Molecular Characterization of *tet*(M) Genes in *Lactobacillus* Isolates from Different Types of Fermented Dry Sausage

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The likelihood that products prepared from raw meat and milk may act as vehicles for antibiotic-resistant bacteria is currently of great concern in food safety issues. In this study, a collection of 94 tetracycline-resistant (Tc^r) lactic acid bacteria recovered from nine different fermented dry sausage types were subjected to a polyphasic molecular study with the aim of characterizing the host organisms and the *tet* genes, conferring tetracycline resistance, that they carry. With the (GTG)$_5$-PCR DNA fingerprinting technique, the Tc^r lactic acid bacterial isolates were identified as *Lactobacillus plantarum*, *L. sakei* subsp. *carnosus*, *L. sakei* subsp. *sakei*, *L. curvatus*, and *L. alimentarius* and typed to the intraspecies level. For a selection of 24 Tc^r lactic acid bacterial isolates displaying unique (GTG)$_5$-PCR fingerprints, *tet* genes were determined by means of PCR, and only *tet*(M) was detected. Restriction enzyme analysis with *Acc*I and *Sca*I revealed two different *tet*(M) allele types. This grouping was confirmed by partial sequencing of the *tet*(M) open reading frame, which indicated that the two allele types displayed high sequence similarities (>99.6%) with *tet*(M) genes previously reported in *Staphylococcus aureus* MRSA 101 and in *Neisseria meningitidis*, respectively. Southern hybridization with plasmid profiles revealed that the isolates contained *tet*(M)-carrying plasmids. In addition to the *tet*(M) gene, one isolate also contained an *erm*(B) gene on a different plasmid from the one encoding the tetracycline resistance. Furthermore, it was also shown by PCR that the *tet*(M) genes were not located on transposons of the Tn*916*/Tn*1545* family. To our knowledge, this is the first detailed molecular study demonstrating that taxonomically and genotypically diverse *Lactobacillus* strains from different types of fermented meat products can be a host for plasmid-borne *tet* genes.

For several decades, studies on the selection and dissemination of antibiotic resistance have focused mainly on clinically relevant bacterial species. More recently, many investigators speculated that commensal bacteria may act as reservoirs of antibiotic resistance genes similar to those found in human pathogens (30; S. B. Levy and A. A. Salyers, Reservoirs of Antibiotic Resistance [ROAR] Network, http://www.healthsci.tufts.edu/apua/Roar/roarhome.htm, 2002). Such commensal reservoir bacteria can be present in the intestines of farmed animals exposed to antibiotics (2, 38) and may subsequently contaminate the raw meat produced from these animals even when hygiene regulations are respected. Several examples of antibiotic-resistant lactic acid bacteria isolated from raw meat exist (20, 26, 28, 37). Fermented foods prepared from raw meat or milk can therefore be considered potential vehicles for the spread of antibiotic-resistant lactic acid bacteria along the food chain to the consumer (35).

Genes conferring resistance to tetracycline, chloramphenicol, erythromycin, and vancomycin have been detected and characterized in *Lactococcus lactis* (27) and enterococci (15, 35) isolated from fermented meat and milk products. In contrast, no molecular data are available on the occurrence of antibiotic resistance genes in lactobacilli present in fermented food products. Members of the genus *Lactobacillus* also constitute an important part of the natural microflora associated with fermented products and are indigenous to the animal and human gastrointestinal tract. These properties make lactobacilli, along with enterococci, interesting indicator organisms with which to study the molecular ecology of antibiotic resistance determinants in food fermentation industries.

Previously, we isolated tetracycline-resistant (Tc^r) strains of various *Lactobacillus* species from different types of modified atmosphere-packed fermented dry sausage (FDS) sold in Belgian retail shops (13). All *Lactobacillus* isolates collected in this way were resistant to at least 64 μg of tetracycline per ml as determined with the de Man, Rogosa, and Sharpe (MRS) agar dilution method (13). The aim of the present investigation was to perform a molecular characterization of the *tet* genes conferring the high-level phenotypic resistance to tetracycline in these isolates. For this purpose, an extended collection of Tc^r *Lactobacillus* isolates was subjected to (GTG)$_5$-PCR fingerprinting (14) to reveal their taxonomic identity and genotypic diversity. Based on these results, a selected subset of different strain types were characterized further to assess the identity, the heterogeneity, and the locations of their *tet* genes.

MATERIALS AND METHODS

Bacterial isolates. In this study, a total of 94 Tc^r lactic acid bacterial isolates recovered from 14 batches of nine different FDS types were included. A type is defined here as a specific product variety distributed under a specific commercial brand. Each type is designated with a number (FDS-01, FDS-02, FDS-06, FDS-07, FDS-08, FDS-09, FDS-11, FDS-12, and FDS-14). A batch is defined as a group of sausages belonging to the same type that were produced at one time and is indicated with a letter from A to E.

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TABLE 1. Primers for PCR detection of *tet, erm,* and *int* genes

Primer pair	Gene(s) targeted	Sequence[a]	Annealing temp (°C)	Amplicon size (bp)	Positive control strain and reference	Reference for primers
DI DII	RPP	5′-GAYACNCCNGGNCAYRTNGAYTT-3′ 5′-GCCCARWANGGRTTNGGNGGNACYTC-3′	45	1,083	pJI3 (22)	7
DI TetM-R	*tet*(M)	5′-GAYACNCCNGGNCAYRTNGAYTT-3′ 5′-CACCGAGCAGGGATTTCTCCAC-3′	55	1,513	pJI3 (22)	7
TetS-FWT 1 TetS-RVT 2	*tet*(S)	5′-ATCAAGATATTAAGGAC-3′ 5′-TTCTCTATGTGGTAATC-3′	55	573	pVP2 (27)	5
TetO-FW 1 TetO-RV 1	*tet*(O)	5′-AATGAAGATTCCGACAATTT-3′ 5′-CTCATGCGTTGTAGTATTCCA-3′	55	781	pAT121 (J. M. Collard, personal communication)	32
TetK-FW 1 TetK-RV 1	*tet*(K)	5′-TTATGGTGGTTGTAGCTAGAAA-3′ 5′-AAAGGGTTAGAAACTCTTGAAA-3′	55	348	pAT102 (P. Courvalin, personal communication)	J. M. Collard, personal communication
TetL-FW 3 TetL-RV 3	*tet*(L)	5′-GTMGTTGCGCGCTATATTCC-3′ 5′-GTGAAMGRWAGCCCACCTAA-3′	55	696	pAT103 (P. Courvalin, personal communication)	J. M. Collard, personal communication
Int-FW Int-RV	*int*	5′-GCGTGATTGTATCTCACT-3′ 5′-GACGCTCCTGTTGCTTCT-3′	50	1,028	Tn*1545* (P. Courvalin, personal communication)	9
ErmB-FW ErmB-RV	*erm*(B)	5′-CATTTAACGACGAAACTGGC-3′ 5′-GGAACATCTGTGGTATGGCG-3′	55	405	Tn*1545* (P. Courvalin, personal communication)	19

[a] N = A, C, G, and T; R = A and G; W = A and T; Y = C and T.

The isolation of 52 Tc^r lactic acid bacterial isolates was reported before (13). This collection was extended with 42 new Tc^r lactic acid bacterial isolates recovered similarly from other batches of fermented dry sausage end products. Isolates were recovered on the basis of colony morphology rather than relative abundance in order to obtain a set of isolates with the highest diversity. All these fermented dry sausages were prepared with a starter culture. All isolates were stored in a bead storage system (Microbank system; Pro-LAB Diagnostics, Wirral, United Kingdom) at −80°C and grown on MRS agar (Difco Co., Detroit, Mich.) at 30°C under microaerophilic conditions (3.75% CO_2–5% O_2–7.5% H_2–83.75% N_2).

(GTG)$_5$-PCR fingerprinting. All Tc^r lactic acid bacterial isolates were identified at the species level and typed at the intraspecies level with high-resolution rep-PCR fingerprinting with the (GTG)$_5$ primer as previously described (14).

Antibiotic susceptibility testing and MIC determination. A modified version of the Kirby-Bauer disk diffusion method (4a), in which Mueller-Hinton medium was replaced with MRS agar, was used for antibiotic sensitivity testing. Oxoid (Basingstoke, United Kingdom) susceptibility test disks of ampicillin (25 µg), chloramphenicol (30 µg), clindamycin (10 µg), erythromycin (10 µg), penicillin G (10 U), rifampin (30 µg), and tetracycline (30 µg) were applied to inoculated MRS plates with the Oxoid disk dispenser. The diameters of the inhibition zones were measured with a digital caliper (digital 2; Mauser, Ludwigsburg, Germany) following 16 to 18 h of incubation of the antibiograms at 30°C. For each of the antibiotics tested, classification of the isolates into sensitive and resistant groups was based on resistance histograms (i.e., number of strains versus size of the inhibition zone). Cutoff values to differentiate among resistant and susceptible groups were defined on the basis of the bimodal distribution of the population in the resistance histograms.

The MIC of tetracycline was determined by applying an Etest strip (AB Biodisk) on an inoculated MRS plate according to the manufacturer's instructions. The Etest strip was read following 16 to 18 h of incubation at 30°C.

DNA preparation and manipulations. Total genomic DNA from each isolate was extracted and purified as described previously (14). Isolation of plasmid DNA was based on the alkaline lysis method of Anderson and McKay (3). Restriction endonuclease digestions of the *tet*(M) gene, agarose gel electrophoresis, and Southern blotting were carried out by standard procedures (31). Labeling of DNA probes with horseradish peroxidase with the ECL direct nucleic acid labeling kit (RPN3000; Amersham Biosciences) was performed according to the manufacturer's instructions.

PCR detection of *tet*, *erm*, and *int* genes. The PCR assay mix (total volume, 50 µl) contained 20 pmol of each primer (Table 1), 1× PCR buffer (Applied Biosystems, Warrington, United Kingdom), each deoxynucleoside triphosphate at a concentration of 200 µM, and 1 U of AmpliTaq DNA polymerase (N808-0160; Applied Biosystems, Warrington, United Kingdom). A 50-ng portion of purified total DNA was used as a template. In a first PCR assay, *tet* genes encoding ribosomal protection proteins (RPP) were detected with degenerate primers DI and DII (7). If positive for RPP genes, additional PCR assays were performed with primers specific for *tet*(M), *tet*(O), and *tet*(S) (Table 1). Next to the RPP *tet* genes, isolates were also tested for the presence of the tetracycline efflux genes *tet*(K) and *tet*(L) and for the transposon integrase gene (*int* gene) of the Tn*916*/Tn*1545* family (Table 1). One strain that expressed erythromycin resistance was analyzed with *erm*(B)-specific primers as described previously (19).

All PCR amplifications were performed in a GeneAmp 9600 PCR system (Perkin-Elmer) with the following temperature program: initial denaturation at 94°C for 5 min; 30 cycles of 94°C for 1 min, annealing temperature (see Table 1) for 1 min, and 72°C for 2 min; and a final extension step at 72°C for 10 min. PCR products (5 µl) were separated by electrophoresis on a 1% agarose gel and visualized by ethidium bromide staining.

Sequencing of PCR products. For *tet*(M)-positive isolates, the purified PCR products obtained with the DI and TetM-R primers were directly sequenced with the primers DI, DII, and TetM-R (Table 1). Sequencing was performed with a BigDye Terminator version 2 Ready Reaction cycle sequencing kit (Applied Biosystems, Warrington, United Kingdom) on an ABI Prism 310 genetic analyzer (Applied Biosystems, Warrington, United Kingdom). On-line similarity searches were performed with the BLAST (Basic Local Alignment Search Tool) family of programs in GenBank.

Nucleotide sequence accession numbers. The sequences of the *tet*(M) genes described in this paper have been assigned GenBank accession numbers AY149574 to AY149597.

RESULTS

Identification of isolates. With (GTG)$_5$-PCR fingerprinting, the 94 Tc^r lactic acid bacterial isolates were identified as *Lactobacillus plantarum* (*n* = 31), *L. sakei* subsp. *carnosus* (*n* =

46), *L. sakei* subsp. *sakei* (*n* = 5), *L. curvatus* (*n* = 7), and *L. alimentarius* (*n* = 5). Only one species was isolated from the majority of the positive samples (64%), whereas in all other cases no more than two species were identified. In some cases, different species compositions were obtained in different batches of the same sausage type, e.g., batches FDS-07A and -07B and FDS-08A and -08C. Numerical analysis of the (GTG)$_5$-PCR fingerprints showed that isolates originating from the same batch often displayed identical banding patterns (results not shown). Based on pattern similarity, the set of isolates was reduced to one isolate per unique (GTG)$_5$-PCR fingerprint type, which resulted in a collection of 24 Tc^r *Lactobacillus* isolates (Fig. 1).

Phenotypic characterization of resistance. Etests revealed that the MIC of tetracycline ranged between 32 and >256 μg/ml (upper limit of test), and the MIC for 50% of strains was >256 μg/ml (Fig. 1). Next to the high-level tetracycline resistance found among the 24 selected isolates, some strains showed an additional phenotypic resistance to erythromycin (*n* = 1), rifampin (*n* = 4), and penicillin (*n* = 1) in disk diffusion susceptibility testing.

Detection, characterization, and localization of resistance genes. Total genomic DNA preparations from all 24 Tc^r *Lactobacillus* isolates were subjected to PCR amplification with the RPP set of primers, with class-specific primers for *tet*(M), *tet*(O), *tet*(S), *tet*(K), and *tet*(L) and with primers for detection of *int* genes of the Tn*916*/Tn*1545* family of transposons. In all 24 isolates, only *tet*(M) was detected, and no *int* genes were found. Restriction enzyme analysis of the *tet*(M) PCR product (74% of the open reading frame) with *Acc*I and *Sca*I revealed two different *tet*(M) allele types, *tet*(M)-*1* and *tet*(M)-*2* (Fig. 1). Most batches contained strains belonging to one allele type, except for batches FDS-02-B, FDS-07-A, and FDS-14, which contained strains displaying both allele types (Fig. 1).

In order to characterize the *tet*(M) genes more profoundly, the open reading frame was partially (74%) sequenced from approximately position 280 (DI primer) to position 1700 (TetM-R primer). Sequence alignments revealed two sequence homology groups and one mosaic gene (Fig. 2). Between the homology groups, a difference of at least 25 bases was found. A first group comprised nine isolates in which the sequenced part of the *tet*(M) gene showed at most five base differences with the *tet*(M) gene of *Neisseria meningitidis* (GenBank accession no. X75073) (12). This group corresponds to *tet*(M)-*1* found by restriction enzyme analysis. A second group of 14 isolates with a maximum of three base differences with the *tet*(M) gene of *Staphylococcus aureus* MRSA 101 (GenBank accession no. M21136) (23) corresponded to *tet*(M)-*2* defined by restriction enzyme analysis.

The *tet*(M) gene of isolate DG 13 exhibited a mosaic structure, combining partial sequences of the two foregoing homology groups. The sequence up to position 1508 of the open reading frame displayed one base difference with the *tet*(M) gene of *Neisseria meningitidis* and was identical to the *tet*(M) gene of *Staphylococcus aureus* MRSA 101 in the remaining part. Restriction enzyme analysis classified the *tet*(M) gene of this strain as a *tet*(M)-*2*. The one isolate expressing phenotypic erythromycin resistance (DG 507) was shown to contain the *erm*(B) gene by PCR and partial sequencing of the PCR product.

Plasmid profiling of the 24 Tc^r *Lactobacillus* isolates showed that 23 isolates (excluding DG 524) contained at least one and usually more than one plasmid (Fig. 1). By Southern blotting and hybridization with a *tet*(M)- and/or *erm*(B)-specific probe, the *tet*(M) genes of 20 isolates and the *erm*(B) gene in isolate DG 507 could be localized on a plasmid. Most of these R-plasmids had a size of approximately 10 kb, and in a few cases the R-plasmid was larger than 25 kb. Most plasmid profiles showed more than one band (up to three) that hybridized with the *tet*(M) probe (Fig. 1), due to hybridization with (low concentrations of) open circular and/or linear plasmid forms. For the remaining four Tc^r *Lactobacillus* isolates that did not carry the *tet*(M) gene on a plasmid (DG 142, DG 484, DG 524, and DG 525), Southern blots of *Eco*RI-digested DNA hybridized to the *tet*(M) probe, with fragments of between 8 and 12 kb (results not shown).

DISCUSSION

To our knowledge, this is the first detailed molecular study of antibiotic resistance genes in *Lactobacillus* species isolated from fermented dry sausages. The presence of antibiotic-resistant *Lactobacillus* species has been documented in wine, cheese (17, 34), poultry, calf, swine (11, 21, 33, 36), pig feces (4, 10, 29), healthy human feces (18), and maize silage (8). In the first phase of the current study, a total of 94 tetracycline-resistant lactic acid bacterial isolates recovered from 14 batches representing nine different fermented dry sausage types were identified by (GTG)$_5$-PCR fingerprinting. All strains could be identified as *Lactobacillus* species commonly associated with fermented meat products, i.e., *L. plantarum*, *L. sakei* subsp. *carnosus*, *L. sakei* subsp. *sakei*, *L. curvatus*, and *L. alimentarius* (16).

In four of the batches investigated, isolates belonged to two different *Lactobacillus* species, and in five batches more than one (GTG)$_5$-PCR fingerprint type per species was found (results not shown). Different batches of one fermented dry sausage type were not found to contain identical (GTG)$_5$-PCR fingerprints, indicating that the source of Tc^r lactobacilli is variable. Interestingly, isolates DG 165, DG 485, and DG 516, originating from three different fermented dry sausage types (FDS-11B, FDS-09B, and FDS-06, respectively), displayed identical DNA fingerprints and harbored identical R-plasmids and *tet*(M) genes, suggesting a possible common source of Tc^r *Lactobacillus* contamination. In order not to select multiple isogenic strains, one strain each of the 24 unique (GTG)$_5$-PCR fingerprint types was selected.

Given the fact that the Tc^r *Lactobacillus* isolates were recovered from nine different fermented dry sausage types and contained 24 (GTG)$_5$-PCR fingerprint types representing five different *Lactobacillus* species, it was somewhat surprising that in all isolates, of the five *tet* genes tested, only *tet*(M) was detected. According to current insights, *tet*(M) is the most widely distributed *tet* gene, being detected in at least eight gram-negative and 18 gram-positive genera, including the lactic acid bacterial genera *Enterococcus*, *Streptococcus*, and *Bifidobacterium* (6). It was suggested that the origin of *tet*(M) is most probably the tetracycline-producing species of *Streptomyces* and that its integration into mobile genetic elements (plas-
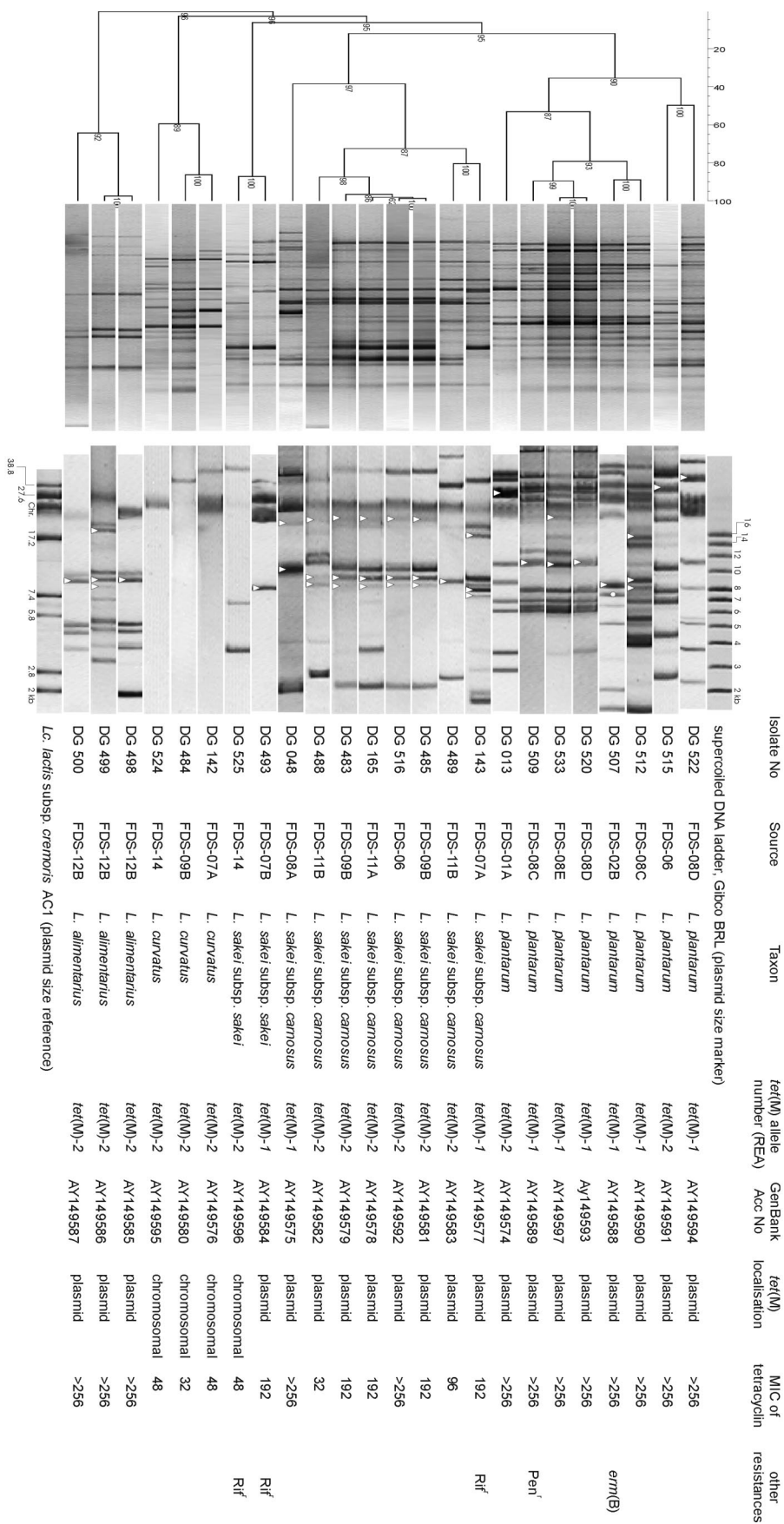
FIG. 1. Composite figure of (GTG)₅-PCR fingerprints and inverted plasmid profiles of the 24 Tcʳ *Lactobacillus* isolates from FDS end products. The dendrogram was constructed after cluster analysis of the digitized (GTG)₅-PCR fingerprints with the unweighted pair group method with arithmetic averages, with correlation levels expressed as a percentage of the Pearson correlation coefficient. Cophenetic correlations (shown on each branch of the dendrogram) indicate how faithfully the dendrogram represents the similarity matrix. A small triangle or circle indicates the place where the *tet*(M) or *erm*(B) probe, respectively, hybridized on the Southern blot of the plasmid DNA. *Lactococcus lactis* subsp. *cremoris* strain AC1 was used as a plasmid size marker (24). The *tet*(M)-1 and *tet*(M)-2 allele types correspond to the *tet*(M) genes found in *Neisseria meningitidis* (X75073) and *Staphylococcus aureus* MRSA 101 (M21136), respectively, based on restriction enzyme analysis (REA) with *Acc*I and *Sca*I. Numerical designations indicate the different batches (type and batch are defined in Materials and Methods). *tet*(M) localization was determined by Southern blot analysis. The MIC of tetracycline was determined by Etest. Chr., chromosomal band.
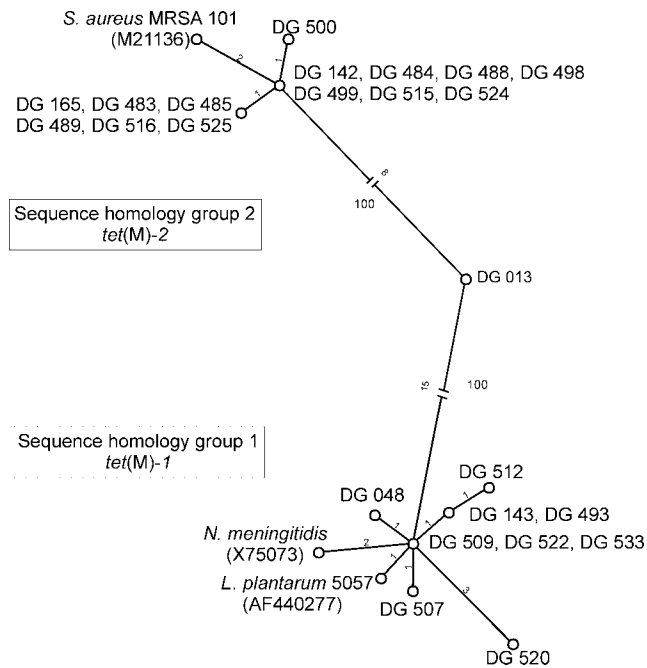
Isolate No	Source	Taxon	*tet*(M) allele number (REA)	GenBank Acc No	*tet*(M) localisation	MIC of tetracyclin	other resistances
		supercoiled DNA ladder, Gibco BRL (plasmid size marker)					
DG 522	FDS-08D	*L. plantarum*	*tet*(M)-1	AY149594	plasmid	>256	
DG 515	FDS-06	*L. plantarum*	*tet*(M)-2	AY149591	plasmid	>256	
DG 512	FDS-08C	*L. plantarum*	*tet*(M)-1	AY149590	plasmid	>256	
DG 507	FDS-02B	*L. plantarum*	*tet*(M)-1	AY149588	plasmid	>256	*erm*(B)
DG 520	FDS-08D	*L. plantarum*	*tet*(M)-1	Ay149593	plasmid	>256	
DG 533	FDS-08E	*L. plantarum*	*tet*(M)-1	AY149597	plasmid	>256	
DG 509	FDS-08C	*L. plantarum*	*tet*(M)-1	AY149589	plasmid	>256	Penʳ
DG 013	FDS-01A	*L. plantarum*	*tet*(M)-2	AY149574	plasmid	>256	
DG 143	FDS-07A	*L. sakei* subsp. *carnosus*	*tet*(M)-1	AY149577	plasmid	192	Rifʳ
DG 489	FDS-11B	*L. sakei* subsp. *carnosus*	*tet*(M)-2	AY149583	plasmid	96	
DG 485	FDS-09B	*L. sakei* subsp. *carnosus*	*tet*(M)-2	AY149581	plasmid	192	
DG 516	FDS-06	*L. sakei* subsp. *carnosus*	*tet*(M)-2	AY149592	plasmid	>256	
DG 165	FDS-11A	*L. sakei* subsp. *carnosus*	*tet*(M)-2	AY149578	plasmid	192	
DG 483	FDS-09B	*L. sakei* subsp. *carnosus*	*tet*(M)-2	AY149579	plasmid	192	
DG 488	FDS-11B	*L. sakei* subsp. *carnosus*	*tet*(M)-2	AY149582	plasmid	32	
DG 048	FDS-08A	*L. sakei* subsp. *carnosus*	*tet*(M)-1	AY149575	plasmid	>256	
DG 493	FDS-07B	*L. sakei* subsp. *sakei*	*tet*(M)-1	AY149584	plasmid	192	Rifʳ
DG 525	FDS-14	*L. sakei* subsp. *sakei*	*tet*(M)-2	AY149596	chromosomal	48	Rifʳ
DG 142	FDS-07A	*L. curvatus*	*tet*(M)-2	AY149576	chromosomal	48	
DG 484	FDS-09B	*L. curvatus*	*tet*(M)-2	AY149580	chromosomal	32	
DG 524	FDS-14	*L. curvatus*	*tet*(M)-2	AY149595	chromosomal	48	
DG 498	FDS-12B	*L. alimentarius*	*tet*(M)-2	AY149585	plasmid	>256	
DG 499	FDS-12B	*L. alimentarius*	*tet*(M)-2	AY149586	plasmid	>256	
DG 500	FDS-12B	*L. alimentarius*	*tet*(M)-2	AY149587	plasmid	>256	
Lc. lactis subsp. *cremoris* AC1 (plasmid size reference)							

FIG. 2. Single most parsimonious tree (unrooted) for *tet*(M) gene relationships of the 24 Tc^r lactic acid bacterial isolates and two reference strains, *Neisseria meningitidis* (X75073) and *Staphylococcus aureus* MRSA 101 (M21136). The recently published *tet*(M) gene of *L. plantarum* 5057 (AF440277) was included (8). The numbers of nucleotide substitutions are indicated on each branch. The bootstrap percentages (500 replicates) are indicated for the separation between the two homology groups.

mids and transposons) has led to its widespread distribution (6, 25).

At the moment of discovery, only *tet*(O) and *tet*(Q) have been reported in members of the genus *Lactobacillus* (6), but recently a *tet*(M) gene was also found in an *L. plantarum* strain (8). Partial sequencing revealed that the *tet*(M) genes in the Tc^r *Lactobacillus* isolates belonged to two homology groups and one individual. The two homology groups correspond to sequences that were published before; group I corresponds with the *tet*(M) found in *Neisseria meningitidis* (12), and group II with *tet*(M) of *Staphylococcus aureus* MRSA 101 (23). Isolate DG 13 represents a new allelic variation, showing high partial similarities with both the *N. meningitidis* and *S. aureus tet*(M) genes. This group may have arisen from homologous recombination and corresponds to the mosaic structures exhibited by the *tet*(M) gene, as previously described (25). The *tet*(M) genes found in these *Lactobacillus* isolates differ from those found in other lactic acid bacteria, including *Enterococcus faecalis* (X56353, M85225, X92947, and X04388), with a base difference ranging between 12 and 115 bases, and *Streptococcus pneumoniae* (X90939), with a base difference ranging between 69 and 86 bases (results not shown).

The *tet*(M) genes of *N. meningitidis* and *S. aureus* MRSA 101 are located on a plasmid and on the chromosome, respectively (12, 23). Within the set of Tc^r *Lactobacillus* isolates, the *tet*(M) genes of sequence homology group I and isolate DG 13 were found exclusively on plasmids (*n* = 10), whereas for sequence homology group II, *tet*(M) genes were localized on the chro-

mosome (*n* = 4) or on a plasmid (*n* = 10). R-plasmids encoding tetracycline, chloramphenicol, gentamicin, and macrolide-lincosamide-streptogramin B resistance have been reported previously in *L. reuteri* (4, 21, 33, 36), *L. fermentum* (10, 18), *L. acidophilus* (36), and *L. plantarum* (1, 8) isolated from raw meat, feces, and maize silage. Most of these R-plasmids were smaller than 10 kb. The tetracycline MIC was significantly lower for the Tc^r *Lactobacillus* isolates with a chromosomal *tet* gene in this study than for those with plasmid-encoded tetracycline resistance, 32 to 48 μg/ml and >192 μg/ml, respectively, with the exception of two *L. sakei* subsp. *carnosus* isolates (DG 488 and DG 489). More research is needed to investigate to what extent this marked difference in phenotypic resistance levels is linked to, e.g., the location of the *tet*(M) gene or the copy number of the R-plasmid.

In conclusion, the results of the current study indicate that *Lactobacillus* species from fermented meat products can harbor acquired tetracycline resistance encoded by a *tet*(M) gene, which is usually located on a plasmid and displays very high genotypic similarities with *tet*(M) genes previously reported in two pathogenic species. Further research may focus on the diversity and transferability of these *Lactobacillus* plasmids into other commensal bacteria and on the source of Tc^r lactobacilli in the production of fermented dry sausages.

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