AAC Journals.ASM.org

Genetic Environment of the *lnu*(B) Gene in a *Streptococcus agalactiae* Clinical Isolate

A. Montilla,[a] A. Zavala,[a] R. Cáceres Cáceres,[b] R. Cittadini,[c] C. Vay,[b,c] G. Gutkind,[a] A. Famiglietti,[b] L. Bonofiglio,[a] M. Mollerach[a]

Cátedra de Microbiología, Facultad de Farmacia y Bioquímica, Universidad de Buenos Aires, Ciudad Autónoma de Buenos Aires, Argentina[a]; Laboratorio de Bacteriología Clínica, Departamento de Bioquímica Clínica, Hospital de Clínicas José de San Martín, Facultad de Farmacia y Bioquímica, Universidad de Buenos Aires, Ciudad Autónoma de Buenos Aires, Argentina[b]; Sanatorio Mater Dei, Ciudad Autónoma de Buenos Aires, Argentina[c]

Specific resistance to lincosamides (L phenotype) is the result of modification and inactivation by lincosamide nucleotidyltransferase enzymes encoded by members of the *lnu* (previously *lin*) gene family (1, 2), of which six different types, *lnu*(A), *lnu*(B), *lnu*(C), *lnu*(D), *lnu*(E), and *lnu*(F), are currently recognized (1, 3–7). In *Streptococcus agalactiae*, the genetic environment in which the gene *lnu*(B) is located has not been explored; in contrast, a genetic element carrying *lnu*(C) has been reported in this species (1), as well as different elements harboring *lnu*(A) and *lnu*(B) in *Staphylococcus aureus* (8, 9), *lnu*(B) in *Enterococcus faecium* (10), *lnu*(C) in *Streptococcus anginosus* (11), and a truncated copy of *lnu*(E) in *Streptococcus suis* (7).

In 2008, an *S. agalactiae* strain (SGB76) was obtained from a pregnant female outpatient at Mater Dei Hospital in Buenos Aires. By Etest, the isolate showed susceptibility to erythromycin (MIC of 0.06 μg/ml) and resistance to clindamycin (MIC of 6 μg/ml). Total DNA was used as the template for PCR screening of *erm*(A), *erm*(B), *mef*(A), and *lnu*(B) genes. Of these, only *lnu*(B) was detected and confirmed by sequencing. To determine the genetic environment of the *lnu*(B) gene, two strategies were used, thermal asymmetric interlaced PCR (TAIL-PCR) in combination with PCR mapping using primers designed based on the previously reported structures from *Enterococcus faecalis* and *S. aureus* (GenBank accession numbers AF408195.1, JQ861958.1, and JX560992) (see Table S1 in the supplemental material).

The 12,076-bp *lnu*(B)-carrying fragment from strain SGB76 contained 11 open reading frames (ORFs) of at least 100 amino acids (accession number KF772204). Other resistance genes detected in this structure include *aadE* (streptomycin resistance), *spw* (spectinoycin resistance), and *lsa*(E) (pleuromutilin, lincosamide, and streptogramin A resistance) (Fig. 1). Basic Local Alignment Search Tool (BLAST) analysis revealed that this sequence exhibited similarity to the *lnu*(B)-containing structures previously identified in *S. aureus* (JQ861959 and JX560992) and *E. faecalis* (AF408195) (Fig. 1) (8, 9). An insertion sequence (IS*1216E*) is located at the left-hand end, similar to IS*1216* in the structure described in *S. aureus* (JX560992) and in a variant of the latter, IS*1216v*, in *E. faecalis*. However, two copies of IS*257* are flanking the *lnu*(B)-carrying element in the structure described in *S. aureus* by Lozano et al. (accession number JQ861959) (9). The region of 5,982 bp located upstream from the *lnu*(B) gene is 99%

Published ahead of print 23 June 2014

Address correspondence to M. Mollerach, mmollera@ffyb.uba.ar.

Supplemental material for this article may be found at http://dx.doi.org/10.1128/AAC.02630-14.

doi:10.1128/AAC.02630-14

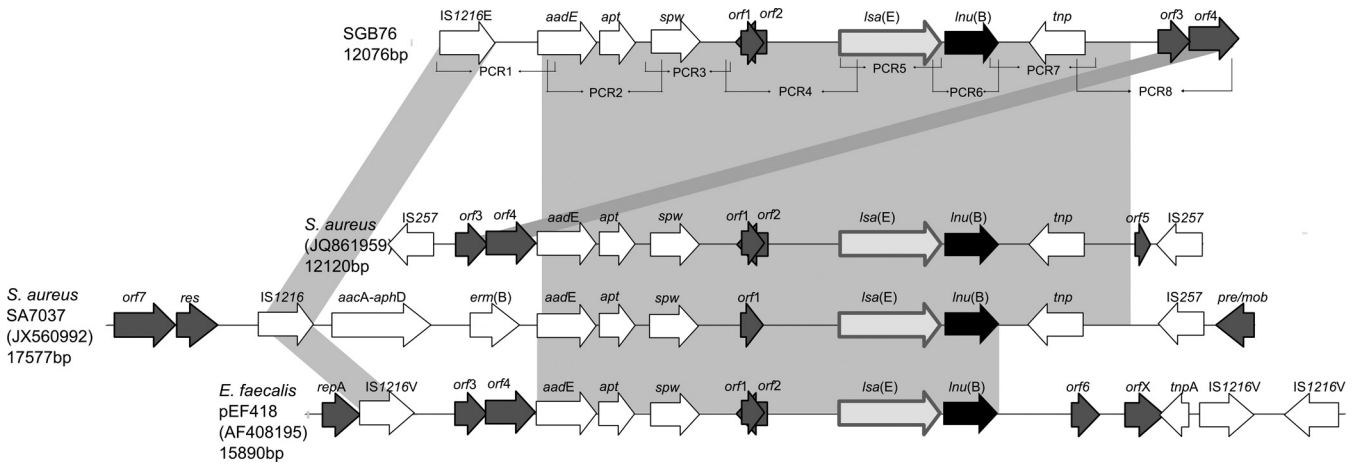

FIG 1 Genetic environment of the *lnu*(B) gene in *S. agalactiae* SGB76 (accession number KF772204) and structural comparison with the corresponding regions identified in *S. aureus* (accession numbers JQ861959 and JX560992) and *E. faecalis* pEF418 (accession number AF408195). Gray areas indicate regions with at least 99% nucleotide sequence identity. The arrows represent the position and orientation of the depicted genes as they were proposed in the original references. The amplicons of the 8 PCRs used to investigate the genetic environment of the *lnu*(B) gene in SGB76 are indicated below the top structure (see Table S1 in the supplemental material). *orf*1 to *orf7* represent open reading frames with either unknown or unconfirmed functions. *aadE*, aminoglycoside adenyltransferase E; *aacA-aphD*, aminoglycoside acetyltransferase A and aminoglycoside phosphotransferase D; *apt*, adenine phosphoribosyltransferase; *erm*(B), methyltransferase; IS, insertion sequence; *lnu*(B), lincosamide nucleotidyltransferase; *lsa*(E), ATP binding protein; *res* and *mob*, mobilization elements; *spw*, spectinomycin resistance gene; *tnp*, transposase.

identical to the structures described in *S. aureus* and *E. faecalis* (Fig. 1). The *lsa*(E) gene located immediately upstream from the *lnu*(B) gene encodes an ABC transporter involved in active efflux of lincosamides, streptogramins A, and pleuromutilins (10, 12). However, the region of 2,331 bp situated downstream from the *lnu*(B) gene showed 99% nucleotide identity to the *S. aureus* (but not to the *E. faecalis*) structure, which includes one copy of the gene encoding a putative transposase of the IS*L3* family (*tnp*) (Fig. 1). Besides the occurrence of this cluster in *E. faecalis* and *S. aureus*, a sequence from an *Enterococcus faecium* isolate of swine origin that was recently released (KF421157.1) (10) shows 99% nucleotide identity.

At the right-hand end, a region containing two genes (*orf3* and *orf4*) is 98% identical to the *orf3* and *orf4* region described in *S. aureus* (JQ861959); however, in the latter it is located at the left-hand end, associated with IS*257*. The description of this multiresistance cluster in *S. agalactiae* represents another example of resistance genes shared by enterococci, staphylococci, and streptococci (10).

Even if *S. agalactiae* SGB76 was susceptible to quinupristin-dalfopristin, as susceptibility to pleuromutilins and streptogramin A could not be assayed, a possible contribution of the *lsa*(E) gene to clindamycin resistance, as was described in *S. aureus*, could not be disregarded (8, 12). Further experiments will assess the contribution of this resistance marker in *S. agalactiae*.

Nucleotide sequence accession number. Sequence data were deposited in the GenBank/EMBL nucleotide databases under accession number KF772204.

ACKNOWLEDGMENTS

This work was supported in part by grants from the University of Buenos Aires, Argentina (number 20020100100510), and CONICET Argentina (PIP, 11220110100707CO). M.M., G.G., and L.B. are members of CONICET.

REFERENCES

1. **Achard A, Villers C, Pichereau V, Leclercq R.** 2005. New *lnu*(C) gene conferring resistance to lincomycin by nucleotidylation in *Streptococcus agalactiae* UCN36. Antimicrob. Agents Chemother. **49:**2716–2719. http://dx.doi.org/10.1128/AAC.49.7.2716-2719.2005.
2. **Roberts MC.** 2008. Update on macrolide-lincosamide-streptogramin, ketolide, and oxazolidinone resistance genes. FEMS Microbiol. Lett. **282:**147–159. http://dx.doi.org/10.1111/j.1574-6968.2008.01145.x.
3. **Bozdogan B, Berrezouga L, Kuo MS, Yurek DA, Farley KA, Stockman BJ, Leclercq R.** 1999. A new resistance gene, linB, conferring resistance to lincosamides by nucleotidylation in *Enterococcus faecium* HM1025. Antimicrob. Agents Chemother. **43:**925–929.
4. **Brisson-Noël A, Delrieu P, Samain D, Courvalin P.** 1988. Inactivation of lincosaminide antibiotics in *Staphylococcus*. Identification of lincosaminide *O*-nucleotidyltransferases and comparison of the corresponding resistance genes. J. Biol. Chem. **263:**15880–15887.
5. **de Azavedo JC, McGavin M, Duncan C, Low DE, McGeer A.** 2001. Prevalence and mechanisms of macrolide resistance in invasive and noninvasive group B streptococcus isolates from Ontario, Canada. Antimicrob. Agents Chemother. **45:**3504–3508. http://dx.doi.org/10.1128/AAC.45.12.3504-3508.2001.
6. **Petinaki E, Guerin-Faublee V, Pichereau V, Villers C, Achard A, Malbruny B, Leclercq R.** 2008. Lincomycin resistance gene *lnu*(D) in *Streptococcus uberis*. Antimicrob. Agents Chemother. **52:**626–630. http://dx.doi.org/10.1128/AAC.01126-07.
7. **Zhao Q, Wendlandt S, Li H, Li J, Wu C, Shen J, Schwarz S, Wang Y.** 2014. Identification of the novel lincosamide resistance gene *lnu*(E) truncated by IS*Enfa5*-*cfr*-IS*Enfa5* insertion in *Streptococcus suis*: *de novo* synthesis and confirmation of functional activity in *Staphylococcus aureus*. Antimicrob. Agents Chemother. **58:**1785–1788. http://dx.doi.org/10.1128/AAC.02007-13.
8. **Li B, Wendlandt S, Yao J, Liu Y, Zhang Q, Shi Z, Wei J, Shao D, Schwarz S, Wang S, Ma Z.** 2013. Detection and new genetic environment of the pleuromutilin-lincosamide-streptogramin A resistance gene *lsa*(E) in methicillin-resistant *Staphylococcus aureus* of swine origin. J. Antimicrob. Chemother. **68:**1251–1255. http://dx.doi.org/10.1093/jac/dkt015.
9. **Lozano C, Aspiroz C, Saenz Y, Ruiz-Garcia M, Royo-Garcia G, Gomez-Sanz E, Ruiz-Larrea F, Zarazaga M, Torres C.** 2012. Genetic environment and location of the *lnu*(A) and *lnu*(B) genes in methicillin-resistant *Staphylococcus aureus* and other staphylococci of animal and human origin. J. Antimicrob. Chemother. **67:**2804–2808. http://dx.doi.org/10.1093/jac/dks320.
10. **Li XS, Dong WC, Wang XM, Hu GZ, Wang YB, Cai BY, Wu CM, Wang Y, Du XD.** 2014. Presence and genetic environment of pleuromutilin-lincosamide-streptogramin A resistance gene *lsa*(E) in enterococci of human and swine origin. J. Antimicrob. Chemother. **69:**1424–1426. http://dx.doi.org/10.1093/jac/dkt502.
11. **Gravey F, Galopin S, Grall N, Auzou M, Andremont A, Leclercq R, Cattoir V.** 2013. Lincosamide resistance mediated by *lnu*(C) (L phenotype) in a *Streptococcus anginosus* clinical isolate. J. Antimicrob. Chemother. **68:**2464–2467. http://dx.doi.org/10.1093/jac/dkt255.
12. **Wendlandt S, Lozano C, Kadlec K, Gomez-Sanz E, Zarazaga M, Torres C, Schwarz S.** 2013. The enterococcal ABC transporter gene *lsa*(E) confers combined resistance to lincosamides, pleuromutilins and streptogramin A antibiotics in methicillin-susceptible and methicillin-resistant *Staphylococcus aureus*. J. Antimicrob. Chemother. **68:**473–475. http://dx.doi.org/10.1093/jac/dks398.