APPLIED AND ENVIRONMENTAL MICROBIOLOGY, May 2009, p. 3358–3361 Vol. 75, No. 10
0099-2240/09/$08.00+0 doi:10.1128/AEM.02538-08

Activation and Transfer of the Chromosomal Phage Resistance Mechanism AbiV in *Lactococcus lactis*▽

Jakob Haaber,[1] Sylvain Moineau,[2] and Karin Hammer[1]*

Center for Systems Microbiology, DTU Systems Biology, Technical University of Denmark, DK-2800 Lyngby, Denmark,[1] and Département de Biochimie et de Microbiologie, Faculté des Sciences et de Génie, Groupe de Recherche en Écologie Buccale, Faculté de Médecine Dentaire, Félix d'Hérelle Reference Center for Bacterial Viruses, Université Laval, Québec, Canada G1V 0A6[2]

Received 6 November 2008/Accepted 9 March 2009

AbiV is a chromosomally encoded phage resistance mechanism that is silent in the wild-type phage-sensitive strain *Lactococcus lactis* subsp. *cremoris* MG1363. Spontaneous phage-resistant mutants of *L. lactis* MG1363 were analyzed by reverse transcriptase PCR and shown to express AbiV. This expression was related to a reorganization in the upstream region of *abiV*. Transfer of *abiV* between two lactococcal strains, most likely by conjugation, was also demonstrated. To our knowledge, this is the first report of natural transfer of a chromosomally encoded phage resistance mechanism.

Industrial milk fermentation is dependent on the well-characterized metabolic features of commercial starter cultures, which contain strains of lactic acid bacteria (6). However, milk fermentation failures due to virulent phages that are infecting these specialized bacterial cultures is a persistent problem for the dairy industry (6, 8, 9, 26). Decades of research have led to the discovery of a number of natural defense systems in *Lactococcus lactis* cells, including the inhibition of phage adsorption (12, 21) and DNA entry (25), restriction/modification systems (1, 12), and abortive infection (Abi) mechanisms (5). These antiviral mechanisms have been used extensively in a relatively small number of industrial strains (7), which has favored the emergence of phage mutants that are insensitive to the natural antiphage barriers (8, 12, 26). This viral evolutionary process has led to a lasting search for new ways to protect cultures against phage attacks. Moreover, to avoid the use of genetic engineering technology, the dairy industry is currently depending on the isolation of novel natural phage resistance barriers in a given wild-type *L. lactis* isolate that can be naturally transferred into industrial starter strains.

Most known lactococcal restriction/modification and Abi systems are plasmid encoded (2, 3, 5, 10, 11, 13, 20, 29, 39), and some of them can be easily transferred from one strain to another through conjugation. This genetic transformation process is universally accepted and has been successfully utilized to create phage-resistant starter cultures (1, 6, 19, 26, 28, 30, 34). Some phage resistance mechanisms are also chromosomally encoded. However, their industrial application is limited because they cannot be transferred into the desired industrial strains without the use of genetic engineering.

Recently, we isolated a novel chromosomally encoded Abi mechanism named AbiV (17) that is active against several lactococcal phages. The *abiV* gene is silent in the phage-sensitive strain *L. lactis* subsp. *cremoris* MG1363, but it can be activated when a promoter is provided (17). Here, we report the isolation of natural bacteriophage-insensitive mutants (BIMs) of *L. lactis* MG1363 that spontaneously express AbiV. Furthermore, we demonstrate that *abiV* can be transferred to lactococcal strains with a protocol used for conjugation assays.

Isolation of mutants of *L. lactis* MB112 spontaneously expressing AbiV. To investigate whether MG1363 could mutate spontaneously to express AbiV, we isolated mutants that could grow in the presence of the virulent phage sk1 (4). Ten independent cultures of *L. lactis* MB112 (fluorouracil-resistant *L. lactis* MG1363, Δ*upp*) (24) were grown exponentially at 30°C in M17 medium (37) supplemented with 0.5% glucose and then mixed with the 936-like phage sk1 at a multiplicity of infection of >1 in the presence of 10 mM $CaCl_2$. The phage-infected bacterial cultures were then incubated for 10 min at room temperature before plating and incubation overnight at 36°C. BIMs that spontaneously gained resistance to sk1 were observed at a frequency of 10^{-8}. Fifty-six colonies were picked randomly among the 10 independent cultures, purified, and cross-streaked (31) against virulent phages sk1, p2 (27), 712 (23), and the AbiV-insensitive mutant p2.1 (18). A BIM expressing AbiV is expected to be resistant to phages sk1 and p2 but sensitive to 712 and p2.1 due to the absence of a functional AbiV target gene (*sav*) in the latter two phages (18). Of the 56 BIMs, one had the expected efficiency of plaquing (EOP) (32) values. The EOP values for this BIM (named *L. lactis* JH-80) were 2×10^{-5} for phage p2, 0.75 for phage 712, and 0.8 for phage p2.1 (17). These values correspond to values obtained with *L. lactis* JH-20 and JH-32 (engineered strains expressing AbiV; see below) (17), suggesting that the natural BIM *L. lactis* JH-80 may now be expressing AbiV.

To verify that the phage resistance phenotype was indeed caused by the production of AbiV, the transcription of *abiV* in *L. lactis* JH-80 was investigated by using reverse transcriptase PCR (RT-PCR) as described previously (17). The RT-PCR was performed on RNA isolated from *L. lactis* JH-80 and also on RNA from *L. lactis* JH-20 (*L. lactis* MB112 containing *abiV* cloned into the expression vector pLC5) (17), JH-54 (*L. lactis*

* Corresponding author. Mailing address: Center for Systems Microbiology, DTU Systems Biology, Matematiktorvet Bldg. 301, Technical University of Denmark, DK-2800 Lyngby, Denmark. Phone: 45 45 25 24 96. Fax: 45 45 93 28 09. E-mail: kha@bio.dtu.dk.

▽ Published ahead of print on 13 March 2009.

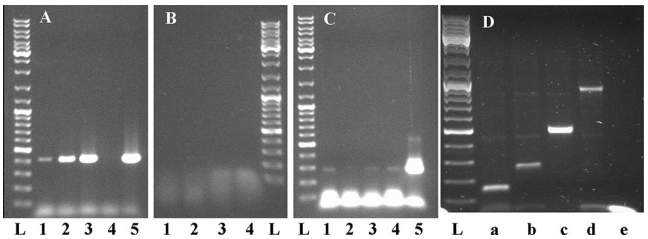

FIG. 1. RT-PCR assays were carried out on RNA isolated from various *L. lactis* strains. (A) Expression of AbiV. (B) Control experiment performed in the absence of RT. (C) Expression of gene *tnp981*. (A to C) Lane 1, *L. lactis* JH-80 (spontaneous BIM); lane 2, *L. lactis* JH-32 (insertional mutant expressing *abiV*); lane 3, *L. lactis* JH-20 (*abiV* gene cloned into an expression vector); lane 4, *L. lactis* JH-54 (empty vector); lane 5, positive PCR control using genomic DNA from *L. lactis* MG1363; lane L, GeneRuler ladder (Fermentas). (D) Detection of increasing lengths of *abiV* transcripts using the same forward primer in *abiV* and progressively more-distant reverse primers. (D) Lane a, forward and reverse (rev) primers located in *abiV*; lane b, rev primer 75 bp upstream of *abiV*; lane c, rev primer 310 bp upstream of *abiV*; lane d, rev primer 727 bp upstream of *abiV*; lane e, rev primer 1,079 bp upstream of *abiV*; lane L, GeneRuler ladder (Fermentas).

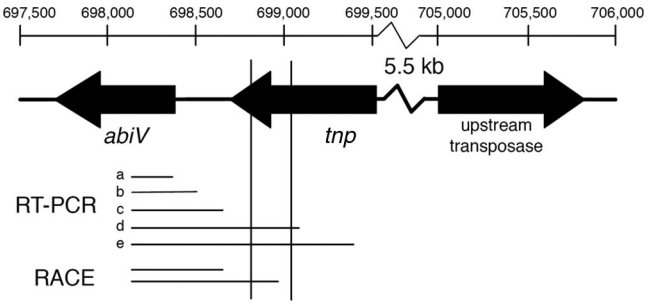

FIG. 2. *abiV* and its upstream region, with *tnp* designating *tnp981*. The *abiV* transcription start site was shown with RT-PCR and RACE. Lines a to d represent the results of the four positive reactions and line e represents the result of the negative reaction shown in Fig. 1D. Two sizes of transcripts were obtained by using RACE. Vertical lines symbolize the range of observed mutations in *tnp981* that were likely caused by a recombination event with the very similar, inversely oriented transposase located 5.5 kb upstream of *tnp981*. Numbers refer to nucleotide numbers in GenBank AM406671. Figure not drawn to scale.

MB112 containing pLC5 without *abiV*) (17), and JH-32 (*L. lactis* MB112 expressing *abiV* due to the integration of pGhost9::IS*S1*) (17). While the levels of *abiV* mRNA in *L. lactis* strains JH-20 and JH-32 were the highest, JH-80 also showed transcription in comparison to undetectable transcription in *L. lactis* JH-54 (Fig. 1A). No PCR products were obtained in control experiments (omitting the RT enzyme), indicating that the RNA preparations were free of contaminating DNA (Fig. 1B). The above data demonstrate that *L. lactis* BIMs that spontaneously express AbiV, thereby conferring phage resistance to the cell without artificial genetic modifications, can be isolated.

In an attempt to elucidate the mutation(s) in the BIM *L. lactis* JH-80 (AbiV$^+$), we PCR amplified a 6,320-bp region (nucleotides [nt] 698427 to 704747 in GenBank AM406671) that included the upstream region of *abiV*. The PCR-amplified DNA fragments of both *L. lactis* JH-80 and reference strain *L. lactis* MB112 were sequenced on both strands. The *L. lactis* MB112 sequence was identical to the *L. lactis* MG1363 sequence. However, four point mutations were found in JH-80, located between 400 bp and 700 bp upstream of *abiV* but within the upstream gene *tnp981* that codes for a putative transposase for the insertion sequence IS*981F* (Fig. 2). Analysis of the region containing the mutations revealed 100% nucleotide identity with the 3′ region (500 nt) of a transposase (nt 705075 to 705926 in GenBank AM406671) which shares 99% nucleotide identity with *tnp981*. This transposase is inversely oriented and located 6,671 bp upstream of *abiV* (Fig. 2). The exact match of the mutated region in *tnp981* to this transposase and the absence of large inversions in the 6-kb region upstream of *abiV* strongly indicate that the mutations were caused by recombination between the two genes.

No terminator structure could be identified between *abiV* and *tnp981*, leaving the possibility that promoter activity upstream or within *tnp981* caused the increased expression of *abiV* in JH-80. We therefore determined the transcription level of *tnp981* in *L. lactis* strains JH-80, JH-32, JH-20, and JH-54. We observed faint bands in all cases, indicating that *tnp981* was transcribed at low levels in the four strains (Fig. 1C). However, nine other transposases that share 99% nucleotide similarity with *tnp981* exist on the genome of *L. lactis* MG1363, thereby complicating the analysis of the expression of *tnp981*. Nevertheless, since the transcription levels of *tnp981* were similar in all strains tested, we concluded that the increased promoter activity upstream of *tnp981* was not the cause of the elevated levels of *abiV* in *L. lactis* JH-80.

To test whether the observed mutations in *L. lactis* JH-80 were involved in *abiV* transcription, we performed RT-PCR (Fig. 1D and Fig. 2). The resulting data indicate that the *abiV* transcripts start between 727 nt and 1,079 nt upstream of the gene. Determination of the *abiV* 5′ mRNA ends was performed by using the rapid amplification of cDNA ends (RACE) method (38). *abiV* mRNA ends were found at 320 nt, as well as at 583 nt, upstream of the gene. Since the *abiV* transcription is initiated upstream of the mutations in *tnp981*, the expression of *abiV* in *L. lactis* JH-80 was probably not caused by increased promoter activity. Instead, the mutations may increase the stability or some antitermination of the transcript from an existing weak promoter and hence increase AbiV expression in the BIM *L. lactis* JH-80. However, our data do not allow us to determine the reason for the weak induction of *abiV* gene expression in the mutant.

Transfer of *abiV* between two *L. lactis* strains. Most known Abi mechanisms are plasmid encoded, though it has been argued that this overrepresentation could be due to the technical advantages of isolating plasmid-encoded Abi systems (5). As indicated above, the *abiV* gene is located on the chromosome of *L. lactis* MG1363 and possibly not readily transferable to another strain, as compared to plasmid-encoded systems. However, conjugation of chromosomal elements, facilitated by the chromosomally encoded sex-factor that is present in, among others, *L. lactis* MG1363 (35, 36), has been previously observed in *L. lactis* (14, 16). This genetic element permits the exchange of genetic material between lactococcal strains by chromosomal transfer and subsequent recombination. Therefore, we investigated whether we could take advantage of this

ability to transfer an active *abiV* gene from one strain to another.

We used the donor strain *L. lactis* JH-32 (AbiV$^+$, erythromycin resistant [Erm^r], and fluorouracil resistant [FU^r]) and the recipient strain *L. lactis* MG1614, an MG1363 derivative that is resistant to rifampin (rifampicin) (Rif^r) and streptomycin (Str^r) (15). In *L. lactis* JH-32, the *abiV* gene is activated by the vector pGhost9::IS*S1* (22) inserted immediately upstream of *abiV* on the bacterial chromosome. We envisioned that if pGhost9::IS*S1* was successfully transferred to *L. lactis* MG1614 by conjugation, *abiV* most likely would be as well, due to their close proximity on the chromosome of *L. lactis* JH-32 (17). Here, as a proof of concept, we used erythromycin resistance (from pGhost9::IS*S1*) as a selection marker.

Briefly, donor and recipient cells were grown separately on GM17 plates, subsequently recovered with saline (0.9% NaCl), and then mixed at ratios of 1:1, 1:3, and 1:9. The mixtures were immediately plated (0.1 ml plate^{-1}) on GM17 and incubated in anaerobic jars overnight at 36°C. This incubation temperature was selected to avoid excision of the integrated pGhost9::IS*S1* in *L. lactis* JH-32 (17, 22). Cells were recovered from GM17 plates with saline and incubated again anaerobically (48 h at 36°C) but on GM17 plates containing erythromycin (3 μg ml^{-1}) and rifampin (100 μg ml^{-1}). These two selection markers were used to select for *L. lactis* MG1614 (rifampin resistant) transconjugants that have acquired pGhost9::IS*S1* (erythromycin resistance). The lactococcal colonies that grew on GM17 plates containing erythromycin and rifampin were then tested for their sensitivity to fluorouracil (0.3 μg ml^{-1}) and their resistance to streptomycin (200 μg ml^{-1}) and phages. By using this phage- and streptomycin-free selection approach, we virtually eliminated the risk of isolating false positives due to spontaneous mutations causing the resistance phenotype. Transconjugants with the phenotype, Rif^r, Str^r, FU^s, Erm^r, and phage resistance, are expected to be derivatives of MG1614 (Rif^r Str^r) that have acquired Erm^r and phage resistance from JH-32 by chromosomal transfer.

Seven putative transconjugants (Erm^r Rif^r) were first isolated after two days of anaerobic incubation (36°C). Five of these mutants were derived from the donor and had acquired a spontaneous Rif^r resistance. One mutant was derived from the recipient, with a spontaneous Erm^r mutation. However, one mutant (*L. lactis* JH-83) had the expected phenotype (Rif^r, Str^r, FU^s, Erm^r, and phage resistance). In fact, phage p2 had an EOP of 10^{-4} on *L. lactis* JH-83. These data strongly suggested that *L. lactis* JH-83 is a transconjugant of *L. lactis* MG1614 that has acquired an activated *abiV* gene by conjugation and recombination. Next, we sequenced the *rpsL* gene of the four strains *L. lactis* MB112, MG1614, JH-32, and JH-83 and found a specific K-to-R amino acid substitution in *rpsL* (data not shown) that is known to cause streptomycin resistance in different bacterial species (33). The same mutation was found in *L. lactis* MG1614 and JH-83, while *L. lactis* MB112 and JH-32 had the wild-type sequence. Since *rpsL* was identical in JH-83 and MG1614 and streptomycin was not used as a selection marker, we concluded that indeed MG1614 was the parental origin of JH-83. To our knowledge, this is the first demonstration of a natural transfer of a chromosomally encoded phage resistance mechanism.

Taken altogether, the above data indicate that the chromosomally encoded *abiV* can be spontaneously activated and also naturally transferred to other lactococcal strains by conjugation. This study suggests that the search for novel chromosomally encoded Abi mechanisms should be revisited and that this may open up new ways to construct naturally phage-resistant strains for large-scale industrial applications.

This work was funded in part by a graduate scholarship from the Technical University of Denmark and a grant from the Proof-of-Concept Consortium to J.H. and a strategic grant from the Natural Sciences and Engineering Research Council (NSERC) of Canada to S.M.

REFERENCES

1. **Allison, G. E., and T. R. Klaenhammer.** 1998. Phage resistance mechanisms in lactic acid bacteria. Int. Dairy J. **8:**207–226.
2. **Bouchard, J. D., E. Dion, F. Bissonnette, and S. Moineau.** 2002. Characterization of the two-component abortive phage infection mechanism AbiT from *Lactococcus lactis*. J. Bacteriol. **184:**6325–6332.
3. **Boucher, I., E. Emond, E. Dion, D. Montpetit, and S. Moineau.** 2000. Microbiological and molecular impacts of AbiK on the lytic cycle of *Lactococcus lactis* phages of the 936 and P335 species. Microbiology **146:**445–453.
4. **Chandry, P. S., S. C. Moore, J. D. Boyce, B. E. Davidson, and A. J. Hillier.** 1997. Analysis of the DNA sequence, gene expression, origin of replication and modular structure of the *Lactococcus lactis* lytic bacteriophage sk1. Mol. Microbiol. **26:**49–64.
5. **Chopin, M. C., A. Chopin, and E. Bidnenko.** 2005. Phage abortive infection in lactococci: variations on a theme. Curr. Opin. Microbiol. **8:**473–479.
6. **Coffey, A., and R. P. Ross.** 2002. Bacteriophage-resistance systems in dairy starter strains: molecular analysis to application. Antonie van Leeuwenhoek **82:**303–321.
7. **Daly, C., G. F. Fitzgerald, L. O'Connor, and R. Davis.** 1998. Technology and health benefits of dairy starter cultures. Int. Dairy J. **8:**195–205.
8. **Daly, C., G. F. Fitzgerald, and R. Davis.** 1996. Biotechnology of lactic acid bacteria with special reference to bacteriophage resistance. Antonie van Leeuwenhoek **70:**99–110.
9. **Deveau, H., S. J. Labrie, M. C. Chopin, and S. Moineau.** 2006. Biodiversity and classification of lactococcal phages. Appl. Environ. Microbiol. **72:**4338–4346.
10. **Durmaz, E., and T. R. Klaenhammer.** 2007. Abortive phage resistance mechanism AbiZ speeds the lysis clock to cause premature lysis of phage-infected *Lactococcus lactis*. J. Bacteriol. **189:**1417–1425.
11. **Emond, E., E. Dion, S. A. Walker, E. R. Vedamuthu, J. K. Kondo, and S. Moineau.** 1998. AbiQ, an abortive infection mechanism from *Lactococcus lactis*. Appl. Environ. Microbiol. **64:**4748–4756.
12. **Forde, A., and G. F. Fitzgerald.** 1999. Bacteriophage defence systems in lactic acid bacteria. Antonie van Leeuwenhoek **76:**89–113.
13. **Garvey, P., C. Hill, and G. F. Fitzgerald.** 1996. The lactococcal plasmid pNP40 encodes a third bacteriophage resistance mechanism, one which affects phage DNA penetration. Appl. Environ. Microbiol. **62:**676–679.
14. **Gasson, M., J. J. Godon, C. J. Pillidge, T. J. Eaton, K. L. Jury, and C. Shearman.** 1995. Characterization and exploitation of conjugation in *lactococcus lactis*. Int. Dairy J. **5:**757–762.
15. **Gasson, M. J.** 1983. Plasmid complements of *Streptococcus lactis* NCDO 712 and other lactic streptococci after protoplast-induced curing. J. Bacteriol. **154:**1–9.
16. **Gireesh, T., B. E. Davidson, and A. J. Hillier.** 1992. Conjugal transfer in *Lactococcus lactis* of a 68-kilobase-pair chromosomal fragment containing the structural gene for the peptide bacteriocin nisin. Appl. Environ. Microbiol. **58:**1670–1676.
17. **Haaber, J., L. C. Fortier, S. Moineau, and K. Hammer.** 2008. AbiV, a novel abortive phage infection mechanism on the chromosome of *Lactococcus lactis* subsp. *cremoris* MG1363. Appl. Environ. Microbiol. **74:**6528–6537.
18. **Haaber, J., G. M. Rousseau, K. Hammer, and S. Moineau.** 2009. Identification and characterization of the phage gene *sav*, involved in sensitivity to the lactococcal abortive infection mechanism AbiV. Appl. Environ. Microbiol. **75:**2484–2494.
19. **Harrington, A., and C. Hill.** 1991. Construction of a bacteriophage-resistant derivative of *Lactococcus lactis* subsp. *lactis* 425A by using the conjugal plasmid pNP40. Appl. Environ. Microbiol. **57:**3405–3409.
20. **Hill, C., L. A. Miller, and T. R. Klaenhammer.** 1990. Nucleotide sequence and distribution of the pTR2030 resistance determinant (*hsp*) which aborts bacteriophage infection in lactococci. Appl. Environ. Microbiol. **56:**2255–2258.
21. **Josephsen, J., and H. Neve.** 2004. Bacteriophage and antiphage mechanisms of lactic acid bacteria, p. 295–350. *In* S. Salminen, A. von Wright, and A. Ouwehand (ed.), Lactic acid bacteria, microbiological and functional aspects. CRC Press, London, United Kingdom.
22. **Maguin, E., H. Prevost, S. D. Ehrlich, and A. Gruss.** 1996. Efficient inser-

tional mutagenesis in lactococci and other gram-positive bacteria. J. Bacteriol. **178:**931–935.
23. **Mahony, J., H. Deveau, S. McGrath, M. Ventura, C. Canchaya, S. Moineau, G. F. Fitzgerald, and D. van Sinderen.** 2006. Sequence and comparative genomic analysis of lactococcal bacteriophages jj50, 712 and P008: evolutionary insights into the 936 phage species. FEMS Microbiol. Lett. **261:**253–261.
24. **Martinussen, J., and K. Hammer.** 1994. Cloning and characterization of *upp*, a gene encoding uracil phosphoribosyltransferase from *Lactococcus lactis*. J. Bacteriol. **176:**6457–6463.
25. **McGrath, S., G. F. Fitzgerald, and D. van Sinderen.** 2002. Identification and characterization of phage-resistance genes in temperate lactococcal bacteriophages. Mol. Microbiol. **43:**509–520.
26. **Moineau, S.** 1999. Applications of phage resistance in lactic acid bacteria. Antonie van Leeuwenhoek **76:**377–382.
27. **Moineau, S., S. A. Walker, E. R. Vedamuthu, and P. A. Vandenbergh.** 1995. Cloning and sequencing of LlaDCHI [corrected] restriction/modification genes from *Lactococcus lactis* and relatedness of this system to the *Streptococcus pneumoniae* DpnII system. Appl. Environ. Microbiol. **61:**2193–2202.
28. **Neve, H., A. Geis, and M. Teuber.** 1987. Conjugation, a common plasmid transfer mechanism in lactic acid streptococci of dairy starter cultures. Syst. Appl. Microbiol. **9:**151–157.
29. **O'Connor, L., A. Coffey, C. Daly, and G. F. Fitzgerald.** 1996. AbiG, a genotypically novel abortive infection mechanism encoded by plasmid pCI750 of *Lactococcus lactis* subsp. *cremoris* UC653. Appl. Environ. Microbiol. **62:**3075–3082.
30. **Romero, D. A., P. Slos, C. Robert, I. Castellino, and A. Mercenier.** 1987. Conjugative mobilization as an alternative vector delivery system for lactic streptococci. Appl. Environ. Microbiol. **53:**2405–2413.
31. **Ross, W., S. H. Shore, and M. M. Howe.** 1986. Mutants of *Escherichia coli* defective for replicative transposition of bacteriophage Mu. J. Bacteriol. **167:**905–919.
32. **Sanders, M. E., and T. R. Klaenhammer.** 1980. Restriction and modification in group N streptococci: effect of heat on development of modified lytic bacteriophage. Appl. Environ. Microbiol. **40:**500–506.
33. **Sreevatsan, S., X. Pan, K. E. Stockbauer, D. L. Williams, B. N. Kreiswirth, and J. M. Musser.** 1996. Characterization of *rpsL* and *rrs* mutations in streptomycin-resistant *Mycobacterium tuberculosis* isolates from diverse geographic localities. Antimicrob. Agents Chemother. **40:**1024–1026.
34. **Steele, J., and L. McKay.** 1989. Conjugal transfer of genetic material in lactococci: a review. J. Dairy Sci. **72:**3388–3397.
35. **Stentz, R., M. Gasson, and C. Shearman.** 2006. The Tra domain of the lactococcal CluA surface protein is a unique domain that contributes to sex factor DNA transfer. J. Bacteriol. **188:**2106–2114.
36. **Stentz, R., K. Jury, T. Eaton, M. Parker, A. Narbad, M. Gasson, and C. Shearman.** 2004. Controlled expression of CluA in *Lactococcus lactis* and its role in conjugation. Microbiology **150:**2503–2512.
37. **Terzaghi, B. E., and W. E. Sandine.** 1975. Improved medium for lactic streptococci and their bacteriophages. Appl. Microbiol. **29:**807–813.
38. **Tillett, D., B. P. Burns, and B. A. Neilan.** 2000. Optimized rapid amplification of cDNA ends (RACE) for mapping bacterial mRNA transcripts. BioTechniques **28:**448–456.
39. **Twomey, D. P., P. J. De Urraza, L. L. McKay, and D. J. O'Sullivan.** 2000. Characterization of AbiR, a novel multicomponent abortive infection mechanism encoded by plasmid pKR223 of *Lactococcus lactis* subsp. *lactis* KR2. Appl. Environ. Microbiol. **66:**2647–2651.