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The *erm*(T) Gene Is Flanked by IS*1216V* in Inducible Erythromycin-Resistant *Streptococcus gallolyticus* subsp. *pasteurianus*

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We investigated the sequence and the genetic context of the *erm*(T) gene in six inducible erythromycin-resistant *Streptococcus gallolyticus* subsp. *pasteurianus* (formerly *S. bovis* biotype II.2) isolates. In all isolates, the *erm*(T) genes were flanked by two IS*1216V*-like elements with the same polarity and were found to be inserted in the chromosome.

Streptococcus gallolyticus subsp. *pasteurianus*, formerly known as *Streptococcus bovis* biotype II.2 (10), can be a cause of endocarditis in elderly people and of septicemia and meningitis in newborns (1, 2, 14). We previously found that *erm*(T) was present in inducible erythromycin-resistant isolates of this species (12). In this study, we determined the sequences of *erm*(T) and its flanking regions from six inducible erythromycin-resistant *S. gallolyticus* subsp. *pasteurianus* isolates and investigated the genetic support of the *erm*(T)-containing elements.

Bacterial strains. Six clinical isolates of *erm*(T)-positive, inducible erythromycin-resistant *Streptococcus gallolyticus* subsp. *pasteurianus* were studied. The six isolates were from blood cultures and collected during the period 2000 to 2003 at the Bacteriology Laboratory, National Taiwan University Hospital, a 2,000-bed teaching hospital in northern Taiwan. One erythromycin-susceptible reference strain (ATCC 43144) of *S. gallolyticus* subsp. *pasteurianus* and two isolates of *erm*(T)-negative *S. gallolyticus* subsp. *pasteurianus* [one was erythromycin resistant due to the presence of an *erm*(B) gene, and the other was erythromycin susceptible] were used as negative controls. The isolates were identified by the API system (bioMérieux Vitek, France), and identification was confirmed by 16S rRNA gene sequences.

Nucleotide sequence of *erm*(T) and flanking regions in isolate NTUH-7421. We have previously determined the partial *erm*(T) sequence in the erythromycin-resistant *S. gallolyticus* subsp. *pasteurianus* NTUH-7421 isolate (12). In this study, we determined the sequence of the entire *erm*(T) gene and its flanking regions by using a long accurate PCR in vitro cloning kit (Takara Shuzo Co. Ltd., Japan). The protocol had been described previously (13). Briefly, a Southern blot analysis (13) was performed with the DNA of NTUH-7421, which was digested with a panel of restriction enzymes and detected with a digoxigenin-labeled *erm*(T)-specific probe prepared by PCR amplification of *erm*(T) by using primers ermT_112F and ermT_684R (Table 1). Probe labeling and detection were carried out by using a commercial kit (Roche Diagnostics GmbH, Penzberg, Germany). This *erm*(T)-specific probe hybridized to a 6.5-kb EcoRI genomic fragment of NTUH-7421 (Fig. 1A). After ligating the EcoRI-digested DNA fragments with cassette adapters, the amplification was performed with cassette primers (C1 for the first PCR and C2 for the second PCR) supplied by the manufacturer and a target gene-specific primer, either ermT_112F or ermT_684R (Table 1). The PCR conditions for long accurate PCR were as described previously (13). Amplification fragments were subsequently sequenced on both strands by an Applied Biosystems Model ABI PRISM 3100 genetic analyzer (Applied Biosystems, Foster City, Calif.) with a *Taq* BigDye-Deoxy Terminator cycle sequencing kit (Applied Biosystems), according to the manufacturer's instructions.

By use of this strategy, the nucleotide sequence of a 4,107-bp fragment containing the entire *erm*(T) and its flanking regions was determined (Fig. 1B). The DNA sequence analysis revealed that the *erm*(T) gene of NTUH-7421 consists of 735 nucleotides, with a very low G+C content (25.4%), and is preceded by an AGGAG ribosome binding site consensus sequence and by a 60-nucleotide leader peptide-encoding gene sequence. The leader peptide-encoding gene sequence encodes a 19-amino-acid peptide, MGIFSIFVINTVHYQPNKK, which was 100% identical to that of *Lactobacillus reuteri* *erm*(T) (11) but differed from that of staphylococcal *erm*(C) (6) by one amino acid (Fig. 2). The *erm*(T) of NTUH-7421 had 99% identity with that of plasmid pGT633 from *L. reuteri* 100-63 or a tylosin-resistant *Lactobacillus* sp. (11, 15).

The inducible erythromycin-resistant phenotype in *S. gallolyticus* subsp. *pasteurianus* NTUH-7421 is probably dependent on the presence of an intact leader peptide sequence located immediately upstream to *erm*(T). This is supported by the finding of Tannock et al. that the *erm*(T) in the plasmid pGT633 of *L. reuteri* contained additional tandem duplication

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TABLE 1. PCR primers used in this study

Description	Primer name	Sequence (5′ to 3′)	Target nucleotide position (nucleotide range)	Annealing temp (°C)	Amplicon size (bp)
erm(T) probe shown in Fig. 1	ermT_112F ermT_684R	GGTTCAGGGAAAGGTCATTTCAC GCTAATATTGTTAAAATCGTCAATTCC	*erm*(T) ~112–134 *erm*(T) ~684–658	52	573
erm(T) probe shown in Fig. 3 and 4	ermT_−93F ermT_down151R	CAACACAGTTCATTATCAACC CATGGAAAGTAATTGCCG	*erm*(T) ~−93–−73 *erm*(T)-down ~134–151	57	978
IS*1216V* probe	IS1216F(55) IS1216R(518)	CCGTGGGCTACTATCTTCGTT AATTTATTGCGTCTCTTTACTGGA	IS*1216V* ~56–76 IS*1216V* ~542–519	52	487
PCR detection of left IS*1216V*	IS1216F(55) ermT_495R	CCGTGGGCTACTATCTTCGTT TGGATGAAAGTATTCTCTAGGG	IS*1216V* ~56–76 *erm*(T) ~495–474	52	1,317
PCR detection of right IS*1216V*	ermT_423F IS1216R(518)	ACTAGCACTATTTTTAATGACAGAAGTTGA AATTTATTGCGTCTCTTTACTGGA	*erm*(T) ~423–452 IS*1216V* ~542–519	52	1,601

of 26-bp direct repeats, which may lead to constitutive expression of erythromycin resistance (11).

Two identical IS*1216V*-like elements were found on both sides of *erm*(T), with the same polarity (Fig. 1B). The IS*1216V* sequence from *S. gallolyticus* is nearly identical (with only one nucleotide difference) to that of a vancomycin-resistant *Enterococcus faecium* (3) (GenBank accession number L40841).

Upstream from the left IS*1216V*-like element, three open reading frames were detected (Fig. 1B). By comparing with the sequences in database, the best matches for the products of these open reading frames were the *rpmF* putative ribosomal protein L32 of *Streptococcus pyogenes* MGAS315 (GenBank accession number AE014172) (98% identity), the *rpmG* putative ribosomal protein L33 of *S. pyogenes* MGAS315 (GenBank accession number AE014172) (98% identity), and the HisS putative histidyl-tRNA synthetases of *Streptococcus thermophilus* CNRZ1066 (GenBank accession number CP000024) (95% sequence identity).

The region downstream of *erm*(T) of *S. gallolyticus* subsp. *pasteurianus* NTUH-7421 was very similar (93% nucleotide identity) to that of a broad-host-range plasmid containing *erm*(T) from a tylosin-resistant *Lactobacillus* sp. over a length of about 300 bp (15). The fragment after the 300-bp sequence and before the other IS*1216V* had no significant homology with known sequences.

Identification of *erm*(T) and IS*1216V*-like element in other clinical isolates. To determine whether IS*1216V* is present on sites other than the neighborhood of *erm*(T) and to determine

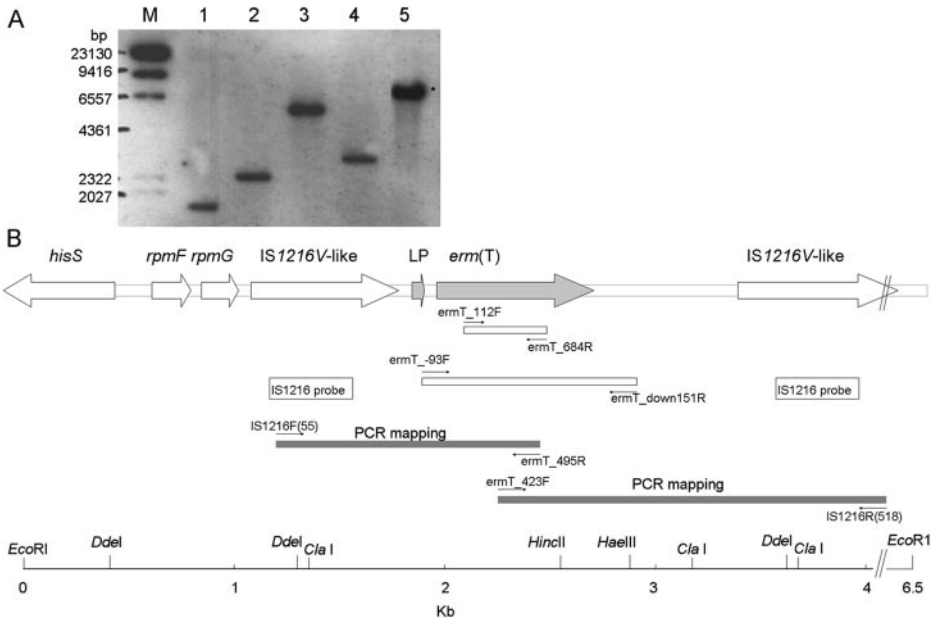

FIG. 1. Genetic organization of *erm*(T) resistance element and flanking regions in S. *gallolyticus* subsp. *pasteurianus* NTUH-7421. (A) Southern blot hybridization of *erm*(T) probe to *S. gallolyticus* subsp. *pasteurianus* NTUH-7421 genomic DNA after digestion with restriction enzymes ClaI, DdeI, HaeIII, HindII, and EcoRI (lanes 1 to 5, respectively). Lane M, DNA marker (digoxigenin-labeled DNA Molecular Weight Marker II′ [Roche]). (B) Genetic organization of *erm*(T) resistance element and flanking regions in *S. gallolyticus* subsp. *pasteurianus* NTUH-7421. Arrows represent putative open reading frames. The restriction sites are also shown.

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                    SD                ⟶Leader peptide
NTUH-7421 ermT      AGGAGAATTAATATATGGGCATTTTTAGTATTTTTGTAAT    40
M64090    ermGT     ----------------------------------------    40
M17990    ermC      -----g-a.--a--------------------------      39

                                                  ___
NTUH-7421 ermT      CAACACAGTTCATTATCAACCAAACAAAAAATAAGTGGTT    80
M64090    ermGT     ----------------------------------------    80
M17990    ermC      --g-------------------------------------    79

NTUH-7421 ermT      AT.......................AATGGATTGTTA       94
M64090    ermGT     --caaccaaacaaaaaataagtggttat------------    120
M17990    ermC      --.........................----a--c----      93
                                                  SD
NTUH-7421 ermT      ATATA.AACATTCATTATAACCTCATAGGAGTGGGTTATA    133
M64090    ermGT     -----.-----------------------------------   159
M17990    ermC      ---agc--a-------------aa--taa--a--------    133
                    ⟶Erm
NTUH-7421 ermT      ATGAACAAAAAAAATATAAAAGATAGTCAAAACTTTATTA    173
M64090    ermGT     ----------------------------------------    199
M17990    ermC      ------g-g------------c-c-----------------   173
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FIG. 2. Alignment of the leader peptide-encoding sequences of *erm*(T) in *S. gallolyticus* subsp. *pasteurianus* NTUH-7421, *erm*(GT) in *Lactobacillus* species (GenBank accession number M64090), and staphylococcal *erm*(C) (GenBank accession number M17990). A dash indicates an identical nucleotide and a dot indicates a gap. TAA (stop codon) is underlined.

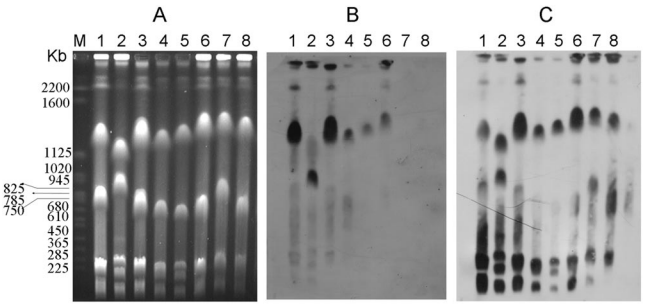

FIG. 4. Localization of *erm*(T) on I-CeuI-generated chromosome fragments of *S. gallolyticus* subsp. *pasteurianus* isolates. (A) I-CeuI fragment restriction patterns separated by pulsed-field gel electrophoresis. (B) Hybridization with a probe specific for the *erm*(T) gene. (C) Hybridization with a 16S rRNA gene-specific probe. Lane M, molecular size standard (*Saccharomyces cerevisiae* chromosomal DNA). Lanes 1 to 6 show *erm*(T)-positive isolates. Lane 1, NTUH-7421; lane 2, NTUH-8819; lane 3, NTUH-7499; lane 4, NTUH-3004; lane 5, NTUH-1043; lane 6, NTUH-4807; lanes 7 and 8, *erm*(T)-negative clinical isolates NTUH-1443 and NTUH-4046, respectively.

whether the other five *erm*(T)-containing isolates contain similar structures, genomic DNAs were digested with EcoRI or HincII and hybridized to the *erm*(T)-specific probe (see above) or an IS*1216V*-specific probe obtained from NTUH-7421 by PCR with primers described in Table 1. The *erm*(T)- and IS*1216V*-specific probes apparently hybridized to the same EcoRI fragment (Fig. 3A, lane 1, and B, lane 1, respectively) in NTUH-7421, confirming the linkage between *erm*(T) and IS*1216V* in this isolate. Moreover, in this isolate, the IS*1216V*-specific probe hybridized to only two HincII fragments, indicating that IS*1216V* is present only in the neighborhood of *erm*(T). In the remaining five *erm*(T)-containing isolates, the

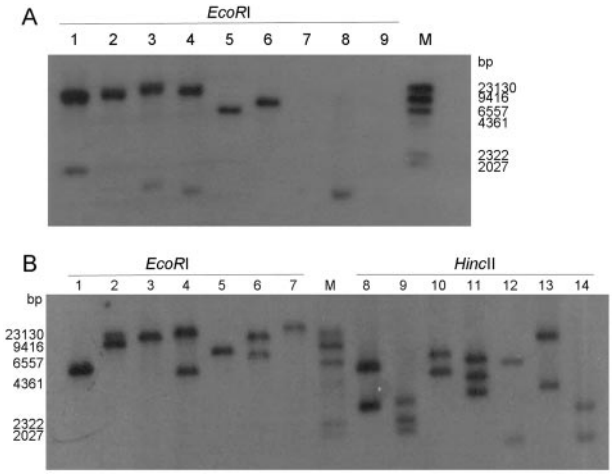

FIG. 3. Southern blot hybridization of *erm*(T) and IS*1216V* probe to *S. gallolyticus* subsp. *pasteurianus* strains. (A) Hybridization with an *erm*(T)-specific probe. Lanes 1 to 6 show *erm*(T)-positive isolates. Lane 1, NTUH-7421; lane 2, NTUH-8819; lane 3, NTUH-7499; lane 4, NTUH-3004; lane 5, NTUH-1043; lane 6, NTUH-4807; lanes 7 and 8, *erm*(T)-negative clinical isolates NTUH-1443 and NTUH-4046, respectively; lane 9, *S. gallolyticus* subsp. *pasteurianus* ATCC 43144; lane M, DNA marker (digoxigenin-labeled DNA Molecular Weight Marker II′ [Roche]). (B) Hybridization with IS*1216V*-specific probe. Lanes 1 to 6 and 8 to 13, *erm*(T)-positive isolates, as in lanes 1 to 6 in panel A. Lanes 7 and 14, *erm*(T)-negative clinical isolate NTUH-1443.

erm(T)- and IS*1216V*-specific probes apparently hybridized to the same EcoRI fragments (Fig. 3). However, additional bands hybridizing to the IS*1216V* probe in *erm*(T)-positive and *erm*(T)-negative isolates were observed (Fig. 3B), indicating that it was not specific to *erm*(T).

We further confirmed that the regions flanking the *erm*(T) gene were identical in all isolates by PCR mapping and sequencing, which were carried out with the primers listed in Table 1 and Fig. 1.

IS*1216*-like modules were found to be associated with antibiotic resistance determinants in enterococcal strains. *Enterococcus hirae* S185R was reported to have a plasmid-borne *pbp3r* gene linked to *erm*(AM), *aadE*, and the *tnp* of IS*1216V* (9). The IS*1216V* element is also part of the Tn*1546*-like elements in vancomycin-resistant enterococci (3, 16). Furthermore, IS*1216V* was proposed to mediate the horizontal spread of the vancomycin resistance transposon Tn*5506* in *E. faecium* (4). IS*1216V* is a member of the IS*S1* family, which includes elements known to be involved in cointegration and recombination processes in *Lactococcus lactis* (8). To our best knowledge, IS*1216V* in *S. gallolyticus* or related species has not been previously reported.

Genetic support of *erm*(T). To determine the location of the *erm*(T) genetic element in *S. gallolyticus*, the DNAs of *S. gallolyticus* isolates were digested with I-CeuI (Fig. 4A) and then hybridized with either a 16S rRNA gene probe or the *erm*(T) probe (Fig. 4B and C). Chromosomal DNA from *S. gallolyticus* was prepared as described previously (7). Pulsed-field gel electrophoresis was performed at 200 V and 14°C with a CHEF-DRII apparatus (Bio-Rad Laboratories), with the pulse times ranging from 60 to 120 s for 24 h.

In all isolates, the chromosomal bands recognized by the *erm*(T) probe (Fig. 4B) were also recognized by the 16S rRNA probe (Fig. 4C), revealing a chromosomal location of the *erm*(T) element in all isolates. The flanking *hisS*, *rpmF*, and *rpmG*-like sequences located upstream from the *erm*(T) element in strain NTUH-7421 further suggested a chromosomal location for the *erm*(T) gene in this isolate.

Concluding remarks. To our best knowledge, *erm*(T) has been found only in *Lactobacillus* and the bovis group of streptococci (5, 11, 12, 15). The presence of *erm*(T) on *S. gallolyticus* subsp. *pasteurianus* is not only found in Taiwan. Lee et al. also reported similar rates of *erm*(T) in their isolates (5). The presence of *erm*(T) and IS*1216V* in *S. gallolyticus* subsp. *pasteurianus* suggests that genetic exchange might occur between *S. gallolyticus* and other gram-positive bacteria, such as *Lactobacillus* or *Enterococcus*.

Nucleotide sequence accession number. The *erm*(T)-containing 4,107-bp nucleotide sequence from *S. gallolyticus* subsp. *pasteurianus* strain NTUH-7421 was deposited in GenBank under accession number AY894138.

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