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Presence of the *Listeria* Tetracycline Resistance Gene *tet*(S) in *Enterococcus faecalis*

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Two hundred thirty-eight tetracycline- and minocycline-resistant clinical isolates of *Enterococcus* and *Streptococcus* spp. were investigated by dot blot hybridization for the presence of nucleotide sequences related to *tet*(S) (first detected in *Listeria monocytogenes* BM4210), *tet*(K), *tet*(L), *tet*(M), *tet*(O), *tet*(P), and *tet*(Q) genes. The *tet*(S) determinant was found in 22 strains of *Enterococcus faecalis*, associated with *tet*(M) in 9 of these isolates and further associated with *tet*(L) in 3 of these strains. *tet*(M) was detected in all strains of *Streptococcus* spp. and in all but 10 isolates of *Enterococcus* spp.; *tet*(L) was found in 93 enterococci and *tet*(O) was found in single isolates of *E. faecalis* and *Streptococcus milleri*. No hybridization with the *tet*(K), *tet*(P), and *tet*(Q) probes was observed. Transfer of *tet*(S) by conjugation to *E. faecalis* or to *E. faecalis* and *L. monocytogenes* was obtained from 8 of the 10 *E. faecalis* strains harboring only this *tet* gene. Hybridization experiments with DNAs of four donors and of the corresponding transconjugants suggested that *tet*(S) was located in the chromosome. These results indicate that the genetic support of *tet*(S) in *E. faecalis* is different from that in *L. monocytogenes*, where it is carried by self-transferable plasmids, and confirm the notion of exchange of genetic information between *Enterococcus* and *Listeria* spp. in nature.

Extensive clinical use of tetracyclines has led to an increase in organisms resistant to these antibiotics in a wide range of gram-positive and gram-negative bacterial species (17). A minimum of 15 hybridization classes of tetracycline resistance determinants that can be assigned to three groups on the basis of the biochemical mechanism of resistance have been described (6). The first group mediates energy-dependent efflux of tetracycline from the cells and includes 10 classes of genes: *tet*(A) to *tet*(E) (33), *tet*(K) (15), *tet*(G) (36), *tet*(H) (12), *tet*(L) (13), and *tet*(P) (31). The second mechanism confers tetracycline and minocycline resistance by ribosomal protection and comprises four gene classes: *tet*(M) (18), *tet*(O) (32), *tet*(Q) (20), and *tet*(S) (5). Recently, a third mechanism, chemical modification of tetracycline, has been discovered in the genus *Bacteroides* and the resistance gene has been designated *tet*(X) (33).

Active efflux seems to be confined to gram-negative bacteria and to a few gram-positive species, whereas ribosomal protection is, by far, the most common tetracycline resistance mechanism in nature (17, 33). Genes *tet*(K) and *tet*(L) have been detected only in gram-positive bacteria (2, 3, 9, 25, 27, 28, 37). The *tet*(P) determinant, which is constituted of two overlapping genes, *tetA*(P) and *tetB*(P), has been found only in *Clostridium* spp. (29, 31). Originally discovered in *Streptococcus agalactiae* (4), *tet*(M) has been subsequently detected in a large variety of aerobic and anaerobic gram-positive and gram-negative bacterial genera, including *Enterococcus* (2, 27, 37), *Streptococcus* (27, 37), *Peptostreptococcus* (26, 27), *Staphylococcus* (3), *Listeria* (9, 25), *Clostridium* (11, 19), *Neisseria* (16), *Kingella* (16), *Gardnerella* (27), *Haemophilus* (26), and *Bacteroides* (1), and in cell wall-less organisms such as *Mycoplasma* and *Ureaplasma* spp. (26). It is the most widely disseminated antibiotic resistance determinant. The *tet*(O) gene, initially found in the gram-negative genus *Campylobacter* (32, 35), was later detected in the gram-positive *Enterococcus* (2, 37), *Streptococcus* (27, 37), *Peptostreptococcus* (27), *Lactobacillus* (27), and *Mobiluncus* spp. (28). So far, the *tet*(Q) gene is apparently confined to *Bacteroides* spp. (20, 33).

We recently determined the sequence of a gene belonging to a new class, *tet*(S), carried by the self-transferable plasmid pIP811 in *Listeria monocytogenes* BM4210 (5). The Tet(S) protein shares 79 and 72% amino acid identities with Tet(M) and Tet(O), respectively, and is more distantly related to Tet(Q) (40% identity). In previous studies, it has been proposed that emergence of multiple-antibiotic resistance in *L. monocytogenes* BM4210 was secondary to acquisition of a replicon originating in the *Enterococcus-Streptococcus* genera (22). Demonstration of a direct exchange of genetic information between *Enterococcus faecalis* and *L. monocytogenes* in the digestive tract of gnotobiotic mice (8) and detection in *L. monocytogenes* of *tet*(M) associated with *int-Tn*, the gene encoding the protein required for the movements of enterococcal conjugative transposons, constitute additional support for this suggestion (25). The *tet*(S) gene was detected in two other clinical isolates of *L. monocytogenes* isolated in France and Switzerland (5) but its occurrence in enterococci and streptococci has not been reported.

To gain an insight into the origin of *tet*(S), we searched for the presence of this gene in tetracycline-minocycline-resistant clinical isolates of *Enterococcus* and *Streptococcus* spp. by DNA-DNA hybridization using a specific probe. In addition, we investigated these strains for the presence of *tet*(K), *tet*(L), *tet*(M), and *tet*(O), already characterized in enterococci and streptococci. Probes specific for *tetA*(P), *tetB*(P), *tet*(Q), and *int-Tn* were also included in this study.

MATERIALS AND METHODS

Bacterial strains. A total of 229 enterococcal and 9 streptococcal clinical isolates resistant to tetracycline and minocycline were collected in 10 hospitals in France. The strains were

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isolated in different geographical locations between 1986 and 1993. Enterococci harboring *tet*(S) were identified by Gram staining, the absence of catalase, the inability to produce gas, the presence of Lancefield antigen group D, and growth in 40% bile, in 6.5% sodium chloride, in 0.1% methylene blue, and at pH 9.6. Species identification (10) was based on the following tests: potassium tellurite reduction, pigmentation, motility at 30°C, and biochemical tests in API 50 CH galleries (bioMérieux, Marcy-l'Etoile, France). *L. monocytogenes* L017 RF (21) and *E. faecalis* JH2-2 (14), both resistant to fusidic acid and rifampin, were used as recipients in conjugation experiments.

Media, culture conditions, and determination of in vitro susceptibility to antibiotics. The bacterial strains were grown in brain heart infusion broth and agar (Difco Laboratories, Detroit, Mich.). Antibiotic susceptibility was tested by diffusion on Mueller-Hinton agar with disks impregnated with antibiotics (Sanofi Diagnostics Pasteur, Marnes-La-Coquette, France). The MICs of antibiotics were determined on brain heart infusion agar with an inoculum of 10^4 CFU per spot by the method of Steers et al. (34). All incubations were done at 37°C.

Genetic techniques. Mating on filters was performed as described earlier (22), and transfer frequencies were expressed as the number of transconjugants per donor CFU after the mating period. The antibiotics and concentrations used for selection of transcipients were as follows: fusidic acid, 10 μg/ml; rifampin, 20 μg/ml; and tetracycline, 5 μg/ml.

Preparation of DNA. Total DNAs from *Enterococcus* and *Streptococcus* spp. were prepared as described previously (25). The presence of DNA was monitored by agarose gel electrophoresis. Aliquots (10 μl) of the crude extracts were denatured for 10 min at 100°C before being spotted onto Nytran membranes (Schleicher & Schuell, Dassel, Germany). Purification of plasmid DNA from *Escherichia coli* (30) and *E. faecalis* (7) was performed by ultracentrifugation in cesium chloride-ethidium bromide as described previously.

Construction of probes and DNA-DNA hybridization. The DNA fragments to be used as probes for hybridization experiments were the 870-bp *Hin*cII fragment of pT181 for *tet*(K) (15), the 310-bp *Cla*I-*Hpa*II fragment of pBC16 for *tet*(L) (13), the 850-bp *Cla*I-*Hin*dIII fragment of Tn*1545* for *tet*(M) (18), the 1,458-bp *Hin*dIII-*Nde*I fragment of pIP1433 for *tet*(O) (32), the 900-bp *Sph*I-*Eco*RI fragment of pCW3 for *tetA*(P) (31), the 1,100-bp *Pst*I-*Eco*RI fragment of pCW3 for *tetB*(P) (31), the 900-bp *Eco*RI-*Eco*RV fragment of pNFD13-2 for *tet*(Q) (20), the 590-bp fragment of pIP811 for *tet*(S) (5), and the 830-bp *Taq*I fragment of Tn*1545* for *int-Tn* (23). Restriction endonuclease-generated fragments were separated by electrophoresis in 0.8% low-melting-temperature agarose type VII (Sigma Chemical Co., St. Louis, Mo.) as described earlier (30) and extracted from the gel by using a commercially available kit (Gene Clean II; Bio 101, Inc., La Jolla, Calif.). The purified fragments were labeled in vitro by nick translation (30) with [α-^{32}P]dCTP. For Southern hybridization, restriction fragments obtained from digestion of plasmid and total DNAs with *Eco*RI endonuclease were separated by 0.8% agarose gel electrophoresis and transferred to a Nytran membrane (Schleicher & Schuell) (30). Dot blot and Southern hybridizations under stringent conditions were performed as follows: prehybridization and hybridization at 68°C for 5 and 18 h, respectively, in 6× SSC (1× SSC is 0.15 M sodium chloride plus 0.015 M sodium citrate) containing 0.5% sodium dodecyl sulfate (SDS) and 0.05% nonfat dry milk. Membranes were washed in 2× SSC–0.1% SDS at room temperature for 30 min each, and in 0.2× SSC–0.1% SDS at 68°C for 45 min. In dot blot experiments, self-hybridization of the probe and hybridization of the probe with total DNA from *E. faecalis* JH2-2 were included as positive and negative controls, respectively.

TABLE 1. Distribution of strains of *Enterococcus* and *Streptococcus* spp. according to their susceptibilities to tetracycline and minocycline and hybridization with *tet* probes

Group or species (no. of strains)	Hybridization with *tet* probes[a]	MIC range (μg/ml) of:	
		Tetracycline	Minocycline
E. faecalis JH2-2	None	1	0.125
Enterococcus spp. (117)	*tet*(M)	64–256[b]	32[b]
Streptococcus group B (7)	*tet*(M)	32–256	16–32
Streptococcus sanguis (1)	*tet*(M)	256	32
Enterococcus spp. (89)	*tet*(L) + *tet*(M)	128–256[c]	16–32[c]
S. milleri (1)	*tet*(M) + *tet*(O)	128	16
E. faecalis (1)	*tet*(L) + *tet*(M) + *tet*(O)	256	32
E. faecalis (10)	*tet*(S)	128–256	32–64
E. faecalis (9)	*tet*(M) + *tet*(S)	128–256	32–64
E. faecalis (3)	*tet*(L) + *tet*(M) + *tet*(S)	128–256	32–64

[a] No hybridization was observed with *tet*(K), *tetA*(P), *tetB*(P), and *tet*(Q) probes.
[b] MICs for 5 of the 117 isolates were determined.
[c] MICs for 3 of the 89 isolates were determined.

Enzymes and chemicals. Restriction endonucleases (Pharmacia Biotech S.A., Saint-Quentin-Yvelines, France) were used according to the recommendations of the manufacturer. Lysozyme was obtained from Sigma Chemical Co. The nick translation reagent kit was obtained from Bethesda Research Laboratories, Inc. (Gaithersburg, Md.). [α-^{32}P]dCTP was purchased from the Amersham Radiochemical Center (Amersham, England). The Raoul DNA molecular weight marker was obtained from Appligene (Illkirch, France). The following antibiotics were provided by the indicated laboratories: chloramphenicol and erythromycin, Roussel-Uclaf (Romainville, France); fusidic acid, Leo (Montigny-Le-Bretonneux, France); minocycline, Lederle (Oullins, France); rifampin, Sigma Chemical Co.; streptomycin, Diamant (Puteaux, France); tetracycline, Rhône-Poulenc Rorer (Vitry-sur-Seine, France).

RESULTS AND DISCUSSION

Properties of the strains studied. The antibiotic susceptibilities of the 229 enterococcal and 9 streptococcal clinical isolates were determined by disk agar diffusion. All the strains were resistant to tetracycline and minocycline and appeared unrelated since they had different antibiotic resistance phenotypes and were isolated in various hospitals in France and at different times between 1986 and 1993.

Distribution of the *tet* determinants. The presence of sequences related to *tet*(K), *tet*(L), *tet*(M), *tet*(O), *tetA*(P), *tetB*(P), *tet*(Q), *tet*(S), and *int-Tn* was determined by dot blot hybridization under high-stringency conditions by using intragenic probes (Table 1). Total DNAs from 117 (51%) enterococcal and 8 (89%) streptococcal isolates displayed homology with the *tet*(M) probe. Sequences related to *int-Tn* were also observed in 105 (90%) and 7 (88%) of these isolates, respectively. Hybridization of both *tet*(L) and *tet*(M) was detected with total DNAs from 89 (39%) enterococcal isolates. In 69 (78%) of these strains, homology with the *int-Tn* probe was also found. DNA from a *Streptococcus milleri* isolate hybridized with *tet*(M), *int-Tn*, and *tet*(O); and that from an *E. faecalis* isolate showed homology with *tet*(L), *tet*(M), and *tet*(O). Ho-

TABLE 2. Properties of *E. faecalis* strains containing *tet*(S)

Strain designation	*tet* determinant(s) in donor	Resistance phenotype[a]	Tc-Mc resistance transferable to[b]:		*tet* gene transferred
			L017	JH2-2	
BM4234	*tet*(S)	Cm Em Sm Tc Mc	+	+	*tet*(S)
BM4235			−	−	−
BM4236			+	+	*tet*(S)
BM4237	*tet*(S)	Cm Sm Tc Mc	−	+	*tet*(S)
BM4238	*tet*(S)	Sm Tc Mc	−	−	−
BM4239			−	+	*tet*(S)
BM4240	*tet*(S)	Tc Mc	−	+	*tet*(S)
BM4241			−	+	*tet*(S)
BM4242			−	+	*tet*(S)
BM4243			−	+	*tet*(S)
BM4244	*tet*(M) + *tet*(S)	Cm Em Sm Tc Mc	+	+	*tet*(M)
BM4245			+	+	*tet*(M)
BM4246			+	+	*tet*(M)
BM4247			+	+	*tet*(M)
BM4248	*tet*(M) + *tet*(S)	Cm Em Tc Mc	+	+	*tet*(M)
BM4249	*tet*(M) + *tet*(S)	Sm Tc Mc	+	−	*tet*(M)
BM4250			+	−	*tet*(M)
BM4251			+	+	*tet*(M)
BM4252	*tet*(M) + *tet*(S)	Tc Mc	−	+	*tet*(M)
BM4253	*tet*(L) + *tet*(M) + *tet*(S)	Cm Em Sm Tc Mc	+	+	*tet*(M)
BM4254			+	+	*tet*(M)
BM4255			+	+	*tet*(M)

[a] Abbreviations: Cm, chloramphenicol resistance; Em, erythromycin resistance; Mc, minocycline resistance; Sm, streptomycin resistance; Tc, tetracycline resistance.
[b] +, positive transfer; −, negative transfer.

mology with the *tet*(S) probe was observed in 22 (10%) *E. faecalis* isolates. In nine (41%) of these strains, *tet*(S) was associated with *tet*(M) and *int-Tn*, while in three (14%) strains it was also associated with *tet*(L). The finding of the *tet*(S) gene in strains of *E. faecalis* constitutes additional support for the notion that plasmid pIP811 from *L. monocytogenes* BM4210 originates in enterococci (22). None of the strains of *Enterococcus* and *Streptococcus* studied displayed homology with the *tet*(K), *tetA*(P), *tetB*(P), and *tet*(Q) probes. Susceptible *E. faecalis* JH2-2 did not hybridize with the probes tested.

Among the strains tested, the *tet*(L) and *tet*(M) genes were widespread, *tet*(O) was rare, and *tet*(K) was absent. The *tet*(K) gene is widely disseminated in *Staphylococcus* spp. (3), rarely detected in *Listeria* spp. (9), and rarely encountered in *Enterococcus-Streptococcus* spp. isolates from Europe (37), whereas it is common in the United States (27). *tet*(L) [which, like *tet*(K), confers resistance to tetracycline but not to minocycline] was found associated with *tet*(M) and, in rare cases, with *tet*(M) and *tet*(O) or *tet*(M) and *tet*(S). In contrast to *tet*(K), the *tet*(L) gene is more common among enterococci and streptococci (37) than among *Staphylococcus* spp. (3) or *L. monocytogenes* (9, 25). The distributions of *tet*(M) and *tet*(O) among gram-positive bacteria differed: the *tet*(M) gene was found, alone or associated with other *tet* genes, in all of the nine streptococcal isolates and in 219 (96%) of the enterococcal isolates analyzed, whereas *tet*(O) was detected associated with other *tet* genes in single isolates of *E. faecalis* and of *S. milleri*. The *tet*(M) gene is common among enterococci and streptococci, where it is part of broad-host-range conjugative transposons and is thus usually associated with *int-Tn*, the gene encoding the integrase of these elements (23, 24). In contrast, *tet*(O) is rarely encountered in these genera, where it is carried by conjugative plasmids (33, 35, 37) or occasionally present in the chromosome of *Streptococcus* spp. (28). In this study, DNAs of 33 of the 219 *Enterococcus* strains and of one of the nine *Streptococcus* strains harboring *tet*(M) alone or associated with other *tet* determinants did not hybridize with the *int-Tn* probe. It is noteworthy that in the genus *Clostridium* *tet*(M) is carried by at least two types of unrelated conjugative transposons. One type shares extensive homology with Tn*916* (11), but the other does not (19). Lack of association of *tet*(M) with *int-Tn* could be due to deletions in conjugative transposons or to the existence of genetic support(s) other than Tn*916*-related elements for this resistance determinant in gram-positive cocci. The *tet*(P) and *tet*(Q) genes were not detected in the strains tested. To date, these genes have been detected only in *Clostridium* spp. (29, 31) and *Bacteroides* spp. (20), respectively. The finding of *tet*(K), *tet*(L), and *tet*(M) in *Listeria* spp. (9, 25) as well as the finding of the *tet*(S) gene in *E. faecalis* (in this study) confirms the notion of easy genetic exchange between gram-positive bacteria under natural conditions. Since DNAs of all the isolates studied hybridized with probes specific for the *tet* genes already described for gram-positive bacteria, we did not detect a strain harboring alone a tetracycline resistance gene not yet characterized.

Susceptibility to tetracycline and minocycline. The MICs of tetracycline and minocycline for 40 strains of *Enterococcus* and *Streptococcus* spp. representative of the various *tet* gene combinations were determined (Table 1). The MICs of tetracycline (32 to 256 μg/ml) and minocycline (16 to 64 μg/ml) were similar for all strains irrespective of their *tet* gene content. No cooperativity of the resistance determinants was observed, as opposed to results in previous studies of *Staphylococcus aureus* (3) and *Enterococcus-Streptococcus* spp. (37).

Antibiotic resistance phenotypes of *E. faecalis* strains harboring *tet*(S). The 22 enterococcus isolates harboring the *tet*(S) determinant, alone or associated with *tet*(L), *tet*(M), and *int-Tn*, were specified as *E. faecalis*. Plasmid pIP811 also

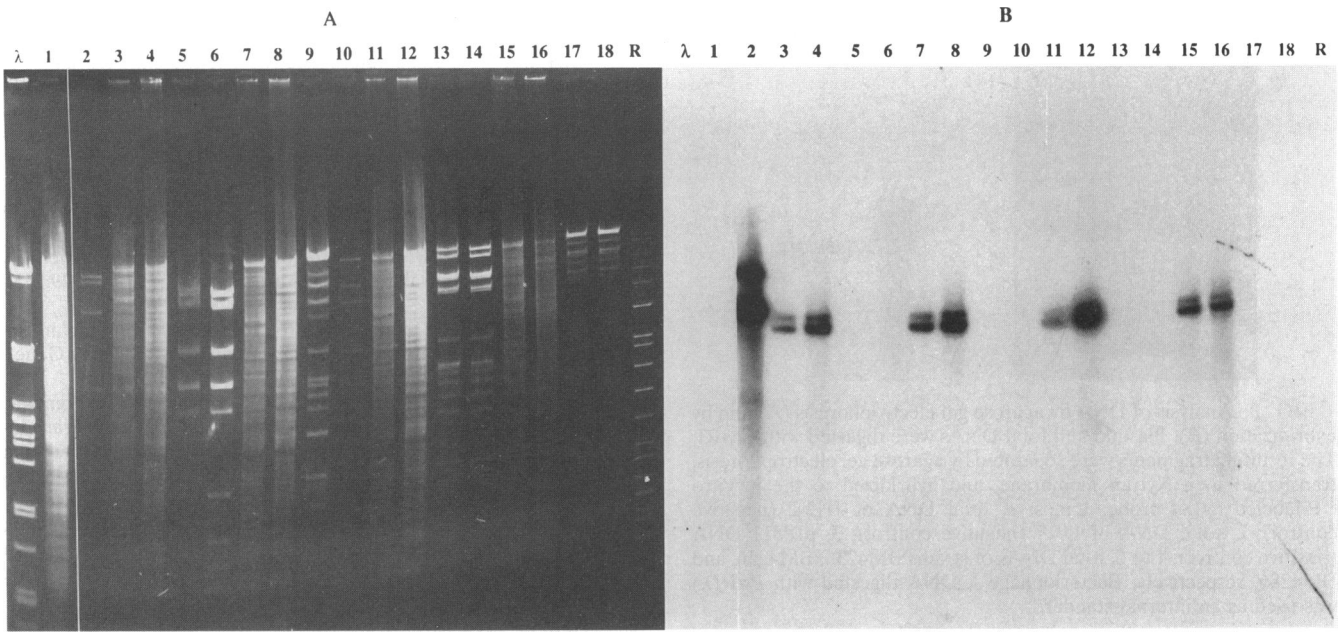

FIG. 1. Analysis of DNA by agarose gel electrophoresis (A) and by hybridization (B). Plasmid and total DNAs were digested with *Eco*RI. The resulting fragments were separated by agarose gel electrophoresis, transferred to a Nytran membrane, and hybridized to the in vitro ^{32}P-labeled *tet*(S) probe. Lanes: 1, total DNA of JH2-2 (negative control); 2, pIP811 DNA (positive control); 3, 4, 7, 8, 11, 12, 15, and 16, total DNAs of strains BM4236, BM4256, BM4237, BM4257, BM4239, BM4258, BM4243, and BM4259, respectively; 5, 6, 9, 10, 13, 14, 17, and 18, plasmid DNAs of strains BM4236, BM4256, BM4237, BM4257, BM4239, BM4258, BM4243, and BM4259, respectively. Bacteriophage λ DNA digested with *Pst*I (λ) and Raoul DNA molecular weight marker (R) served as internal standards.

confers resistance to chloramphenicol, erythromycin, and streptomycin (22), and these isolates can be assigned to five groups according to their resistance to these antibiotics in addition to tetracycline-minocycline (Table 2). One group consisted of 10 strains that were resistant to chloramphenicol, erythromycin, and streptomycin and contained *tet*(S) alone or associated with *tet*(M) and *int-Tn* or with *tet*(M), *int-Tn*, and *tet*(L). A strain harboring *tet*(S) was resistant to chloramphenicol and streptomycin. One isolate was resistant to chloramphenicol and erythromycin and contained the *tet*(M), *int-Tn*, and *tet*(S) genes. Another group was composed of five strains resistant to streptomycin that carried *tet*(S) alone or in association with *tet*(M) and *int-Tn*. The last group consisted of five isolates harboring *tet*(S), alone or with *tet*(M) and *int-Tn*, that were tetracycline-minocycline resistant only.

Filter mating experiments. Transfer of tetracycline resistance from the 22 *E. faecalis* strains harboring *tet*(S) was studied by filter mating. The strains fell into three groups according to the combinations of *tet* genes they harbored and to their resistance phenotypes (Table 2). Twenty strains transferred tetracycline-minocycline resistance to one of the plasmid-free strains *L. monocytogenes* L017 and *E. faecalis* JH2-2, or to both (Table 2), at frequencies ranging from 10^{-4} to 10^{-9}. Selection for transfer of tetracycline resistance did not reveal cotransfer of any other resistance. The conjugation frequencies from *E. faecalis* containing *tet*(S) alone or associated with other *tet* determinants to *E. faecalis* JH2-2 and to *L. monocytogenes* L017 were similar. The MICs of tetracycline and minocycline for *E. faecalis* and *L. monocytogenes* transconjugants were similar to those for the wild strains (data not shown).

Among the nine strains harboring *tet*(S), *tet*(M), and *int-Tn*, six transferred tetracycline-minocycline resistance to both *L. monocytogenes* and *E. faecalis*, two transferred resistance to *L. monocytogenes* only, and one isolate transferred resistance to *E. faecalis* but not to *L. monocytogenes* ($<10^{-9}$). The three isolates containing *tet*(S) associated with *tet*(L), *tet*(M), and *int-Tn* transferred resistance to both *L. monocytogenes* and *E. faecalis*. Total DNAs extracted from the 21 transconjugants hybridized with *tet*(M) and *int-Tn* probes but not to *tet*(S). The acquisition of *tet*(M) and *int-Tn* but not of *tet*(S) by these transconjugants suggests that *tet*(M) was transferred more efficaciously than *tet*(S).

Of the 10 isolates containing *tet*(S) alone, 2 strains transferred tetracycline-minocycline resistance to both *L. monocytogenes* and *E. faecalis* and 6 strains transferred resistance to *E. faecalis* only (Table 2). Transfer from the two remaining strains to either recipient was not detected in three independent experiments. In the 10 transconjugants obtained, acquisition of tetracycline resistance correlated with acquisition of the *tet*(S) gene, as revealed by dot blot hybridization. Four donors (BM 4236, BM4237, BM4239, and BM4243) and the corresponding *E. faecalis* transconjugants (BM4256, BM4257, BM4258, and BM4259) were selected for further studies. Plasmid and total DNAs of these strains were purified, digested with *Eco*RI, and analyzed by agarose gel electrophoresis and by Southern hybridization with a *tet*(S) intragenic probe (Fig. 1). Comparative analysis revealed that the *Eco*RI-generated restriction profiles of plasmid DNA of a donor and of the corresponding transconjugant were indistinguishable but that they differed between the various donor-transconjugant pairs. The plasmids that varied from approximately 40 to 160 kb did not hybridize with the *tet*(S) probe. In contrast, two restriction fragments of total DNA of similar size in all the donors and the *E. faecalis* transconjugants hybridized with the probe, suggesting that *tet*(S) was located in the chromosome. The *tet*(S) fragment used as a probe contains an *Eco*RI site (5), and the presence in each strain of two hybridizing fragments indicated that a single copy of the resistance determinant was present. Total DNAs

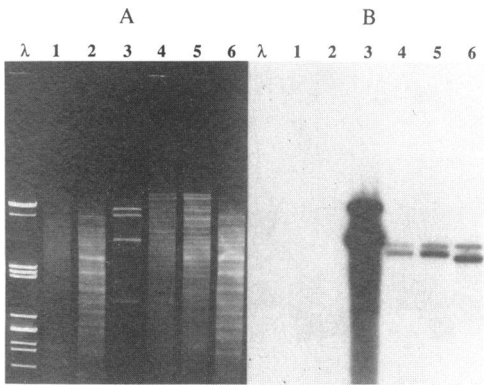

FIG. 2. Analysis of DNA by agarose gel electrophoresis (A) and by hybridization (B). Plasmid and total DNAs were digested with *Eco*RI. The resulting fragments were separated by agarose gel electrophoresis, transferred to a Nytran membrane, and hybridized to the in vitro ^{32}P-labeled *tet*(S) probe. Lanes: 1, total DNA of JH2-2 (negative control); 2, total DNA of L017 (negative control); 3, pIP811 DNA (positive control); 4 to 6, total DNAs of strains BM4236, BM4256, and BM4286, respectively. Bacteriophage λ DNA digested with *Pst*I (λ) was used as an internal standard.

from *E. faecalis* BM4236 and from the corresponding *E. faecalis* BM4256 and *L. monocytogenes* BM4286 transconjugants were analyzed similarly (Fig. 2). One of the two hybridizing fragments in BM4286 differed in size from those in BM4236 and in BM4256, confirming that transfer of *tet*(S) was not due to transfer of a replicon en bloc. The stability of tetracycline resistance in BM4236, BM4256, and BM4286 was assessed by replica plating after growth for 100 generations in antibiotic free broth. All the clones tested (approximately 300 colonies of each strain) were found to be resistant, a finding which is compatible with a chromosomal rather than a plasmid location of *tet*(S). Further studies are required to characterize the putative mobile genetic element(s) carrying this resistance determinant. The sizes of the two hybridizing restriction fragments in the various donors and transconjugants were different from those of the two bands of pIP811, suggesting that the genetic environment of *tet*(S) in *E. faecalis* studied here and in *L. monocytogenes* BM4210 (5) differed.

L. monocytogenes is a frequent inhabitant of the gastrointestinal tracts of humans and animals, where various *Enterococcus-Streptococcus* species harboring conjugative resistance plasmids and transposons are also common. It has thus been proposed that the intestinal ecosystem is the most probable site for genetic transfer between these bacterial genera (8). Our findings constitute further support that emergence of antibiotic resistance in *Listeria* spp. represents an extension of the gene pool of enterococci and streptococci.

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