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Analysis of a Region from the Bacteriophage Resistance Plasmid pCI528 Involved in Its Conjugative Mobilization between *Lactococcus* Strains

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A 10-kb *Hin*dIII fragment of pCI528 cloned into the nonconjugative shuttle vector pCI3340 could be transferred by conjugative mobilization from *Lactococcus lactis* subsp. *lactis* MG1363, whereas other *Hin*dIII fragments of pCI528 or the vector alone were nonmobilizable. Subcloning of this 10-kb region identified a 4.4-kb *Bgl*II-*Eco*RI fragment which contained all the DNA essential for transfer. Sequence analysis of a 2-kb region within this 4.4 kb-segment revealed a region rich in inverted repeats and two potential overlapping open reading frames, one of which demonstrated homology to mobilization proteins of two nonconjugative staphylococcal plasmids.

Conjugation has proven to be a useful gene transfer system in furthering the genetic analysis of lactococci (14). In early plasmid biology studies, it was a key element in allowing specific phenotypic traits to be assigned to particular plasmid molecules (21, 31). The analysis of transconjugants at a phenotypic and molecular level has led to the identification of the widespread distribution and role of insertion (IS) elements in mediating recombination and cointegration events in these bacteria (2, 37). In addition, conjugation has been exploited as a food-grade mechanism for the construction of phage-resistant starter cultures for use in commercial cheese fermentations (46).

While self-transmissible plasmids are capable of mediating their own transfer, it is also clear that other non-self-transmissible molecules can be transferred between donor and recipient lactococcal hosts. Steele and McKay (50) have described two types of conjugative mobilization in lactococci: (i) conduction, in which a cointegrate is formed between a self-transmissible plasmid and a mobilizable plasmid, and (ii) donation, in which the mobilizable plasmid enters the recipient cell without cointegration between the two plasmids occurring. Anderson and McKay (2) have provided a clear description of conduction involving the mobilization of the lactose (Lac) plasmid pSK08 by the sex factor plasmid pRS01 in *Lactococcus lactis* subsp. *lactis* ML3 in which the ISS*1* element on pSK08 acted as the agent mediating cointegration (37). Similarly, Gasson has described a very closely related system in *L. lactis* subsp. *lactis* 712, the parent strain of MG1363 (14). In this instance, the sex factor was chromosomally located and, as was observed with pRS01 and pSK08, the 712 sex factor mediated mobilization of the resident Lac plasmid pLP712, giving rise to enlarged plasmids in the transconjugant. Romero and Klaenhammer (42, 43) have also described IS-mediated cointegration between the mobilizing phage resistance plasmid pTR2030 and nonconjugative vectors such as pSA3. Mobilization of the lactose-proteinase plasmid pCI301 by conduction using pAMβ1 has also been reported, although in this case, IS elements did not appear to be involved (17).

Reports of donation among the lactococci are relatively uncommon, probably because of the difficulty in eliminating with certainty undetected cointegration events. A potential example of donation was described by Coffey et al. (7) and involved conjugal mobilization of the Lac plasmid, pCI842, by the conjugative phage resistance plasmid pCI829 in *L. lactis* subsp. *lactis* UC811. Transconjugants which harbored the Lac plasmid only, in an apparently unaltered form, were obtained. Nonetheless, even in this instance, the possibility of IS-mediated transfer cannot be ruled out, since it is known that copies of certain IS elements are located on pCI829 (5).

Van der Lelie et al. (55) recently described the conjugal transfer of the non-self-transmissible *Streptococcus agalactiae* plasmid, pMV158, from *Enterococcus faecalis* to *L. lactis* subsp. *lactis* IL1403 (mediated by either pAMβ1 or pIP501) or between IL1403 strains (mediated by a sex-factor-type element located on the chromosome of IL1403 [54]). This required the presence of a particular region of the molecule encoding a protein which showed homology to the Pre proteins of some class I *Staphylococcus aureus* plasmids (15, 34). These Pre proteins are needed for a recombination function which acts at specific sites termed RS_A/RS_B (35) causing interplasmid recombination normally leading to the formation of stable cointegrates. However, cointegrate formation could not be detected in transconjugants following mobilization of pMV158 by pAMβ1 or pIP501, and van der Lelie et al. suggested that either (i) cointegration was not required for mobilization or (ii) cointegrates did form but resolved rapidly, leaving the size of the mobilized plasmid unaltered (55). Mobilization of pMV158 by pIP501 was also described by Priebe and Lacks (38).

The plasmid pCI528 from *L. lactis* subsp. *cremoris* UC503 encodes a powerful mechanism of phage resistance which involves inhibition of phage adsorption through the production of a cell surface polymer (8, 25). Consequently, it was of interest to introduce the plasmid into a range of lactococcal starter strains by using food-grade conjugal strategies to generate isolates with enhanced phage resistance properties. The donor strain used in these mating experiments was *L. lactis* subsp. *lactis* UC505, an MG1363 strain into which pCI528 was introduced by a series of cotransformation, conjugation, and plasmid-curing experiments (8). However,

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TABLE 1. Strains and plasmids

Strain or plasmid	Characteristic(s)	Source or reference
Strains		
L. lactis subsp. *lactis*		
MG1363	Plasmid-free derivative of 712	13
UC505	MG1363 harboring pCI528	8
952	Lactococcal dairy starter strain	Christian Hansens Laboratory
		Christian Hansens Laboratory, Hørsholm, Denmark
869	Lactococcal dairy starter strain	Christian Hansens Laboratory, Hørsholm, Denmark
IL1403(pIL204)	IL1403 harboring Em^r pIL204	48
ML1611	MG1363 harboring pCI1611	24
ML1619	952 harboring pCI1611	This study
ML1616	MG1363 harboring 14.6-kb *Eco*RI fragment of pCI528 cloned in pAM401	This study
E. coli		
V517	Size reference plasmids	29
TG1	(*lac-pro*) *supE thi hsdD5*/F′, *traD36 proA*$^+$ *B*$^+$ *lacI*^{q} *lacZ M15*	6
Plasmids		
pAM401	10.4-kb Cm^r *E. coli-Lactococcus* shuttle vector	59
pCI372	5.7-kb Cm^r *E. coli-Lactococcus* shuttle vector	18
pCI518	5.4-kb *Eco*RI fragment of pCI528 cloned into pAM401	24
pCI519	14.6-kb *Eco*RI fragment of pCI528 cloned into pAM401	24
pCI520	8.0-kb *Hin*dIII-*Eco*RI fragment of pCI1611 cloned into pCI3340	This study
pCI521	6.3-kb *Bgl*II-*Hin*dIII fragment of pCI1611 cloned into pCI3340	This study
pCI522	5-kb *Hin*dIII-*Sac*I fragment of pCI1611 cloned into pCI3340	This study
pCI523	5-kb *Sac*I-*Hin*dIII fragment of pCI1611 cloned into pCI3340	This study
pCI528	46-kb plasmid encoding resistance to bacteriophage, isolated from *L. lactis* subsp. *cremoris* UC503	8
pCI530	10-kb + 1.5-kb *Hin*dIII fragments of pCI528 cloned into pUC18	24
pCI533	4.4-kb *Bgl*II-*Eco*RI fragment cloned into pCI372	This study
pCI1611	10-kb *Hin*dIII fragment of pCI528 cloned into pCI3340	24
pCI1620	14.5-kb *Hin*dIII fragment of pCI528 cloned into pCI3340	24
pCI1621	5.3-kb *Hin*dIII fragment of pCI528 cloned into pCI3340	24
pCI3340	5.7-kb Cm^r *E. coli-Lactococcus* shuttle vector	18
pUC18	2.69 kb, Amp^r LacZ	60

initial attempts to introduce the plasmid into commercial lactococcal cultures were frustrated by the apparent inability to detect transfer from this donor. The only recipient into which pCI528 could be readily transferred was *L. lactis* subsp. *lactis* 952, and surprisingly, subsequent transfer from this to a broad number of hosts could be achieved (20a). Detailed analysis of *L. lactis* subsp. *lactis* 952 has recently revealed that it belongs to the 712-ML3 class of strains and appears to harbor a sex-factor-type element apparently similar to the equivalent elements in 712-ML3 (26). Although not proven, the apparent inability to conjugate pCI528 from hosts other than 952 (20a) and UC505 (in this latter case only into 952) suggests that pCI528 is not self-transmissible but mobilizable. Consequently, the plasmid is referred to as being non-self-transmissible throughout this paper.

To date, there is no information available regarding mobilization functions encoded by lactococcal plasmids. When molecular analysis of pCI528 resulted in it being subcloned as *Hin*dIII fragments into the nonconjugative vector pCI3340 (24), it was found that the recombinant plasmid harboring a 10-kb *Hin*dIII fragment, pCI1611, could be readily transferred by conjugation from MG1363, unlike the vector alone or other *Hin*dIII subclones. In this study, we describe the characterization of this 10-kb *Hin*dIII region of pCI528 in order to elucidate the nature of plasmid mobilization in lactococci.

Interestingly, the replication region of pCI528 was also localized within the 10-kb *Hin*dIII region and exhibited extensive homology with the replication region of the proposed theta-replicating plasmid pCI305 (18, 27).

MATERIALS AND METHODS

Bacterial strains, plasmids, and culture conditions. Strains and plasmids used in this study are listed in Table 1. Lactococcal cultures were grown at 30°C in M17 supplemented with 0.5% glucose (51). *Escherichia coli* cultures were propagated in Luria-Bertani broth and incubated at 37°C (30). M13 phage was propagated as outlined by Maniatis et al. (30). Ampicillin (50 μg/ml) was used to maintain pUC18 in *E. coli*. When required, chloramphenicol was added at 25 and 5 μg/ml to *E. coli* and lactococcal cultures, respectively.

Plasmid preparation and analysis. The lysis procedure of Anderson and McKay (1) was used to isolate plasmid DNA from lactococcal strains. *E. coli* plasmid and M13 double-stranded phage DNAs were isolated according to the method of Birnboim and Doly (4), and large-scale volumes were purified by cesium chloride-ethidium bromide density gradient ultracentrifugation in a Beckman VTi65 rotor.

Restriction endonucleases and molecular cloning techniques. Restriction enzymes and T4 DNA ligase were obtained from Boehringer Corp., Dublin, Ireland. Generally, DNA digestions and clonings were performed as outlined by

Sambrook et al. (45). DNA fragments were isolated from agarose gels by using the GeneClean kit II (Bio 101, La Jolla, Calif.).

Conjugation strategy. Solid-surface conjugation experiments were performed according to the method of McKay et al. (32) using lactose indicator agar (33) when required. The ratio of donor to recipient was 1:1.

Hybridization experiments. DNA was digested with restriction endonucleases, electrophoresed on 0.7% agarose gels, and transferred onto Hybond N^+ filters by the method of Southern (49), as modified by Wahl et al. (57). DNA was labelled by using the Enhanced Chemiluminescence Gene Detection System (Amersham, Little Chalfont, Buckinghamshire, England). Hybridization conditions were arranged and washing steps were carried out according to manufacturer's instructions.

Electroporation of bacteria. Electroporation of lactococcal strains was done according to the procedure of Holo and Nes (19) with a Bio-Rad Gene Pulser apparatus (Bio-Rad Corp., Richmond, Calif.). *E. coli* transformation was performed under conditions outlined in the manufacturer's instruction manual.

Nucleotide sequence analysis. Relevant DNA fragments were cloned into M13mp18 and M13mp19 vectors (60). The nucleotide sequence was determined by using both single-stranded M13 and alkali-denatured pUC18 templates with the Sequenase 2.0 kit (US Biochemical, Cleveland, Ohio). Sequencing was initiated with commercial M13 primers and continued with specific synthetic 17-mer primers prepared by using a DNA synthesizer (PCR-MATE; Applied Biosystems, Foster City, Calif.). Sequencing gels were run according to the instructions outlined by Bio-Rad. Sequence data were analyzed by the GeneJockey software program (Apple Computer, Inc., Cupertino, Calif.).

RESULTS

Identification of a region of pCI528 required for conjugal transfer. It had previously been observed that the phage resistance plasmid, pCI528, could be conjugally transferred from an MG1363 donor only to a 952 recipient, while transfer to other dairy starter strains could never be detected (20a). However, subsequent transfer from the 952 strain to a range of other lactococcal hosts could be readily achieved, which suggested that there were both host- and plasmid-specific factors responsible for mediating pCI528 transfer. Therefore, to identify the specific region of the plasmid which was involved in mediating its conjugative mobilization, a number of *Hin*dIII fragments of pCI528, cloned into the nonconjugative shuttle vector pCI3340, were electroporated into MG1363 and examined for their ability to be transferred by conjugation into wild-type *L. lactis* subsp. *lactis* 952. Cm^r transconjugants were detected at a frequency of 10^{-4} when ML1611 was used as donor. This is an MG1363 host containing pCI1611 (i.e., pCI3340 into which a 10-kb *Hin*dIII fragment of pCI528 has been cloned). No transconjugants were obtained from donors harboring pCI1620, pCI1621, or pCI3340 (Table 1). Because of the occurrence of open circular forms of other native plasmids, the presence of pCI1611 in 952 could not be visualized by plasmid profile analysis, and therefore hybridization was used to confirm that the plasmid had transferred (Fig. 1).

The ability of pCI1611 to be transferred by conjugation into and out of a variety of lactococcal strains was monitored. The results obtained are presented schematically in Fig. 2. As expected, pCI1611 could be readily transferred

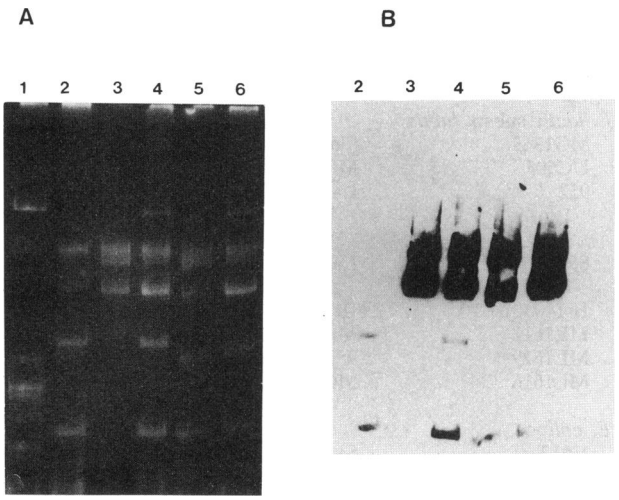

FIG. 1. Hybridization analysis (B) of plasmid profiles of *L. lactis* subsp. *lactis* 952-derived transconjugants (A). The probe used was enhanced chemiluminescence-labelled pCI1611. Lane 1, *E. coli* V517 harboring the size reference plasmids; lanes 2, 952; lanes 3, ML1611; lanes 4 to 6, 952 transconjugants harboring pCI1611.

between 952 and MG1363 isolates. Significantly, while the plasmid could be transferred into the unrelated *L. lactis* subsp. *lactis* IL1403(pIL204) and 869 strains from ML1619 and ML1611 (952 and MG1363 harboring pCI1611, respectively), attempts to transfer the plasmid from the new IL1403(pIL204) donors proved unsuccessful. It was also interesting that although pCI1611 could be transferred into IL1403(pIL204) and 869 from ML1611, the MG1363 host into which the plasmid had initially been electroporated, no transfer was observed from MG1363(Sm) into which the plasmid had been conjugally introduced. Restriction analysis of pCI1611 and DNA isolated from two of these MG1363(Sm)-derived transconjugants revealed that no de-

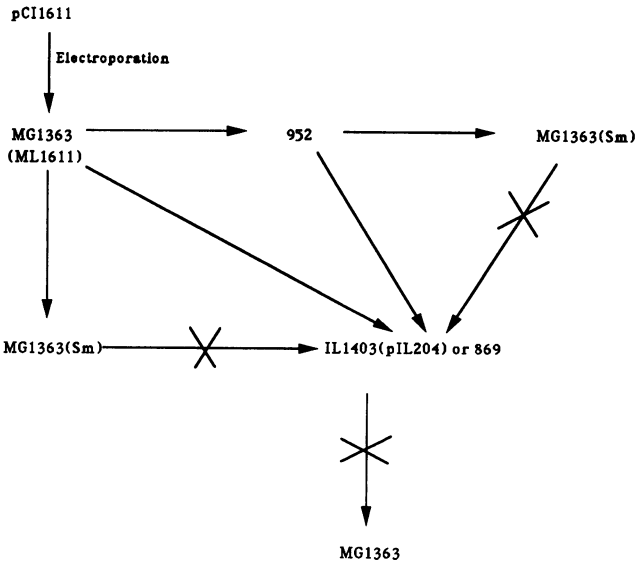

FIG. 2. Diagrammatic representation of transfer of pCI1611 among lactococcal strains. →, transfer of pCI1611; —X→, no transfer detected. In all cases where transfer occurred, frequencies of transfer were approximately 10^{-4} per recipient.

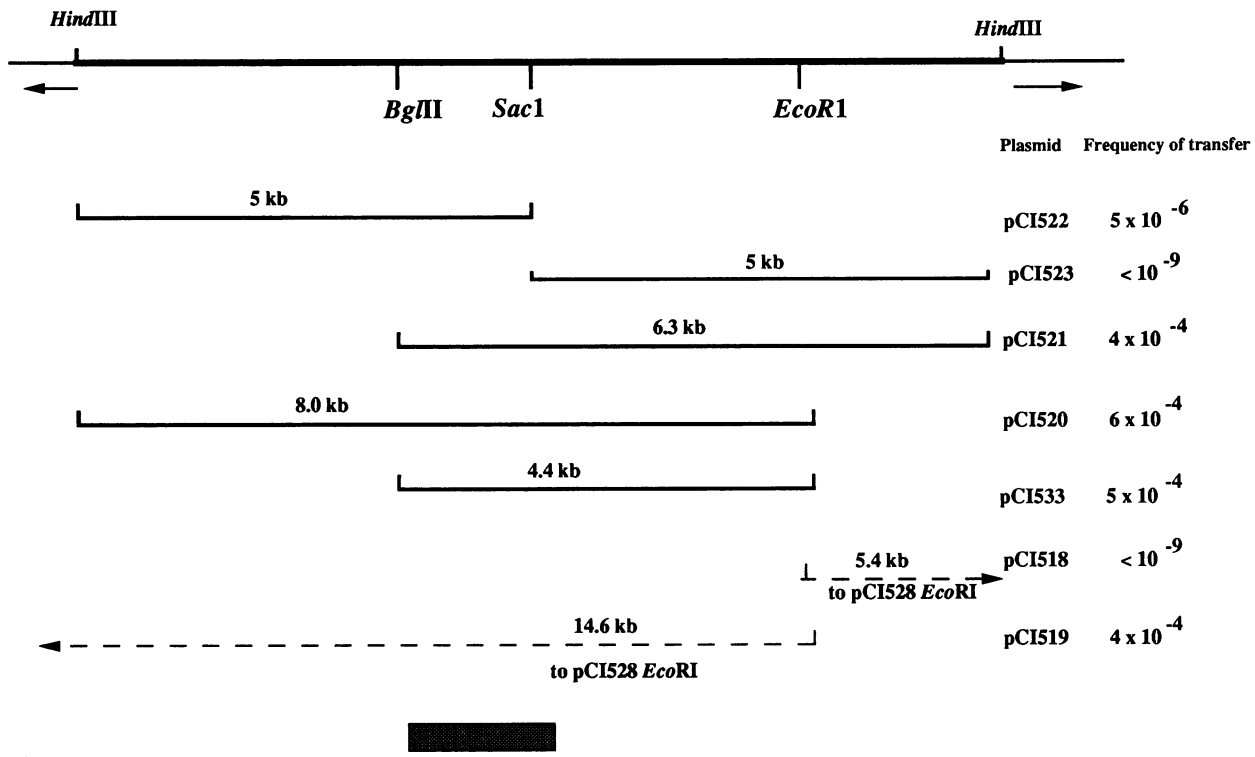

FIG. 3. Restriction map of 10-kb *Hin*dIII fragment of pCI528. ▬, pCI528 DNA; —, pCI3340/pCI372 DNA. Solid line segments, subfragments of the 10-kb fragment of pCI528 cloned into pCI3340/pCI372; broken line segments, flanking *Eco*RI fragments cloned into pAM401; ▩, consensus transfer region. Transfer frequencies of subclones are listed.

tectable alterations or rearrangements had occurred as a consequence of shuttling the plasmid between the MG1363 isolates (data not shown).

Localization of the transfer function on pCI528 to a 4.4-kb *Bgl*II-*Eco*RI fragment. Hybridization analysis localized the 10-kb *Hin*dIII fragment to a region within a 20-kb *Stu*I segment of pCI528 which overlaps with part of both the 14.6- and 5.4-kb *Eco*RI fragments (data not shown). Since these *Eco*RI fragments had previously been cloned in pAM401 (24), mating experiments were performed with MG1363 containing each of these recombinant clones as donors. Transconjugants were recovered at a frequency comparable with that obtained for pCI1611 with ML1616 (MG1363 harboring the 14.6-kb *Eco*RI recombinant clone, pCI519 [Fig. 3]), which allowed further delineation of the region of pCI528 required for transfer. No transconjugants were obtained with the clone containing the 5.4-kb *Eco*RI fragment (Fig. 3).

Further subcloning experiments with vectors pCI3340 and pCI372 generated a range of constructs whose ability to be conjugally transferred could be monitored (Fig. 3). Conjugation into either MG1363 or 952 at frequencies similar to those observed with pCI1611 was observed when MG1363 harboring pCI520, pCI521, and pCI533 were used as donors. Interestingly, pCI522 was consistently mobilized at a 100-fold lower frequency than any of these latter plasmids. From subclone analysis, it therefore appears that all the DNA required for transfer is contained within the 4.4-kb *Bgl*II-*Eco*RI fragment and that the 1.3-kb *Bgl*II-*Sac*I fragment harbors certain functions essential for transfer.

Analysis of transconjugant plasmids. Attempts to recover cointegrate plasmids in transconjugants derived from the initial mating experiments with donors harboring pCI1611 proved unsuccessful. Similarly, as described earlier, restriction digest analysis of DNA from pCI1611 and two of the transconjugant plasmids in MG1363(Sm) indicated that no significant amount of extra DNA was present in the latter (data not shown). These results suggest that if plasmid recombination had occurred during transfer, for example, between pCI528 and sex factor DNA, resolution was such that detection of cointegrate structures was not possible. Probing of pCI1611 with IS*904* and IS*981* (11, 36) indicated no DNA homology (data not shown). Interestingly, when ISS*1* (37) was used as a probe, weak homology did occur with pCI1611 but not with the 4.4-kb *Bgl*II-*Eco*RI subclone, pCI533, which harbors sufficient genetic material to mediate efficient transfer.

Sequence analysis. The 1.3-kb *Bgl*II-*Sac*I fragment, which was required for transfer of pCI528, was subcloned from pCI533 from an *Xba*I site located on the vector pCI372 and the *Sac*I site located on pCI528 DNA into M13mp18 and M13mp19 and sequenced. Preliminary analysis indicated that the first 300 bases harbored at least three large inverted-repeat regions (Fig. 4) with high ΔG values. A large (14-bp), almost perfect (one mismatch) direct repeat (Fig. 4) and several smaller, less perfect structures (data not shown) were also identified downstream of these inverted repeats. Computer analysis of the sequence of the 1.3-kb fragment revealed a potential open reading frame (ORF1), initiating at nucleotide (nt) 178 and terminating at nt 672, capable of encoding a protein of a molecular weight of 19,553. However, no suitable ribosome binding site could be located upstream of ORF1 and no homology to any sequences in

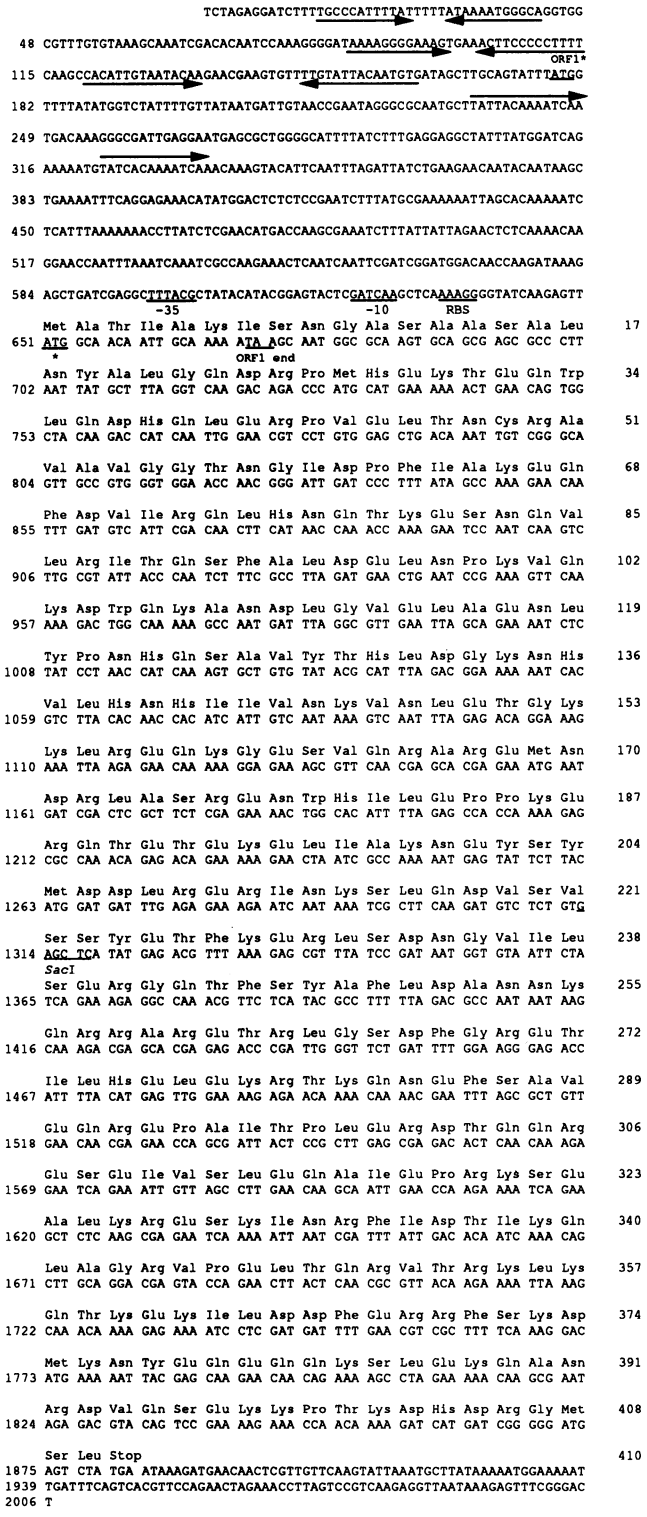

FIG. 4. Nucleotide sequence of the region within the 10-kb *Hin*dIII fragment of pCI528 involved in conjugative mobilization. Inverted- and direct-repeat regions are indicated by arrows below and above the sequence, respectively. Only the beginning and end of ORF1, initiating at nt 178 and terminating at nt 672, are indicated. The deduced amino acid sequence for ORF2 is shown. The putative promoter sites, the ribosome binding site (RBS), and the start codon (*) are indicated by lines under the sequence.

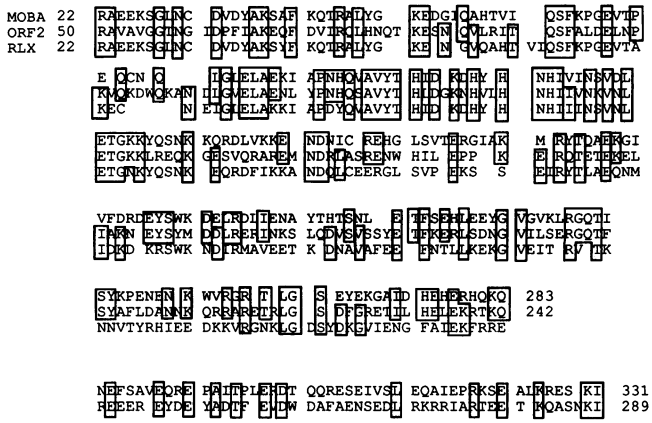

FIG. 5. Amino acid sequence alignment of the homologous regions of ORF2, MobA (pC221) and Rlx (pS194). Homologous amino acids are boxed.

GenBank was detected. Therefore, the function of ORF1, if any, is unclear.

Further analysis of the 1.3-kb *Xba*I-*Sac*I fragment using the GeneJockey program indicated the presence of a second larger incomplete ORF which overlapped slightly with ORF1 and appeared to stretch past the *Sac*I site. Thus the relevant region of the adjacent *Sac*I-*Eco*RI fragment was cloned, sequenced, and combined with the 1.3-kb *Xba*I-*Sac*I sequence to reveal a second complete ORF, ORF2, of 1,233 bp, only 668 of which were located within the 1.3-kb *Xba*I-*Sac*I fragment. ORF2 initiated at nt 651 and terminated at nt 1883 and had a capacity to encode a protein with a molecular weight of 47,660. A potential ribosome binding site (AAAGG) which is complementary to the 3′ end of 16S rRNA of gram-positive and -negative bacteria was located 13 bp upstream of the ATG start codon (28, 47). A putative −10 promoter sequence was found 5 bp upstream of the ribosome binding site, and this was separated by 19 bp from the −35 sequence. The −10 region (GATCAA) conforms weakly with the *E. coli* consensus promoter region (TATAAT), while the −35 sequence (TTTACG) closely resembles the consensus −35 region (TTGACA) (44). A TG motif regularly observed 1 bp upstream of the lactococcal −10 region (56) was not present.

GenBank analysis indicated that ORF2 exhibited homology with particular regions of hypothetical protein A (MobA) and relaxation protein A (Rlx) of two *S. aureus* plasmids, pC221 and pS194, respectively (39, 41). Homology began at amino acid (aa) 50 of ORF2 and terminated at aa 283 and aa 331 for pC221 and pS194, which therefore exhibited 40 and 33% homology over lengths of 234 and 282 aa, respectively (Fig. 5). Significantly, both the MobA and Rlx proteins are known to be involved in conjugative mobilization of their respective plasmids (39, 41).

DISCUSSION

We have previously described how *L. lactis* subsp. *lactis* 952 can mediate the conjugative mobilization by conduction of the nonconjugative vector pGB301 (26). While an IS element responsible for cointegrate formation could not be identified, the system appeared to be broadly similar to that described by Romero and Klaenhammer (42, 43), who mobilized the vector pSA3 using the phage resistance plasmid pTR2030. In this paper, we describe a potentially different

type of plasmid mobilization, in which cointegrate formation was never observed. The phage resistance plasmid pCI528 could be transferred by conjugation into and out of the donor strain 952 without any obvious alteration in its gross structure. Similarly, the clone harboring a 10-kb *Hin*dIII fragment of pCI528 also transferred without the formation of any detectable cointegrate event. Thus, it is possible that this type of transfer may be an example of donation. Nonetheless, the role of conduction cannot be eliminated at this time.

It is notable that while initial attempts to conjugally transfer pCI528 from MG1363 into dairy starter strains proved unsuccessful except when 952 was used as the recipient (20a), in this study, the transfer of clones harboring the 10-kb fragment of pCI528 from MG1363 to a number of different strains was achievable. The reason for this discrepancy is as yet unclear but may perhaps be related to plasmid size and the difficulty in mobilizing larger plasmids from MG1363 into unrelated strains. Interestingly, although transfer of pCI1611 from the MG1363 strain into which the plasmid had been introduced by electroporation was observed, no transfer was detected from MG1363(Sm) into which pCI1611 was introduced by conjugation. Restriction analysis of plasmid DNA isolated from these strains indicated no gross alteration in DNA structure. A possible explanation for this observation has been provided by Gasson (14a), who has recently suggested that the sex factor element present in MG1363 may be cured at high frequency. Thus, the inability of both of the MG1363(Sm) isolates to act as conjugal donors of pCI1611 (Fig. 2) could be due to loss of the sex factor element during the isolation of the streptomycin-resistant derivatives of the original MG1363 host, meaning that this element is no longer available to mobilize pCI1611.

Conjugal transfer of plasmid DNA in gram-negative bacteria involves nicking and initiation of transfer at a site termed *oriT*, separation of the two plasmid DNA strands, strand transfer, DNA synthesis in the donor and recipient, and recircularization of the plasmid molecule. For conjugal transfer to occur successfully, a *cis*-acting *oriT* and a number of *trans*-acting functions necessary for processes such as cell-to-cell contact and mating bridge formation are required (58). In the *E. coli* F plasmid system, at least 20 genes stretching over 33 kb of DNA are necessary for plasmid transfer (20).

The fact that conjugation between gram-negative and gram-positive bacteria can occur (53) may indicate similarities between the conjugation mechanisms in both types of host. Thomas and Archer (52) have identified a 14-kb region of DNA on the 52-kb *S. aureus* plasmid pGO1 encoding all essential conjugal functions. Tn*551* and Tn*917* mutagenesis revealed that unlike F-plasmid transfer, however, a single major polycistronic transcript encoding all transfer-associated proteins was not present in pGO1. Analysis of the conjugal transfer determinants on the broad-host-range plasmid pIP501 identified two separate regions of 7.5 and 8.8 kb that were necessary for transmission of the plasmid (22) and the genes for three essential proteins, *cjnA*, *cjnB*, and *cjnC*, were shown to be transcribed as a single polycistronic mRNA. The *oriT* of pIP501 has recently been localized to a 1.6-kb fragment (23). A lactococcal vector into which this fragment was cloned could be mobilized at high frequency between lactococci when transfer functions were provided in *trans* on a conjugal derivative of pIP501. A 200-aa ORF, which exhibited no homology with data bank sequences, was identified, but its role in mobilization, if any, is not yet confirmed. Three palindromic sequences were also identified, the largest of which was proposed as the nick target site of pIP501 transfer proteins.

While there is relatively little information regarding the genetic requirements for conjugal mobilization of a plasmid by donation, the mobilizable plasmid must nevertheless contain an origin of transfer (*oriT*) and encode mobilizing proteins which recognize this site and cause the initiation of transfer. *oriT* regions described for gram-negative conjugative and nonconjugative plasmids, e.g., RK2, ColE1, and RSF1010, consist of short regions (100 to 300 bp) rich in inverted and direct repeats and containing a nick relaxation site (3, 9, 16). Detailed study of the mobilization locus of the nonconjugative gram-negative RSF1010 plasmid identified a 1.8-kb region within which were included *oriT* and three genes encoding proteins of 9, 16, and 65 kDa, two of which were overlapping and all of which were essential for mobilization (9, 10). A similar type of locus was observed with the *S. aureus* pC221 nonconjugative plasmid on which two partially overlapping genes, *mobA* and *mobB*, and an *oriT* were all required intact for mobilization (39, 40). No sequence homology between the previously mentioned ColE1 and the pC221 mobilization regions was observed, although their functions were related to each other.

We have shown that the DNA sequence reported in this study encompassed a segment of pCI528 necessary for conjugative mobilization of this plasmid. Located within the first 300 bp, there was a region rich in inverted repeats of high ΔG value, suggesting the possible presence of stem-loop structures, which are typical of *oriT* regions, in addition to one significant direct repeat.

Computer analysis revealed at least one large ORF downstream of the inverted repeat region, encoding a protein of 47 kDa. A computer search of GenBank sequences revealed homology with two *S. aureus* proteins, MobA and Rlx (39, 41), involved in conjugative mobilization of their respective plasmids, pC221 and pS194, which is circumstantial evidence that ORF2 may play an important role in the conjugative mobilization of pCI528. This region of homology flanked the *Sac*I site of ORF2. As described above, 565 bp of ORF2 is located outside the 1.3-kb *Bgl*II-*Sac*I fragment essential for transfer, which may explain why clones harboring only this region transfer less efficiently than those incorporating all of ORF2. Nevertheless, it is surprising that the protein is still functional at all, considering the extent of the deletion. Previously, it was reported that a truncated product of a gene encoding a mobilization protein on RSF1010 was still functional (10). As described earlier, the role of the overlapping ORF1, if any, has not yet been elucidated. Significantly, the two ORFs involved in mobilization on pC221 and pS194 were also found to overlap.

From the data available at present, it is likely that the region of pCI528 which has been sequenced may encode the *oriT* region and at least one *mob* gene. Considering that both the 952 and MG1363 strains harbor sex-factor-type elements (12, 26), it is presumed that the conjugative functions required for this type of mobilization are provided by these elements. This report presents the first description of at least part of the molecular basis for plasmid mobilization in *Lactococcus* strains, and it provides new information which will contribute to our understanding of how conjugation systems operate in these bacteria.

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