ANTIMICROBIAL AGENTS AND CHEMOTHERAPY, Dec. 2006, p. 4189–4190 Vol. 50, No. 12
0066-4804/06/$08.00+0 doi:10.1128/AAC.00657-06

Molecular Analysis of a Chromosome-Carried *erm*(B) Gene and Its Flanking Insertion Points in *Lactobacillus johnsonii* G41▽

Ana Belén Flórez, Mohammed Salim Ammor, Susana Delgado, and Baltasar Mayo*

Instituto de Productos Lácteos de Asturias (CSIC), Carretera de Infiesto s/n, 33300-Villaviciosa, Asturias, Spain

Received 30 May 2006/Returned for modification 28 July 2006/Accepted 12 September 2006

An *erm*(B) gene carried on the *Lactobacillus johnsonii* G41 chromosome and the upstream and downstream regions were fully sequenced. Apparently, a 1,495-bp segment of pRE25 from *Enterococcus faecalis* carrying the *erm*(B) gene became inserted, by an unknown mechanism, into the *L. johnsonii* chromosome.

The possession of no acquired resistance to antibiotics is a key safety criterion that candidate probiotic microorganisms should fulfill besides functional and technological properties (6). A collection of intestinal lactic acid bacteria and bifidobacteria of human origin was investigated for antibiotic resistance during a survey for new probiotic strains (1). Among them, one *Lactobacillus johnsonii* strain (G41) was suspected of being resistant to erythromycin. Characterization of antibiotic resistance genes and the elements involved in their transfer might help to reduce the spread of antibiotic resistance by the food chain (2, 8). Besides mutations in the 23S rRNA molecule, more than 40 genes encoding efflux proteins, methylases, and inactivating enzymes have been described previously (4).

The MIC of erythromycin for strain G41 was found to be 256 μg ml^{-1} by Etest (AB Biodisk, Solna, Sweden). A MIC of 4 μg ml^{-1} has been recently proposed as the microbiological breakpoint to separate susceptible from resistant strains (2). In addition, the MIC of clindamycin for this strain was also 256 μg ml^{-1}.

Identification and location of the *erm*(B) determinant. The genes *ermA*, *erm*(B), *erm*(C), *erm*(F), and *mef*(A) are widely distributed among gram-positive and gram-negative organisms (3, 5). Using as a template total DNA from *L. johnsonii* G41, positive amplification was only obtained for *erm*(B); the primers and PCR conditions used were reported elsewhere (5). The sequence of this amplicon proved to be identical to *erm*(B) sequences from *Streptococcus*, *Enterococcus*, and *Lactobacillus* sp. strains.

As no plasmid DNA was detectable in *L. johnsonii* G41, hybridization experiments, using as a probe a digoxigenin-labeled *erm*(B) internal segment and high-stringency conditions, located the *erm*(B) determinant on the bacterial chromosome (Fig. 1).

Sequence and analysis of the *erm*(B) gene and the surrounding regions. Total DNA from *L. johnsonii* was digested with EcoRI and HindIII, self-ligated, and used as a template for inverse PCR with primers erm(B)1f (5′-CATCAAGCAATG AAACACG-3′) and emrB2r (5′-GTCTGTTTCAAAACAGT AGATG-3′), which are based on *erm*(B) internal sequences. An amplicon of around 5.5 kbp was obtained with the EcoRI-digested ligation, from which the complete sequence of *erm*(B) and the upstream and downstream regions were obtained (GenBank accession no. DQ518904).

Two partial open reading frames (ORFs) with high homology to two contiguous genes on the *L. johnsonii* NCC 533 chromosome (GenBank accession no. NC_005362) were observed at the extremes of the segment (Fig. 2). The first (Δ5′-*orf1* in Fig. 2) encoded the C-terminal part of a phosphoenolpyruvate phosphotransferase component (protein identification no. ABF70486.1). A second ORF split into two parts by the *erm*(B) region (5′-*orf2* and Δ3′-*orf2* in Fig. 2) may code for a hypothetical protein (protein identification no. AAS09591.1).

The inserted sequence comprised 1,495 bp identical to those corresponding to the *erm*(B) locus in *Enterococcus faecalis* plasmid pRE25 (7) (positions 12,577 to 14,022 of the sequence with GenBank accession no. X92945). This segment included three complete ORFs related to the MLS phenotype [*orf3*, encoding the putative MLS leader peptide; the *erm*(B) gene (*orf4*); and *orf5*, a second small ORF present at most *erm*(B) loci (3, 5)] and some adjacent pRE25 sequences.

The insertion is flanked by a duplicated 9-bp sequence (AA AGAAAAA) (Fig. 2), which is originally present once at the

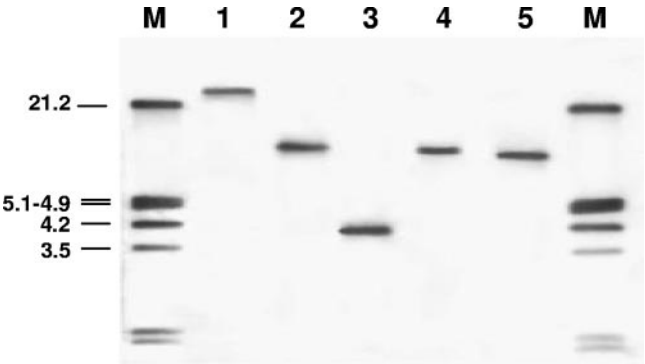

FIG. 1. Southern blot analysis of total genomic DNA from *L. johnsonii* G41 left undigested or digested with EcoRI, BlgII, PstI, or ClaI (lanes 1 through 5, respectively) and hybridized with an internal segment of the *erm*(B) gene obtained by PCR and labeled with digoxigenin (Roche [Hoffmann-La Roche Ltd., Basel, Switzerland]). Lanes M, molecular size marker (digoxigenin-labeled EcoRI-HindIII-digested lambda DNA [Roche]). The values on the left are molecular sizes in kilobase pairs.

* Corresponding author. Mailing address: Baltasar Mayo, Instituto de Productos Lácteos de Asturias (CSIC), Carretera de Infiesto s/n, 33300-Villaviciosa, Spain. Phone: 34 985 89 21 31. Fax: 34 985 89 22 33. E-mail: baltasar.mayo@ipla.csic.es.

▽ Published ahead of print on 25 September 2006.

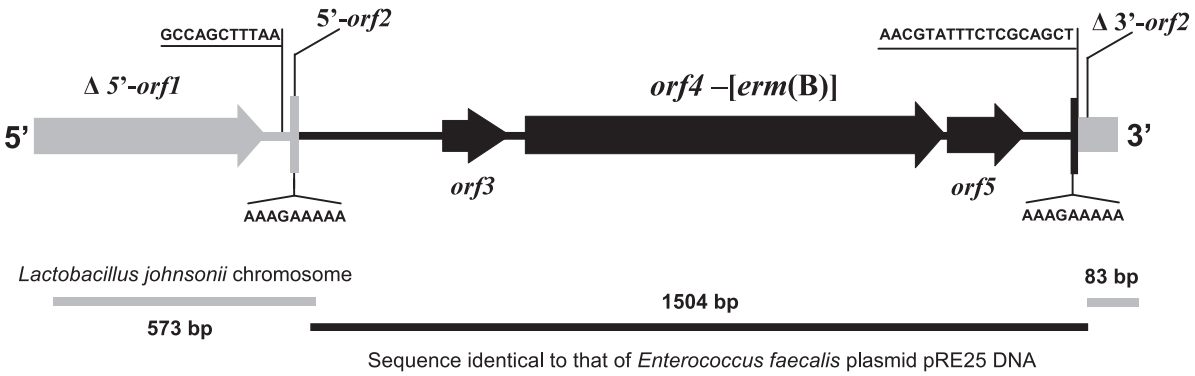

FIG. 2. Diagram of the *erm*(B) gene of *L. johnsonii* G41 and the sequences and structures at its chromosomal integration position. Gray and black bars indicate the segment homologous to the *L. johnsonii* NC 533 chromosome (GenBank accession no. NC_005362) and that identical to the *erm*(B) locus in *E. faecalis* plasmid pRE25 (positions 12,527 to 14,022 of the sequence with GenBank accession no. X92945), respectively. ORFs and sequences are as indicated in the text. This diagram is not drawn to scale.

start of corresponding *orf2* in the *L. johnsonii* NCC 533 genome and twice and in the same place and orientation in the pRE25 sequence. Chromosomal and plasmid sequences might provide homology for the integration of pRE25 in a manner similar to phage integration by *attB* and *attP* sites. However, two sequences with no homology to other DNA sequences appeared around the junction points (Fig. 2): one at the 3′ end (AACGTATTTCTCGCAGCT), immediately downstream of the 9-bp sequence, and a second one at the 5′ end (GCCAG CTTTAA), 75 bp upstream of the 9-bp sequence. These sequences suggest further DNA rearrangement during or after integration.

In conclusion, this work reports on the molecular characterization of an *erm*(B) gene encoding erythromycin and clindamycin resistance in *L. johnsonii* G41. The gene was found to be identical to that present in many gram-positive bacteria. Analysis of the surrounding regions suggested that a segment of the *erm*(B) locus from enterococcal plasmid pRE25 was integrated and rearranged into the *L. johnsonii* genome via unknown mechanisms.

This work was supported by an EU project within the sixth Framework Programme (ACE-ART, reference no. 506214). M. S. Ammor was awarded a postdoctoral fellowship from the Secretaría de Estado de Universidades e Investigación of the Spanish Ministry of Education and Science (reference no. SB2004-0165).

REFERENCES

1. **Delgado, S., A. B. Flórez, and B. Mayo.** 2005. Antibiotic susceptibility of *Lactobacillus* and *Bifidobacterium* species from the human gastrointestinal tract. Curr. Microbiol. **50:**202–207.
2. **European Commission.** 2005. Opinion of the Scientific Panel on Additives and Products in Substances used in Animal Feed on the updating of the criteria used in the assessment of bacteria for resistance to antibiotics of human and veterinary importance. EFSA J. **223:**1–12.
3. **Jensen, L. B., N. Frimodt-Moller, and F. M. Aarestrup.** 1999. Presence of *erm* gene classes in gram-positive bacteria of animal and human origin in Denmark. FEMS Microbiol. Lett. **170:**151–158.
4. **Leclercq, R.** 2002. Mechanisms of resistance to macrolides and lincosamides: nature of the resistance elements and their clinical implications. Clin. Infect. Dis. **34:**482–492.
5. **Roberts, M. C., W. O. Chung, D. Roe, M. Xia, C. Marquez, G. Borthagaray, W. L. Whittington, and K. K. Holmes.** 1999. Erythromycin-resistant *Neisseria gonorrhoeae* and oral commensal *Neisseria* spp. carry known rRNA methylase genes. Antimicrob. Agents Chemother. **43:**1367–1372.
6. **Saarela, M., G. Mogensen, R. Fonden, J. Mättö, and T. Mattila-Sandholm.** 2000. Probiotic bacteria: safety, functional and technological properties. J. Biotechnol. **84:**197–215.
7. **Schwarz, F. V., V. Perreten, and M. Teuber.** 2001. Sequence of the 50-kb conjugative multiresistance plasmid pRE25 from *Enterococcus faecalis* RE25. Plasmid **46:**170–187.
8. **Teuber, M., L. Meile, and F. Schwarz.** 1999. Acquired antibiotic resistance in lactic acid bacteria from food. Antonie Leeuwenhoek **76:**115–137.