APPLIED AND ENVIRONMENTAL MICROBIOLOGY, Dec. 2002, p. 6451–6456 Vol. 68, No. 12
0099-2240/02/$04.00+0 DOI: 10.1128/AEM.68.12.6451–6456.2002
Copyright © 2002, American Society for Microbiology. All Rights Reserved.

Identification of a Replicon from pTXL1, a Small Cryptic Plasmid from *Leuconostoc mesenteroides* subsp. *mesenteroides* Y110, and Development of a Food-Grade Vector

Franck Biet,[1]* Yves Cenatiempo,[1] and Christophe Fremaux[2]

Institut de Biologie Moléculaire et d'Ingénierie Génétique, Equipe d'Accueil 2224, Université de Poitiers, 86022 Poitiers Cedex,[1] and Rhodia Food, Zone d'Activités de Buxières, 86220 Dangé Saint-Romain,[2] France

Received 29 August 2002/Accepted 5 September 2002

A 2,665-bp cryptic plasmid, pTXL1, isolated from *Leuconostoc mesenteroides* subsp. *mesenteroides* Y110 was identified. This plasmid harbors a replicon localized on a 1,300-bp fragment. Two observations suggested that pTXL1 does not belong to rolling-circle replication (RCR)-type plasmids and most likely replicates via a theta mechanism. These hypotheses are supported by the observation that no detectable single-stranded intermediate was found for the replicon and that, unlike in RCR-type plasmids, the pTXL1 replicon sequence lacks an open reading frame encoding a replicase. The small-sized pTXL1 plasmid is stable and, according to its origin, can be considered in the "generally recognized as safe" category. Its ability to replicate in several lactic acid bacteria was exploited to develop a vector producing mesentericin Y105, a class II anti-*Listeria* bacteriocin. With this new vector, a recombinant industrial *Leuconostoc cremoris* strain able to produce mesentericin Y105 was constructed.

Plasmids from lactic acid bacteria (LAB) have become a focus of numerous studies, thus leading to the development of families of cloning vectors. Most vectors for LAB are rolling-circle replication (RCR) plasmids, which replicate by using single-stranded intermediates similar to those from other gram-positive bacteria (2, 15, 22). While these plasmids have been indispensable in designing new gene cloning systems in LAB, problems linked to their stability are well documented (14, 21). Cloning vectors that replicate without the use of RCR display several advantages, as exemplified by those derived from the enterococcal pAMβ1 plasmid (9). The replication of pAMβ1 and other LAB plasmids, such as pWV02 (20) and pCI305 (17), by the theta mechanism was reviewed by Jannière et al. (19).

Leuconostoc spp. are a diverse group of heterofermentative LAB of considerable industrial importance. Many *Leuconostoc* species harbor one or more native plasmids of various sizes, but to date, only a few reports have dealt with RCR plasmids in species of the *Leuconostoc* genus (5, 7, 10, 11, 30) and none concerns identification of a non-RCR plasmid. The aim of the present study was to analyze the mode of replication of the *Leuconostoc* plasmid pTXL1 (6) in order to develop families of vectors designed for specific industrial applications. Plasmid pTXL1 was rather small and stable during attempts to cure its host strain, *Leuconostoc mesenteroides* subsp. *mesenteroides* Y110, with novobiocin treatment. Moreover, it seemed compatible with various vectors derived either from the pWV01 replicon or from the pAMβ1 replicon (data not shown), and we consequently hypothesized that pTXL1 might be a good candidate for elaborating new food-grade vectors for *Leuconostoc*.

Bacterial strains and plasmids used in this study are listed in Table 1.

L. mesenteroides subsp. *mesenteroides* Y110, which originated from goats' milk, was isolated and grown in MRS (Difco) broth or agar (1.2%, wt/vol) at 30°C. It contains five plasmids (6). The smallest, pTXL1 (2.6 kb) (6), was inserted into the unique *Sal*I site of pBSSKII$^+$ to yield pFBYC050. General genetic techniques used were as previously described (25, 28). *Escherichia coli* competent cells were prepared and transformed according to the method of Hanahan (16). The resulting transformant strains were propagated at 37°C in LB (28) broth or on agar (1.5%, wt/vol) containing ampicillin at a final concentration of 100 μg ml^{-1}. The sequences of pTXL1 were determined on both strands with an Auto-read sequencing kit (Pharmacia) and with appropriate primers by using an automated laser fluorescence DNA sequencer (Pharmacia). Sequence analyses were performed with the Genetics Computer Group sequence analysis software package (University of Wisconsin). The G+C content in pTXL1 is 34%, in accordance with the G+C percentage of *Leuconostoc* subsp. chromosomes and plasmids previously described (10, 12). Surprisingly, sequence analysis revealed no similarities with genes encoding known proteins involved in replication, such as the replication initiation protein required for RCR plasmids. Generally, small cryptic plasmids replicate by an RCR mechanism and their sequence bears a gene encoding their replicase (19). No sequence resembling a double-strand origin or a single-strand origin involved in the conversion of single-strand DNA (ssDNA) intermediates into a double-stranded DNA plasmid molecule was identified in the pTXL1 nucleotidic sequence.

To locate the region required for pTXL1 replication, the erythromycin cassette originating from pGhost9:IS*S1* was inserted into pFBYC050 (Fig. 1). Plasmid pFBC050E was there-

* Corresponding author. Present address: LBE/IBL Institut Pasteur de Lille, 1 rue du Prof. Calmette, B.P. 245, 59019 Lille Cedex, France. Phone: 33-3-2087 1195. Fax: 33-3-2087 1192. E-mail: franck.biet@pasteur-lille.fr.

TABLE 1. Bacterial strains and plasmids

Strain or plasmid	Relevant characteristic(s)	Source or reference[a]
Strains		
Escherichia coli DH5α	*recA endA1 gyrA96 thi-1 hsdR17 supE44* Δ*lac* U169(ϕ80 Δ*lacZ*ΔM15) *deoR* F^- λ^-	Gibco-BRL
Leuconostoc mesenteroides subsp. *mesenteroides*		
Y105	Wild-type strain	18
Y110	Wild-type strain	CNCM I-1936[a]
FR52	Wild-type strain	24
Leuconostoc mesenteroides subsp. *dextranicum* DSM20484	Wild-type strain	DSMZ[b]
Leuconostoc cremoris LC	Wild-type industrial strain	Rhodia Food
Lactococcus lactis		
IL1403	Lab strain	8
MG1363	Lab strain	13
RD230	Wild-type industrial strain	Rhodia Food
Lactobacillus sake 23K	Plasmid cured	3
Listeria ivanovii BUG 497	Lab strain	Pasteur Institute
Pediococcus acidilactici P120	Wild-type industrial strain	Rhodia Food
Plasmids		
pBSSKII+	pBluescript SK II+ cloning vector, 2.96 kb, Ap^r *lacZ*	Stratagene
pGhost9:IS*S1*	pWV01 derivative replicon	23
pFR18	1.8-kb cryptic plasmid from *L. mesenteroides* FR52	5
pFBYC018	pBSSKII+::1.8-kb *Hin*dIII pFR18, 4.8 kb, Ap^r Em^s	5
pFBYC018E	pFBYC018::1.1-kb *Bam*HI pGhost9:IS*S1* (Em^r), 5.9 kb, Ap^r Em^r	5
pFBYC18E	pFBYC018E with 2.5-kb *Pvu*II pBSSKII+ deleted, 3.5 kb, Em^r	5
pTXL1	2.66-kb cryptic plasmid from *L. mesenteroides* Y110	5
pFBYC050	pBSSKII+::2.66-kb *Sal*I pTXL1, 5.62 kb, Ap^r Em^s	This study
pFBYC051	pBSSKII+::2.66-kb *Spe*I pTXL1, 5.62 kb, Ap^r Em^s	This study
pFBYC052	pBSSKII+::1.8-kb *Ssp*I pFR18, 4.8 kb, Ap^r Em^s	This study
pFBYC050E	pFBYC050::1.1-kb *Bam*HI pGhost9:IS*S1*, 7.72 kb, Ap^r Em^r	This study
pFBYC50E	pFBYC050E with 2.5-kb *Pvu*II fragment deleted, Em^r	This study
pFBYC065	pFBYC050E with 312-kb ExoIII[c] fragment deleted, Ap^r Em^r	This study
pFBYC060K	pFBYC050E with 482-kb *Kpn*I-*Kpn*I fragment deleted, Ap^r Em^r	This study
pFBYC062S	pFBYC050E with 1.4-kb *Sal*I-*Ssp*I fragment deleted, Ap^r Em^r	This study
pFBYC068S	pFBYC050E with 1.9-kb *Sal*I-*Afl*III fragment deleted, Ap^r Em^r	This study
pFBYC051E	pFBYC051::1.1-kb *Bam*HI pGhost9:IS*S1*, 7.72 kb, Ap^r Em^r	This study
pFBYC06	pRW5.6 with *Hin*dIII deleted	4
pFBYC069	pFBYC051E::669-bp P59, *dvnA* fragment from pFBYC06	This study
pFBYC070	pFBYC069::485-bp *dvnA::mesY*, *mesI* PCR product	This study

[a] CNCM, Collection Nationale de Cultures de Microorganisms, France.
[b] DSMZ, Deutsche Sammlung von Mikroorganismen and Zellkulturen, Germany.
[c] ExoIII, exonuclease III.

fore constructed by insertion of the 1.1-kb *Bam*HI cassette into the unique *Bam*HI site in the multiple cloning site of pFBYC050. Erythromycin-resistant transformants of *E. coli* were selected on brain heart infusion agar plates (Difco) containing 150 μg of erythromycin ml^{-1}. Erythromycin-resistant transformants of *Leuconostoc* subsp. strains were grown at 30°C in MRS (Difco) broth or agar (1.2%, wt/vol) containing erythromycin (5 μg ml^{-1}). As this construct was able to transform *Leuconostoc mesenteroides* subsp. *dextranicum* DSM20484 to Em^r, a series of subclones was constructed to further locate the minimal region, which allowed replication (Fig. 1). We found that the minimal replicon required for the replication of pTXL1 resides within the 1.3-kb *Sal*I-*Ssp*I fragment (Fig. 1). Within this minimum replicon three palindromic structures designated IRI, IRII, and IRIII were identified (Fig. 2). IRI, -II, and -III could form hairpin loops, with calculated changes in the free energy of formation (ΔG^o) of −15, −22, and −10 kcal mol^{-1}, respectively. Three direct repeats, DRI, DRII, and DRIII, were identified (Fig. 1). IRI and DRI may be required for plasmid replication because the construct pFBYC065, from which these repeats were deleted (Fig. 1), was not able to replicate in *Leuconostoc*. Sequence analysis revealed two small open reading frames, ORF1 and ORF2, extending from positions 1001 to 1339 and from positions 883 to 644, respectively. By scanning data banks for sequences deduced from ORF1 (80 amino acids) and ORF2 (113 amino acids), we did not find

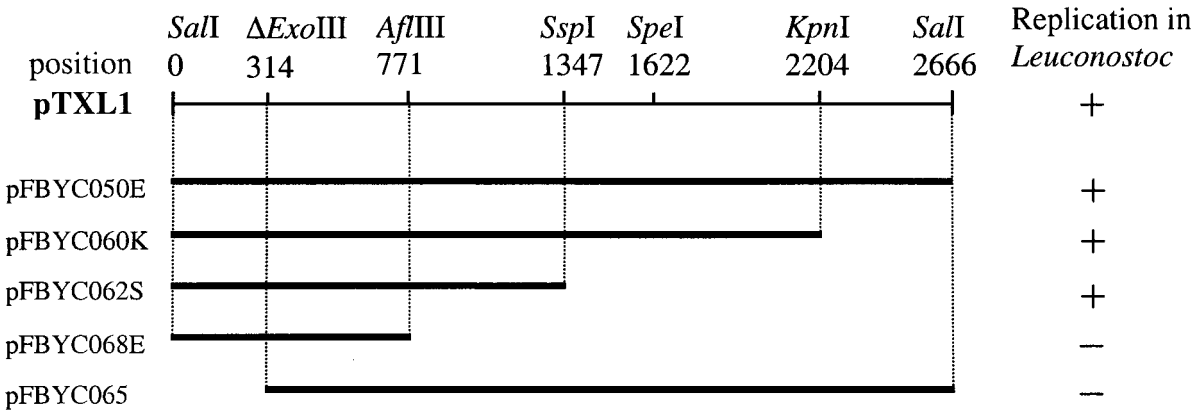

FIG. 1. Identification of the minimal replicon from pTXL1. The restriction map of pTXL1 is shown with the positions of the restriction sites. Partial enzymatic deletion by exonuclease III is denoted by ΔExoIII with the size of the deletion.

homology to any known protein. A deletion of ORF1 and ORF2 in the construct pFBYC068E abolished plasmid replication in *Leuconostoc*, suggesting that both ORFs may be involved in plasmid replication.

Generally, small LAB plasmids use RCR. RCR implicates the formation of an ssDNA intermediate of the plasmid (29). Therefore, the ability of pTXL1 to generate ssDNA was examined as described previously (5). *Leuconostoc* cells were grown in MRS medium. Rifampin was added at 100 μg ml^{-1} in order to inhibit RNA polymerase (22). Whole-cell lysates were prepared as described previously (25, 26). S1 nuclease (Gibco-BRL) treatment was performed according to the method of Noirot-Gros et al. (26). ssDNA was detected by nonalkaline Southern blot hybridization. DNA transfer from agarose gel to a Hybond-N^+ nylon membrane (Amersham) was adapted from the work of Sambrook et al. (28). The DNA denaturation step was omitted. DNA probes consisted of purified DNA restriction fragments that were specific for each plasmid and labeled by use of a random priming kit (Gibco-BRL) and [α-^{32}P]dCTP (Amersham). Hybridization of the transferred DNA with the labeled probes was performed according to the method of te Riele et al. (29). Nonspecific hybridizations were avoided by washing the membrane at 62°C in 1× SSC (1× SSC is 0.15 M NaCl plus 0.015 M sodium citrate)–0.1% sodium dodecyl sulfate, after which the membrane was exposed to an autoradiography film.

As a control, we used strains of *L. mesenteroides* subsp. *dextranicum* DSM20484 containing pFBYC18E (Fig. 3a) or pGhost9:IS*S1* (Fig. 3b), two well-described RCR plasmids (5, 23). In addition to hybridizing to the open circular forms and covalently closed circular forms of the plasmids, the probes specific for pFBYC18E (Fig. 3a) and pGhost9:IS*S1* (Fig. 3b) hybridized to a faint fast-migrating band suspected of being an ssDNA intermediate. Their sensitivity to S1 nuclease demonstrated their single-stranded nature. Similarly, whole-cell DNA from *L. mesenteroides* subsp. *dextranicum* DSM20484 containing pFBYC50E was tested with pTXL1 as a probe (Fig. 3c). The hybridization failed to detect any single-stranded intermediate of pTXL1. This result strongly suggests that pTXL1 does not belong to the RCR plasmid family. This indirect proof, added to the absence of any ORF encoding a replicase as well as the lack of single-strand and double-strand origin sequences, is consistent with a mode of replication differing from the rolling-circle mechanism.

RCR plasmids are less stable when they contain foreign DNA (20), which limits their use as cloning vectors. To investigate the stability of pTXL1 as a cloning vector, single colonies of *Leuconostoc* transformants carrying various endogenous plasmids were used to inoculate MRS broth without erythromycin. The cultures were maintained in mid-log phase and serially transferred until approximately 100 generations were obtained. Plasmid stability was then estimated by comparing the numbers of CFU on selective (5 μg of erythromycin ml^{-1}) and nonselective (without antibiotic) agar plates. Results presented in Table 2 show that, in contrast to what occurs with pGhost9:IS*S1* or pFR18 derivatives, the segregational stability of pTXL1 derivatives was not affected by a large insertion of foreign DNA. In addition, Southern blot analysis of plasmid DNA prepared from erythromycin-resistant colonies cultivated for 100 generations without selection pressure revealed no structural change in the plasmid (data not shown).

Taking into account the overall properties of the pTXL1 replicon described above, it appeared to be a good alternative for use in developing *Leuconostoc* and other LAB cloning vectors. Indeed, the pTXL1 replicon was shown to be able to replicate in various *Leuconostoc* species (*cremoris* LC; *mesenteroides* subsp. *mesenteroides* Y110, Y105, and FR52; and *mesenteroides* subsp. *dextranicum* DSM20484), *Pediococcus acidilactici* P120, and *Lactobacillus sakei* 23K but not in *E. coli* (data not shown). Preparation of electrocompetent LAB cells and electroporation were as described previously (27). *P. acidilactici* P120 was grown in MRS (Difco) broth or agar (1.2%, wt/vol) at 30°C. MRS medium supplemented with 1% glucose was used for *Lactobacillus sakei* 23K propagation at 30°C. Interestingly, the transformation frequencies observed with the pTXL1 derivatives (pFBYC050E and pFBYC50E) were similar to those observed with the control RCR plasmid pGhost9:IS*S1*. In *Leuconostoc* strains, the transformation frequencies per microgram quantities of pTXL1 derivatives or pGhost9/IS*S1* were about 10^5 transformants in *L. mesenteroides* subsp. *dextranicum* DSM20484 and about 10^1 in *L. mesenteroides* subsp. *mesenteroides* Y110. In the *Lactobacillus sakei* 23K and

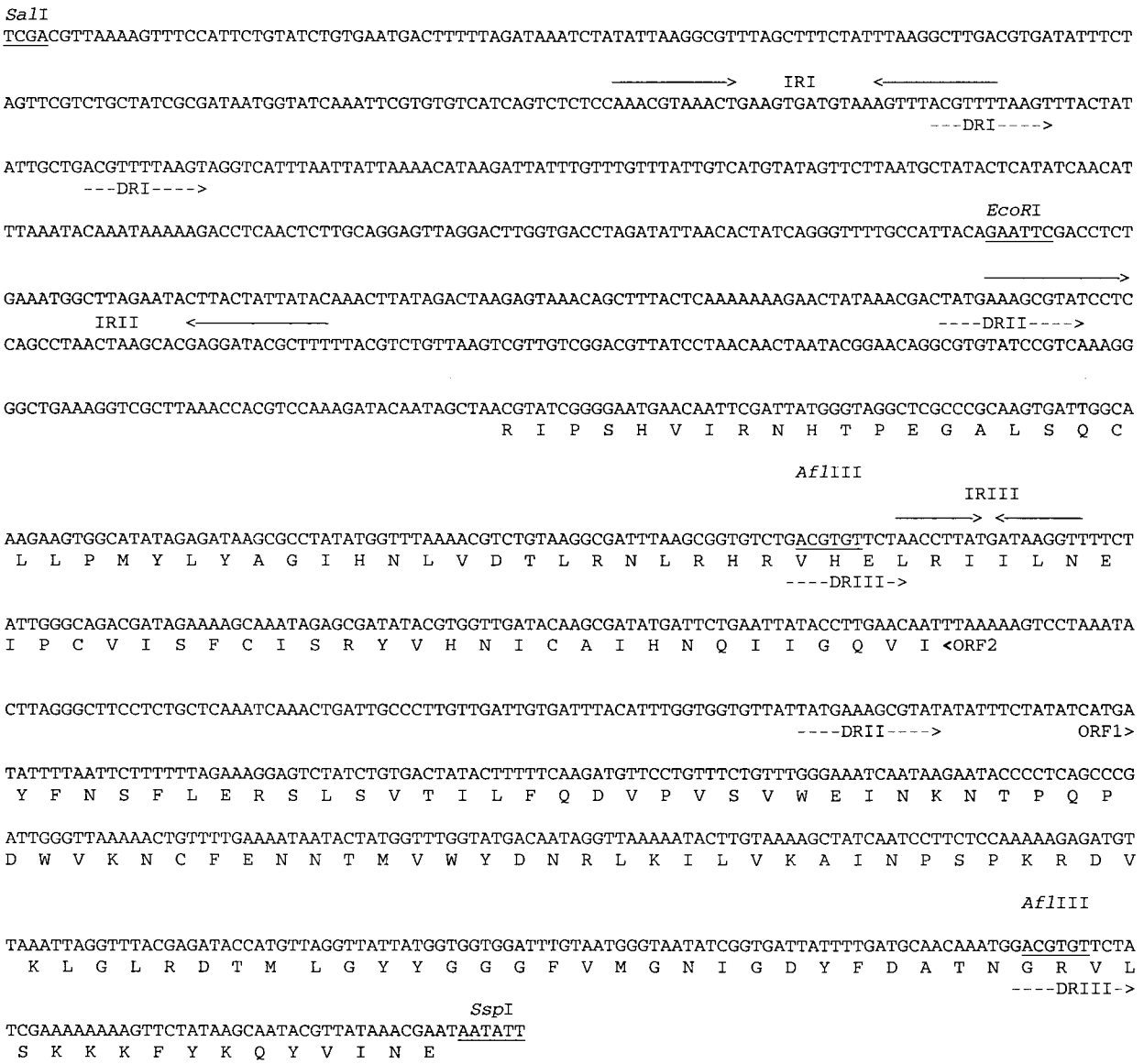

FIG. 2. Nucleotide sequences of the pTXL1 minimum replicon-linearized sequence and the deduced amino acid sequences for putative ORF1 and ORF2. The arrows with solid lines indicate inverted repeats (IRI, IRII, and IRIII), and the arrows with dashed lines indicate direct repeats (DRI, DRII, and DRIII).

P. acidilactici P120 strains, the transformation frequencies were 10^5 for both plasmids. In addition, this replicon was compatible with the pWV01 replicon that served as a basis for most genetic tools devoted to LAB. Consequently, pTXL1 was used to design a narrow-host-range food-grade vector.

We decided to transform an industrial *L. cremoris* strain aiming at the heterologous production of the anti-*Listeria* bacteriocin mesentericin Y105, originally produced by *L. mesenteroides* subsp. *mesenteroides* Y110.

A 669-bp *Sma*I-*Hin*dIII restriction fragment containing the constitutive lactococcal p59 promoter, a ribosome-binding site, and part of the divergicin A signal peptide gene (4) was cloned into pFBYC051 (Table 1) that had been restricted with *Sma*I and *Hin*dIII enzymes. This construct, named pFBYC069, was restricted with *Hin*dIII and ligated with a 518-bp *Hin*dIII restriction fragment containing the mesentericin Y105 structural gene (*mesY*) and its immunity gene (*mesI*). The resulting plasmid, pFBYC070, was introduced into the *L. mesenteroides* subsp. *dextranicum* DSM20484 strain devoid of anti-*Listeria* activity and sensitive to mesentericin Y105 and into the industrial *L. cremoris* LC strain devoid of anti-*Listeria* activity. Production of mesentericin Y105 by recombinant *L. mesenteroides* subsp. *dextranicum* DSM20484 and recombinant *L. cremoris* LC were examined by a rapid purification method (4) and characterized by mass spectroscopy analysis (data not shown). Bacteriocin activity was assayed against *Listeria ivanovii* BUG 497 as described previously (1). Figure 4 shows that recombinant *L. mesenteroides* subsp. *dextranicum* DSM20484 and *L.*

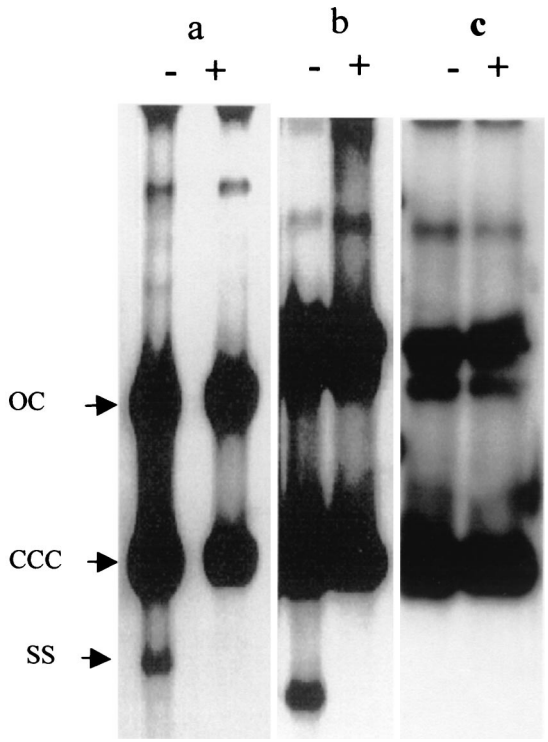

FIG. 3. Detection of single-stranded intermediates of pFBYC18E (a), pGhost9:IS*S1* (b), and the pTXL1 derivative (c) in *L. mesenteroides* subsp. *dextranicum* DSM20484. A nonalkaline Southern blot hybridization analysis was performed with radiolabeled pFBYC18E (a), pGhost9:IS*S1* (b), and pTXL1 (c) probes. Whole-cell DNA was prepared from MRS cultures containing rifampin. DNA preparations treated with endonuclease S1 are indicated by a +, and untreated preparations are indicated by a −. OC, open circular DNA; CCC, covalently closed circular DNA; SS, ssDNA.

cremoris LC producing mesentericin Y105 displayed bactericidal activity against *Listeria*, unlike nonrecombinant *Leuconostoc* strains.

These results proved that the *L. mesenteroides* subsp. *dextranicum* DSM20484 strain and the industrial strain *L. cremoris* LC were able to express the bacteriocin from the pTXL1 derivative vector at a level similar to that of the natural producer.

We described here a new *Leuconostoc* plasmid, the first small non-RCR-type *Leuconostoc* plasmid identified to date. Its food-grade origin combined with its small size, high stability, and narrow host range makes pTXL1 a food-grade vector of interest. Derivatives of pTXL1 should be useful for constructing *Leuconostoc* starter strains with improved fermentative, flavor-enhancing, and bacteriocin-producing abilities.

TABLE 2. Stability of pTXL1 derivatives during extended growth under nonselective conditions

Plasmid	Replicon	% of DSM20484 clones containing the plasmid after 100 generations[a]	Reference
pGhost9:IS*S1*	pWV01	76	23
pFBYC050E	pTXL1	100	This study
pFBYC50E	pTXL1	100	This study
pFBYC018E	pFR18	3	5
pFBYC18E	pFR18	100	5

[a] Percentages are averages of results from duplicate experiments and were determined as percentages of erythromycin-resistant colonies.

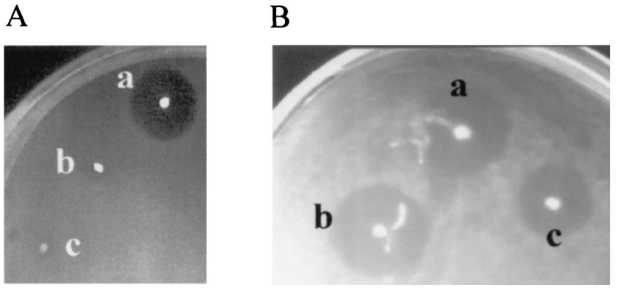

FIG. 4. Bacteriocin activity assays against *Listeria ivanovii* BUG 497 overlaying *Leuconostoc* strains. (A) *L. mesenteroides* subsp. *mesenteroides* Y105 (a); nonrecombinant *L. mesenteroides* subsp. *dextranicum* DSM20484 (b), and nonrecombinant *L. cremoris* LC (c); (B) *L. mesenteroides* subsp. *mesenteroides* Y105; (a) *Leuconostoc mesenteroides* subsp. *dextranicum* DSM20484 containing pFBYC070 (b), and *Leuconostoc cremoris* LC containing pFBYC070 (c).

Nucleotide sequence accession number. The EMBL accession number for the complete nucleotide sequence of pTXL1 linearized at its unique *Sal*I site reported in this paper is AJ272077.

This work was achieved as part of the program BIOAVENIR (contract 780227), supported by Rhône-Poulenc with the participation of the Ministère de la Recherche et de l'Espace and the Ministère de l'Industrie et du Commerce Extérieur.

We are grateful to Monique Zagorec for her contribution to the transfer of plasmids in *Lactobacillus sakei*. We thank Laurent Jannière for helpful discussion on single-stranded DNA detection.

REFERENCES

1. **Ahn, C., and M. E. Stiles.** 1990. Plasmid-associated bacteriocin production by a strain of *Carnobacterium piscicola* from meat. Appl. Environ. Microbiol. **56:**2503–2510.
2. **Bates, E. E., and H. J. Gilbert.** 1989. Characterization of a cryptic plasmid from *Lactobacillus plantarum*. Gene **85:**253–258.
3. **Berthier, F., M. Zagorec, M. C. Champomier-vergès, and S. D. Ehrlich.** 1996. Efficient transformation of *Lactobacillus sake* by electroporation. Microbiology **142:**1273–1279.
4. **Biet, F., J. M. Berjeaud, R. W. Worobo, Y. Cenatiempo, and C. Fremaux.** 1998. Heterologous expression of the bacteriocin mesentericin Y105 using the dedicated transport system and the general secretion pathway. Microbiology **144:**2845–2854.
5. **Biet, F., Y. Cenatiempo, and C. Fremaux.** 1999. Characterization of pFR18, a small cryptic plasmid from *Leuconostoc mesenteroides* subsp. mesenteroides FR52, and its use as a food grade vector. FEMS Microbiol. Lett. **179:**375–383.
6. **Biet, F., Y. Cenatiempo, and C. Fremaux.** May 1999. Non RCR *Leuconostoc* plasmid capable of being transferred into lactic acid bacteria; use as cloning and expressing tool. French patent WO9924591.
7. **Brito, L., G. Vieira, M. A. Santos, and H. Paveia.** 1996. Nucleotide sequence analysis of pOg32, a cryptic plasmid from *Leuconostoc oenos*. Plasmid **36:** 49–54.
8. **Chopin, A., M. C. Chopin, A. Moillo-Batt, and P. Langella.** 1984. Two plasmid-determined restriction and modification systems in Streptococcus lactis. Plasmid **11:**260–263.
9. **Clewell, D. B., Y. Yagi, G. M. Dunny, and S. K. Schultz.** 1974. Characterization of three plasmid deoxyribonucleic acid molecules in a strain of *Streptococcus faecalis*: identification of a plasmid determining erythromycin resistance. J. Bacteriol. **117:**283–289.
10. **Coffey, A., A. Harrington, K. Kearney, C. Daly, and G. Fitzgerald.** 1994. Nucleotide sequence and structural organization of the small, broad-host-range plasmid pCI411 from *Leuconostoc lactis* 533. Microbiology **140:**2263–2269.
11. **Fremaux, C., M. Aigle, and A. Lonvaud-Funel.** 1993. Sequence analysis of *Leuconostoc oenos* DNA: organization of pLo13, a cryptic plasmid. Plasmid **30:**212–223.
12. **Garvie, E. I., V. Zezula, and V. A. Hill.** 1974. Guanine plus cytosine content

of the deoxyribonucleic acids of the leuconostocs and some heterofermentative lactobacilli. Int. J. Syst. Bacteriol. **24:**248–251.
13. **Gasson, M. J.** 1983. Plasmid complements of *Streptococcus lactis* NCDO 712 and other lactic streptococci after protoplast-induced curing. J. Bacteriol. **154:**1–9.
14. **Gruss, A., R. Dabert, S. Sozhamannan, and S. D. Ehrlich.** 1990. Replication of ssDNA plasmids is sensitive to DNA content. *In* R. P. Novick (ed.), Molecular biology of the staphylococci. VCH Publishers, New York, N.Y.
15. **Gruss, A., and S. D. Ehrlich.** 1989. The family of highly interrelated single-stranded deoxyribonucleic acid plasmids. Microbiol. Rev. **53:**231–241.
16. **Hanahan, D.** 1983. Studies on transformation of *Escherichia coli* with plasmid DNA. J. Mol. Biol. **166:**557–580.
17. **Hayes, F., P. Vos, G. F. Fitzgerald, W. M. de Vos, and C. Daly.** 1991. Molecular organization of the minimal replicon of novel, narrow-host-range, lactococcal plasmid pCI305. Plasmid **25:**16–26.
18. **Hechard, Y., B. Derijard, F. Letellier, and Y. Cenatiempo.** 1992. Characterization and purification of mesentericin Y105, an anti-Listeria bacteriocin from *Leuconostoc mesenteroides*. J. Gen. Microbiol. **138:**2725–2731.
19. **Jannière, L., A. Gruss, and S. D. Ehrlich.** 1993. *Bacillus subtilis* and other gram-positive bacteria: biochemistry, physiology, and molecular genetics, p. 625–644. *In* A. L. Sonenshein, J. A. Hoch, and R. Losick (ed.), Plasmids. American Society for Microbiology, Washington, D.C.
20. **Kiewiet, R., S. Bron, K. de Jonge, G. Venema, and J. F. Seegers.** 1993. Theta replication of the lactococcal plasmid pWVO2. Mol. Microbiol. **10:**319–327.
21. **Kiewiet, R., J. Kok, J. F. Seegers, G. Venema, and S. Bron.** 1993. The mode of replication is a major factor in segregational plasmid instability in *Lactococcus lactis*. Appl. Environ. Microbiol. **48:**726–731.
22. **Leenhouts, K. J., B. Tolner, S. Bron, J. Kok, G. Venema, and J. F. Seegers.** 1991. Nucleotide sequence and characterization of the broad-host-range lactococcal plasmid pWVO1. Plasmid **26:**55–66.
23. **Maguin, E., H. Prevost, S. D. Ehrlich, and A. Gruss.** 1996. Efficient insertional mutagenesis in lactococci and other gram-positive bacteria. J. Bacteriol. **178:**931–935.
24. **Mathieu, F., I. S. Suwandhi, N. Rekhif, J. B. Milliere, and G. Lefebvre.** 1993. Mesenterocin 52, a bacteriocin produced by Leuconostoc mesenteroides ssp. mesenteroides FR 52. J. Appl. Bacteriol. **74:**372–379.
25. **Muriana, P. M., and T. R. Klaenhammer.** 1987. Conjugal transfer of plasmid-encoded determinants for bacteriocin production and immunity in *Lactobacillus acidophilus* 88. Appl. Environ. Microbiol. **53:**553–560.
26. **Noirot-Gros, M. F., V. Bidnenko, and S. D. Ehrlich.** 1994. Active site of the replication protein of the rolling circle plasmid pC194. EMBO J. **13:**4412–4420.
27. **Raya, R. R., C. Fremaux, G. L. De Antoni, and T. R. Klaenhammer.** 1992. Site-specific integration of the temperate bacteriophage ϕadh into the *Lactobacillus gasseri* chromosome and molecular characterization of the phage (*attP*) and bacterial (*attB*) attachment sites. J. Bacteriol. **174:**5584–5592.
28. **Sambrook, J., E. F. Fritsch, and T. Maniatis.** 1989. Molecular cloning: a laboratory manual, 2nd ed. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
29. **te Riele, H., B. Michel, and S. D. Ehrlich.** 1986. Single-stranded plasmid DNA in *Bacillus subtilis* and *Staphylococcus aureus*. Proc. Natl. Acad. Sci. USA **83:**2541–2545.
30. **Zuniga, M., I. Pardo, and S. Ferrer.** 1996. Nucleotide sequence of plasmid p4028, a cryptic plasmid from *Leuconostoc oenos*. Plasmid **36:**67–74.