ANTIMICROBIAL AGENTS AND CHEMOTHERAPY, Nov. 2002, p. 3690–3691 Vol. 46, No. 11
0066-4804/02/$04.00+0 DOI: 10.1128/AAC.46.11.3690–3691.2002

The Macrolide Efflux Genetic Assembly of *Streptococcus pneumoniae* Is Present in Erythromycin-Resistant *Streptococcus salivarius*

Macrolide resistance in pneumococci and oral streptococci is a common phenomenon (8). The macrolide resistance determinants *erm*(B) and *mef*(A) are widespread (4, 7, 9). We characterized the erythromycin resistance determinant of a *Streptococcus salivarius* strain isolated from a healthy person with no exposure to antibiotics 6 months prior to isolation. It was identified as *mef*(A) conferring an erythromycin MIC of 6 μg/ml (6). *mef*(A) was part of a macrolide efflux genetic assembly (mega) recently identified in *Streptococcus pneumoniae* (GenBank accession no. AF274302 [3]) in the United States. The nucleotide sequence of the mega from *S. salivarius* Sp6 (GenBank accession no. AJ318993) was 99.8% identical to that of the pneumococcal element (Fig. 1A). Deletions resulted in a 5,511-bp element in *S. salivarius* compared to 5,532 bp in *S. pneumoniae. orf1* is identical to *mef*(A) from *S. pneumoniae* (U83667 [11]); *orf2* encodes an ABC transporter identical to *mel* in the mega of *S. pneumoniae.* The open reading frames *orf1* to *-5* of the *S. salivarius* mega are homologous to *orf4* to *-8* from Tn*1207.1*, a *mef*(A)-carrying defective transposon of *S. pneumoniae* (AF227520 [10]). *orf6* is not present in Tn*1207.1*. Upstream of *mef*(A) of the *S. salivarius* mega is a 16-bp gap that eliminates a direct repeat in the mega of *S. pneumoniae* (AF274302). This gap is also present in Tn*1207.1* and in megas in Italian pneumococcal strains (2). Contrary to Orf4 in the mega of *S. pneumoniae,* Orf4 in the *S. salivarius* sequence is 19 amino acids larger due to a single base pair deletion but is the same size as Orf7 in Tn*1207.1.* Another deletion in Orf5 of the mega in *S. salivarius* causes a shortened open reading frame.

The mega in *S. salivarius* was located on the chromosome. Downstream of the *S. salivarius* mega, three open reading frames with homologies to a regulator protein (orfb), an ABC transporter (orfc), and a transmembrane protein (orfd) were found.

The mega from *S. salivarius* Sp6 was transformed in two independent experiments with low but significant frequency (two transformants per 10^7 recipient cells and 100 ng of *S. salivarius* DNA) into erythromycin-susceptible *S. pneumoniae* strain R800 by in vitro transformation using accepted protocols and control DNA (1, 5). No spontaneous mutation of the recipient cells was obtained. The MIC for the transformants was comparable to that for the donor strains. *Sma*I-digested genomic DNA of the recipient strain and of transformants of *S. pneumoniae* R800 was analyzed by pulsed-field gel electrophoresis. The mega integrated into a 291-kbp fragment to yield a 388-kbp fragment detected by Southern hybridization (Fig. 1B).

The mega was detected by PCR with specific primers for nucleotides 1 to 2723 and 2291 to 5510 in oral streptococci from six healthy persons, including *S. salivarius* (10 strains), *Streptococcus mitis* (six strains), *Streptococcus parasanguis* (five strains), and *Streptococcus cristatus* (three strains).

This is the first report of the pneumococcal mega in *S. salivarius* and of a successful transformation of *S. pneumoniae* with *S. salivarius* DNA. The mechanism of worldwide dissemination of the mega in oral streptococci remains to be established.

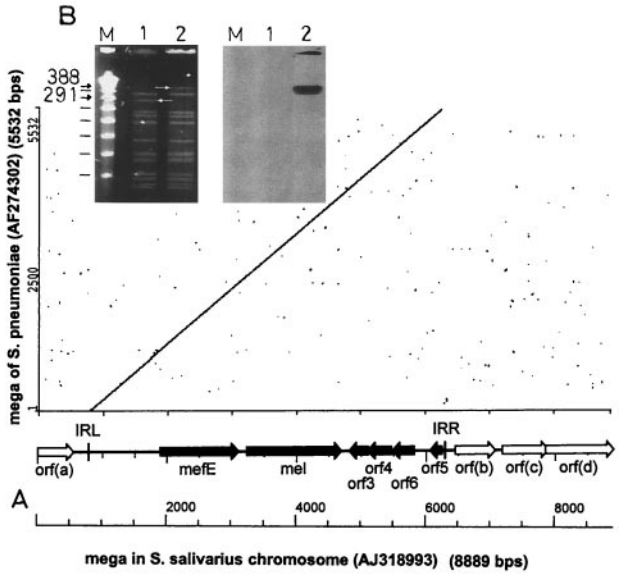

FIG. 1. (A) Comparison of the mega of *S. salivarius* (GenBank accession no. AJ3188993) and *S. pneumoniae* (GenBank accession no. AF274302) in a dot plot analysis. IR, inverted repeats described by Gay and Stephens (3). (B) Identification of the *S. pneumoniae* R800 chromosomal DNA fragment containing the *S. salivarius* mega by digestion with *Sma*I, pulsed-field gel electrophoresis, and Southern hybridization. Lanes M, Lambda Ladder PFG Marker (in kilobases) (Biolabs); lanes 1, *S. pneumoniae* R800; and lanes 2, R800-transformant RSp6-II. Arrows indicate the fragment into which mega and adjacent sequences integrated.

REFERENCES

1. **Alloing, G., B. Martin, C. Granadel, and J. P. Claverys.** 1998. Development of competence in Streptococcus pneumoniae: pheromone autoinduction and control of quorum sensing by the oligopeptide permease. Mol. Microbiol. **29:**75–83.
2. **Del Grosso, M., F. Iannelli, C. Messina, M. Santagati, N. Petrosillo, S. Stefani, G. Pozzi, and A. Pantosti.** 2002. Macrolide efflux genes *mef*(A) and *mef*(E) are carried by different genetic elements in *Streptococcus pneumoniae.* J. Clin. Microbiol. **40:**774–778.
3. **Gay, K., and D. S. Stephens.** 2001. Structure and dissemination of a chromosomal insertion element encoding macrolide efflux in *Streptococcus pneumoniae*. J. Infect. Dis. **184:**56–65.
4. **Ioannidou, S., P. T. Tassios, A. Kotsovili-Tseleni, M. Foustoukou, N. J. Legakis, and A. Vatopoulos.** 2001. Antibiotic resistance rates and macrolide resistance phenotypes of viridans group streptococci from the oropharynx of healthy Greek children. Int. J. Antimicrob. Agents **17:**195–201.
5. **Martin, B., M. Prudhomme, G. Alloing, C. Granadel, and J. P. Claverys.** 2000. Cross-regulation of competence pheromone production and export in the early control of transformation in *Streptococcus pneumoniae.* Mol. Microbiol. **38:**867–878.
6. **NCCLS.** 1997. Methods for dilution antimicrobial susceptibility tests for bacteria that grow aerobically, p. M100–S107, 4th ed., vol. 17. NCCLS, Wayne, Pa.
7. **Perez-Trallero, E., D. Vicente, M. Montes, J. M. Marimon, and L. Pineiro.** 2001. High proportion of pharyngeal carriers of commensal streptococci resistant to erythromycin in Spanish adults. J. Antimicrob. Chemother. **48:** 225–229.
8. **Roberts, M. C.** 1998. Antibiotic resistance mechanisms in bacteria of oral and upper respiratory origin. Int. J. Antimicrob. Agents **9:**255–267.

9. **Rodriguez-Avial, I., C. Rodriguez-Avial, E. Culebras, A. Benitez, and J. J. Picazo.** 2001. Distribution of *mef*(A) and *erm*(B) genes in macrolide-resistant blood isolates of viridans group streptococci. J. Antimicrob. Chemother. **47:**727–728.
10. **Santagati, M., F. Iannelli, M. R. Oggioni, S. Stefani, and G. Pozzi.** 2000. Characterization of a genetic element carrying the macrolide efflux gene mef(A) in *Streptococcus pneumoniae.* Antimicrob. Agents Chemother. **44:** 2585–2587.
11. **Tait-Kamradt, A., J. Clancy, M. Cronan, F. Dib-Hajj, L. Wondrack, W. Yuan, and J. Sutcliffe.** 1997. *mefE* is necessary for the erythromycin-resistant M phenotype in *Streptococcus pneumoniae*. Antimicrob. Agents Chemother. **41:**2251–2255.

Christina Stadler
Michael Teuber*
Laboratory of Food Microbiology
ETH Zurich
CH-8092 Zurich, Switzerland

*Phone: 41-1-632-33-33
Fax: 41-1-632-12-66
E-mail: teuber@ilw.agrl.ethz.ch