JOURNAL OF BACTERIOLOGY, June 1984, p. 954–962
0021-9193/84/060954-09$02.00/0

Vol. 158, No. 3

Genetic and Physical Characterization of Recombinant Plasmids Associated with Cell Aggregation and High-Frequency Conjugal Transfer in *Streptococcus lactis* ML3†

DOUGLAS G. ANDERSON[1] AND LARRY L. McKAY[2]*

Department of Microbiology, University of Minnesota, Minneapolis, Minnesota 55455,[1] and Department of Food Science and Nutrition, University of Minnesota, St. Paul, Minnesota 55108[2]

Received 8 June 1983/Accepted 24 February 1984

Restriction mapping was employed to characterize the 104-kilobase (kb) cointegrate lactose plasmids from 15 independent transconjugants derived from *Streptococcus lactis* ML3 as well as the 55-kb lactose plasmid (pSK08) and a previously uncharacterized 48.4-kb plasmid (pRS01) from *S. lactis* ML3. The data revealed that the 104-kb plasmids were cointegrates of pSK08 and pRS01 and were structurally distinct. The replicon fusion event occurred within adjacent 13.8- or 7.3-kb *Pvu*II fragments of pSK08 and interrupted apparently random regions of pRS01. Correlation of the transconjugants' clumping and conjugal transfer capabilities with the interrupted region of pRS01 identified pRS01 regions coding for these properties. In the 104-kb plasmids, the pRS01 region was present in both orientations with respect to the pSK08 region. The replicon fusion occurred in recombination-deficient (Rec^-) strains and appeared to introduce a 0.8 to 1.0-kb segment of DNA within the junction fragments. The degeneration of the cointegrate plasmids was monitored by examining the lactose plasmids from nonclumping derivatives of clumping transconjugants. These plasmids displayed either precise or imprecise excision of pRS01 sequences or had dramatically reduced copy numbers. Both alterations occurred by *rec*-independent mechanisms. Alterations of a transconjugant's clumping phenotype also occurred by *rec*-independent inversion of a 4.3-kb *Kpn*I-*Pvu*II fragment within the pRS01 sequences of the cointegrate plasmid.

Lactose metabolism in group N streptococci is a plasmid-linked characteristic (1, 10, 14, 20) and, depending upon the strain, is associated with a single plasmid ranging from 30 to 52 megadaltons (1, 10, 17). The conjugal transfer of lactose-fermenting ability (Lac^+) has also been demonstrated in these bacteria, and in many cases the Lac plasmid isolated from transconjugants is different from the Lac plasmid present in the donor strain (26, 29). For example, Walsh and McKay (29) showed that Lac^+ transconjugants resulting from matings between *Streptococcus lactis* ML3 and *S. lactis* LM2301 possessed a single plasmid of 104 kilobases (kb), which was nearly twice the size of the 55-kb Lac plasmid in the donor ML3. Unlike the parental strains, the majority of these Lac^+ transconjugants aggregated in broth and transferred lactose-fermenting ability at a frequency of 10^{-1} to 10^{-2} per donor. *S. lactis* ML3 transferred Lac at a frequency of 10^{-8} to 10^{-9} per donor. Lac^+ transconjugants of *S. lactis* 712 also clumped, transferred lactose metabolism at a high frequency, and possessed a plasmid larger than the plasmids observed in the donor strain (10, 12).

Walsh and McKay (29, 30) speculated that the genes responsible for clumping (*clu*) and high-frequency conjugal transfer (*tra*) in ML3 resided on a chromosomal transfer factor and that translocation of this factor to the Lac plasmid resulted in the formation of the recombinant plasmids observed in the transconjugants. This was based on the observation that the 104-kb plasmid appeared to be a recombinant between the 55-kb Lac plasmid and an unidentified segment of DNA. In addition, Lac^+ Clu^- derivatives of Lac^+ Clu^+ transconjugants were isolated that possessed either a 55-kb plasmid or a 104-kb plasmid. Transconjugants that possessed the 104-kb plasmid and displayed neither the Clu^+ phenotype nor high-frequency conjugation could also be isolated. These results indicated the clumping capability was lost either by deletion of the proposed transfer factor or by an undefined alteration of the 104-kb plasmid. Restriction analysis of the 55-kb Lac plasmids from ML3 and Clu^- derivatives from Clu^+ transconjugants indicated that they were identical, suggesting that precise excision or deletion of the proposed transfer factor had occurred (30). Plasmid curing experiments also linked the clumping and high-frequency conjugal transfer capabilities to the 104-kb plasmid in Lac^+ Clu^+ transconjugants.

To date, restriction mapping of the lactose plasmids in *S. lactis* ML3 and its transconjugants has been limited by the inability to isolate the required quantities of plasmid DNA. We recently developed a methodology that overcomes this limitation (2). As a result, this paper presents detailed restriction maps of the plasmids that reside in *S. lactis* ML3 and are involved with the formation of the plasmids isolated from transconjugants of *S. lactis* ML3. Restriction maps of clumping and nonclumping transconjugants of *S. lactis* ML3 and their respective derivatives are also presented. Additionally, the extent to which the host recombination system is involved with the formation of the recombinant 104-kb lactose plasmids is examined through matings with Rec^- mutants of *S. lactis* ML3. The results identified the origin of the 104-kb plasmids in Lac^+ transconjugants of ML3 and, in addition, identified an inversion element that affected the clumping phenotype.

* Corresponding author.
† Paper no. 13406 of the Scientific Journal Series of the Minnesota Agricultural Experiment Station. The research was conducted in part under Minnesota Agricultural Experiment Station Project no. 18–62.

TABLE 1. Bacterial strains and plasmids

Strain	Derivation	Size of resident plasmids (kb)	Plasmid designation	Reference[a]
S. lactis				
ML3	Lac$^+$ parent culture	104, 55, 48.4, 8.5, 3.0, 1.5	pSK08 (55 kb), pRS01 (48.4 kb)	(17)
MMS36	Lac$^+$ recombination-deficient mutant of *S. lactis* ML3	Same as ML3		
MMS361	Lac$^+$ streptomycin-resistant derivative of MMS36	Same as ML3		
MMS362	Spontaneous Lac$^-$ derivative of MMS361	48.4, 8.5, 3.0, 1.5		
MMS365	Rifampin-resistant derivative of MMS36	Same as ML3		
MMS366	Spontaneous Lac$^-$ derivative of MMS365	Same as MMS362		
LM2301	Lac$^-$ streptomycin-resistant derivative of LM0230	None		(8)
LM2345	Lac$^-$ rifampin- and spectinomycin-resistant derivative of LM0230	None		
S. faecalis				
JH2-2		26.5	pAMβ_1	(13)

[a] Strains with no reference were developed in this study.

MATERIALS AND METHODS

Bacterial strains and plasmids. The resident plasmids in the parental bacterial strains employed are presented in Table 1. Strains being investigated were maintained by biweekly transfer at 32°C in M17 broth containing 0.5% glucose or lactose (28).

Isolation of mutants. Mutants resistant to streptomycin (600 μg/ml), rifampin (150 μg/ml), or spectinomycin (300 μg/ml) were isolated by mutagenesis with ethyl methanesulfonate as previously described (3). Spontaneous Clu$^-$ derivatives of Clu$^+$ Lac$^+$ transconjugants were isolated by repetitive transfer at 32°C in M17 broth containing 0.5% lactose (29).

Solid surface analysis. Solid surface matings were performed by a modification of the method of McKay et al. (21). With a 2% inoculum, the Lac$^+$ donor strains were propagated in M17 lactose broth, and the Lac$^-$ recipients were propagated in M17 glucose broth. Strains were incubated at 32°C for 4 h before mating. Matings were performed by spreading 80 μl of the recipient strain and 20 μl of the appropriately diluted donor strain on the surface of a plate containing 5% nonfat dry milk, 1% glucose, and 1.5% agar. Donor strains were also diluted and plated on bromocresol purple medium (22) containing 0.5% lactose to determine total CFU. After 16 h at 32°C, the mating plates were replica plated onto bromocresol purple-lactose medium containing an antibiotic to select against the donor strain. Independent Lac$^+$ antibiotic-resistant transconjugants appeared in 36 h. The conjugal transfer frequency was expressed as the number of Lac$^+$ transconjugants per input donor cell.

Plasmid purification. Plasmid DNA was prepared (2) and was purified by cesium chloride-ethidium bromide density gradient centrifugation (15). After the removal of ethidium bromide, the purified plasmid DNA was dialyzed against 10 mM Tris-1 mM EDTA (pH 7.8) and stored frozen at −20°C. Purification of a specific plasmid from a preparation containing several plasmid species was accomplished by sucrose gradient centrifugation. Preparations containing the 55- or 104-kb plasmids were centrifuged in a Beckman SW40 Rotor at 36,000 rpm for 3 h at 20°C through a 10 to 40% sucrose gradient prepared in 1.0 M NaCl–20 mM Tris–5 mM EDTA (pH 8.0). Gradients were fractionated, and plasmid DNA was detected by agarose gel electrophoresis as described below. Fractions containing the desired plasmid were dialyzed against 10 mM Tris–1 mM EDTA (pH 7.8) and precipitated with ethanol. The plasmid DNA was dissolved in 10 mM Tris–1 mM EDTA (pH 7.8) and stored at −20°C.

Copy number determinations. Plasmid DNA was purified from cells present in 1.5 or 3.0 ml of a 4-h lysis broth culture as described above. The number of CFU present in the broth culture was determined by dilution and plating on bromocresol purple-lactose medium. Diluted plasmid preparations were loaded onto a horizontal agarose gel containing 0.6% agarose (SeaKem; ME grade) in 40 mM Tris–20 mM sodium acetate–2 mM EDTA (pH 8.1). Electrophoresis was performed for 6 h at 80 V (2.9 V/cm). Lambda DNA was utilized as the DNA concentration standard. The gels were stained with a solution of ethidium bromide (0.5 μg/ml) and photographed under UV illumination. Negatives of the photographs were scanned on a Joyce-Loebl densitometer, and the concentration of DNA present was determined by calculating the area under the peaks. Knowing the molecular weight of a plasmid, the dilution factor utilized to determine the concentration of the plasmid, and the number of cells lysed in preparation of the plasmid DNA, the number of plasmid molecules per cell was calculated. Calculated copy numbers represent estimates of the average number of a given plasmid species within the population of cells subjected to the plasmid purification protocol employed.

Restriction analysis. Restriction enzymes were obtained from Bethesda Research Laboratories, Inc. Restriction digests were performed as described by Davis et al. (6). Restriction fragments were separated by agarose gel electrophoresis as described above. Restriction fragments generated by a *Hin*dIII digestion of lambda DNA provided DNA size standards. Restriction mapping was performed by sequential single, double, and triple digestions.

RESULTS

Plasmid content of *S. lactis* ML3. Previous reports indicated that *S. lactis* ML3 contains four plasmids of 55, 8.5, 3.0, and 1.5 kb (17). The ability to metabolize lactose was linked

to the 55-kb plasmid, designated pSK08 (17). We estimated that approximately 10.7 ± 1.2 copies of pSK08 are present per cell. The restriction map of pSK08 presented in Fig. 1A indicates the locations of single sites for *Sal*I and *Sst*II, two sites each for *Bam*HI, *Sst*I, and *Xho*I, and five *Kpn*I sites.

Two additional plasmids having sizes of approximately 104 and 48 kb were identified in *S. lactis* ML3 (Fig. 2). The 104-kb plasmid appeared to be present at approximately 0.06 copies per cell and was only observed in the presence of pSK08. These observations suggested that the 104-kb plasmid is a recombinant derivative of pSK08 which is either formed at low efficiency or resolved at high efficiency. Due to its low copy number, this plasmid was not characterized by restriction mapping.

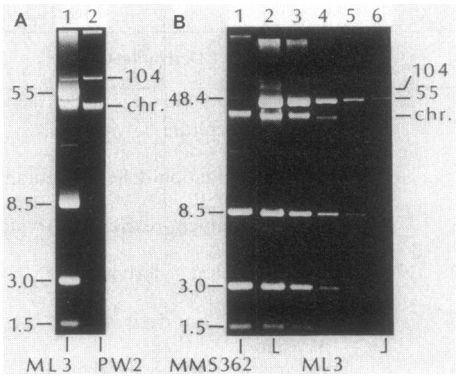

FIG. 2. (A) Plasmid DNA from *S. lactis* ML3 (lane 1) and Lac$^+$ transconjugant PW2 (lane 2). (B) Plasmid DNA from Lac$^-$ MMS362 (lane 1) and Lac$^+$ *S. lactis* ML3 (lanes 2 through 6). Lanes 1 and 2 represent the quantity of plasmid DNA purified from 3.0 ml of cells. Lanes 3, 4, 5, and 6 represent the quantity of plasmid DNA purified from 1.5, 0.5, 0.17, and 0.057 ml of cells, respectively.

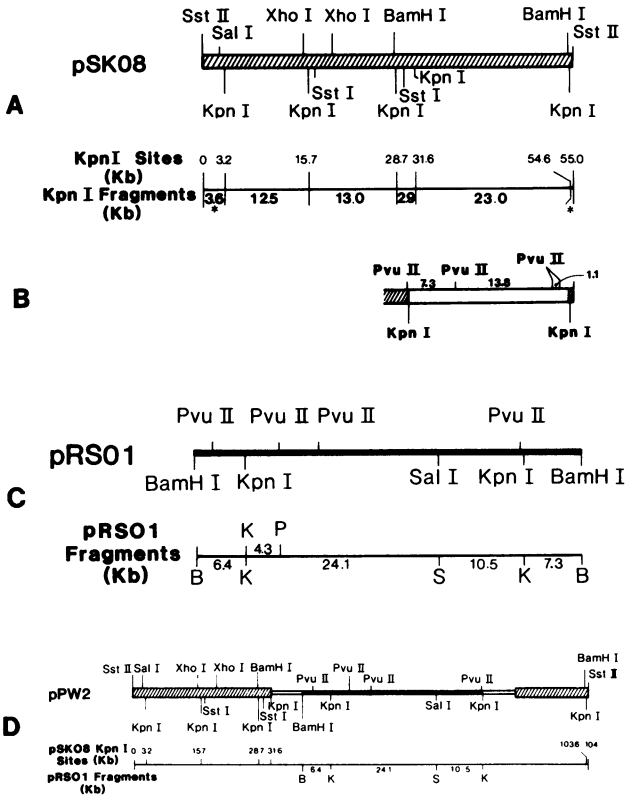

FIG. 1. Restriction maps of pSK08, pRS01, and pPW2. (A) Restriction map of pSK08. Below the restriction map, the locations of the *Kpn*I sites are designated as kb from the single *Sst*II site on this plasmid. The fragments generated by these *Kpn*I sites are also presented. The asterisk identifies a 3.6-kb *Kpn*I fragment that was interrupted by the *Sst*II site. (B) Restriction map of the *Pvu*II sites in the vicinity of the 23.0-kb *Kpn*I fragment of pSK08. The digestion of the 23.0-kb *Kpn*I fragment with *Pvu*II generated a 7.0-kb *Kpn*I-*Pvu*II fragment, whereas a *Pvu*II digestion of pSK08 generated a 7.3-kb *Pvu*II fragment (Fig. 4). These observations identified a *Pvu*II site 0.3 kb outside the 23.0-kb *Kpn*I fragment. (C) Restriction map of pRS01. Below the restriction map, the sizes of restriction fragments defined by *Bam*HI (B), *Kpn*I (K), *Sal*I (S), and *Pvu*II (P), which are mentioned in the text, are identified. (D) Restriction map of pPW2. Regions of pPW2 identical to pSK08 are represented by a wide hatched bar, and regions of pPW2 identical to pRS01 are represented by a solid bar. Junction fragments generated by the replicon fusion event are represented by open bars. The replicon fusion interrupted the 23.0-kb *Kpn*I fragment of pSK08 and the 7.3-kb *Kpn*I-*Bam*HI fragment of pRS01. The uninterrupted pSK08 *Kpn*I fragments and the uninterrupted pRS01 fragments are shown below the map of pPW2.

Plasmid content of Lac$^+$ transconjugants. The majority (>96%) of the Lac$^+$ transconjugants of *S. lactis* ML3 contained a single plasmid of 104 kb. A restriction map of one of these plasmids, designated pPW2, is presented in Fig. 1D. It possessed restriction fragments identified in both pSK08 and pRS01, indicating that pPW2 was formed by the fusion of pSK08 and pRS01. Also, two additional fragments that were not present in pSK08 or pRS01 were observed. These restriction fragments were probably formed at the junctions of pSK08 and pRS01. Also, the 23.0-kb *Kpn*I fragment of pSK08 (Fig. 1A) was interrupted in the formation of pPW2. This fragment was interrupted in 14 of 15 of the 104-kb plasmids characterized. This observation suggested that a site(s) within this fragment was involved in the fusion of pSK08 and pRS01.

Figure 3 presents three distinct classes of restriction maps of 15 independent 104-kb plasmids. The major class was similar to pPW2 (Fig. 1D). All were cointegrates of pSK08 and pRS01, and the fusions occurred within the 23.0-kb *Kpn*I fragment of pSK08. However, these cointegrate plasmids were diverse with respect to the order of the restriction sites, indicating that the fusions occurred at many sites within pRS01. The pRS01 sites also mapped in both orientations with respect to the *Kpn*I sites at the ends of the 23.0-kb *Kpn*I fragment, i.e., pPW2 and pDA11. These observations suggested that no specific site(s) within pRS01 mediated the fusion of pSK08 and pRS01.

The restriction maps of pDA08 and pDA42 (Fig. 3B) indicated that the replicon fusions also occurred within the 23.0-kb *Kpn*I fragment of pSK08. However, some of the

The 48-kb plasmid was present at approximately 0.25 molecules per cell and was initially observed only in Lac$^-$ derivatives of *S. lactis* ML3. It is likely that this plasmid is the 27-megadalton plasmid intermittently observed by Snook and McKay (26). The restriction map of the 48.4-kb plasmid, designated pRS01, is presented in Fig. 1C. Plasmid pRS01 possessed two *Kpn*I sites, single sites for *Bam*HI and *Sal*I, four *Pvu*II sites, and no sites for *Sst*I, *Sst*II, and *Xho*I. It appeared that pRS01 was not detected in the Lac$^+$ strain because its copy number was low and it tended to comigrate with pSK08 on agarose gels (Fig. 2B).

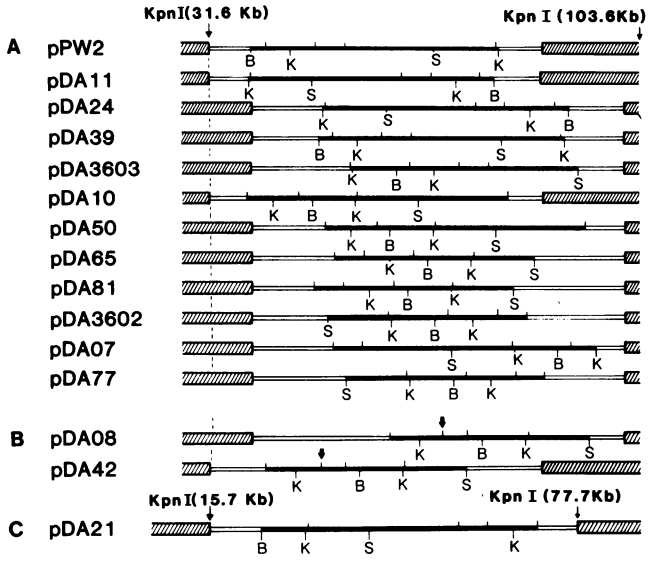

FIG. 3. Restriction maps of recombinant plasmids isolated from 15 independent Lac$^+$ transconjugants. To simplify the presentation, pSK08 sites common to the transconjugants are not presented. Consequently, the maps represent the pRS01 restriction sites present within the appropriate *Kpn*I fragment of pSK08. The pSK08, pRS01, and junction regions are represented as described in Fig. 1D. The pRS01 restriction sites are identified as follows: *Kpn*I (K), *Bam*HI (B), and *Sal*I (S). Small lines above the pRS01 region identify *Pvu*II sites. (A) Class of plasmids in which the replicon fusion event interrupted the 23.0-kb *Kpn*I fragment of pSK08 and different fragments of pRS01. Uninterrupted pRS01 fragments were identical to those in panel C. (B) Class of plasmids similar to those in panel A, except the uninterrupted pRS01 fragments are not identical to those in panel C. The *Pvu*II site marked with an arrow defined a 4.3-kb *Kpn*I-*Pvu*II fragment that was adjacent to the 6.4-kb *Kpn*I-*Bam*HI fragment in pRS01, but was within the 10.5-kb *Kpn*I-*Bam*HI fragment in pDA08 and pDA42. (C) Plasmid in which the replicon fusion event interrupted the 13.0-kb *Kpn*I fragment of pSK08 and the 6.4-kb *Bam*HI-*Kpn*I fragment of pRS01.

nonjunction fragments were of a different size than those in pRS01. The 6.4-kb *Kpn*I-*Bam*HI fragment in pRS01 was absent, and a 10.5-kb *Kpn*I-*Bam*HI fragment was observed. Since the 7.3-kb *Bam*HI-*Kpn*I fragment and the 10.5-kb *Kpn*I-*Sal*I fragment of pRS01 were still present in pDA08 and pDA42, it appeared that the location of the *Kpn*I site determining the size of the 6.4-kb *Kpn*I-*Bam*HI fragment had been altered. Examination of *Pvu*II sites in the vicinity of the altered *Kpn*I site revealed that a *Pvu*II site that defined a 4.3-kb *Pvu*II-*Kpn*I fragment adjacent to the 6.4-kb *Kpn*I-*Bam*HI fragment in pRS01 (Fig. 1C) mapped within the 10.5-kb *Kpn*I-*Bam*HI fragment in pDA08 and pDA42. These data suggested that the 4.3-kb *Kpn*I-*Pvu*II fragment in pDA08 and pDA42 was present in an inverted orientation with resepct to its orientation in pRS01.

In pDA21 (Fig. 3C), the fusion of pSK08 and pRS01 occurred within the 13.0-kb *Kpn*I fragment of pSK08. This was the only plasmid characterized in which the replicon fusion event did not occur within the 23.0-kb *Kpn*I fragment of pSK08.

Since all but one of the replicon fusions occurred within the 23.0-kb *Kpn*I fragment, it was characterized to determine the site(s) at which the plasmids were joined. The 23.0-kb *Kpn*I fragment was purified by electroelution (18) and digested with *Pvu*II. The locations of three *Pvu*II sites are presented in Fig. 1B. The *Kpn*I site at 31.6 kb interrupted a 7.3-kb *Pvu*II fragment, and a 13.8-kb *Pvu*II fragment mapped within the 23.0-kb fragment. *Pvu*II digestions of the 104-kb plasmids from Lac$^+$ transconjugants revealed that either the 7.3-kb *Pvu*II fragment (pPW2, pDA11, pDA10, and pDA42) or the 13.8-kb *Pvu*II fragment (i.e., pDA24, etc.) of pSK08 was interrupted in the formation of cointegrate plasmids (Fig. 3A and B). This identified at least two separate regions of the 23-kb *Kpn*I fragment that can be involved in formation of pSK08-pRS01 cointegrates.

Mapping the *Pvu*II sites in the 23.0-kb *Kpn*I fragment of pSK08 (Fig. 1B) and in pRS01 (Fig. 1C) permitted the identification of both the parental *Pvu*II fragments which were interrupted during formation of cointegrate plasmids and the resultant junction fragments in the cointegrate plasmids. A comparison of parental and junction *Pvu*II fragments for seven 104-kb plasmids is presented in Table 2. The sum of the junction *Pvu*II fragment sizes appeared to be 0.8 to 1.0 kb longer than the sum of the parental *Pvu*II fragment sizes. When the fusion of two plasmids are mediated by a transposition event, the transposable element is replicated, and one copy of the transposable element resides at each junction (24). Therefore, the sum of the junction fragment sizes appear to exceed the sum of the interrupted parental fragment sizes by the size of one copy of the transposable element. Although the data presented in Table 2 are consistent with this mechanism of cointegrate plasmid formation, the data do not specifically imply the additional 0.8 to 1.0 kb of DNA in the cointegrate plasmids resulted from the duplication of a 0.8- to 1.0-kb transposable element. The size difference, however, is within the range reported for insertion sequences (16). The observations that two distinct regions of the 23-kb fragment are involved in cointegrate formation and no analogous regions are identified in pRS01 suggested that one or more insertion sequences resided on pSK08. The hypothesis that pSK08-pRS01 cointegrate plasmids are formed by the transposition of an insertion sequence(s) resident on pSK08 to pRS01 was supported by the restriction data demonstrating insertion of pSK08 into many sites in pRS01 in both possible orientations.

Transconjugant formation in a recombination-deficient *S. lactis* ML3 mutant. Transposition of an insertion sequence occurs by a mechanism which is independent of host-mediated homologous recombination (16). To determine whether

TABLE 2. A comparison of the sizes of interrupted parental *Pvu*II fragments with the resultant cointegrate junction *Pvu*II fragments

Plasmid	*Pvu*II fragment size (kb)			Difference[a]
	Interrupted parental fragments		Cointegrate junction fragments	
	pSK08	pRS01		
pDA10	7.3	4.9	7.0, 6.1	+0.9
pDA11	7.3	9.9	11.4, 6.8	+1.0
pPW2	7.3	9.9	9.6, 8.5	+0.9
pDA07	13.8	8.4	13.5, 9.6	+0.9
pDA39	13.8	9.9	15.9, 8.6	+0.8
pDA24	13.8	9.9	11.8, 12.7	+0.8
pDA50	13.8	4.9	7.2,12.5	+1.0

[a] The difference was calculated as the sum of the sizes of the cointegrate junction *Pvu*II fragments minus the sum of the interrupted parental *Pvu*II fragments. Sizes were estimated by agarose gel electrophoresis.

pSK08-pRS01 cointegrate plasmids were formed in the absence of the host recombination system, a recombination-deficient mutant of *S. lactis* ML3, designated MMS36, was isolated (3). Lac$^+$ transconjugants containing a 104-kb plasmid were isolated, and the restriction maps of two independent 104-kb plasmids, designated pDA3602 and pDA3603, are presented in Fig. 3A. Both pDA3602 and pDA3603 were cointegrate plasmids resembling those isolated from transconjugants of matings performed in Rec$^+$ strains. This suggested that the fusion of pSK08 and pRS01 occurred by the same mechanism in both Rec$^+$ and Rec$^-$ strains, and that the mechanism was *rec* independent.

Since the observations described above indicated that a putative insertion sequence(s) resided on pSK08, the formation of cointegrate plasmids containing pSK08 and another resident plasmid might be expected. This was tested by mating pAMβ_1 into MMS36. Plasmid pAMβ_1 displays conjugal transfer capability and expresses resistance to erythromycin (Em^r) (11). Cointegrates of pSK08-pAMβ_1 were detected by the cotransfer of lactose metabolism and erythromycin resistance to a Lac$^-$ Em^s Str^r derivative of MMS36. The isolation of plasmid DNA from three independent transconjugants revealed that they carried cointegrate plasmids and the fusions occurred within the 13.8-kb *Pvu*II fragment of pSK08 (Fig. 1B). These cointegrate plasmids also displayed different junction fragments, indicating the fusion events occurred at different sites within pAMβ_1. The isolation of pSK08-pAMβ_1 cointegrates from transconjugants formed in a Rec$^-$ strain provided strong evidence that the region within the 13.8-kb *Pvu*II fragment of pSK08 is mediating replicon fusion events by a *rec*-independent mechanism (Fig. 4). The observation that the five *rec*-independent replicon fusions have all involved recombination within the 13.8-kb *Pvu*II fragment suggests that the 7.3-kb *Pvu*II fragment of pSK08 either cannot mediate *rec*-independent replicon fusions or does so at a significantly lower frequency.

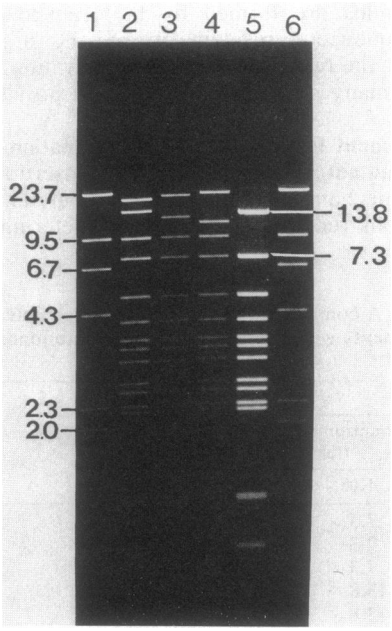

FIG. 4. *Pvu*II digestions of pSK08 (lane 5) and the following three independent pSK08-pAMβ_1 cointegrate plasmids; pDA3605 (lane 2), pDA3620 (lane 3), and pDA3626 (lane 4). The 13.8-kb *Pvu*II fragment of pSK08 was interrupted in the formation of the pSK08-pAMβ_1 cointegrate plasmids. *Hin*dIII digestion of lambda DNA (lanes 1 and 6) were employed as size standards.

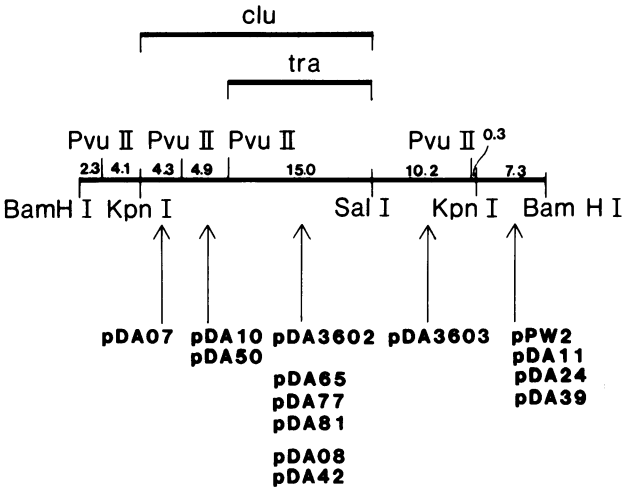

FIG. 5. Restriction fragments of pRS01 that were interrupted in the formation of pSK08-pRS01 cointegrate plasmids. Correlation of the interrupted pRS01 fragment with the strains clumping and conjugal transfer capabilities identified restriction fragments coding for these phenotypes.

Functional characteristics of pRS01. In the formation of pSK08-pRS01 cointegrate plasmids, sequences of pRS01 were interrupted. Since both clumping and conjugal transfer capabilities are linked with the pRS01 segment of DNA (29), sequences coding for these phenotypes may be interrupted by the formation of pSK08-pRS01 cointegrates. This provided a method for mapping the locations of genes involved with the clumping and conjugal transfer phenotypes on pRS01. The resolution of this method was low because the precise insertion sites of pSK08 into pRS01 are unknown. The restriction fragments of pRS01 that were interrupted in the pSK08-pRS01 cointegrates are shown in Fig. 5. Table 3 documents the clumping phenotype and the conjugal transfer capability of the strains carrying the respective cointegrate plasmids.

Strains carrying cointegrate plasmids with interrupted 4.9-kb *Pvu*II or 4.3-kb *Kpn*I-*Pvu*II fragments were Clu$^-$. However, interruptions of the 15.0-kb *Sal*I-*Pvu*II fragment generated cointegrate plasmids whose strains displayed either a clumping or a nonclumping phenotype. Strains carrying cointegrate plasmids with interrupted 7.3-kb *Bam*HI-*Kpn*I or 10.2-kb *Pvu*II-*Sal*I fragments displayed a clumping phenotype. It therefore appeared that an interruption in any of three contiguous restriction fragments can result in the inactivation of genes responsible for the clumping phenotype.

Strains possessing cointegrate plasmids with interruptions in the 15.0-kb *Sal*I-*Pvu*II fragment displayed either the ability or inability to transfer plasmid-linked lactose metabolism by conjugation. Interruptions in other restriction fragments did not adversely affect conjugal transfer. It appeared that genes required for conjugal transfer are located within the 15.0-kb *Sal*I-*Pvu*II fragment.

Instability of the clumping phenotype. To examine the event(s) that occurs when a Clu$^+$ strain spontaneously loses the ability to clump, independent Clu$^-$ Lac$^+$ strains were isolated from Clu$^+$ Lac$^+$ strains by repetitive transfer. This

TABLE 3. Clumping phenotype and Lac transfer frequency of Lac$^+$ transconjugants carrying cointegrate plasmids

Plasmid	pRS01 restriction fragment interrupted in cointegrate plasmids	Clu phenotype	Transfer frequency (Lac$^+$ transconjugants/donor)
Lac$^+$ ML3 × LM2301 transconjugants			
pDA07	4.3-kb *Kpn*I-*Pvu*II	−	3.92×10^{-6}
pDA10	4.9-kb *Pvu*II	−	4.60×10^{-5}
pDA50	4.9-kb *Pvu*II	−	1.37×10^{-4}
pDA65	15.0-kb *Pvu*II-*Sal*I	+	$<7.69 \times 10^{-8}$[a]
pDA77	15.0-kb *Pvu*II-*Sal*I	+	$<2.50 \times 10^{-7}$[a]
pDA81	15.0-kb *Pvu*II-*Sal*I	+	$<1.47 \times 10^{-7}$[a]
pDA08	15.0-kb *Pvu*II-*Sal*I	−	5.81×10^{-7}
pDA42	15.0-kb *Pvu*II-*Sal*I	−	$<3.39 \times 10^{-8}$[a]
pPW2	7.3-kb *Kpn*I-*Bam*HI	+	1.85×10^{-2}
pDA11	7.3-kb *Kpn*I-*Bam*HI	+	1.19×10^{-1}
pDA24	7.3-kb *Kpn*I-*Bam*HI	+	6.15×10^{-2}
pDA39	7.3-kb *Kpn*I-*Bam*HI	+	5.04×10^{-2}
Lac$^+$ MMS36 × MMS362 transconjugants			
pDA3602	15.0-kb *Pvu*II-*Sal*I	+	4.28×10^{-2}
pDa3603	10.2-kb *Sal*I-*Kpn*I	+	6.50×10^{-2}

[a] No Lac$^+$ transconjugants were observed in any mating experiments.

event occurred at a frequency of approximately 2.5% per generation (29; D. G. Anderson and L. L. McKay, unpublished data). The plasmid content of Clu$^-$ Lac$^+$ derivatives was determined by agarose gel electrophoresis and found to be diverse (Table 4).

The predominant class of Clu$^-$ derivatives possessed fewer copies of the 104-kb plasmid than did their respective Clu$^+$ parent strains. In some strains the presence of the 104-kb plasmid was not detected unless the plasmid preparations were first concentrated by cesium chloride-ethidium bromide density gradient centrifugation. In strains possessing a detectable 104-kb plasmid, the observed copy numbers for the 104-kb plasmid in Clu$^+$ DA3602 and its nonclumping derivative DA0201 were 1.9 and 0.04 copies per cell, respectively. In spite of its low copy number, strain DA0201 stably maintained the Lac$^+$ phenotype associated with the 104-kb plasmid.

The second class of Clu$^-$ derivatives possessed plasmids smaller than the 104-kb plasmids, and restriction analysis was employed to characterize them. When digested with *Pvu*II, the fragments generated from the majority of these plasmids were identical to those generated by a *Pvu*II digestion of pSK08. This indicated that pRS01 was precisely excised from the pSK08-pRS01 cointegrate plasmid. Examination of overloaded *Pvu*II digests of these plasmids also revealed the presence of pRS01 *Pvu*II fragments. Compared with pSK08 fragments, the substantially lower intensity of the pRS01 fragments suggested that both plasmids were present at their normal copy numbers. Similar *Pvu*II digestions documented the presence of both pSK08 and pRS01 in Lac$^+$ *S. lactis* ML3 and in 3 to 4% of the independently isolated Lac$^+$ transconjugants that did not possess cointegrate plasmids (D. G. Anderson and L. L. McKay, unpublished data).

A 56.7-kb plasmid was isolated from strain DA1104, a Clu$^-$ derivative of Clu$^+$ DA11 (derived from an ML3 × LM2301 mating) in which the excision of pRS01 was not precise. Although this plasmid was slightly larger than pSK08, digestion with *Pvu*II revealed that a PvuII site present in pSK08 was missing in pDA1104. The nature of the recombination event resulting in the formation of this plasmid is unknown.

Another strain, designated DA0307, was isolated as a Clu$^-$ derivative of Clu$^+$ DA3603 (derived from an MMS36 × MMS362 mating). This strain was unique in two respects; first, it possessed a 48.2-kb plasmid, designated pDA0307, which appeared to be present in very high copy number; second, the 8.5-kb plasmid normally residing in DA3603 was missing. It appeared that pDA0307 was a recombinant between the pSK08-pRS01 cointegrate and the 8.5-kb plasmid; restriction mapping of pDA0307 confirmed this hypothesis (4). All of the restriction sites associated with the 8.5-kb plasmid were present, and all *Pvu*II restriction fragments associated with pRS01 were absent in pDA0307. In addition, three *Pvu*II sites present in pSK08 were absent. The formation of this recombinant plasmid in an Rec$^-$ strain suggested

TABLE 4. Plasmid content of spontaneous Clu$^-$ derivatives of Clu$^+$ transconjugants

Size/copy no. of Lac plasmid		Observed alteration[a] of 104-kb plasmid	Mechanism (n = 28)	Frequency (n = 28)
Clu$^+$ transconjugant	Clu$^-$ derivative			
104 kb/1.9	104 kb/≤0.04	Reduced copy no.	Chromosomal integration[b]	0.464
104 kb/1.9	55 kb/4.6	Reduced size	Precise excision	0.178
	56.7 kb/ND[c]		Imprecise excision	0.036
	48.2 kb/ND[c]		Recombination and excision	0.036
104 kb/1.0	104 kb/1.9	None	Inversion of pRS01 sequences	0.286

[a] Observed alteration of 104-kb plasmid determined by examining covalently closed circular plasmid DNA by gel electrophoresis.
[b] Proposed mechanism.
[c] ND, not determined.

that the recombination event was mediated by a transposition event.

Plasmids isolated from the third class of spontaneous Clu$^-$ derivatives possessed the 104-kb plasmid in the same copy number as the plasmids within their parental strains. Restriction maps of these plasmids revealed that the locations of a *Kpn*I and a *Pvu*II site were different and in an inverted orientation with respect to their locations on their parental plasmids. A comparison of pDA11, pDA24, and pDA3602 and their respective plasmids from Clu$^-$ derivatives is presented in Fig. 6A. The observations that all other restriction sites were identical and that no detectable size change of the plasmid occurred implied that the fragment of DNA containing these *Kpn*I and *Pvu*II sites had undergone an inversion event. This event adversely affected the clumping phenotype of the strain. The restriction maps of pDA0202 and pDA0203 were structurally similar to those of pDA08 and pDA42 (Fig. 3B and 6B). Both DA08 and DA42 did not clump. These observations were consistent with the hypothesis that the orientation of the 4.3-kb *Kpn*I-*Pvu*II fragment can affect expression of genes involved with the clumping phenotype.

The results obtained from transconjugants of DA08 were also consistent with this hypothesis. Normally, Lac$^+$ transconjugants of Clu$^+$ strains clump, and Lac$^+$ transconjugants of Clu$^-$ strains do not clump. However, the Clu$^-$ DA08 transferred lactose metabolism to LM2345 at a frequency of 5.8×10^{-7} per donor, and the resulting transconjugants clumped. Two Clu$^+$ transconjugants were isolated, DA0816 and DA0810. The restriction maps of pDA0816 and pDA0810 were identical and are presented in Fig. 6B. As above, only the locations of the *Kpn*I and *Pvu*II sites defining a 4.3-kb fragment were altered, and their orientation was opposite from that observed in pDA08. Spontaneous Clu$^-$ derivatives of DA0816 and DA0810 were isolated, and the restriction maps of their respective plasmids are presented in Fig. 6B. The locations of the *Kpn*I and *Pvu*II sites are identical to those of pDA08. These data clearly demonstrated that the inversion of the DNA segment containing the 4.3-kb *Kpn*I-*Pvu*II fragment was a conservative, reversible event, and that the clumping capability of DA08 derivatives correlated precisely with the orientation of this 4.3-kb *Kpn*I-*Pvu*II fragment. It therefore appeared that pRS01 contained an inversion element whose orientation affected the expression of the clumping phenotype in strains carrying pSK08-pRS01 cointegrate plasmids. This observation permitted the examination of the effect of clumping on the frequency of conjugal transfer of lactose metabolism. In Fig. 6, Clu$^+$ strains carrying the designated plasmids transferred lactose-fermenting ability at frequencies of 1.1×10^{-2} to 9.9×10^{-2} per donor, whereas the Clu$^-$ derivatives transferred Lac at frequencies of 5.5×10^{-5} to 7.0×10^{-7} per donor. These results indicate that the loss of clumping ability reduced the conjugal transfer frequency by a factor of approximately 10^4, and that the acquisition of clumping ability increased the conjugal transfer frequency by a factor of approximately 10^4. It appeared that the clumping phenotype of a strain significantly affects the frequency of conjugal transfer of lactose metabolism. Similar relationships between a clumping phenotype and conjugal transfer frequency have been observed by Dunny et al. (7) in *Streptococcus faecalis* and by Gasson and Davies (11) in *S. lactis* 712.

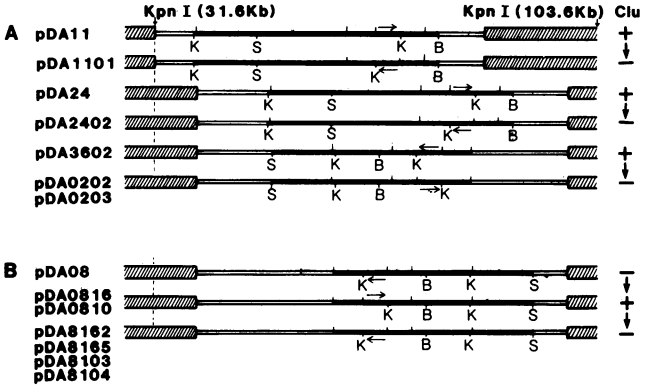

FIG. 6. Retriction maps of plasmids isolated from Clu$^+$ and Clu$^-$ transconjugants. (A) Plasmids from Clu$^+$ transconjugants DA11, DA24, and DA3602 and their spontaneous Clu$^-$ derivatives. These plasmids differed in the orientation of the *Kpn*I-*Pvu*II fragment marked with an arrow. (B) Plasmids from Clu$^-$ transconjugants of DA08, two Clu$^+$ transconjugants of DA08, and their Clu$^-$ derivatives.

DISCUSSION

The characteristics of the recombination event between pSK08 and pRS01 have been described and can be summarized as follows: the recombination event was *rec* independent, occurred at apparently random regions of pRS01, and altered the phenotypic expression of characteristics associated with pRS01. These three characteristics describe recombination events known to occur when a transposable element interacts with a target site to generate a cointegrate molecule (25), suggesting that the formation of the pSK08-pRS01 cointegrate plasmid was mediated by a transposable element. The apparent introduction of an additional 0.8- to 1.0-kb fragment is consistent with transposition and suggests that the transposable element is 0.8 to 1.0 kb in size and thus is similar to known insertion sequences (16). That cointegration always occurred within a limited region of pSK08 suggests that the insertion sequence was located on pSK08. Based on our results and these interpretations, we propose that the transposition of an insertion sequence on pSK08 to pRS01 generates a pSK08-pRS01 cointegrate plasmid as an intermediate of the transposition event.

The concept that these cointegrate plasmids are transposition intermediates is consistent with the observed instability of these plasmids and provides a framework for discussing the diverse plasmids that were characterized in Clu$^-$ derivatives of Clu$^+$ strains. One class of Clu$^-$ strains displayed a stable Lac$^+$ phenotype while maintaining the 104-kb plasmid at an apparent copy number of 0.04 or less. The stable Lac$^+$ phenotype could be accounted for by the transposition-mediated integration of the plasmid into the chromosome, and the low copy number of the plasmid may reflect the frequency at which the integrated plasmid is excised from the chromosome. A second class of Clu$^-$ strains possessed plasmids appearing identical to pSK08 and pRS01 at the copy number observed in *S. lactis* ML3. These plasmids could result from the resolution of a cointegrate plasmid. A third class of Clu$^-$ strains carried plasmids in which pSK08 and pRS01 sequences were deleted. The formation of pDA1104 may have occurred by an intramolecular transposition event, whereas the formation of pDA0307 may have occurred by sequential intermolecular and intramolecular transposition events. It appears that alterations in either plasmid size or copy number that affect the clumping phenotype can be accounted for by events involving the putative insertion sequences located in the cointegrate plasmids.

Another mechanism of altering the expression of the clumping phenotype involving an invertible 4.3-kb *Kpn*I-*Pvu*II fragment was identified whose orientation was directly correlated with the clumping phenotype. This invertible region was mapped within the pRS01 sequences of the cointegrate plasmids. The inversion was *rec* independent, which is consistent with observations of other inversion elements (23; E. M. Lederberg and B. A. D. Stocker, Bacteriol. Proc., p. 35, 1970). Additional experimentation will be required to characterize the structure of this inversion element and to determine the mechanism by which it modulates the expression of the clumping phenotype.

The identification of the inversion element described above suggests that genes involved in the clumping phenotype may be located on a specific fragment of pRS01. Analysis of the pRS01 fragments that were interrupted in the formation of cointegrate plasmids confirmed the involvement of the 4.3-kb *Kpn*I-*Pvu*II fragment as well as two other contiguous fragments in the expression of the clumping phenotype. These restriction fragments define a 23.1-kb region of pRS01 (Fig. 1C). The identification of the genes involved with the expression of the clumping phenotype should facilitate the analysis of the regulation of this trait. It is puzzling that *S. lactis* ML3 carried pRS01 with the inversion element primarily in the Clu^+ orientation and displayed a Clu^- phenotype, whereas Lac^+ transconjugants that carried uninterrupted *clu* genes with the inversion element in the Clu^+ orientation displayed a Clu^+ phenotype. The *clu* genes within these strains differed with respect to *clu* gene dosage and the replicon upon which the *clu* genes resided. In *S. lactis* ML3, the *clu* genes resided on pRS01 at approximately 0.2 copies per cell. The low copy number of pRS01 may be accounted for by a chromosome-plasmid transition of this plasmid similar to that described for bacteriophage P1 (27). In the Lac^+ transconjugants, the *clu* genes resided on a pSK08-pRS01 replicon at approximately two copies per cell. Additionally, the expression of the *clu* genes in the pSK08-pRS01 replicon may be affected by promoters at or near the pSK08-pRS01 junctions.

The analysis of the pRS01 fragments that were interrupted in the formation of the cointegrate plasmid also identified a restriction fragment associated with conjugal transfer ability. The restriction fragment defining the *tra* region is shown in Fig. 5. However, the presence of transfer-deficient plasmids in transconjugants presumably reflects the fact that they could be mobilized by a competent transfer system in the donor cell.

The mapping of *tra* genes to pRS01 and the observation that greater than 96% (69 of 72) of the Lac^+ transconjugants carried pRS01 sequences as pSK08-pRS01 cointegrate plasmids suggested that the transfer of the *lac* genes was mediated by the conjugal transfer capability of pRS01. The results discussed above suggested that the fusion of pRS01 to pSK08 was mediated by the transposition of an insertion sequence located on pSK08 to pRS01. The resultant cointegrate plasmid would represent a transposition intermediate whose structural instability was characterized in Lac^+ transconjugants and their derivatives. It is reasonable to assume that the same degree of structural instability also occurred within the cells in which these cointegrates are formed. Therefore, the 104-kb plasmid identified in *S. lactis* ML3 probably was not a structurally unique plasmid, but a population of independent, structurally diverse cointegrate plasmids. The low copy number of the 104-kb plasmid would reflect the combined frequencies of cointegrate plasmid formation and disintegration within the cell population.

The characteristics of the Lac^+ transconjugants isolated from matings with *S. lactis* 712 are remarkably similar to those of the Lac^+ transconjugants of *S. lactis* ML3. The plasmid DNA isolated from Lac^+ transconjugants of *S. lactis* 712 was sometimes larger than the parental Lac plasmid and appeared to contain a novel insertion (10). The Lac^+ transconjugants could also display a clumping phenotype, which was denoted as Lax^- (12). A comparison of restriction digests of the Lac plasmid from *S. lactis* 712, pLP712 (9, 10), with pSK08 indicate that these two plasmids are very similar, if not identical. This suggests that the mechanism for the conjugal transfer of lactose metabolism in *S. lactis* 712 may be the same as that in *S. lactis* ML3.

Although pSK08 and pLP712 display significant physical similarities, these plasmids generate deletions at different frequencies. No deletions of pSK08 have been directly isolated (D. G. Anderson and L. L. McKay, unpublished data), whereas deleted derivatives of pLP712 are frequently isolated (9). It is significant that most of the deletions of pLP712 that have been mapped originated within a region that is analogous to the regions of pSK08 that contain putative insertion sequences. This suggests that the deletion behavior observed in pLP712 may be caused by the intramolecular transposition of an insertion element. The difference in the deletion frequencies of pSK08 and pLP712 might be accounted for by the different stabilities of each Lac plasmid in their respective host cell or the presence of mutations in the host background which affect transposition frequencies (5).

In conclusion, evidence for the presence of an insertion sequence(s) on the lactose plasmid of *S. lactis* ML3 has been presented. The ability of this element to mediate replicon fusion events accounts for the formation of a cointegrate plasmid that can transfer the plasmid-linked *lac* genes by conjugation. The prevalence of lactose plasmids of 50 to 60 kb within the lactic streptococci suggest that conjugation may be responsible for the dissemination of this characteristic within these strains. If conjugal transfer occurred by the mechanism described in *S. lactis* ML3, the lactose plasmid in many strains of lactic streptococci may possess an insertion sequence. Transposition of this element to a chromosomal site may stabilize the Lac phenotype (19), whereas intramolecular transposition-deletion events may shorten the Lac plasmid to a size that can be transduced (10, 20). The identification of *rec*-independent recombination events in a strain of *S. lactis* is significant, and further characterization will elucidate the scope and impact of these events in the lactic streptococci.

ACKNOWLEDGMENTS

This work was supported in part by an educational grant to D.G.A. from Kelco, Division of Merck and Company, Inc., San Diego, Calif. Research support was provided by the Biotechnology Group, Miles Laboratories, Inc., Elkhart, Ind.

We also thank S. Falkow for helpful discussions and D. Clewell for sending us a strain carrying pAMβ_1.

LITERATURE CITED

1. **Anderson, D. G., and L. L. McKay.** 1977. Plasmids, loss of lactose metabolism and appearance of partial and full lactose-fermenting revertants in *Streptococcus cremoris* B_1. J. Bacteriol. **129**:367–377.
2. **Anderson, D. G., and L. L. McKay.** 1983. A simple and rapid method for isolating large plasmid DNA from lactic streptococci. Appl. Environ. Microbiol. **46**:549–552.
3. **Anderson, D. G., and L. L. McKay.** 1983. Isolation of a recombination deficient mutant of *Streptococcus lactis* ML3. J.

Bacteriol. **155**:930–932.
4. **Anderson, D. G., and L. L. McKay.** 1984. In vivo cloning of *lac* genes in *Streptococcus lactis* ML3. Appl. Environ. Microbiol. **47**:245–249.
5. **Clements, M. B., and M. Syvanen.** 1981. Isolation of a pol A mutation that affects transposition of insertion sequences and transposons. Cold Spring Harbor Symp. Quant. Biol. **45**:201–204.
6. **Davis, R. W., D. Botstein, and J. R. Roth.** 1980. A manual for genetic engineering-advanced bacterial genetics, p. 227. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
7. **Dunny, G., B. Brown, and D. B. Clewell.** 1978. Induced cell aggregation and mating in *Streptococcus faecalis*. Evidence for a bacterial sex pheromone. Proc. Natl. Acad. Sci. U.S.A. **75**:3479–3483.
8. **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.
9. **Gasson, M. J.** 1982. Identification of the lactose plasmid in *Streptococcus lactis* 712, p. 217. *In* D. Schlessinger (ed.), Microbiology—1982. American Society for Microbiology, Washington, D.C.
10. **Gasson, M. J.** 1983. Plasmid complements of *Streptococcus lactis* NCDO712 and other lactic streptococci after protoplast-induced curing. J. Bacteriol. **154**:1–9.
11. **Gasson, M. J., and F. L. Davies.** 1980. Conjugal transfer of the drug resistance plasmid pAMβ1 in the lactic streptococci. FEMS Microbiol. Lett. **7**:51–53.
12. **Gasson, M. J., and F. L. Davies.** 1980. High-frequency conjugation associated with *Streptococcus lactis* donor cell aggregation. J. Bacteriol. **143**:1260–1264.
13. **Jacob, A., and S. J. Hobbs.** 1974. Conjugal transfer of plasmid-borne multiple antibiotic resistance in *Streptococcus faecalis* var. *zymogenes*. J. Bacteriol. **117**:360–372.
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**:1041–1043.
15. **Klaenhammer, T. R., L. L. McKay, and K. A. Baldwin.** 1978. Improved lysis of group N streptococci for isolation and rapid characterization of plasmid DNA. Appl. Environ. Microbiol. **35**:592–600.
16. **Kleckner, N.** 1981. Transposable elements in prokaryotes. Annu. Rev. Genet. **15**:341–404.
17. **Kuhl, S. A., L. D. Larsen, and L. L. McKay.** 1979. Plasmid profiles of lactose-negative and proteinase-deficient mutants of *Streptococcus lactis* C10, ML3, and M18. Appl. Environ. Microbiol. **37**:1193–1195.
18. **Maniatis, T., E. F. Fritsch, and J. Sambrook.** 1982. Molecular cloning, p. 164. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
19. **McKay, L. L., and K. A. Baldwin.** 1978. Stabilization of lactose metabolism in *Streptococcus lactis* C2. Appl. Environ. Microbiol. **36**:360–367.
20. **McKay, L. L., K. A. Baldwin, and J. D. Efstathiou.** 1976. Transductional evidence for plasmid linkage of lactose metabolism in *Streptococcus lactis* C2. Appl. Environ. Microbiol. **32**:45–52.
21. **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.
22. **McKay, L. L., A. Miller III, W. E. Sandine, and P. R. Elliker.** 1970. Mechanisms of lactose utilization by lactic acid streptococci: enzymatic and genetic analyses. J. Bacteriol. **102**:804–809.
23. **Murphy, E., and R. P. Novick.** 1979. Physical mapping of *Staphylococcus aureus* penicillinase plasmid pI525: characterization of an invertible region. Mol. Gen. Genet. **175**:19–30.
24. **Ohtsubo, E., M. Zenilman, H. Ohtsubo, M. McCormick, C. Machida, and Y. Machida.** 1981. Mechanism of insertion and cointegration mediated by IS 1 and Tn3. Cold Spring Harbor Symp. Quant. Biol. **45**:283–295.
25. **Shapiro, J. A.** 1979. Molecular model for the transposition and replication of bacteriophage Mu and other transposable elements. Proc. Natl. Acad. Sci. U.S.A. **76**:1933–1937.
26. **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.
27. **Sternberg, N., D. Hamilton, S. Austin, M. Yarmolinsky, and R. Hoess.** 1981. Site-specific recombination and its role in the life cycle of bacteriophage P1. Cold Spring Harbor Symp. Quant. Biol. **45**:297–309.
28. **Terzaghi, B. E., and W. E. Sandine.** 1975. Improved medium for lactic streptococci and their bacteriophages. Appl. Microbiol. **29**:807–813.
29. **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.
30. **Walsh, P. M., and L. L. McKay.** 1982. Restriction endonuclease analysis of the lactose plasmid in *Streptococcus lactis* ML3 and two recombinant lactose plasmids. Appl. Environ. Microbiol. **43**:1006–1010.