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Staphylococcus sciuri Gene *erm*(33), Encoding Inducible Resistance to Macrolides, Lincosamides, and Streptogramin B Antibiotics, Is a Product of Recombination between *erm*(C) and *erm*(A)

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A gene which mediates inducible resistance to macrolides, lincosamides, and streptogramin B antibiotics, designated *erm*(33), was detected on the *Staphylococcus sciuri* plasmid pSCFS1. Analysis of the *erm*(33) reading frame suggested that this gene was the product of a recombination between an *erm*(C) gene and an *erm*(A) gene. Such a recombination event is a novel observation for *erm* genes.

Staphylococcus sciuri, a common inhabitant of the skin of rodents and other mammals, has been reported to carry a number of resistance plasmids, such as the tetracycline resistance plasmid pSTS9 (12), the chloramphenicol resistance plasmid pSCS13 (10), and also the chloramphenicol-streptomycin resistance plasmid pSCS12 (11), which differ in size and/or structure from the resistance plasmids commonly found in staphylococci. More recently, the first and, to date, only known staphylococcal chloramphenicol-florfenicol resistance plasmid was isolated from a bovine *S. sciuri* isolate (13). Analysis of this plasmid, designated pSCFS1, showed that it also mediated inducible resistance to macrolides, lincosamides, and streptogramin B antibiotics (MLS_B antibiotics). Based on the results of PCR analysis, the MLS_B resistance gene of plasmid pSCFS1 was considered to be an *erm* gene of class C (13). Since *erm*(C) genes are commonly located on small 2.3- to 4.3-kb plasmids (1–3, 16) and have very rarely been detected on larger plasmids, we decided to clone and sequence the *erm* gene and its adjacent regions of the ca. 17-kb plasmid pSCFS1. This approach should provide information on how the *erm*(C) gene has become part of plasmid pSCFS1.

To localize the *erm* gene in plasmid pSCFS1, hybridization studies were conducted with a gene probe that consisted of the 378-bp *Sac*I-*Bcl*I fragment of plasmid pSES5 (3). This gene probe comprised the entire *erm*(C) translational attenuator and the first 219 bp of the *erm*(C) gene. The smallest hybridizing fragment was an *Eco*RI-*Pst*I fragment of ca. 2.2 kb. This fragment was cloned into pBluescript II SK(+), and the recombinant plasmid was transformed into *Escherichia coli* JM107. Sequence analysis on both strands was performed by primer walking, starting with the M13 universal and reverse primers.

The sequence of this *Eco*RI-*Pst*I fragment consisted of 2,196 bp. At the *Eco*RI end, the first 602 bp represented the 5′ end of a reading frame whose product showed similarity to plasmid-borne recombination-mobilization proteins of gram-positive bacteria (Fig. 1). The amino terminal 200 amino acids (aa) of this reading frame were most closely related to the corresponding parts of a recombination-mobilization protein of *Listeria monocytogenes* (U40997) with 82% amino acid identity, the recombination protein of the *Bacillus* plasmid pTB913 (X15670; 81% amino acid identity), the mobilization protein of *Geobacillus stearothermophilus* (M63891; 81% amino acid identity), and the recombination-mobilization protein of *Staphylococcus cohnii* (AF015628; 79% amino acid identity). Sequence homology to the expected *erm*(C) region started about 340 bp from the start codon of the aforementioned reading frame. A 66-bp region that showed similarity to the *pre-mob* upstream region as well as to the *erm*(C) upstream region indicated the junction of both sequences and might have served as a putative recombination site. This site did not exhibit similarity to the staphylococcal recombination sites RS_A (on which Pre acts) or RS_B (6, 7) previously found on small *erm*(C)-carrying plasmids (6, 7). The 8-bp sequence GTA TTCTT within this region did not occur in the two partner sequences (Fig. 1). It represented an imperfect tandem duplication which might have arisen during the recombination process.

Further downstream, a small reading frame for a peptide of 19 aa and two pairs of inverted repeated sequences were found to precede a reading frame for a 243-aa protein (Fig. 1). This region corresponded closely to the translational attenuators seen upstream of the inducibly expressed *erm*(C) genes of plasmids pT48 (1), pE194 (2) or pSES5 (3). The inverted repeats appear to form different mRNA secondary structures in the presence or absence of inducing macrolides, thereby allowing or preventing translation of the *erm*(C) transcripts (15). The regulatory region of the *erm* gene of plasmid pSCFS1 differed only by four base pair exchanges and three base pair deletions from the translational attenuators of inducibly expressed *erm*(C) genes (1–3). All these sequence alterations were located in the noncoding parts of the regulatory region and also did not affect the inverted repeats. The 243-aa protein represented a rRNA methylase that mediates MLS_B resistance. A comparison of its amino acid sequence with those of

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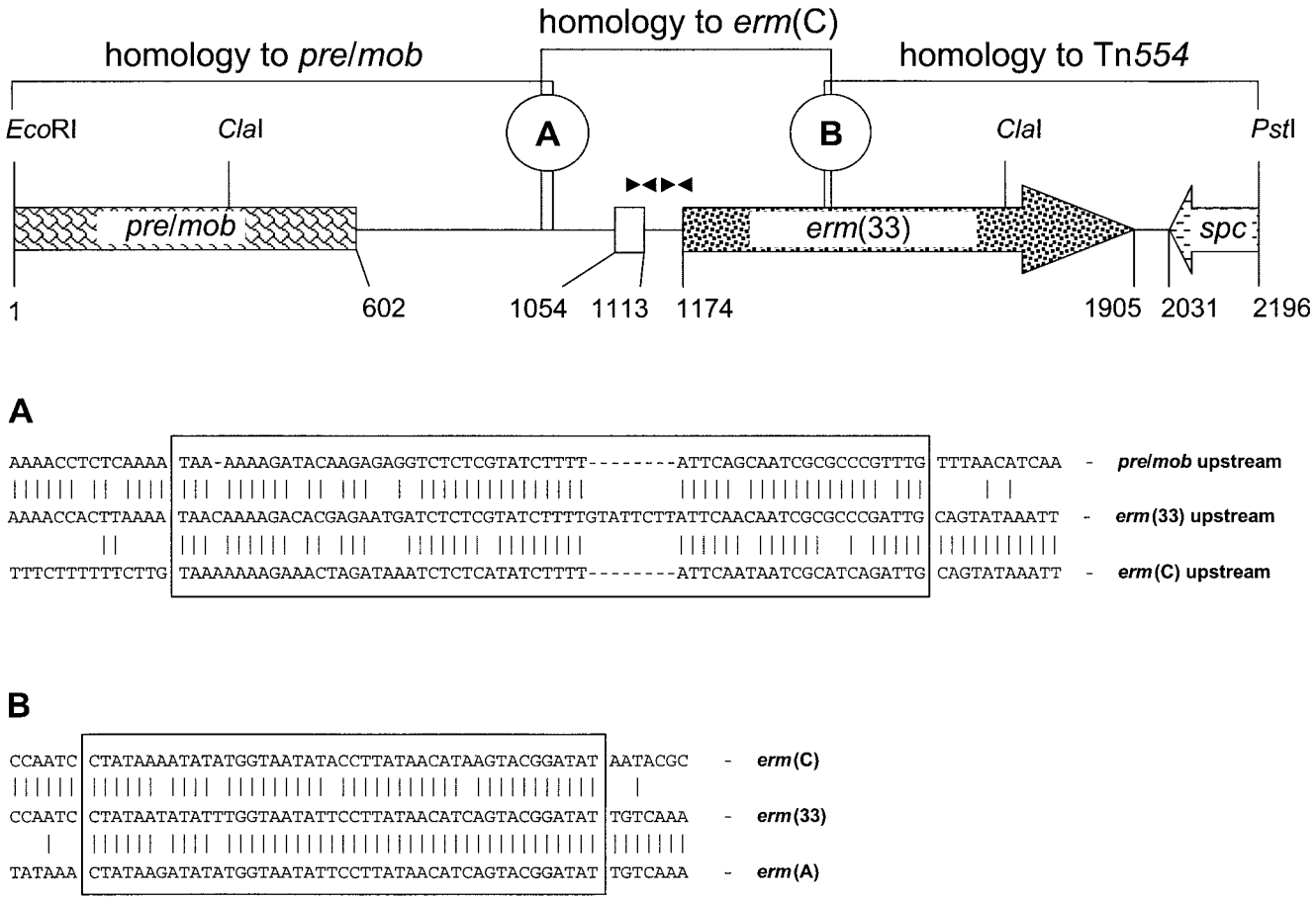

FIG. 1. Restriction map and structural organization of the 2.2-kb *Eco*RI-*Pst*I fragment of plasmid pSCFS1. The 5′ end of a *pre-mob*-like gene is shown as a box. The white box indicates the small reading frame for the 19-aa regulatory peptide. The black triangles represent the two pairs of inverted repeated sequences detected in the *erm*(33) translational attenuator. The *erm*(33) gene and the 3′ end of the *spc* reading frame are displayed as arrows, with the arrowhead showing the direction of transcription. The sequences of two potential recombination sites A and B (displayed as boxes) and their adjacent sequences are shown in detail below the map.

other rRNA methylase proteins revealed highest overall identity of 79% to Tn*554*-analogous Erm(A) proteins (A25101, AP003137, AP003363, and AB037671) (Table 1), but only 71% amino acid identity to the Erm(A) from *Streptococcus pyogenes* (AF002716), formerly known as Erm(TR) (14). Identity to the Erm(C) proteins found on small plasmids from *Bacillus* and *Staphylococcus* varied between 73% [Erm(C) of the *Staphylococcus hominis* plasmid pSES5 (Y09001)] and 76% [Erm(C) of the *Staphylococcus hyicus* plasmid pSES21 (Y09003)] (Table 1). Identity of the Erm protein of plasmid pSCFS1 to Erm(T) of the *Lactobacillus reuteri* plasmid pGT633 (M64090) was only 67% and was even lower at 65% to Erm(Y) of *Staphylococcus aureus* (AB014481) and Erm(G) of *Bacillus sphaericus* (M15332). Since this Erm protein exhibited ≤79% amino acid identity to the next most closely related Erm proteins (8), it received the designation Erm(33) from the Nomenclature Center for MLS Genes (http://faculty.washington.edu/marilynr/; M. Roberts, personal communication). Further analysis of the *erm*(33) gene

TABLE 1. Comparison of different parts of the *erm*(33) gene and Erm(33) protein sequences with the respective parts of the most closely related *erm* genes and Erm proteins

Sequence	% Identity							
	erm(33) gene				Erm(33) protein			
	5′ end (284 bp)	Recombination area (45 bp)	3′ end (403 bp)	Entire gene (732 bp)	N terminus (95 aa)	Recombination area (15 aa)	C terminus (133 aa)	Entire protein (243 aa)
erm(A) Tn*554*	59.5	95.6	100.0	84.0	50.5	86.7	100.0	79.4
erm(C) pSES21	99.3	88.9	58.9	76.5	97.9	86.7	59.4	76.1
erm(C) pE194	95.4	91.1	60.4	76.0	94.7	86.7	58.3	74.5
erm(C) pSES5	94.7	93.3	59.6	75.4	93.3	93.3	57.9	73.7

showed that the first 284 bp at the 5′ end of the reading frame were almost identical to those of *erm*(C) genes (12 base pair exchanges that caused five amino acid alterations), while the 403 bp at the 3′ end of *erm*(33) were indistinguishable from those of *erm*(A) from Tn*554* (Table 1). At the junction of *erm*(C)-homologous and *erm*(A)-homologous sequences, a stretch of 45 bp was seen which displayed sequence identities of 95.6% to *erm*(A) and 93.3% to *erm*(C) (Fig. 1; Table 1). Assuming that *erm*(33) resulted from a recombination between an *erm*(C) and an *erm*(A) gene, it is most probable that this 45-bp sequence had served as the site for the recombination. The knowledge of the entire *erm*(33) sequence also explains the initial misidentification of the gene as an *erm*(C) gene (13) since the PCR primers and the *erm*(C) gene probe used (3) bound exclusively in the *erm*(C)-homologous part of *erm*(33).

The 125 bp immediately downstream of *erm*(33) corresponded exactly to the noncoding sequence downstream of *erm*(A) in Tn*554* (5). Furthermore, the final 166 bp of the *Eco*RI-*Pst*I fragment represented the 3′ end of the *spc* gene of Tn*554* coding for a spectinomycin adenyltransferase (5). The observation that additional Tn*554*-homologous sequences were detected in the sequenced part downstream of *erm*(33) confirmed the involvement of a Tn*554*-associated *erm*(A) gene (4) in the development of *erm*(33).

To the best of our knowledge, this is the first report of a natural recombination between two known *erm* genes resulting in the formation of a functionally active new *erm* gene. Both genes *erm*(A) and *erm*(C) which show ca. 62% identity in their nucleotide sequences and 58% identity in their deduced amino acid sequences are widespread among human and animal staphylococci and occasionally have been detected in the same isolates (9). Under such conditions, a recombination might have occurred by chance. Since Erm(33) did not differ in its substrate spectrum, nor in the MICs as determined for inducibly expressed Erm(A) or Erm(C) proteins (9), there is no biological need for the bacteria to develop a recombination product such as Erm(33). However, in PCR-directed studies on the occurrence of different *erm* genes in staphylococci, misidentification of *erm*(33) or failure to detect this gene by using previously described PCR assays must be taken into consideration. Therefore, a suitable primer system that specifically recognizes this gene should be used for the correct identification of *erm*(33).

Nucleotide sequence accession number. The sequence of a the *erm*(33) gene has been deposited with the EMBL database under accession number AJ313523.

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