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New Plasmid-Borne Antibiotic Resistance Gene Cluster in *Pasteurella multocida*

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A new antibiotic resistance gene cluster comprising genes for sulfonamide (*sul2*), streptomycin (*strA-strB*), and tetracycline [*tetR-tet*(H)] resistance was detected on plasmid pVM111 from *Pasteurella multocida*. The *tetR-tet*(H) gene region was inserted between *sul2* and *strA*, possibly by illegitimate recombination. Two potential recombination sites of 18 and 25 bp were identified.

The first tetracycline resistance (*tet*) gene of hybridization class H was detected in 1993 on plasmid pVM111 (4) from a *Pasteurella multocida* isolate obtained in 1975 from the tissues of a turkey in California that had died of avian cholera (5). Later, *tet*(H) genes were also detected in porcine and bovine *P. multocida* and *Mannheimia haemolytica* isolates (3). In 1998, the *tet*(H) gene was identified as part of the composite transposon Tn*5706* from *P. multocida* (12). In recent years, three types of *tet*(H)-carrying plasmids, designated pPMT1 (12), pPAT1 (8), and pMHT1 (7), have been analyzed in detail. All these plasmids were detected in either *P. multocida*, *Pasteurella aerogenes*, or various *Mannheimia* sp. isolates from cattle or pigs. They were 4.4 to 6.8 kb in size and mediated only tetracycline resistance. While restriction maps and sequence data for the regions flanking the *tetR-tet*(H) gene region were available for these plasmids, the corresponding data are still missing for pVM111. Since plasmid pVM111 has been found to be larger than the other *tet*(H)-carrying plasmids known so far and has also been found to mediate sulfonamide and streptomycin resistance by genes that have not been further specified (4), we analyzed plasmid pVM111 for its resistance genes and their organization with regard to cotransfer of the resistance genes and its impact on the development and spread of multiresistance.

Plasmid pVM111 was transformed into *Escherichia coli* JM109 by the $CaCl_2$ method, and transformants were selected on Luria-Bertani agar supplemented with 20 μg of tetracycline/ml. Pure plasmid DNA suitable for restriction mapping, PCR analysis, cloning experiments, and sequence analysis was obtained by alkaline lysis with subsequent purification by affinity chromatography on Midi columns (Qiagen, Hilden, Germany) (9). Restriction mapping already suggested that pVM111 does not carry a complete copy of Tn*5706*. Confirmation of the presence of the *tet*(H) gene was done by PCR (3). PCR-directed analysis of the genes responsible for sulfonamide and streptomycin resistance (10, 11) revealed the presence of the genes *sul2* and *strA* in pVM111.

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In previous studies with isolates of *P. aerogenes*, *M. haemolytica*, *Mannheimia varigena*, and *Mannheimia* taxon 10 from cattle and swine, the primers suitable for the detection of a linkage of the genes *sul2* and *strA* led to the identification of a first resistance gene cluster in which a *catA3* gene for chloramphenicol resistance was inserted into the noncoding spacer between *sul2* and *strA* (10, 11). The same approach with the primer combination sul1 and str2 (the sequence of the forward primer was from the 5′ end of *sul2*, and the sequence of the reverse primer was from the 3′ end of *strA*) (10) and pVM111 DNA as the template resulted in an amplicon of ca. 3.5 kb. When this amplicon was used as a template for PCR analysis, positive results were seen not only for *sul2* and *strA* but also for *tet*(H), suggesting that a *tet*(H) segment might have been inserted between *sul2* and *strA*.

For sequence analysis, the two ca. 4.5-kb *Eco*RI-*Pst*I fragments and the 0.8-kb *Pst*I fragment (Fig. 1) were cloned into pBluescript II SK(+) (Stratagene, Amsterdam, The Netherlands). Both strands of the small *Pst*I fragment and the *Eco*RI-*Pst*I fragment which contained the *tetR-tet*(H) region as well as the *strA-strB* genes were sequenced completely, while fragments of approximately 850 bp at both ends of the second *Eco*RI-*Pst*I fragment were sequenced (Fig. 1). In total, the sequence of a ca. 7-kb segment of the 9.8-kb plasmid pVM111 was determined by primer walking starting with M13 universal and reverse primers (Stratagene). Analysis of the sequence showed the arrangement of the resistance genes displayed in Fig. 1. Studies of the sequence at the junctions between the *tetR-tet*(H) gene region and the adjacent *sul2* and *strA* upstream sequences suggested that integration of the *tet* gene region might have occurred by illegitimate recombination. Two possible recombination sites of 18 and 25 bp were identified (Fig. 2). Recombination between the noncoding region downstream of *tet*(H) in Tn*5706* with the terminal *sul2* sequence resulted in an extension of the *sul2* reading frame by one codon, in addition to the change of the final three codons of *sul2* in pVM111 (Fig. 2). Variations in the terminal parts of the *sul2* reading frame which caused either an extension or a shortening of the *sul2* reading frame but which did not affect the biological activity of the corresponding dihydropteroate synthase have previously been observed in plasmids pLS88 from *Haemophilus ducreyi* (2) and pYFC1 from "*Pasteurella haemo-*

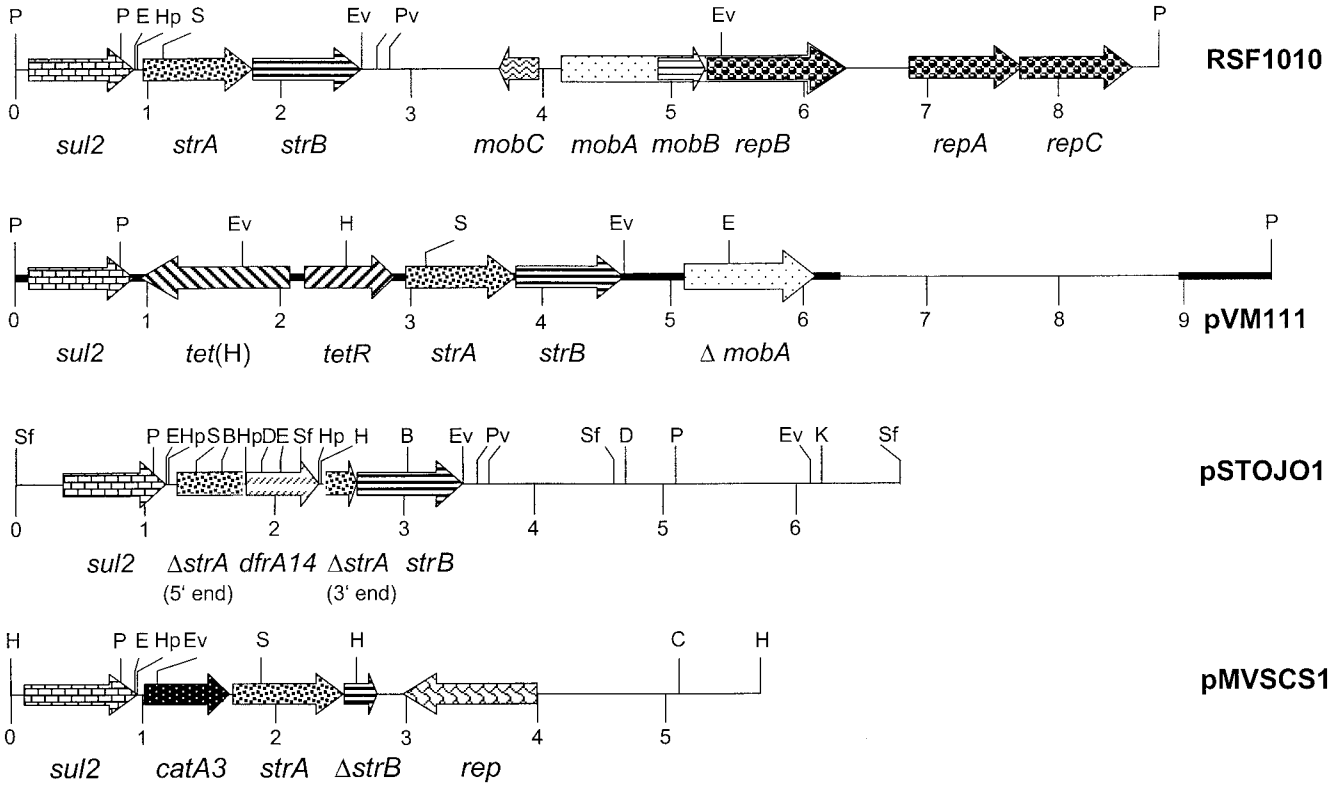

FIG. 1. Comparative analysis of restriction maps and structural organizations of plasmids RSF1010 (14), pVM111 (this study), pSTOJO1 (13), and pMVSCS1 (11). Restriction endonucleases are abbreviated as follows: B, *Bcl*I; D, *Dra*I; E, *Eco*RI; Ev, *Eco*RV; H, *Hin*dIII; Hp, *Hpa*I; K, *Kpn*I; P, *Pst*I; Pv, *Pvu*II; S, *Sac*I; and Sf, *Sfu*I. A distance scale (in kilobases) is presented below each map. The reading frames for genes *sul2*, *strA*, Δ*strA*, *strB*, Δ*strB*, *tet*(H), *tetR*, *dfrA14*, *catA3*, *mobA* to *mobC*, Δ*mobA*, *rep*, and *repA* to *repC* are shown as arrows, with the direction of transcription indicated by the arrowhead. The black bar in the map of pVM111 indicates the sequenced part.

lytica" (1). The sequence of the 18-bp recombination site (Fig. 2) showed 67% identity to the sequence of *sul2-strA-strB*-carrying plasmid RSF1010 (14) and 78% identity to the corresponding part of the Tn*5706* sequence (12). The second recombination site of 25 bp was located in the noncoding spacer downstream of *tetR* and upstream of *strA*. It showed 64% identity to the RSF1010 sequence and 76% identity to the noncoding region downstream of *tetR* in Tn*5706*. The sequence

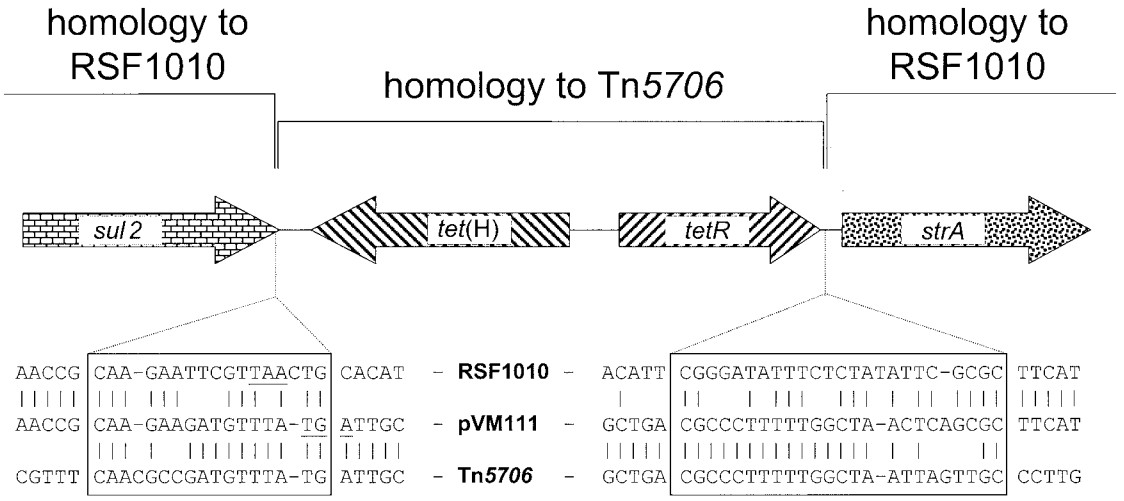

FIG. 2. Presentation of the two potential recombination sites downstream of *tet*(H) and *tetR* probably involved in the integration of a Tn*5706*-like *tetR-tet*(H) gene region in an RSF1010-like spacer region between *sul2* and *strA*. Identical bases with respect to the pVM111 sequence are indicated by vertical bars. The original translational stop codon of the *sul2* gene in the RSF1010 sequence and the alternative stop codon in the pVM111 sequence are underlined. The two putative recombination sites are displayed as boxes.

of the *tetR-tet*(H) gene region of pVM111 corresponded exactly to that previously described by Hansen et al. (4). The sequences of the flanking parts corresponded closely to the sequence of broad-host-range plasmid RSF1010 (14).

Multiresistance gene clusters are of particular importance since they confer resistance to several different antimicrobials or classes of antimicrobials, e.g., sulfonamides, chloramphenicol, and streptomycin (10, 11) or sulfonamides, tetracyclines, and streptomycin. When such resistance gene clusters are located on plasmids, they are easily spread among strains, species, and sometimes even genera. The spread of a plasmid carrying a multiresistance gene cluster bears the danger of the coselection and persistence of resistance genes even without direct selective pressure. Previous studies have shown that *sul2-strA*-carrying plasmids are widespread among gram-negative bacteria (10, 16, 17) and that they are capable of accepting other resistance genes, such as *catA3* (10, 11) and *dfrA14* (13), to form new resistance gene clusters (Fig. 1). These observations were extended in this study by the description of a new type of plasmid-borne antibiotic resistance gene cluster in *P. multocida*. Resistance to sulfonamides, streptomycin, or tetracyclines had been reported to occur at high frequencies of 72.6, 50.0, and 40.5%, respectively, among *P. multocida* isolates from Germany (6); and resistance to these antimicrobials represented the most prevalent type of resistance in these bacteria. PCR screening of a large number of independent *Pasteurella* and *Mannheimia* isolates from food-producing animals confirmed that this new type of resistance gene cluster has not yet been observed in European *Pasteurella* and *Mannheimia* isolates (10, 11). However, the finding that approximately one-third of the *sul2-strA*-carrying isolates identified in recent studies (10, 11) also harbored *tet*(H) genes (7–9) might indicate the potential for the development of such a cluster in European isolates as well. For this, the widespread use of tetracyclines, which account for almost two-thirds of all antimicrobials used in veterinary medicine in the European Union member states and Switzerland (15), might represent a relevant selective force. In addition, the observation that plasmids from *Pasteurella* and *Mannheimia* carrying any type of *sul2-strA*-based resistance gene cluster known so far can replicate and express their resistance genes not only in their original hosts but also in *E. coli* underlines the importance of these plasmids in the spread of antimicrobial multiresistance.

Nucleotide sequence accession number. The sequence of the 7,006-bp segment of pVM111 has been deposited in the EMBL database under accession number AJ514834.

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