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Conjugal Transfer of Lactose-Fermenting Ability Among *Streptococcus cremoris* and *Streptococcus lactis* Strains†

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Streptococcus cremoris C3 was found to transfer lactose-fermenting ability to LM2301, a *Streptococcus lactis* C2 lactose-negative streptomycin-resistant (Lac^- Str^r) derivative which is devoid of plasmid deoxyribonucleic acid (DNA); to LM3302, a Lac^- erythromycin-resistant (Ery^r) derivative of *S. lactis* ML3; and to BC102, an *S. cremoris* B_1 Lac^- Ery^r derivative which is devoid of plasmid DNA. *S. cremoris* strains R1, EB_7, and Z8 were able to transfer lactose-fermenting ability to LM3302 in solid-surface matings. Transduction and transformation were ruled out as mechanisms of genetic transfer. Chloroform treatment of donor cells prevented the appearance of recombinant clones, indicating that viable cell-to-cell contact was responsible for genetic transfer. Transfer of plasmid DNA was confirmed by agarose gel electrophoresis. Transconjugants recovered from EB_7 and Z8 matings with LM3302 exhibited plasmid sizes not observed in the donor strains. Transconjugants recovered from R1, EB_7, and Z8 matings with LM3302 were able to donate lactose-fermenting ability at a high frequency to LM2301. In *S. cremoris* R1, EB_7, and Z8 matings with LM2301, streptomycin resistance was transferred from LM2301 to the *S. cremoris* strains. The results confirm genetic transfer resembling conjugation between *S. cremoris* and *S. lactis* strains and present presumptive evidence for plasmid linkage of lactose metabolism in *S. cremoris*.

The instability of lactose metabolism in lactic streptococci was reported as early as 1937 by Yawger and Sherman (31) in *Streptococcus lactis* and by Sherman and Hussong (24) in *Streptococcus cremoris*. Consecutive transfers in milk or broth containing fermentable carbohydrates other than galactose or lactose were observed to result in the appearance of organisms unable to ferment lactose. More recently, researchers demonstrated that the occurrence of lactose-negative (Lac^-) mutants could be increased by consecutive transfers in broth containing acridine dyes (1, 12, 16, 21), ethidium bromide (7, 15, 16), or by growing the culture at elevated temperatures which were restrictive to growth (1, 7, 15, 16, 21). Plasmid linkage of lactose metabolism was demonstrated in *S. lactis* and *S. lactis* subsp. *diacetylactis* by the concomitant losses of plasmid deoxyribonucleic acid (DNA) and lactose-fermenting ability (7, 12, 15, 21). Although similar studies were conducted with *S. cremoris* strains (16, 21), only the atypical *S. cremoris* strain B_1 was observed to simultaneously lose lactose metabolic capability and plasmid DNA (1).

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Studies directed toward confirming plasmid linkage to phenotypic properties have often employed genetic transfer systems (2, 6, 13, 20, 27, 29). Conjugation of lactose metabolism has been demonstrated in *S. lactis* 712 (9) and has provided evidence that lactose metabolism is linked to plasmid DNA in *S. lactis* ML3 (20) and in *S. lactis* subsp. *diacetylactis* 18-16 (13), DRC3, and 11007 (20). Therefore, genetic transfer in *S. cremoris* strains was investigated to determine whether plasmid linkage of metabolic traits could be demonstrated.

MATERIALS AND METHODS

Bacterial strains. All strains used in this study were maintained in our stock culture collection by biweekly transfer at 30°C in M17 broth (26) containing 0.5% glucose or lactose. Antibiotic-resistant derivatives were obtained by selecting spontaneous mutants after consecutive transfers at 30°C in M17 broth containing increased concentrations of streptomycin-sulfate or erythromycin (Sigma Corp.). Antibiotic-resistant mutants were maintained by biweekly transfer at 30°C in M17 broth containing 600 μg of streptomycin-sulfate per ml or 15 μg of erythromycin per ml. *Escherichia coli* K-12 strains J5, J53, and C600 were propagated in brain heart infusion broth as described previously (14).

Solid-surface matings. Sixteen *S. cremoris*

strains (HP, E8, TR, KH, Z8, ML_1, Wg_2, AM_2, 202, C-11, 9596, W, C3, EB_9, R1, and EB_7) were screened as potential donors of lactose-fermenting ability. Table 1 lists the relevant phenotype and plasmid composition of recipients and donors used in this study. Fresh, overnight cultures of donor and recipient were inoculated (2%) into M17-lactose (M17-L) and M17-glucose (M17-G) broth, respectively, and were incubated at 30°C for 4 to 8 h for *S. cremoris* strains or 4 to 5 h for *S. lactis* strains. Mating trials were performed as described previously (20). Lac^+ transconjugants were selected on bromocresol purple lactose (BCP-lac) indicator agar containing 600 μg of streptomycin per ml, 15 μg of erythromycin per ml, or both antibiotics. To rule out genetic transfer by systems other than conjugation, donor cells were treated with chloroform, donor cell filtrates were mixed with recipient cells, or donor cells were grown in the presence of 100 μg of deoxyribonuclease (DNase) per ml followed by the addition of 500 μg of DNase per ml in subsequent mating steps.

Plasmid analysis. Plasmid DNA was prepared for agarose gel electrophoresis by two methods. A modification of the method described by Klaenhammer et al. (14) was used, in which cultures were grown for 4 to 8 h at 30°C and treated with lysozyme for 5 to 8 min before the addition of 5% sodium dodecyl sulfate to effect lysis. Alternatively, a previously described modification (30) of a lysis procedure, involving a 5-min lysozyme treatment, high sodium dodecyl sulfate, alkaline denaturation, and polyethylene glycol concentration of plasmid DNA (11), was used. Agarose gel electrophoresis was performed as described previously (14).

RESULTS

Attempted mating trials. To demonstrate the possible presence of conjugal transfer systems in *S. cremoris* strains, three Lac^- strains (LM2301, LM3302, and BC102) were used as potential recipients. Of the *S. cremoris* strains screened, only four (C3, R1, EB_7, and EB_9) formed Lac^+ Str^r clones when mated with LM2301. No Lac^+ revertants were observed from LM2301 platings, and no spontaneous Str^r colonies were observed on the donor control plates. Lac^+ Str^r recombinants were recovered at a frequency of about 1 per 10^7 donor cells for R1, EB_7, and EB_9. However, examination of the plasmid profiles from these recombinants showed that they resembled the Lac^+ donor from which they were derived (data not shown). The results implied that the *str* marker was

TABLE 1. *Strains of S. lactis and S. cremoris used*

Strain designation	Relevant phenotype	Plasmid composition (Mdal)	Derivation/reference
S. lactis LM2301	Lac^- Prt^- Str^r	None	Formerly designated Str^r LM0230 (20)
S. lactis LM2302	Lac^- Prt^- Str^r Ery^r	None	Spontaneous mutant of LM2301 (30)
S. lactis LM3302	Lac^- Prt^- Ery^r	27, 5.5, 2, 1	Spontaneous Ery^r mutant of a Lac^- derivative of *S. lactis* ML3 (25, 30)
S. cremoris BC102	Lac^- Prt^+ Ery^r	None	Spontaneous Ery^r mutant of acriflavine-induced plasmid-cured mutant of *S. cremoris* B_1 (30)
S. cremoris C3	Lac^+ Prt^+ Str^s Ery^s	34, 27, 21, 16, 12.5, 2.8, 2.0	This study and (16)
S. cremoris R1	Lac^+ Prt^+ Str^s Ery^s	34, 30, 27, 23, 17, 15, 11, 6.5, 2.0, 1.8, 1.5	This study and (16)
S. cremoris EB_7	Lac^+ Prt^+ Str^s Ery^s	42, 40, 30, 27, 20, 9, 5, 4, 1.5, 1.2	This study and (16)
S. cremoris Z8	Lac^+ Prt^+ Str^s Ery^s	27, 17, 11, 7.8, 2.6, 1.5	This study
S. cremoris CS34	Lac^+ Prt^+ Str^r	55, 34, 27, 12.5, 2.8, 2.0	Lac^+ colony originating from C3 × LM2301 matings (this paper)
S. lactis CC101	Lac^+ Str^r	34, 27	Lac^+ transconjugant of C3 × LM2301 mating (this paper)
S. lactis CM307	Lac^+ Ery^r	34, 27	Lac^+ transconjugant of C3 × LM3302 mating (this paper)
S. lactis RM101	Lac^+ Ery^r	34, 6.5	Lac^+ transconjugant of R1 × LM3302 mating (this paper)
S. lactis RM108	Lac^+ Ery^r	34	Lac^+ transconjugant of R1 × LM3302 mating (this paper)
S. lactis EB101	Lac^+ Ery^r	56, 27, 5.5, 2, 1	Lac^+ transconjugant of EB_7 × LM3302 mating (this paper)
S. lactis ZM803	Lac^+ Ery^r	30, 27, 5.5. 2, 1	Lac^+ transconjugant of Z8 × LM3302 mating (this paper)

being transferred from the Lac^- to the Lac^+ strain; therefore these recombinants were not examined further. Of the 16 *S. cremoris* strains examined as potential donors of *lac* to BC102, only C3 matings resulted in recovery of Lac^+ Ery^r transconjugants. In contrast, Lac^+ Ery^r transconjugants were recovered from C3, R1, EB_7, and Z8 matings with LM3302. The Lac^+ transconjugants recovered from the various matings were subsequently examined further to confirm the presence of plasmid conjugal transfer systems in *S. cremoris*.

***S. cremoris* C3 matings.** Lac^+ transconjugants were recovered at an average frequency of 1×10^{-5}, 1.5×10^{-6}, and 1.3×10^{-4} when LM2301, BC102, and LM3302, respectively, were used as recipients. Genetic transfer by cell-to-cell contact was substantiated by the absence of Lac^+ colonies on donor control plates, by using chloroform-treated cells, or when donor cell filtrates were used in plating mixtures. The frequency of *lac* transfer decreased by almost 50% in the presence of DNase when LM2301 was the recipient (data not shown). However, 20 mM $MgSO_4$ alone decreased the transfer frequency to approximately the same extent. Similar effects of $MgSO_4$ on transfer efficiency were previously reported by LeBlanc et al. (17).

Agarose gel electrophoresis of plasmid DNA isolated from these Lac^+ transconjugants revealed that when LM2301 was the recipient, three different plasmid profiles were observed. Five transconjugants contained a 34- and 27-megadalton (Mdal) plasmid, three did not contain detectable plasmid DNA, and three transconjugants contained a single 34-Mdal plasmid (Table 2). *S. cremoris* C3 possessed plasmids of approximately 34, 27, 21, 16, 12.5, 2.8, and 2.0 Mdal (Fig. 1). A 27- and a 34-Mdal plasmid were detected in Lac^+ transconjugants derived from C3 × BC102 and C3 × LM3302 matings (Table 2).

To test the stability of the 34- and 27-Mdal plasmids in LM2301, a Lac^+ transconjugant containing both plasmids was continuously transferred at 30°C in M17-G broth containing 15 μg of acriflavine per ml. After four transfers, 16% of the population was Lac^-, and of four mutants examined, all were observed to have lost both plasmids.

To determine whether the Lac^+ transconjugants could serve as donors of *lac* genes, CC101 and CM307 were examined as potential donors. Recipients included BC102 and LM3302 for matings with CC101, and LM2301 for matings with CM307. Lac^+ transconjugants were recovered from these matings at frequencies of 4.4×10^{-7}, 3.1×10^{-5}, and 1.2×10^{-4}, respectively. Table 2 shows the plasmid sizes transferred. Plasmid profiles in CC101 × LM3302 matings were of two types: those containing both the 34- and 27-Mdal plasmids and those containing a single 27-Mdal plasmid (Fig. 1). Similarly, the 27-Mdal or both the 34- and 27-Mdal plasmids were detected in Lac^+ transconjugants derived from CC101 × BC102 matings. In CM307 × LM2301 matings one type of Lac^+ transconjugant contained a 34- and a 27-Mdal plasmid, and the second type contained a 34- and a 25-Mdal plasmid (Table 2).

Seventy-five Lac^+ transconjugants from the C3 × LM2301 mating were screened for cotransfer of proteinase (Prt) activity by testing for their ability to coagulate 11% reconstituted nonfat dry milk at 21°C within 20 h. Only one was found to be Prt^+, and it was designated CS34. However, CS34 was shown to be sensitive to C3 lytic phage and unable to hydrolyze arginine when grown in Niven broth (22). Examination of CS34 for plasmid DNA revealed a modified C3 plasmid profile (Fig. 2), in which a 55-Mdal plasmid was present but the 21- and 16-Mdal plasmids present in C3 were absent. This strain is therefore a variant of C3 in which streptomycin resistance may have been transferred from LM2301 to C3. In CS34 × LM2302 and CS34 × LM3302 matings, Lac^+ Ery^r recombinants were recovered at a frequency of 5×10^{-6} and 6.8×10^{-5}, respectively. When LM2302 was used as the recipient, four transconjugants contained the 34-Mdal plasmid, one contained the 27-Mdal plasmid, and no plasmid DNA was detectable in another 15 transconjugants (Table 2). All Lac^+ transductants examined from LM3302 matings were Ery^r Str^r and possessed the CS34 plasmid profile.

***S. cremoris* R1 matings with LM3302.** Conjugal transfer of lactose metabolism in R1 × LM3302 matings occurred at a frequency of 5.3×10^{-7} per donor. A 34- and a 6.5-Mdal plasmid were demonstrated in the Lac^+ Ery^r transconjugants (Fig. 3). Nine of ten Lac^+ transconjugants acquired only the 34-Mdal plasmid (Table 2), which suggests that lactose-fermenting ability is linked to this plasmid. This type of transconjugant was designated RM108, and the other, containing the 34- and 6.5-Mdal plasmids, was designated RM101.

To determine whether RM101 and RM108 could serve as donors of *lac* genes, each was mated with LM2301. Two Lac^+ transconjugants recovered from RM101 matings contained a 34- and a 5.5-Mdal plasmid, whereas two recovered from RM108 matings contained only the 34-Mdal plasmid (Table 2). The origin of the 5.5-Mdal plasmid was not determined.

TABLE 2. *Conjugal transfer of a lactose plasmid derived from S. cremoris strains*

Donor	Recipient	No. of transconjugants examined for plasmid DNA	Size of plasmids transferred to recipient (Mdal)	Transconjugant Designation
S. cremoris C3	*S. lactis* LM2301	11	34, 27 (5)[a]	CC101
			34 (3)	
			None detectable (3)	
S. cremoris C3	*S. lactis* LM3302	12	34, 27	CM307
S. cremoris C3	*S. cremoris* BC102	4	34, 27	
S. cremoris CS34	*S. lactis* 2302	20	34 (4)	
			27 (1)	
			None detectable (15)	
S. lactis CC101	*S. lactis* LM3302	4	34, 27 (2)	
			27 (2)	
S. lactis CC101	*S. cremoris* BC102	4	34, 27 (2)	
			27 (2)	
S. lactis CM307	*S. lactis* LM2301	5	34, 27 (4)	
			34, 25 (1)	
S. cremoris R1	*S. lactis* LM3302	10	34 (9)	RM108
			34, 6.6 (1)	RM101
S. lactis RM101	*S. lactis* LM2301	2	34, 5.5 (2)	
S. lactis RM108	*S. lactis* LM2301	2	34 (2)	
S. cremoris EB_7	*S. lactis* LM3302	2	42	EB102
			56	EB101
S. lactis EB101	*S. lactis* LM2301	4	2	
S. cremoris Z8	*S. lactis* LM3302	12	27, 20 (3)	
			34, 20 (3)	
			34 (4)	
			30 (1)	ZM803
			30, 7.8 (1)	
S. lactis ZM803	*S. lactis* LM2301	2	56	
			34	

[a] The number in parentheses represents the number of Lac^+ transconjugants examined possessing the indicated transferred plasmids.

***S. cremoris* EB_7 matings with LM3302.** Only two Lac^+ transconjugants were recovered from four EB_7 × LM3302 matings. One, designated EB102, contained a transferred plasmid of 42 Mdal, which suggests that it is the lactose plasmid in EB_7, and the other, designated EB101, contained a 56-Mdal plasmid, in addition to the other resident plasmids in the recipient (Table 2). The largest plasmid in EB_7 is 42 Mdal.

In EB101 × LM2301 matings no plasmid DNA larger than 2 Mdal was observed in any of the transconjugants examined (Table 2). To determine Lac^+ stability in these transconjugants, they were consecutively transferred at 30°C in M17-G with and without 6 μg of acriflavine per ml. After 10 transfers, one exhibited a 3% loss of lactose-fermenting ability in both the presence and absence of acriflavine, and no Lac^- isolates were detected from the other.

***S. cremoris* Z8 matings with LM3302.** Five types of plasmid profiles were observed in 12 Lac^+ Ery^r transconjugants screened from Z8 × LM3302 matings (Table 2). Although the largest detectable plasmid in Z8 was 27 Mdal, plasmid species larger than 27 Mdal were observed in some of the transconjugants.

To confirm the linkage of *lac* in one of these recombinant plasmids, a Lac^+ transconjugant containing a 30-Mdal plasmid (ZM803) was mated with LM2301. Of 20 transconjugants, 19 formed cell aggregates when grown in M17 broth. A clumping and a nonclumping transconjugant were then examined for plasmid DNA. The former contained a single 56-Mdal plasmid, and the latter contained a 34-Mdal plasmid (data not shown). Aggregation was readily lost with successive transfer at 30°C; however, the plasmid profiles of these isolates were not determined.

High-frequency conjugation by Lac^+ transconjugants used as donors. *S. cremoris* C3 was the only *S. cremoris* strain tested which was capable of transferring lactose-fermenting ability directly to LM2301 in conjugal matings. The indirect transfer of lactose-fermenting ability from R1, EB_7, and Z8 to LM2301 was accomplished by first transferring the *lac* genes (plasmid linked) to LM3302. The Lac^+ transconjugants recovered from the R1, EB_7, and Z8 matings with LM3302 were capable of transferring the lactose plasmid to LM2301 at a high frequency (Table 3). There was no significant dif-

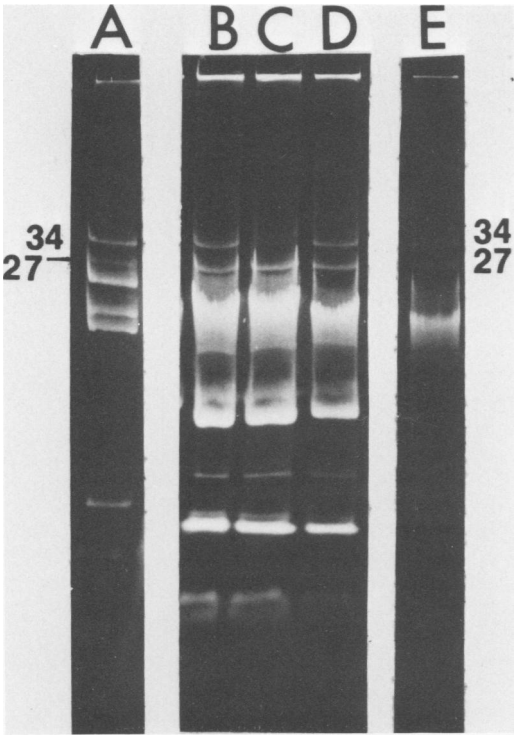

FIG. 1. *Agarose gel electrophoretic patterns of plasmid DNA isolated from S. cremoris C3 and CC101 and from Lac$^+$ transconjugants recovered from CC101 matings with LM3302. (A) CsCl-ethidium-bromide-gradient-purified plasmid DNA from S. cremoris C3 demonstrating the 34- and 27-Mdal plasmids. (E) Plasmid profile of CC101 containing the 34- and 27-Mdal plasmids. (B and D) Lac$^+$ transconjugants containing both the 34- and 27-Mdal plasmids. (C) Lac$^+$ transconjugant containing only the 27-Mdal plasmid. Plasmid DNAs in wells B through E were isolated from ethanol-precipitated cleared lysates, and molecular weights were determined by electrophoresis with lysates containing plasmids of known molecular weights (data not shown).*

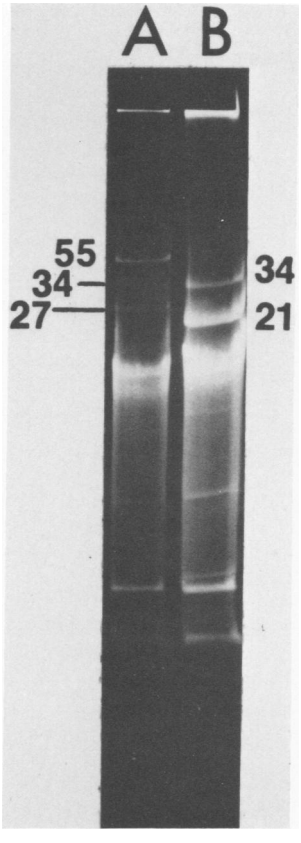

FIG. 2. *Agarose gel electrophoretic patterns of plasmid DNA isolated from ethanol-precipitated cleared lysates. (A) CS34, containing a 55-Mdal plasmid. (B) S. cremoris C3, containing the 34- and 21-Mdal plasmids; the 27-Mdal plasmid was not observed.*

ference in the frequency of *lac* plasmid transfer to LM2301 when C3 or Lac$^+$ transconjugants recovered from C3 × LM3302 matings were used as donors of lactose-fermenting ability.

DISCUSSION

Plasmid-mediated transfer of lactose metabolism from *S. lactis* subsp. *diacetylactis* 18-16 to *S. lactis* LM2301 (13) was the first reported demonstration of conjugal transfer in group N streptococci. Since then, the *lac* genes have been transferred by conjugation from *S. lactis* (9, 20) and other *S. lactis* subsp. *diacetylactis* strains (20) to *S. lactis* recipients. Other reports of plasmid-mediated transfer in group N streptococci have included the transfer of an antibiotic resistance plasmid, pAMβ1, from *S. faecalis* to *S. lactis* 712. Transconjugants recovered from this mating were able to donate the antibiotic resistance plasmid to other lactic streptococci (8). The results presented here provide additional evidence for intra- and interspecies genetic transfer among group N streptococci.

Examination for plasmid DNA in Lac$^+$ transconjugants recovered from conjugal matings revealed that *S. cremoris* EB$_7$ could transfer at least one plasmid, R1 and C3 could transfer at least two plasmid species, and Z8 could transfer three different plasmids. From these matings, lactose metabolism could be presumptively linked to the 34-Mdal plasmid in R1 and to the 42-Mdal plasmid in EB$_7$. The presence of a 34-Mdal plasmid, a 27-Mdal plasmid, or both plasmids in Lac$^+$ transconjugants recovered from

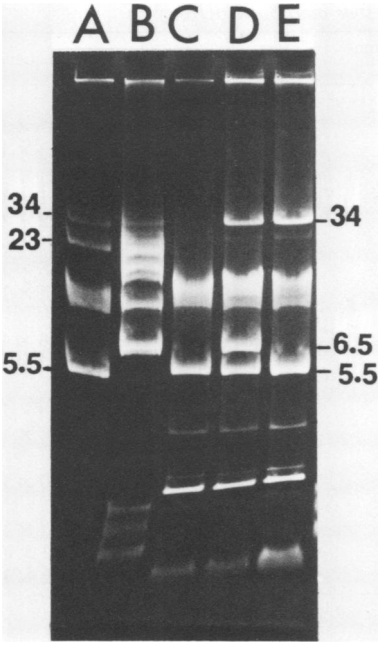

FIG. 3. *Agarose gel electrophoretic patterns of ethanol-precipitated cleared lysates isolated from S. cremoris R1, LM3302, and Lac⁺ transconjugants recovered from R1 matings with LM3302. (A) E. coli reference mobility markers (top to bottom) RP4, Sa, and RSF1010. (B) CsCl-ethidium bromide-density-gradient-purified plasmid DNA from R1 containing two transmissible plasmids with sizes of 34 and 6.5 Mdal. (C) LM3302. (D) Lac⁺ transconjugant RM101 containing the transferred 34- and 6.5-Mdal plasmids. (E) Lac⁺ transconjugant RM108 containing the transferred 34-Mdal plasmid.*

C3, CC101, and CM307 matings suggests that lactose metabolism may be linked to one or both plasmids in C3. The presence of a 25-Mdal plasmid in a Lac⁺ transconjugant from mating CM307 with LM2301 may be due to integration or deletion of a portion of one of these plasmids.

No *lac* plasmid DNA was observed in some of the Lac⁺ transconjugants recovered from conjugal matings of *S. cremoris* C3 and EB101 with LM2301 or CS34 with LM2302. When Lac⁺ transconjugants from matings of EB101 with LM2301 were screened for stability of *lac*, Lac⁻ isolates were observed at a frequency of 3% for one, whereas no Lac⁻ colonies were isolated from the other. These findings indicate that the *lac* genes may have integrated into the recipient LM2301 chromosome in the second transconjugant tested, as was demonstrated for stabilized transductants of *S. lactis* C2 (19). Similarly, the absence of detectable plasmid DNA in transconjugants recovered from C3 or CS34 matings with LM2301 or LM2302, respectively, may be due to the labilization of the plasmid DNA or integration of the *lac* genes into the recipient chromosome. Inability to recover plasmid DNA in transconjugants has been reported previously (10, 17, 20).

Mobilization of chromosomal DNA appeared to occur during conjugal matings. Streptomycin resistance appeared to be transferred to the Lac⁺ *S. cremoris* strains R1, EB_9, and EB_7 from LM2301 since no Lac⁺ Str^r clones were observed on control plates. Also, the frequency of recovery of these recombinants was within the range previously reported for conjugal transfer in lactic streptococci (20). Lac⁺ transconjugants recovered from CS34 matings with LM3302 contained the plasmid profile of CS34. Since Lac⁺ transconjugants were recovered at a relatively high frequency and all recombinants tested were also Str^r, erythromycin resistance may have been transferred from LM3302 to CS34. McKay et al. (20) previously reported mobilization of streptomycin resistance from LM2301 to *S. lactis* C2 in conjugal matings and suggested that this marker was mobilized by chromosomally integrated plasmid DNA.

Plasmid recombination during conjugation was observed when *S. cremoris* C3, Z8, and EB_7 were used as donors of *lac* genes. In the Lac⁺ Str^r transconjugant CS34, a 55-Mdal plasmid was observed, which may have been generated when streptomycin resistance was mobilized from LM2301 to C3. Since no Lac⁺ transconjugants contained a 55-Mdal plasmid, the presence of *lac* or streptomycin resistance genes in the 55-Mdal plasmid could not be determined by conjugal matings. Some Lac⁺ transconjugants recovered from *S. cremoris* Z8 and EB_7 matings with LM3302 also contained plasmids larger than those observed in the Lac⁺ donor. When these transconjugants were used as donors, *lac* genes were transferred to LM2301 at a high frequency. Examination for *lac* plasmid transfer in the Lac⁺ transconjugants recovered from ZM803 matings with LM2301 showed even larger plasmids than were observed in the Lac⁺ donor. Whether the larger plasmids contained in some Lac⁺ transconjugants resulted from the formation of cointegrate intermediates in conjugal transfer (3, 4, 23, 27, 28) or resulted from the donation of plasmid DNA which recombined with recipient DNA upon entry is unknown.

During conjugal matings, some Lac⁺ transconjugants recovered from matings of ZM803 with LM2301 grew as aggregates in broth. Similar cell aggregation was observed with Lac⁺ transconjugants recovered from matings of a Lac⁺ transductant with LM3302 (25). High-frequency

TABLE 3. *Frequency of transfer of lactose metabolic activity*

Donor	*S. lactis* recipient	Transconjugant designation	Transconjugants/ml	Frequency[a]
S. cremoris				
C3	LM2301	CC101	215	1.7×10^{-5}
C3	LM3302	CM307	1,661	1.3×10^{-4}
S. lactis				
CC101	LM3302		530	3.1×10^{-5}
CM307	LM2301		6.4×10^{3}	1.2×10^{-4}
S. cremoris				
R1	LM2301		None	$<1.8 \times 10^{-8}$
R1	LM3302	RM101, RM108	9	5.3×10^{-7}
S. lactis				
RM101	LM2301		9.7×10^{4}	1.6×10^{-3}
RM108	LM2301		6.6×10^{4}	1.0×10^{-3}
S. cremoris				
EB_7	LM2301		None	$<2.8 \times 10^{-8}$
EB_7	LM3302	EB101, EB102	—[b]	$<2.6 \times 10^{-7}$
S. lactis				
EB101	LM2301		2.1×10^{7}	2.0×10^{-1}
S. cremoris				
Z8	LM2301		None	$<3.3 \times 10^{-8}$
Z8	LM3302	ZM803	19	3.1×10^{-7}
ZM803	LM2301		4.2×10^{4}	5.2×10^{-3}

[a] Calculated as transconjugants per donor.
[b] —, Obtained two transconjugants from four mating trials.

transfer by donors exhibiting cell aggregation has been reported in *S. faecalis* by Dunny et al. (5), and in *S. lactis* by Gasson and Davies (9) and by Walsh and McKay (30). The relationship between previous reports of cell aggregation and aggregation in transconjugants from ZM803 matings is currently being investigated.

In strains other than B_1 (1), previous attempts to isolate Lac^- variants of *S. cremoris* lacking corresponding plasmid DNA were unsuccessful (16, 21), even when acridine dyes and elevated temperatures were used. These findings were confirmed by attempts made in this study to isolate *S. cremoris* variants which had lost lactose-fermenting ability (data not shown). These results are in contrast to the ease of Lac^- variant isolation from *S. lactis* (7, 15, 21) and *S. lactis* subsp. *diacetylactis* (12). Larsen and McKay (16) proposed that lactose metabolism in *S. cremoris* might be stabilized by the integration of *lac* genes into the *S. cremoris* chromosome. However, results of genetic transfer trials indicate that lactose metabolism may be linked to plasmid DNA in some strains. Macrina and Ballbinder (18) described an F′ *lac* plasmid which was stable to conventional curing techniques. When this plasmid was transferred to recombination-deficient *Salmonella typhimurium* and *E. coli* strains, the genetic stability of the plasmid to acridine dyes was maintained, whereas the wild-type plasmid in the same genetic background was readily lost with the same treatment. Macrina and Ballbinder suggested that genetic stability in the altered plasmid was due to a plasmid-linked characteristic. Since lactose-fermenting ability was not readily lost in the *S. cremoris* strains examined, but could be transferred to *S. lactis*, the stability of these plasmids in *S. lactis* was tested. Consecutive transfer of Lac^+ transconjugants in the presence of acriflavine resulted in the recovery of Lac^- variants at a frequency comparable to that obtained with curing of *S. lactis* strains (21). Therefore, the stability of lactose metabolism to acridine dyes in *S. cremoris* may be a result of decreased permeability of *S. cremoris* to these dyes as compared to *S. lactis*. Alternatively, *S. cremoris* may have evolved a system of stabilizing the lactose plasmid without having the *lac* genes integrated into the chromosome.

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