similar to that found for the BM4242 donor but differed to that found for the JH2-2 recipient (Table 4). In BM4337 the 29- and 30 kb *NotI* fragments of the recipient were replaced by a fragment of 53 kb (Figs 3 and 4). The latter hybridized with the BF3 probe, as was the case with the BM4242 donor (Table 3).

Since the size of certain *NotI* fragments in the transconjugants was found to be similar to that in the donor but differed from that in the recipient, this suggested the exchange of large pieces of chromosome. The origin of the DNA was studied by analysis of *EcoRI* and *HindIII* fragment length polymorphism (RFLP) (data not shown).

In the OG1RF-derived transconjugant BM4336, the *tet(S)* and *npr* probes hybridized with a *NotI* fragment with a size of 160 kb similar to that of fragment $F1_{BM}$ (Fig. 3, Table 3). A second 220 kb fragment, that hybridized with F_{OG} but not with the *tet(S)* probe, was also present. The size of this fragment differed from that of $F2_{BM}$ (180 kb), implying that one of the junctions of

Table 3. Comparison of *NotI* RFLP

Only differently sized fragments in the recipient and in the transconjugants are indicated. The fragments that hybridize with the probes in the donor, the recipient and the transconjugants are indicated.

Donor BM4242	Size of <i>NotI</i> fragments (kb)		Fragments hybridizing with:
	Recipient OG1RF	Transconjugants	
		BM4336 BM4337	
			390
	350		<i>tet(S)</i> , <i>npr</i> , F_{OG}
		220	<i>npr</i> , F_{OG}
180			F_{OG}
160		160	F_{OG}
53			<i>tet(S)</i> , <i>npr</i> , F_{OG}
		53	BF3
	30	30	BF3
	29	29	BF3

Table 4. Comparison of *NotI* RFLP

Only differently sized fragments in the recipient and in the transconjugants are indicated. The fragments that hybridize with the probes in the donor, the recipient and the transconjugants are indicated.

Donor BM4242	Size of <i>NotI</i> fragments (kb)		Fragments hybridizing with:
	Recipient JH2-2	Transconjugants	
		BM4333 BM4335	
		350	<i>tet(S)</i> , <i>npr</i> , F _{OG}
	310		<i>npr</i> , F _{OG}
	200	200	<i>nox</i> , J _{JH} , BF3
181		181	F _{OG}
180		180	<i>nox</i> , J _{JH} , BF3
170		170	BF1, BF2
	161	161	BF1, BF2
160		160	<i>tet(S)</i> , <i>npr</i> , F _{OG}
53		53	BF3
	30	30	BF3
	16	16	BF3

Fig. 5. *NotI* and *SmaI* restriction maps of the chromosomal regions flanking the insertion site of *tet(S)* in *E. faecalis* OG1RF, JH2-2 and BM4242. The maps were established by hybridization with the markers first localized on the physical map of OG1RF (*npr*, *nox*, λ clones BF1, BF2 and BF3) and with fragments F_{OG} and J_{JH}. The relative order of cloned fragments BF1 and BF2 in the *NotI* and *SmaI* fragments is not known and is arbitrarily represented.

the transferred DNA was within this fragment (Fig. 6). The RFLP obtained with the λ BF1, λ BF2, λ BF3, and *nox* probes and *NotI*-, *HindIII*- or *EcoRI*-restricted BM4336 DNA were similar to that of the OG1RF recipient (data not shown). These results indicate that the DNA acquired by BM4336 contained a *NotI* site and that its borders were within the F fragment (Fig. 6). In the second OG1RF-derived transconjugant, BM4337, the size of the F-hybridizing fragment (390 kb) corresponded to that in the recipient (350 kb) plus 40 kb corresponding to the acquisition of the *tet(S)* element (Fig. 3, Table 3). Since the RFLP observed with the λ BF1

OG1RF-derived transconjugants

JH2-2-derived transconjugants

Fig. 6. Schematic representation of transferred DNA. N, *NotI* site. The size of the *NotI* fragments is indicated in parentheses. ■, DNA from the BM4242 donor. □, DNA from OG1RF or JH2-2 recipient. ■, 40 kb element carrying *tet(S)*. The *npr*, BF1, BF2, BF3 and *nox* probes used in *HindIII* and *EcoRI* RFLP studies are indicated in bold when the profile is similar to that of the donor and in non-bold when similar to that of the recipient. The *npr* probe is not indicated for BM4336 and BM4337 since there was no difference between the *HindIII* and *EcoRI* profiles of the donor and the recipient. The relative order of cloned fragments BF1 and BF2 in the *NotI* restriction fragment is not known and is arbitrarily represented.

and λ BF2 probes were respectively similar to those in the recipient (data not shown) and since the acquired DNA did not contain a *NotI* site, we hypothesize that

limits were within the F fragment, upstream from the *NotI* site that separates F1 and F2. Surprisingly, the RFLP obtained with the λ BF3 probe was similar to that of BM4242, indicating that a second DNA exchange took place (Fig. 6).

More precise information on the extent of DNA transferred was obtained by the analysis of the JH2-2-derived transconjugants. In BM4333, the size of the F-hybridizing fragment corresponded to that of F_{JH} with a 40 kb increase, indicating that one limit of the acquired DNA lay inside $F1_{BM}$ (Table 4). However, the RFLP obtained with the *npr*, λ BF1, λ BF2, λ BF3 and *nox* probes were similar to those in the donor (data not shown), implying an exchange of homologous DNA between the donor and the recipient. This suggests that a chromosomal fragment encompassing more than 400 kb, with one junction in fragment F and the other beyond fragment J, was transferred (Fig. 6).

In BM4335, the size of the two F-hybridizing fragments was similar to that of $F1_{BM}$ and $F2_{BM}$ in the donor (Table 4). In contrast, the RFLP with the *npr*, λ BF1, λ BF2, λ BF3 and *nox* probes was similar to that of the recipient (data not shown). These data suggest that the acquired DNA extended from within F1 to beyond F2 (Fig. 6).

In the BM4333, BM4335, BM4336 and BM4337 transconjugants one border of the transferred DNA lay inside the first 160 kb of fragment F. This border, when analysed by using rare-cutting endonucleases, was found to carry *tet(S)* (Fig. 6). The second border was located inside the F fragment in the OG1RF-derived transconjugants but outside in the JH2-2-derived transconjugants (more than 400 kb in BM4333 and more than 180 kb in BM4335) (Fig. 6). In the JH2-2-derived transconjugants, two markers provided information on the region flanking the insertion site: study of RFLP with the *npr* probe and *HindIII*- or *EcoRI*-digested DNA and the presence of a *NotI* site inside fragment F, characteristic of DNA originating in the donor. Analysis of these markers showed that the RFLP with the *npr* probe was similar to that in the donor when the transferred DNA extended towards fragment J (as in BM4333), and that, in contrast, the additional *NotI* site inside fragment F was present when the transferred DNA extended towards F2 (as in BM4335) (Fig. 6). Combined with the RFLP observed with the λ BF1, λ BF2, λ BF3 and *nox* probes, this indicated that one border of the transferred DNA was located between the *npr* gene and the *NotI* site that separates $F1_{BM}$ and $F2_{BM}$ and that the *tet(S)* element was adjacent to this border. In OG1RF-derived transconjugants, hybridization patterns of the *npr* probe after *HindIII* or *EcoRI* digestion did not show any polymorphism between the donor and the recipient.

Since in every transconjugant homologous DNA adjacent to the *tet(S)* element was exchanged, we used as a recipient UV202, a recombination-deficient derivative of JH2-2 (Yagi & Clewell, 1980), to study the involvement of homologous recombination in the DNA transfer. The

tet(S) determinant transferred from BM4242 to UV202 at a frequency of 10^{-9} , as opposed to 5×10^{-7} to JH2-2 (Table 2). This is in contrast with the results obtained with conjugative transposon Tn916 which transfers at similar frequencies to both strains (Franke & Clewell, 1981). These results suggest that homologous recombination is involved in the acquisition of DNA by the recipients. Analysis of the restriction profiles obtained with *SmaI* and *NotI* restriction enzymes in two UV202-derived transconjugants revealed acquisition of the 40 kb *tet(S)* element in chromosomal F fragment (data not shown). As observed with JH2-2, the extent of DNA transferred differed in the two transconjugants since one of them acquired an additional *NotI* site and the other displayed modifications in the *NotI* physical map outside the F fragment (data not shown).

Transfer of *tet(S)* from other *E. faecalis* isolates

E. faecalis BM4236, BM4239, BM4240 and BM4241 that could transfer *tet(S)* by conjugation at frequencies ranging from 10^{-4} to 10^{-7} were analysed by ZIFE after *SfiI* and *NotI* digestion (data not shown). All the strains appeared different and contained plasmid DNA that hybridized with the pIP825 probe. Analysis of *NotI*- and *SmaI*-generated restriction profiles of total DNA, and hybridization patterns with the *tet(S)* and *npr* probes were carried out on every isolate and on a transconjugant after mating with JH2-2 (data not shown). In the four transconjugants, insertion of a 40 kb fragment was detected in *NotI* fragment F_{JH} and in two of them, the acquired DNA contained an additional *NotI* site. In the donors and corresponding transconjugants, the *tet(S)* and *npr* probes hybridized to *NotI* and *SmaI* fragments of similar size, confirming that insertion of *tet(S)* occurred in the same chromosomal region. These results suggest that these wild strains conjugate *tet(S)* by a mechanism similar to that of BM4242.

Conjugation is likely to be responsible for the transfer of *tet(S)* among *E. faecalis* strains, since the presence of pIP825 was required for transfer to take place. In Gram-positive bacteria, mobilization of chromosomal DNA has been obtained via conjugative transposons or conjugative plasmids (Torres *et al.*, 1991; Stout & Iandolo, 1990). Chromosomal mobilization by conjugative transposon Tn925 has been shown in *B. subtilis* (Torres *et al.*, 1991). In *E. faecalis*, evidence of conjugal transfer of several chromosomal determinants by plasmids pAD1, pAMy1 and pOB1 has been reported (Franke *et al.*, 1978). In *Staphylococcus aureus*, mobilization of the chromosome has been obtained with a conjugative plasmid carrying transposon Tn551, which was also present in the chromosome (Stout & Iandolo, 1990). In the mobilization of chromosomal DNA from BM4242, pIP825 could act by two types of mechanisms: mobilization *in cis*, implying formation of a co-integrate between a plasmid and the chromosome, or *in trans*, which could imply the presence of an origin of transfer (*oriT*) in the transferred DNA (Reimann & Haas, 1993). We favour the former hypothesis since most examples of

chromosomal mobilization reported in Gram-negative bacteria occur *in cis* (Reimann & Haas, 1993) and, to the best of our knowledge, chromosomal mobilization *in trans* was reported only by genetic manipulation, using Tn5 and mini-Mu derivatives carrying *oriT_{REK2}* (Yakobson & Guiney, 1984; Groisman & Casadaban, 1986). Formation of co-integrate is well-documented in Gram-negative Hfr bacteria (Lloyd & Buchman, 1995). The recombination sites between the F factor and the chromosome are insertion sequences (Umeda & Ohtsubo, 1989), and such sequences might also be present in the *tet(S)* element. Chromosomal integration of the transferred DNA in the recipient is likely to involve homologous recombination since there is a significant decrease in transfer frequency in the *rec⁻* derivative of *E. faecalis* JH2-2. Moreover homologous recombination could account for the restricted host range of *tet(S)* transfer. Further characterization of the *tet(S)* element will be of interest to characterize the type of movement involved in the transfer of tetracycline resistance.

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