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DNA-DNA Homology among Lactose- and Sucrose-Fermenting Transconjugants from *Lactococcus lactis* Strains Exhibiting Reduced Bacteriophage Sensitivity†

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DNA-DNA homology between a reduced bacteriophage sensitivity (Rbs$^+$) probe and DNA from both Rbs$^+$ and Rbs$^-$ *Lactococcus lactis* strains was examined. Homology was detected between the probe and five plasmids (pCI750, pCC34, pEB56, pNP2, and pJS88) isolated from lactose-positive Rbs$^+$ transconjugants and between the probe and genomic DNA of a sucrose-positive Rbs$^+$ transconjugant. Additionally, hybridizations conducted between the probe and plasmids reported to encode abortive bacteriophage infection indicated homology with pTR2030 but not with pBF61 and pGBK17. The results suggest that a common genetic determinant(s) may be present in a variety of lactococcal plasmids coding for Rbs$^+$.

When 10 previously described lactose-positive (Lac$^+$) transconjugants and one sucrose-positive (Suc$^+$) transconjugant from the genus *Lactococcus* were tested for reduced bacteriophage sensitivity (Rbs$^+$), 6 of the 11 transconjugants exhibited an Rbs$^+$ phenotype (21). Four of the Lac$^+$ transconjugants (AB001, CC101, JS30, and WW4) possess a similar Rbs$^+$ phenotype in that small isometrically headed phage do not form any detectable plaques on these hosts, whereas phage with prolate heads exhibit a reduced plaque size and efficiency of plating (EOP) at 21, 30, and 37°C. One Lac$^+$ transconjugant (EB101) and the Suc$^+$ transconjugant (JS21) allow small isometrically headed phage to plaque with reduced EOP and plaque size. The Suc$^+$ transconjugant differs from all the Lac$^+$ Rbs$^+$ transconjugants in that its Rbs$^+$ phenotype is ineffective against phage with prolate heads. The evidence reported for a common Rbs$^+$ genetic determinant(s) is only phenotypic.

The Rbs$^+$ phenotype is associated with a distinct plasmid in the five Lac$^+$ Rbs$^+$ transconjugants (21). Examination of the mechanism of Rbs$^+$ for one of these plasmids (pCI750) indicated that an abortive bacteriophage infection (Abi$^+$) is involved. The cloning of an Rbs$^+$-encoding DNA fragment from pCI750 (M. C. Murphy, Ph.D. dissertation, University College Cork, Ireland, 1988) made available an Rbs$^+$ DNA probe. The Rbs$^+$ clone contains the 13.9- and 1.8-kilobase (kb) *Bcl*I fragments of pCI750 inserted into the *Bcl*I site of pGB301(pMM1). Transformants containing pGB301 with only the 1.8-kb *Bcl*I fragment are Rbs$^-$. No transformants containing pGB301 with only the 13.9-kb *Bcl*I fragment were isolated. Restriction data indicated that these two fragments are not contiguous on pCI750, which suggests that the Rbs$^+$ gene(s) of pMM1 is located on the 13.9-kb *Bcl*I fragment. The Rbs$^+$ phenotype expressed by pMM1 differs from that of pCI750. Phage c2, with a prolate head, plaques on MM1 at 32°C with an EOP of 1.0, while on AB001, the EOP is 9.2×10^{-3}. The c2 plaque size is reduced on both strains. The 13.9-kb *Bcl*I fragment isolated from pMM1 was chosen as the probe because of its involvement with the Rbs$^+$ phenotype against small isometrically headed bacteriophages. However, its size also suggests the presence of DNA sequences involved in other traits.

In this report, we describe hybridizations between this Rbs$^+$ probe and the 11 transconjugants previously examined for Rbs$^+$ (21). Three other plasmids reported to code for an Abi$^+$ phenotype were also tested for homology with the Rbs$^+$ probe.

Lactococcus lactis strains (Table 1) were maintained by biweekly transfer at 32°C in M17 broth containing 0.5% glucose, lactose, or sucrose (26). Plasmid and chromosomal DNA were extracted by the methods of Anderson and McKay (1) and Gawron-Burke and Clewell (11), respectively. Plasmid DNA was purified by using cesium chloride-ethidium bromide density gradient centrifugation (19). Purification of the 56-megadalton plasmid from a CsCl-ethidium bromide EB101 plasmid pool preparation was accomplished by sucrose gradient centrifugation (2). The Southern transfer technique of Davis et al. (6) was used. For labeling experiments, the 13.9-kb *Bcl*I fragment of pMM1 was isolated from low-melting-point agarose (19). Multiprime DNA labeling kit 1601Y (Amersham Corp., Arlington Heights, Ill.) was used with [α-^{32}P]dCTP (800 Ci/mmol; New England Nuclear Corp., Boston, Mass.) to generate a radioactive probe from the 13.9-kb *Bcl*I DNA fragment. Hybridizations were carried out using the SSC buffer (8.765 g of NaCl, 4.41 g of sodium citrate per liter of distilled water, pH 7.0) procedure of Barinaga et al. (4). Autoradiography was performed as described by Baldwin and McKay (3).

For genomic DNA, protoplasts were formed from 15 ml of a culture (optical density at 590 nm, 0.25) of JS21, LM2306, or MM1 and suspended in 2.5 ml of SMMB buffer (0.5 M sucrose, 20 mM maleate, 20 mM $MgCl_2$, 1% bovine serum albumin, pH 6.5) by the method of Kondo and McKay (17), as modified by Froseth et al. (9). Nitrocellulose (BA85; 0.45-μm pore size; Schleicher & Schuell, Inc., Keene, N.H.) and two pieces of 3-MM paper (Whatman, Inc., Clifton, N.J.) were cut to fit the dot blot manifold. The nitrocellulose and two pieces of 3-MM paper were saturated with 2 ml of

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TABLE 1. Strains of *L. lactis* subsp. *lactis* used in this study

Strain	Derivation[a]	Plasmid content (MDa)[b]	Plasmid designation (MDa)[b]	Reference
LM0230	Plasmid-free derivative of *L. lactis* subsp. *lactis* C2	None		7
LM2306	Mal$^-$ Str^r Ery^r derivative of LM0230	None		25
MM1	LM0230 containing pMM1 which is pGB301 with inserted 13.9- and 1.8-kb *BclI* fragments from pCI750	17	pMM1	M. C. Murphy[c]
JK301	Transformant of LM0230 containing only pGB301	6.5	pGB301	17
EB101	Lac$^+$ Rbs$^+$ transconjugant from *L. lactis* subsp. *cremoris* EB7 × *L. lactis* subsp. *lactis* LM3302	56, 27, 5.5, 2.0, 1.0	pEB56 (56)	24
CC101	Lac$^+$ Rbs$^+$ transconjugant from *L. lactis* subsp. *cremoris* C3 × *L. lactis* subsp. *lactis* LM2301	34, 27	pCC34 (34)	24
CC102	Lac$^-$ Rbs$^+$ derivative of CC101	34	pCC34	21
AB001	Lac$^+$ Rbs$^+$ transconjugant from *L. lactis* subsp. *cremoris* UC653 × *L. lactis* subsp. *lactis* MG1614	50, 26	pCI750 (50)	5
AB002	Lac$^-$ Rbs$^+$ derivative of AB001	50	pCI750	21
JS30	Lac$^+$ Rbs$^+$ transconjugant from *L. lactis* subsp. *lactis* 11007 × LM2336	88, 32	pJS88 (88)	Unpublished data
JS31	Lac$^-$ Rbs$^+$ derivative of JS30	88	pJS88	Unpublished data
WW4	Lac$^+$ Rbs$^+$ transconjugant from *L. lactis* subsp. *lactis* WM4 × LM2301	88, 83	pNP2 (88)	23
CS1	Lac$^-$ Rbs$^+$ derivative of WW4	88	pNP2	23
KC1	Lac$^+$ Rbs$^-$ transconjugant from *L. lactis* subsp. *lactis* C_2O × LM2301	Two plasmids		20
PW1	Lac$^+$ Rbs$^-$ transconjugant from *L. lactis* subsp. *lactis* ML3 × LM2301	60	pPW1	27
RM108	Lac$^+$ Rbs$^-$ transconjugant from *L. lactis* subsp. *cremoris* R1 × *L. lactis* subsp. *lactis* LM3302	34, 27, 5.5, 2.0, 1.0		24
ZM803	Lac$^+$ Rbs$^-$ transconjugant from *L. lactis* subsp. *cremoris* Z8 × LM3302	30, 27, 5.5, 2.0, 1.0		24
GK4101	Lac$^+$ Rbs$^-$ transconjugant from *L. lactis* subsp. *lactis* 18-16 × LM2301	41		14
JS21	Suc$^+$ Nip$^+$ Rbs$^+$ transconjugant from *L. lactis* subsp. *lactis* 11454 × LM2306	ND[d]		25
T-RS1a	Lac$^-$ Rbs$^+$ derivative of T-RS1 containing only pTR2030	30	pTR2030	16
BF26	Transformant of LM0230 containing only pBF61	26	pBF61	8
GBK17	Transformant of LM0230 containing only pGBK17	17.8	pGBK17	18

[a] Lac$^+$, lactose fermenting; Lac$^-$, lactose negative; Rbs$^+$, reduced bacteriophage sensitivity; Mal$^-$, maltose negative; Suc$^+$, sucrose fermenting; Nip$^+$, nisin producer; Str^r, streptomycin resistant; Ery^r, erythromycin resistant.

[b] MDa, Megadaltons.

[c] Ph.D. thesis, University College, Cork, Ireland, 1988.

[d] ND, None detected. Although no plasmid DNA was detected, data suggesting the involvement of plasmid DNA with the Suc$^+$ phenotype have been reported (25).

1× SSC buffer and assembled onto the manifold. After a vacuum was applied, 15 μl of the protoplast preparations was added to each well. The nitrocellulose was removed and placed on 3-MM paper saturated with 1.5 M NaCl–0.2 N NaOH for 4 min to denature the DNA. The nitrocellulose filter was neutralized by placing it on 3-MM paper saturated with 0.5 M Tris hydrochloride (pH 7.4)–3 M NaCl for 4 min. The nitrocellulose was placed on 3-MM paper saturated with 2× SSC, removed, and baked at 80°C for 2 h. Hybridization and autoradiography were as described above.

To determine if DNA-DNA homology existed between the Rbs$^+$ probe and the Lac$^+$ Rbs$^+$ transconjugants, the plasmid pools of eight Lac$^+$ transconjugants (EB101, CC101, AB001, ZM803, RM108, PW1, GK4101, and KC1) and the Lac$^-$ derivatives of two Lac$^+$ transconjugants (JS31 and CS1) were isolated and probed. Hybridization with the five Rbs$^+$ transconjugants (Fig. 1) demonstrated homology among the five plasmids phenotypically associated with Rbs$^+$ (21). These plasmids were pEB56, pCI750, pCC34, pNP2, and pJS88 from EB101, AB001, CC101, CS1, and JS31, respectively. No homology was observed with the other plasmids present in these strains or with the plasmid DNA from the five Lac$^+$ Rbs$^-$ transconjugants (data not shown).

To assess the extent of homology, restriction digests of the five Rbs$^+$ plasmids were hybridized with the Rbs$^+$ probe. Hybridization results (Fig. 2) verified that homology existed among these plasmids; a fragment of approximately 4.1-kb was present in all the Rbs$^+$ plasmids. The number of additional fragments exhibiting homology with the Rbs$^+$ 13.9-kb DNA probe of pMM1 ranged from 1 to 3. The high degree of homology between pCI750 and the probe was expected since the 13.9-kb *BclI* fragment was derived from pCI750. In addition to the 4.1-kb fragment, homologous fragments of 2.4, 3.0, and 6.8 kb were present in pCI750, pNP2, and pJS88, indicating a high degree of homology among these plasmids. When the 4.1-kb *Eco*RI fragment of pCI750 was cloned into pSA3, the resulting plasmid was Rbs$^-$ (Murphy, Ph.D. thesis). This suggested that although the 4.1-kb *Eco*RI fragment may contain DNA sequence(s) involved in the Rbs$^+$ phenotype, the complete Rbs$^+$ genetic locus is not present. These results strongly support the previously reported phenotypic data that pEB56, pCC34, pCI750, pNP2, and pJS88 contain a common genetic determinant(s) involved in their Rbs$^+$ phenotypes.

Reports that the Suc$^+$ nisin-producing (Nip$^+$) element codes for an Rbs$^+$ phenotype (12, 21) and the availability of an Rbs$^+$ probe (this study) provided an approach for examining the genetic basis of the Suc$^+$ Nip$^+$ phenotypes. Phe-

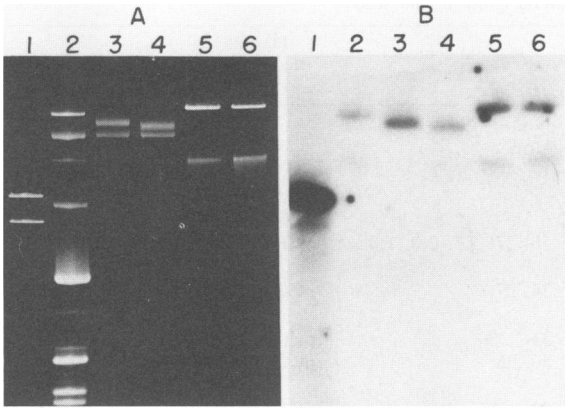

FIG. 1. (A) Plasmid profiles of Rbs^+ transconjugants. (B) Autoradiogram prepared after hybridization with ^{32}P-labeled 13.9-kb *Bcl*I fragment of pMM1. Lanes: 1, pMM1 digested with *Bcl*I; 2, EB101 plasmid pool; 3, AB001 plasmid pool; 4, CC101 plasmid pool; 5, CS1 plasmid pool; 6, JS31 plasmid pool.

notypic and genetic evidence implicating involvement of plasmid DNA with the Suc^+ Nip^+ phenotypes has been reported (12, 25); however, no definitive physical evidence has been presented. Hybridizations were attempted to determine if DNA homologous to the probe was present in the chromosome of JS21 (Fig. 3). No homology was detected, which suggests that the conjugally transferred Suc^+ Nip^+ Rbs^+ element is not present in the chromosome of JS21. To determine if the DNA responsible for the Rbs^+ phenotype in JS21 is lost during the DNA isolation procedure, total genomic DNA was analyzed for homology with the Rbs^+ probe. Homology was detected in MM1 and JS21 but not in LM2306 (Fig. 3C). These results suggest that the putative Suc^+ Nip^+ Rbs^+ plasmid is lost during some stage of the plasmid isolation procedure. The recent report by Kaletta and Entian (13) suggests that the plasmid is large, since a nearly immobile band on a 0.7% agarose gel, when restricted, contained a fragment coding for nisin production.

Abi^+ is thought to be the mechanism involved in the Rbs^+ phenotypes of pCI750 (21), pTR2030 (16), pBF61 (8), and

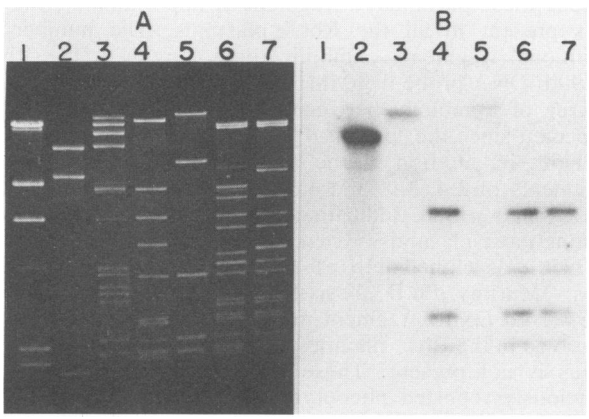

FIG. 2. (A) *Eco*RI digestions of Rbs^+ plasmids. (B) Autoradiogram prepared after hybridization with ^{32}P-labeled 13.9-kb *Bcl*I fragment of pMM1. Lanes: 1, λ DNA digested with *Hin*dIII; 2, pMM1 digested with *Bcl*I; 3, pEB56 digested with *Eco*RI; 4, pCI750 digested with *Eco*RI; 5, pCC34 digested with *Eco*RI; 6, pNP2 digested with *Eco*RI; 7, pJS88 digested with *Eco*RI.

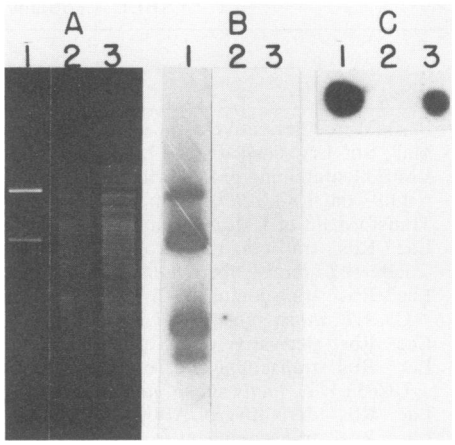

FIG. 3. (A) pMM1 and chromosomal DNA from a Suc^+ transconjugant and its recipient digested with *Eco*RI. (B) Autoradiogram prepared after hybridization with ^{32}P-labeled 13.9-kb *Bcl*I fragment of pMM1. (C) Autoradiogram of dot blots of genomic DNA prepared after hybridization with ^{32}P-labeled 13.9-kb *Bcl*I fragment of pMM1. Lanes: 1, pMM1 (A and B) and MM1 genomic DNA (C); 2, LM2306 chromosomal DNA (A and B) and LM2306 genomic DNA (C); 3, JS21 chromosomal DNA (A and B) and JS21 genomic DNA (C).

pGBK17 (18) and other plasmids (10). Of these plasmids, pTR2030, pBF61, and pGBK17 were examined for homology with the Rbs^+ probe. Hybridization results suggested that homology exists only with pTR2030 (Fig. 4). The homologous band in pGBK17 corresponds with the cloning vector pGB301. This was most likely due to incomplete separation of the 13.9-kb *Bcl*I fragment from the pGB301 portion of pMM1 during preparation of the probe. A band corresponding to pGB301 also appeared in the pMM1 lane of the autoradiogram (Fig. 4, lane 2).

The results presented suggest that a common genetic determinant(s) may be involved in the Rbs^+ phenotypes of pTR2030 and the five Rbs^+ transconjugants previously examined. This supports the proposal of Klaenhammer (15) that pTR2030 and pCI750 code for similar Rbs^+ phenotypes

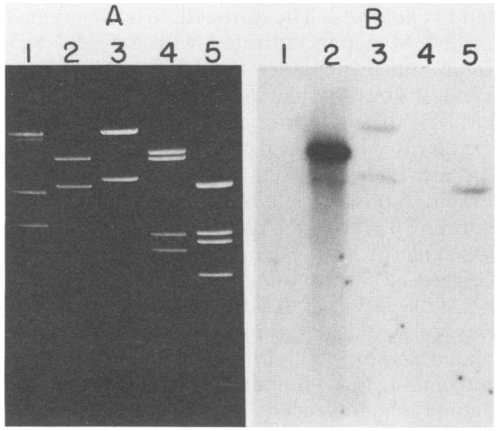

FIG. 4. (A) Restriction endonuclease digestions of plasmids reported to code for Abi^+. (B) Autoradiogram prepared after hybridization with ^{32}P-labeled 13.9-kb *Bcl*I fragment of pMM1. Lanes: 1, λ DNA digested with *Hin*dIII; 2, pMM1 digested with *Bcl*I; 3, pTR2030 digested with *Eco*RI; 4, pBF61 digested with *Eco*RI; 5, pGBK17 digested with *Hpa*II and *Eco*RI.

and the comment by Sanders (22) that the Abi$^+$ "genes are widespread, and may be isolatable from many different strains and harbored on different plasmids." The lack of homology to pBF61 and pGBK17 suggests that different Abi$^+$ mechanisms and genetic loci may exist.

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LITERATURE CITED

1. **Anderson, D. G., and L. L. McKay.** 1983. Simple and rapid method for isolating large plasmid DNA from lactic streptococci. Appl. Environ. Microbiol. **46:**549–552.
2. **Anderson, D. G., and L. L. McKay.** 1984. Genetic and physical characterization of recombinant plasmids associated with cell aggregation and high-frequency conjugal transfer in *Streptococcus lactis* ML3. J. Bacteriol. **158:**954–962.
3. **Baldwin, K. A., and L. L. McKay.** 1987. Spontaneous release of temperate phage by relysogenized lactose-positive transductants of *Streptococcus lactis* C2. J. Dairy Sci. **70:**2005–2012.
4. **Barinaga, M., R. Franco, J. Meinkoth, E. Ong, and G. M. Wahl.** 1981. Methods for the transfer of DNA, RNA, and protein to nitrocellulose and diazotized paper solid supports. Schleicher & Schuell, Inc., Keene, N.H.
5. **Baumgartner, A., M. Murphy, C. Daly, and G. F. Fitzgerald.** 1986. Conjugative co-transfer of lactose and bacteriophage resistance plasmids from *Streptococcus cremoris* UC653. FEMS Microbiol. Lett. **35:**233–237.
6. **Davis, R. W., D. Botstein, and J. R. Roth (ed.).** 1980. Advanced bacterial genetics. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
7. **Efstathiou, J. D., and L. L. McKay.** 1977. Inorganic salts resistance associated with a lactose-fermenting plasmid in *Streptococcus lactis*. J. Bacteriol. **130:**257–265.
8. **Froseth, B. R., S. K. Harlander, and L. L. McKay.** 1988. Plasmid-mediated reduced bacteriophage sensitivity in *Streptococcus lactis* KR5. J. Dairy Sci. **71:**275–284.
9. **Froseth, B. R., R. E. Herman, and L. L. McKay.** 1988. Cloning of nisin resistance determinant and replication origin on 7.6-kilobase *Eco*RI fragment of pNP40 from *Streptococcus lactis* subsp. *diacetylactis* DRC3. Appl. Environ. Microbiol. **54:**2136–2139.
10. **Gautier, M., and M.-C. Chopin.** 1987. Plasmid-determined systems for restriction and modification activity and abortive infection in *Streptococcus cremoris*. Appl. Environ. Microbiol. **53:**923–927.
11. **Gawron-Burke, C., and D. B. Clewell.** 1984. Regeneration of insertionally inactivated streptococcal DNA fragments after excision of transposon Tn*916* in *Escherichia coli*: strategy for targeting and cloning of genes from gram-positive bacteria. J. Bacteriol. **159:**214–221.
12. **Gonzalez, C. F., and B. S. Kunka.** 1985. Transfer of sucrose fermenting ability and nisin production phenotypes among lactic streptococci. Appl. Environ. Microbiol. **49:**627–633.
13. **Kaletta, C., and K. D. Entian.** 1989. Nisin, a peptide antibiotic: cloning and sequencing of the *nisA* gene and postranslational processing of its peptide product. J. Bacteriol. **171:**1597–1601.
14. **Kempler, G. M., and L. L. McKay.** 1979. Genetic evidence for plasmid-linked lactose metabolism in *Streptococcus lactis* subsp. *diacetylactis*. Appl. Environ. Microbiol. **37:**316–323.
15. **Klaenhammer, T. R.** 1987. Plasmid-directed mechanisms for bacteriophage defense in lactic streptococci. FEMS Microbiol. Rev. **46:**313–325.
16. **Klaenhammer, T. R., and R. B. Sanozky.** 1985. Conjugal transfer from *Streptococcus lactis* ME2 of plasmids encoding phage resistance, nisin resistance and lactose-fermenting ability: evidence for a high frequency conjugative plasmid responsible for abortive infection of virulent bacteriophage. J. Gen. Microbiol. **131:**1531–1541.
17. **Kondo, J. K., and L. L. McKay.** 1984. Plasmid transformation of *Streptococcus lactis* protoplasts: optimization and use in molecular cloning. Appl. Environ. Microbiol. **48:**252–259.
18. **Laible, N. J., P. L. Rule, S. K. Harlander, and L. L. McKay.** 1987. Identification and cloning of plasmid deoxyribonucleic acid coding for abortive phage infection from *Streptococcus lactis* ssp. *diacetylactis* KR2. J. Dairy Sci. **70:**2211–2219.
19. **Maniatis, T., E. F. Fritsch, and J. Sambrook.** 1982. Molecular cloning: a laboratory manual. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
20. **McKay, L. L., K. A. Baldwin, and P. M. Walsh.** 1980. Conjugal transfer of genetic information in group N streptococci. Appl. Environ. Microbiol. **40:**84–91.
21. **Murphy, M. C., J. L. Steele, C. Daly, and L. L. McKay.** 1988. Concomitant conjugal transfer of reduced bacteriophage sensitivity mechanisms with lactose- and sucrose-fermenting ability in lactic streptococci. Appl. Environ. Microbiol. **54:**1951–1956.
22. **Sanders, M. E.** 1988. Phage resistance in lactic acid bacteria. Biochimie **70:**411–421.
23. **Scherwitz, K. M., K. A. Baldwin, and L. L. McKay.** 1983. Plasmid linkage of a bacteriocin-like substance in *Streptococcus lactis* subsp. *diacetylactis* strain WM4: transferability to *Streptococcus lactis*. Appl. Environ. Microbiol. **45:**1506–1512.
24. **Snook, R. J., and L. L. McKay.** 1981. Conjugal transfer of lactose-fermenting ability among *Streptococcus cremoris* and *Streptococcus lactis* strains. Appl. Environ. Microbiol. **42:**904–911.
25. **Steele, J. L., and L. L. McKay.** 1986. Partial characterization of the genetic basis for sucrose metabolism and nisin production in *Streptococcus lactis*. Appl. Environ. Microbiol. **51:**57–64.
26. **Terzaghi, B. E., and W. E. Sandine.** 1975. Improved medium for lactic streptococci and their bacteriophages. Appl. Microbiol. **29:**807–813.
27. **Walsh, P. M., and L. L. McKay.** 1981. Recombinant plasmid associated with cell aggregation and high-frequency conjugation of *Streptococcus lactis* ML3. J. Bacteriol. **146:**937–944.