Physical and Genetic Map of the *Lactococcus lactis* subsp. *cremoris* MG1363 Chromosome: Comparison with That of *Lactococcus lactis* subsp. *lactis* IL 1403 Reveals a Large Genome Inversion†

PASCAL LE BOURGEOIS,¹ MARTINE LAUTIER,¹ LOIC VAN DEN BERGHE,¹
MICHAEL J. GASSON,² AND PAUL RITZENTHALER^{1*}

Laboratoire de Microbiologie et Génétique Moléculaire, Institut de Biologie Cellulaire et de Génétique du Centre National de la Recherche Scientifique, 31062 Toulouse, France,¹ and Agriculture and Food Research Council Institute of Food Research, Norwich Research Park, Colney, Norwich NR4 7UA, United Kingdom²

Received 28 November 1994/Accepted 9 March 1995

A physical and genetic map of the chromosome of the *Lactococcus lactis* subsp. *cremoris* reference strain MG1363 was established. The physical map was constructed for *NotI*, *ApaI*, and *SmaI* enzymes by using a strategy that combines creation of new rare restriction sites by the random-integration vector pRL1 and ordering of restriction fragments by indirect end-labeling experiments. The MG1363 chromosome appeared to be circular and 2,560 kb long. Seventy-seven chromosomal markers were located on the physical map by hybridization experiments. Integration via homologous recombination of pRC1-derived plasmids allowed a more precise location of some lactococcal genes and determination of their orientation on the chromosome. The MG1363 chromosome contains six rRNA operons; five are clustered within 15% of the chromosome and transcribed in the same direction. Comparison of the *L. lactis* subsp. *cremoris* MG1363 physical map with those of the two *L. lactis* subsp. *lactis* strains IL1403 and DL11 revealed a high degree of restriction polymorphism. At the genetic organization level, despite an overall conservation of gene organization, strain MG1363 presents a large inversion of half of the genome in the region containing the rRNA operons.

Lactococcus lactis is a gram-positive mesophilic bacterium that is used extensively in starter cultures in the manufacture of dairy products. There has been much research over the last decade to investigate its genetics. Because of the industrial importance of plasmids and bacteriophages, initial genetic studies tended to focus on these extrachromosomal elements. More recently, there has been an increased interest in chromosomal genes, and more than 80 markers have now been characterized. In contrast, little information is available about the structure and the organization of the chromosome of *Lactococcus* strains, mainly because, as for the majority of bacteria, study of the genome of lactococci cannot be done by the classical genetic mapping systems such as interrupted conjugation, transformation, and transduction. The physical and genetic map of the chromosome of various lactococci would be valuable for studying the organization and phylogeny of the lactococcal genome and to indicate the relatedness between the different species and subspecies of the lactococci. Pulsed-field gel electrophoresis (PFGE) is a powerful tool for genome characterization and has led to the construction of the physical map of more than 80 bacterial chromosomes.

PFGE was used in several studies of the genome of *L. lactis* to estimate the genome size and for genome fingerprinting (54, 58, 84), to analyze plasmid stability (92), and to study the integration sites of transposon Tn917 derivatives (41). However, only two complete restriction maps of *L. lactis* have been published. The first map constructed was a *NotI* and *SmaI* physical map of the *L. lactis* subsp. *lactis* DL11 strain (86), a proteinase-negative (Prt⁻) derivative of the nisin-producing

strain ATCC 11454, and the second was a combined physical and genetic map of the commonly used laboratory strain *L. lactis* subsp. *lactis* IL1403 (49), a plasmid-free derivative of IL594 (12).

A prime candidate for chromosome mapping is the lactococcal reference strain *L. lactis* subsp. *cremoris* MG1363 (29). MG1363 is a plasmid-free derivative of strain NCDO 712, which is the ancestor (16) of the well-studied group of lactococcal strains NCDO 763 (ML3), NCDO 505, NCDO 2031, C2, and all strains derived therefrom, including the plasmid-free strain LM2301 (65). These strains constitute the most extensively studied group of lactococcal strains in terms of genetics and molecular biology.

According to phenotypic characteristics, the NCDO 712 group was formerly classified as *L. lactis* subsp. *lactis*, but DNA-DNA reassociation studies indicated that this group was not representative of the majority of the subspecies *lactis* (42). Recent data from 16S rRNA sequencing (43, 80) and Southern analysis with probes specific for the subspecies *lactis* (34) has demonstrated that the NCDO 712 group belongs to the subspecies *cremoris*.

We present the construction of the *ApaI*, *SmaI*, *NotI*, and *I-CeuI* physical map of the *L. lactis* subsp. *cremoris* MG1363 chromosome and the localization of various chromosomal genes. Comparison of this map with the genome map of the two *L. lactis* subsp. *lactis* strains DL11 and IL1403 gives information about the chromosomal genetic organization at the subspecies level.

(A preliminary account of part of this work was presented previously [52].)

MATERIALS AND METHODS

Bacterial strains and plasmids. *L. lactis* subsp. *cremoris* MG1363 (29) and *L. lactis* subsp. *lactis* IL1403 (12) were grown at 30°C in GM17 (M17 broth [85] supplemented with 0.4% glucose). *Escherichia coli* TG1 [*supE* *hsd*Δ5 *thi* Δ(*lac*-

* Corresponding author. Mailing address: Laboratoire de Microbiologie et Génétique Moléculaire, IBCG, 118 route de Narbonne, 31062 Toulouse, France. Phone: (33) 61 33 58 25. Fax: (33) 61 33 58 86. Electronic mail address: ritzenth@ibgc.biotoul.fr.

† This article is dedicated to Isabelle Kremper.

FIG. 1. PFGE restriction patterns of MG1363 and IL1403 chromosomes. (A) Typical combination of *ApaI*, *SmaI*, and *NotI* digestions. Lanes 1 to 5, MG1363 chromosome; 1, *ApaI/NotI*; 2, *ApaI*; 3, *ApaI/SmaI*; 4, *SmaI*; 5, *SmaI/NotI*. Lanes 6 to 10, IL1403 chromosome; 1, *ApaI/NotI*; 2, *ApaI*; 3, *ApaI/SmaI*; 4, *SmaI*; 5, *SmaI/NotI*. Lanes λ , λ DNA concatemers; lane L, 1-kb DNA ladder. Running conditions were 10 V/cm, 7.5 s of pulse time, for 13 h. (B) Digestion with *I-CeuI* and *NotI*. Lanes: 1, MG1363 chromosome digested with *I-CeuI*; 2, MG1363 digested with *I-CeuI* and *NotI*; 3, IL1403 chromosome digested with *I-CeuI*; 4, IL1403 digested with *I-CeuI* and *NotI*; lane λ , λ DNA concatemers. Running conditions were 7.3 V/cm, 45 s of pulse time, for 24 h. The sizes of the *I-CeuI* fragments of strain IL1403 are: Ce1, 1,480 kb; Ce2, 560 kb; Ce3, 240 kb; Ce4, 80 kb; Ce5, 45 kb; and Ce6, 18 kb.

(i) **Random insertion of *ApaI*, *SmaI*, and *NotI* restriction sites.** The integrative plasmid pRL1 integrates randomly into the chromosome of *Lactococcus* strains, creating additional *ApaI*, *SmaI*, and *NotI* restriction sites at the insertion point (50). Briefly, this integration uses the replicative transposition activity of the functional insertion sequence ISS/RS (39) and allows rapid unambiguous location of *ApaI*, *SmaI*, and *NotI* restriction sites close to the integration site by Southern analysis. The ISS/RS insertion sequence is also able to transpose efficiently in *Enterococcus faecalis* and *Streptococcus thermophilus* (59).

Fifty-one sites of integration of pRL1 were mapped precisely on the MG1363 chromosome (Table 2 and Fig. 2). Although some integration sites were clustered (the groups 8, 11, 17, and 53 and 2, 26, 37, 43, and 45 are each located within less than 50 kb), pRL1 integration sites seems to be distributed equally along the chromosome. Random insertion site distribution has already been observed in *L. lactis* subsp. *lactis* IL1403 (49). Analysis of only 35 integration sites (minimal set) allowed the unambiguous location of 51% of the *ApaI* and 54% of the *SmaI* restriction fragments.

(ii) **Completion of the physical map by indirect end labeling.** The remaining unmapped restriction sites were assigned on the MG1363 chromosome by the indirect end-labeling strategy (7), which allows the order of restriction fragments to be established relative to a fixed point. Indirect end-labeling experiments with 16 pRL1 derivative strains of MG1363 (Fig. 2 and Table 2) yielded a physical map of the MG1363 chromosome for these restriction sites except for the location of four *ApaI* restriction fragments (one of the three Ap24 fragments, one of the two Ap27 fragments, and the Ap30 and Ap31 fragments) and two *SmaI* fragments (Sm18 and Sm19).

Construction of the genetic map. The physical map was used as a guide for localizing genetic markers (Table 3) on the *L. lactis* subsp. *cremoris* MG1363 chromosome. Hybridization results with several of these probes helped improve the resolution of the restriction map. The genetic map of the *L. lactis* subsp. *cremoris* MG1363 chromosome was constructed by Southern hybridization analysis with homologous and heterol-

ogous probes. For some lactococcal genetic markers, accurate location and gene orientation on the chromosome could be obtained by homologous recombination using the integrative vector pRC1 (50).

(i) **Ribosomal operons (*rrn*).** The chromosome of lactococci contains six ribosomal operons (44, 49, 86). The complete sequence of one ribosomal operon of the *L. lactis* subsp. *lactis* IL1403 strain is available (10). The organization of this operon is similar to that described in the general model for eubacteria (i.e., 16S rRNA-23S rRNA-5S rRNA). Sequence analysis of this *rrn* operon also revealed the presence of unique recognition sites for three restriction endonucleases used for bacterial genome mapping: the *ApaI* and *SmaI* sites in the 16S rRNA gene (*rrs*), and the *I-CeuI* site in the 23S rRNA gene (*rrl*) (56).

The six ribosomal operons were localized on the physical map of MG1363 strain by hybridization with probes specific for the 5' end of the 16S rRNA gene and for the 3' end of the 23S rRNA gene, derived from *Clostridium perfringens* (27). Each probe revealed six PFGE-separated fragments. Both the *ApaI* and *SmaI* hybridization patterns were different with the 16S and 23S probes, suggesting that each *rrn* operon contains one *ApaI* and one *SmaI* site. Ribosomal operon mapping allowed the location of the small restriction fragments Sm18 and Sm19 on the physical map. Sm18 hybridized only with the *rrl* probe, and Sm19 hybridized only with the *rrs* probe. Presumably, Sm18 maps between Sm11 and Sm2, and Sm19 maps between Sm13 and Sm10.

The position of each of the six rRNA operons on the chromosome, based on the *ApaI* and *SmaI* physical map, was also confirmed by using the intron-encoded endonuclease *I-CeuI* (56). This enzyme, which recognizes a 26-bp sequence, cleaves specifically in the 23S rRNA gene of a wide range of organisms. The chromosomes of strains MG1363 and IL1403 were cleaved six times by *I-CeuI* (Table 1 and Fig. 1), and the distance between each *rrn* operon, corresponding to each PFGE-separated fragment, was in agreement with their locations on the *ApaI* and *SmaI* physical map.

(ii) **Mobile genetic elements.** Four different insertion sequences in *L. lactis* have been described: ISS1 (73), IS904 (21),

TABLE 2. Analysis of pRL1 integration sites in the strain MG1363 chromosome^a

Strain	<i>Apa</i> I fragments		<i>Sma</i> I fragments		<i>Not</i> I fragments	
	Absent	Present (kb) ^a	Absent	Present (kb)	Absent	Present (kb)
CL56-1 ^b	Ap1A	200 + 6	Sm8	65 + 43	No1	(1,905) ^c + 310
CL56-2 ^b	Ap4	55 + 95	Sm1	425 + 190	No2	65 + 170
CL56-3	Ap20	17 + 20	Sm3	195 + 95	No1	250 + (1,965)
CL56-4 ^b	Ap13	11 + 55	Sm7	50 + 80	No1	(1,635) + 580
CL56-6	Ap16	4 + 46	Sm2	40 + 290	No1	(1,585) + 630
CL56-7 ^b	Ap5	70 + 50	Sm2	130 + 200	No1	(1,730) + 485
CL56-8	Ap3	90 + 80	Sm5A	75 + 110	No1	(1,415) + 800
CL56-10 ^b	Ap6	85 + 20	Sm3	115 + 175	No1	(1,980) + 235
CL56-11	Ap3	155 + 10	Sm5A	25 + 160	No1	(1,365) + 850
CL56-12	Ap10A	45 + 35	Sm5B	20 + 165	No1	760 + (1,455)
CL56-13	Ap10B	55 + 25	Sm1	165 + 450	No1	(2,115) + 100
CL56-14	Ap1A	100 + 105	Sm1	40 + 580	No1	(1,995) + 220
CL56-15	Ap1A	50 + 160	Sm1	90 + 530	No1	(2,050) + 165
CL56-17 ^b	Ap3	140 + 25	Sm5A	40 + 145	No1	(1,385) + 830
CL56-18	Ap1A	175 + 35	Sm1	490 + 115	No1	135 + (2,080)
CL56-20 ^b	Ap14A	26 + 31	Sm10	28 + 27	No1	910 + (1,285)
CL56-21 ^b	Ap6	8 + 95	Sm3	180 + 115	No1	235 + (1,980)
CL56-22	Ap14A	21 + 35	Sm10	34 + 23	No1	(1,295) + 920
CL56-23	Ap6	29 + 75	Sm3	160 + 135	No1	215 + (2,000)
CL56-24 ^b	Ap2	23 + 162	Sm5B	37 + 145	No1	(1,315) + 900
CL56-25	Ap10B	55 + 25	Sm6	55 + 80	No1	(1,760) + 455
CL56-26	Ap4	75 + 75	Sm1	410 + 205	No2	45 + 185
CL56-27	Ap7	40 + 50	Sm1	300 + 320	No3	47 + 75
CL56-28	Ap1B	91 + 125	Sm1	23 + 590	No2	10 + 225
CL56-29 ^b	Ap14B	48 + 7	Sm8	75 + 35	No1	350 + (1,865)
CL56-30 ^b	Ap2	115 + 55	Sm5B	178 + 7	No1	930 + (1,285)
CL56-34	Ap10C	55 + 25	Sm1	415 + 200	No1	60 + (2,155)
CL56-35	Ap17	10 + 35	Sm1	390 + 225	No1	30 + (2,185)
CL56-36	Ap8	74 + 11	Sm9	35 + 30	No1	720 + (1,495)
CL56-37	Ap4	100 + 50	Sm1	375 + 220	No2	25 + 210
CL56-38 ^b	Ap10A	6 + 75	Sm9	28 + 35	No1	(1,490) + 725
CL56-39	Ap1A	170 + 50	Sm1	515 + 100	No1	150 + (2,065)
CL56-40	Ap12B	54 + 18	Sm2	55 + 275	No1	(1,615) + 600
CL56-41	Ap1B	80 + 130	Sm16	8 + 13	No1	22 + (2,193)
CL56-42 ^b	Ap1B	20 + 190	Sm3	35 + 265	No1	85 + (2,130)
CL56-43	Ap4	85 + 65	Sm1	400 + 220	No2	35 + 200
CL56-45	Ap4	55 + 100	Sm1	230 + 360	No2	210 + 25
CL56-46	Ap12A	60 + 20	Sm2	30 + 300	No1	360 + (1,855)
CL56-47	Ap12B	55 + 15	Sm2	60 + 260	No1	(1,605) + 610
CL56-48	Ap10C	73 + 7	Sm1	400 + 220	No1	50 + (2,165)
CL56-49 ^b	Ap10B	65 + 20	Sm6	55 + 80	No1	(1,765) + 450
CL56-50	Ap7	30 + 60	Sm1	300 + 305	No3	55 + 65
CL56-51	Ap2	125 + 55	Sm4	160 + 60	No1	(1,235) + 980
CL56-52	Ap7	77 + 11	Sm1	285 + 335	No3	35 + 80
CL56-53	Ap3	35 + 130	Sm5A	130 + 55	No1	850 + (1,365)
CL56-54 ^b	Ap1B	8 + 200	Sm1	110 + 495	No2	90 + 145
CL56-55	Ap7	15 + 75	Sm1	325 + 290	No3	70 + 50
CL56-56	Ap19	18 + 22	Sm14	20 + 6	No1	(1,217) + 1,000
CL56-57	Ap21	17 + 12	Sm4	115 + 110	No1	1,060 + (1,155)
CL56-58	Ap9	49 + 38	Sm5B	85 + 105	No1	835 + (1,380)
CL56-59 ^b	Ap12A	61 + 22	Sm2	35 + 295	No1	370 + (1,845)

^a The second fragment of each pair gave a strong signal when it was hybridized with pRL1.^b Strain used for indirect end-labeling experiments.^c Parentheses indicate that the fragment size was deduced from the size of *Not*I fragment No1 (2,215 kb).

IS905 (22), and IS981 (72). We attempted to map two of these insertion elements on the chromosome of strain MG1363. The first was the ISS1RS from the lactose protease plasmid pUCL22 of *L. lactis* subsp. *lactis* Z270 (39), which is very similar to ISS1. Previous results indicate that there are two chromosomal copies of ISS1 in strain MG1363 (20). However, hybridization experiments revealed only one *Apa*I (Ap10) and one *Sma*I (Sm6) fragment. It was possible that the two copies were localized on the same *Apa*I and *Sma*I fragment. Therefore, Southern analysis by conventional agarose electrophore-

sis with *Eco*RI, *Hind*III, *Pst*I, or *Hae*III endonuclease was performed, and only one fragment hybridized with the probe. These results strongly suggested that the MG1363 chromosome contains only one copy of ISS1. The second IS element mapped on the MG1363 chromosome was IS1076 from plasmid pUCL22 (40), which is closely related to the IS904 element. Eight PFGE-separated bands scored positive after *Apa*I or *Sma*I digestion and hybridization experiments with IS1076 as the probe, indicating that at least eight copies were present on the chromosome.

TABLE 3. Genetic markers localized on the IL1403 and MG1363 maps

Element ^a	Description ^b	Source (species or <i>L. lactis</i> strain ^c)	Reference
Recurrent sequences, mobile elements, and bacteriophages			
5' <i>rrnA</i>	16S rDNA (<i>rrs</i> gene)	<i>Clostridium perfringens</i>	27
3' <i>rrnI</i>	23S rDNA (<i>rrl</i> gene)	<i>Clostridium perfringens</i>	27
ISS1RS (iso-ISS1)	Insertion sequence	<i>L. lactis</i> subsp. <i>lactis</i> Z270	39
IS1076 (iso-IS904)	Insertion sequence	<i>L. lactis</i> subsp. <i>lactis</i> Z270	40
Tn5276 target site 1	Nisin-sucrose transposon	<i>L. lactis</i> NIZO R5	75
φT712	Temperate bacteriophage	<i>L. lactis</i> subsp. <i>cremoris</i> NCDO 712	16
Lactococcal genes			
<i>adk</i> *	Adenylate kinase	MG1363	45
<i>aldB</i> *	Acetolactate decarboxylase	MG1363 and IL1403	11
<i>als</i>	α-Acetolactate synthase	DSM 20384	62
<i>amdA</i>	Amidase	MG1363	63
<i>bglR</i>	Positive regulator	IL1403	4
<i>cluA</i>	Sex factor aggregation	MG1363	35
<i>dnaE</i> *	DNA primase	ML3	1
<i>dnaJ</i>	Heat shock response	NIZO R5	89
<i>dnaK</i>	Heat shock response	MG1363	25
<i>fpg</i> *	Formamidopyridine-DNA-glycosylase	ML3	24
<i>gap</i>	Glyceraldehyde-3-phosphate dehydrogenase	NIZO R5	19
<i>gerC</i> *	Germination of <i>B. subtilis</i>	C2	31
<i>grpE</i>	Heat shock response	MG1363	25
<i>hisCGDBHAFIE</i>	Histidine biosynthesis	NCDO 2118	17
<i>hisRF</i> *	Histidyl-tRNA synthetase	ML3	76
<i>ilvDBNCA</i>	BCAA biosynthesis	NCDO 2118	33
<i>infA</i> *	Initiation factor 1	MG1363	45
<i>lcnCD</i>	Lactococcin biosynthesis	IL1403	46
<i>ldh</i>	Lactate dehydrogenase	NIZO R5	19
<i>leuABCD</i>	BCAA biosynthesis	NCDO 2118	33
<i>mleR</i>	MLF activator	IL1403	77
<i>mleS</i>	MLF enzyme (Mlep)	IL1403	18
<i>oppDFBCA</i>	Oligopeptide transport system	SSL135	87
<i>pepC</i>	Thiol aminopeptidase	AM2	9
<i>pepN</i>	Lysine aminopeptidase	Wg2	83
<i>pepO</i>	Endopeptidase	SSL135	87
<i>pepT</i>	Tripeptidase	MG1363	67
<i>pepX</i>	X-prolyl dipeptidyl aminopeptidase	ML3	69
<i>pip</i>	Phage infection protein	C2	31
<i>recA</i>	Homologous recombination	ML3	24
<i>rplO</i> *	Ribosomal protein L15	MG1363	45
<i>rpmJ</i> *	Ribosomal protein B	MG1363	45
<i>rpoD</i>	σ ⁴³ factor of RNA polymerase	ML3	1, 26
<i>rpsM</i> *	Ribosomal protein S13	MG1363	45
<i>rrg</i> *	Glucosyltransferase regulator	C2	31
<i>secY</i>	Protein translocation	MG1363	45
<i>sod</i>	Superoxide dismutase	MG1363	81
<i>thyA</i>	Thymidylate synthase	NCDO 712	79
<i>trpEGDCFBA</i>	Tryptophan biosynthesis	IL1403	3
<i>trxB</i>	Thioredoxin reductase	NCDO 2118	76
<i>usp45</i>	Secreted protein	MG1363	88
<i>uvrC</i>	Excision nuclease	IL1403	32
Heterologous genes			
<i>gyrA</i>	DNA gyrase (subunit A)	<i>Clostridium perfringens</i>	7
" <i>rec</i> -like"	DNA recombination	<i>Streptococcus thermophilus</i>	66
<i>rpoA</i>	α subunit of RNA polymerase	<i>Escherichia coli</i>	7
<i>rpsL</i>	Ribosomal protein S12	<i>Escherichia coli</i>	7
<i>uncD</i>	β subunit of ATP synthase F1	<i>Escherichia coli</i>	91

^a The names of the ORFs marked with an asterisk are based on computerized protein sequence similarity searches. The *hisCGDBHAFIE*, *leuABCD-ilvDBNCA-aldB*, and *trpEGDCFBA-bglR* operons were localized by using the *hisF*, *ilvD*, and *trpC* genes as probes, respectively. The genes *dnaE* and *rpoD* are clustered (*rpoD* operon) (1) and were localized by using *rpoD* as the probe. The genes *rplO*, *secY*, *adk*, *infA*, *rpmJ*, and *rpsM* are clustered (45) and were localized by using *secY* as the probe. The genes *fpg* and *recA* are clustered (24) and were localized by using *recA* as the probe. The genes *pip*, *gerC*, and *rrg* are clustered (31) and were localized by using *pip* as the probe. The genes *grpE* and *dnaK* are clustered (25) and were localized by using *dnaK* as the probe. The genes *oppDFBCA* and *pepO* are clustered (87) and were localized by using *pepO* as the probe.

^b BCAA, branched-chain amino acids; MLF, malolactic fermentation.

^c Strains MG1363, ML3, C2, AM2, Wg2, and NCDO 712 are *L. lactis* subsp. *cremoris*; NIZO R5 is *L. lactis*; all other strains are *L. lactis* subsp. *lactis*.

FIG. 2. Chromosomal locations of pRL1 integration sites (IS). IS-*x* corresponds to CL56-*x* in Table 2, where *x* is a number from 1 to 59. In order to facilitate locating the integration sites, the positions of the three *NotI* sites and of the positions of the six ribosomal operons are indicated.

Many industrial strains of *L. lactis* produce antimicrobial peptides, and the genes responsible for the production of and resistance to these compounds have been found on conjugative transposons (21, 75). Rauch et al. demonstrated that the nisin-sucrose transposon Tn5276 was able to integrate at various locations in the MG1363 chromosome but showed a preference for insertion into a single target site, designated site 1. Preliminary PFGE analysis showed that Tn5276 insertion site 1 was located on 200-kb *SmaI* and on 12-kb *EagI* fragments (75). By using a 4.3-kb *HindIII* chromosomal DNA fragment of strain MG1614 containing site 1 as the probe, it was possible to assign integration site 1 to fragments Ap2 and Sm5B.

(iii) Integration sites of temperate bacteriophages. The location of prophage DNA on the bacterial chromosome can be of interest for the study of interactions at the DNA level between bacteriophages and the host cell. Various studies show that temperate bacteriophages are responsible for the PFGE restriction fragment length polymorphism of the *Staphylococcus aureus* (82), *Clostridium perfringens* (8), and *Salmonella typhimurium* (95) chromosomes.

The attachment site (*attB*) of the lactococcal temperate phage TP901.1 was precisely located on the MG1363 and IL1403 chromosomes. The integrative plasmid pBC143 (13) contains the TP901.1 *attP* site, the integrase gene, and single *ApaI* and *SmaI* restriction sites. When introduced into lactococcal cells, it integrates into the chromosome via specific recombination. The parental restriction fragment (*ApaI* or *SmaI*) disappears and is replaced by two new fragments, which can be revealed by hybridization with the pBC143 probe.

The putative attachment site of the temperate bacteriophage

φT712 was located by using a DNA fragment of the phage as the probe (data not shown).

(iv) Lactococcal and heterologous genetic markers. Thirty-three cloned lactococcal or heterologous genetic markers were located on the MG1363 physical map by Southern hybridization under high-stringency (homologous genes) or low-stringency (heterologous genes) conditions with PFGE-separated restriction fragments after combinations of single and double digestions with *ApaI*, *SmaI*, and *NotI* (Fig. 1A). Some lactococcal genes were more precisely located on the chromosome by another procedure. The positions of the lactococcal *ilvD*, *thyA*, *rpoD*, *ldh*, *pepX*, and *pepC* genes were determined by using the integrative vector pRC1. When a lactococcal gene is introduced into plasmid pRC1, the resulting plasmid can integrate into the chromosome via homologous recombination, and this insertion allows the accurate mapping of the gene (50). In addition, if the transcription direction of the gene is known, the gene orientation (5' → 3') on the chromosome can be deduced from hybridization data. The *recA* gene was localized by using an MG1363 *recA* strain (23) constructed by insertional mutagenesis with a thermosensitive integrative plasmid (60). As this mutagenic event creates new *ApaI*, *SmaI*, and *NotI* sites at the insertion point, the strategy described above was used to locate the *recA* gene and to deduce its orientation of transcription on the MG1363 chromosome. The *cluA* gene contains an *ApaI* site (32). This gene was thus precisely localized on the MG1363 physical map by classical Southern hybridization, using probes specific for the 5' end and for the 3' end of the *cluA* gene, and confirmed the location of the Ap26 fragment between the Ap1B and Ap4 fragments.

As many mapped genes were either organized in operons (*ilv*, *his*, and *trp* operons) or clustered with other characterized open reading frames (ORFs) (*secY*, *rpoD*, and *pip* genes), the 33 probes used in this study allowed a total of 77 chromosomal genetic markers to be mapped.

A combined physical and genetic map of the *L. lactis* subsp. *lactis* MG1363 chromosome is shown in Fig. 3 and is compared with that of *L. lactis* subsp. *lactis* IL1403 in Fig. 4. Overall, the gene organization is conserved. However, strain MG1363 presents a large inversion of one half of the genome in the region containing the rRNA operons.

One interesting feature of the *Lactococcus* chromosome is the location of genes encoding the subunits of RNA polymerase. When the *rpoA* gene of *E. coli* was used as the probe, two *ApaI* and *SmaI* fragments were revealed with the same intensity, suggesting the presence of two copies of the *rpoA* gene in MG1363. IL1403 also contains two copies of the *rpoA* gene (49), although one copy hybridized weakly with the probe. One copy (arbitrarily named *rpoA*-1) mapped between the *rrnA* and *rrnF* operons, and the second mapped near the cluster of genes *secY*-*adk*-*infA*-*rpmJ*-*rpsM*, as found on the chromosomes of *E. coli* (2) and *Bacillus subtilis* (71). The precise location of the *rpoD* gene was determined by homologous recombination and this gave further information on the gene organization near this ORF. It was previously observed that the lactococcal *rpoD* gene was associated with the *dnaG* gene in an operon (1). In *E. coli* and *B. subtilis*, the *rpoD* gene is located in an operon called the macromolecular synthesis (MMS) operon (90). This operon contains the gene coding for DNA primase (*dnaG* in *E. coli* and *dnaE* in *B. subtilis*) and either a gene coding for S21 ribosomal protein (*rpsU*) in *E. coli* or a gene of unknown function (P23) in *B. subtilis*. In contrast to the position of the "rpoD operon" in *E. coli* and *B. subtilis*, which is very distant from any *rrn* operon, the *rpoD* operon is very closely associated with the *rrnF* operon (less than 3 kb) in the MG1363 chromosome.

FIG. 4. Comparison of the chromosome maps of (A) *L. lactis* subsp. *lactis* IL1403 and (B) *L. lactis* subsp. *cremoris* MG1363. Genes that were precisely located on the physical maps are shown in boldface. Arrows indicate the 5' → 3' orientation of the gene. Parentheses indicate genes which hybridized weakly with the corresponding fragment. Shaded regions indicate the genome inversion between the two strains. Solid boxes correspond to unmapped *Apa*I fragments. Asterisks indicate the ambiguous locations of the probes. Abbreviations: Ap, *Apa*I; Ce, *Ceu*I; No, *Not*I; Sm, *Sma*I.

Neisseria gonorrhoeae, *Campylobacter* spp., and *Brucella melitensis* (gram-negative bacteria), and *Enterococcus faecalis*, *Listeria monocytogenes*, and *Staphylococcus aureus* (gram-positive bacteria).

The physical map of the *L. lactis* subsp. *cremoris* MG1363

chromosome was compared with the previously published map of the two *L. lactis* subsp. *lactis* strains IL1403 (Fig. 4) and DL11. In contrast to the good correspondence observed along 60% of the DL11 and IL1403 physical maps (49), the physical map of the MG1363 chromosome is very different, as seen

when comparing the *ApaI* and *SmaI* restriction profile with those of these two reference strains (Fig. 1A). These polymorphisms could be explained by the nucleotide sequence divergence between some coding regions of the subspecies *lactis* and *cremoris*, which has been estimated to be between 20 and 30% (34). This difference is of the same order of magnitude as that observed between *E. coli* and *S. typhimurium*, which separated about 100 millions years ago (70). We suggest that for strains belonging to the same subspecies (i.e., *lactis* for the DL11 and IL1403 strains), the restriction polymorphism observed was due mainly to genome rearrangements rather than point mutations. For strains of different subspecies (i.e., *cremoris* and *lactis* for MG1363 and IL1403), this polymorphism may be caused by sequence divergence as well as genome rearrangements. A recent study (36) demonstrated that restriction polymorphism between different isolates of *E. faecalis* was much more frequently due to DNA rearrangements than to point mutations.

The construction of an accurate genetic map is valuable because it provides access to the genome organization and gives better clues to genome rearrangements than does a physical map. The backbone of a bacterial chromosome can be defined with a limited set of key housekeeping genes, including ribosomal operons (*rm*). The strain MG1363 chromosome contains six *rm* operons; five are clustered into 15% of the chromosome and are transcribed in the same direction, and the sixth (arbitrarily named *rmF*) is transcribed in the opposite direction. The six operons are located on 36% of the genome and organized in the same manner as the *rm* operons of *L. lactis* subsp. *lactis* IL1403 and DL11. This unequal distribution is analogous to that found in most prokaryotes, in which *rm* operons are located on 37 to 52% of the chromosome and generally found to be within two groups that are transcribed divergently, away from the origin of replication (*oriC*). If the position of *oriC* with respect to the *rm* operons is conserved in the *Lactococcus* genome, the chromosomal replication origin should be located near the *rmA* operon in MG1363 and IL1403. Moreover, the lactococcal genes that were precisely mapped were transcribed in the same direction as the *rm* operons in both strains MG1363 and IL1403 (Fig. 4) except for the *pepX* gene on the MG1363 chromosome. These observations are in agreement with those for *E. coli* and *B. subtilis*, in which most genes are transcribed in the same direction as replication occurs (6, 96). The *I-CeuI* endonuclease, which cleaves a sequence of the *rhl* gene, which is strongly conserved among bacteria and the organelles of many eukaryotes (56), was used to compare directly the number and location of ribosomal operons in MG1363 and IL1403 (Fig. 1B). The position of each *rm* in the two strains is conserved, since there was little or no size difference in the *I-CeuI* fragments, especially fragments Ce5, Ce4, and Ce3.

Knowledge of the positions of mobile genetic elements on the bacterial chromosome contributes to our understanding of chromosomal dynamics. In *E. coli*, IS elements are involved in genome rearrangements such as DNA amplifications (68), small deletions (5), and large chromosomal inversions (47). It has been proposed that during protoplasts fusion in *L. lactis* subsp. *cremoris*, insertion sequences are also implicated in rearrangement of genetic material (93). The presence of only one chromosomal copy of the *ISS1* insertion sequence in MG1363, together with its absence from the IL1403 chromosome, strongly suggests that *ISS1* is not primarily responsible for genomic rearrangements in these two *Lactococcus* strains. The number and location of the *IS1076* elements are not conserved between the MG1363 and IL1403 chromosomes, since the nine copies are located in one half of the IL1403 chromo-

some, whereas the seven copies present in the MG1363 strain are distributed all along the chromosome.

Comparison of the genetic maps of the two strains provides information on genome dynamics in *L. lactis* strains. Despite a good correlation between the gene orders on the two chromosomes, they differ by the presence of a large chromosomal inversion between IL1403 and MG1363 that covers about 50% of the genome and contains the six *rm* operons (Fig. 4). The borders of this inversion are located near the *uncD* and *rpoD* genes. However, the position of the *oppDFBCA-pepO* gene cluster, located close to one side of the inversion, is not conserved between the IL1403 and MG1363 chromosomes, probably indicating that the genome rearrangement observed between the two strains is more complex than a single inversion event. Interestingly, close analysis of the integration sites of pRL1 in the MG1363 (Fig. 2) and IL1403 (49) chromosomes indicates that the borders of the inversion are located in regions that are relatively devoid of integration events, between IS-4 and IS-25 or between IS-59 and IS-47 on the MG1363 map.

Genome comparison between different species of bacteria reveals that gene organization is relatively conserved even for organisms which diverged 100 million years ago, as for *E. coli* and *S. typhimurium*, suggesting that selective forces or mechanistic restrictions act to preserve gene order. In addition, among the different types of genome rearrangements (i.e., amplification, deletions, and inversions), naturally occurring large inversions are rarely found in bacterial chromosomes. At the species level, two chromosomal inversions including the replication terminus have been reported: the well-characterized inversion between *E. coli* and *S. typhimurium* (78), and an inversion between *Salmonella enteritidis* and *S. typhimurium* (55). At the intraspecies level, only a few inversions between *E. coli* laboratory strains have been described, and all involved recombination between two similar, oppositely oriented sequences, such as the *rm* operons for strain W3110 (38) and IS elements for strains 1485IN (47), BHB2600 (15), and LN1091 (57). Recently, genome map comparisons based on gene location revealed the presence of a chromosomal inversion between two serovars of *Leptospira interrogans* (97) and on the chromosome of different strains of *Mycoplasma hominis* (48). In *L. lactis*, the chromosomal inversion between IL1403 and MG1363 does not involve homologous recombination between two *rm* operons, since all are located inside the inverted region or between two *IS1076* insertion sequences, since no copy of this element could be found close to the borders of the inversion.

The presence of this large chromosomal inversion raises the question of the degree of plasticity in the species *L. lactis*. The frequency inversion has to be evaluated: is it of common occurrence and observable even in isogenic strains, or is it the result of an ancestral event that took place before the divergence into the two subspecies *lactis* and *cremoris*?

ACKNOWLEDGMENTS

We are grateful to the colleagues who have generously provided lactococcal genes and integrative plasmids, many prior to publication. We sincerely thank Michèle Coddeville, Hafed Nadjari, and Nicolas Guichard for skillful assistance with experimental work. P. Le Bourgeois is particularly indebted to M. J. Pillaire for her support while writing the manuscript.

This work was supported by grants from the Centre National de la Recherche Scientifique (UPR 9007), from the EEC-BRIDGE program, and from the Région Midi-Pyrénées.

REFERENCES

- Araya, T., N. Ishibashi, S. Shimamura, K. Tanaka, and H. Takahashi. 1993. Genetic and molecular analysis of the *rpoD* gene from *Lactococcus lactis*. Biosci. Biotech. Biochem. **57**:88–92.
- Bachmann, B. J. 1990. Linkage map of *Escherichia coli* K-12, edition 8. Microbiol. Rev. **54**:130–197.
- Bardowski, J., S. D. Ehrlich, and A. Chopin. 1992. Tryptophan biosynthesis genes in *Lactococcus lactis* subsp. *lactis*. J. Bacteriol. **174**:6563–6570.
- Bardowski, J., S. D. Ehrlich, and A. Chopin. 1994. BglR protein, which belongs to the BglG family of transcriptional antiterminators, is involved in β -glucoside utilization in *Lactococcus lactis*. J. Bacteriol. **176**:5681–5685.
- Birkenbihl, R. P., and W. Vielmetter. 1989. Cosmid-derived map of *E. coli* strain BHB2600 in comparison to the map of strain W3110. Nucleic Acids Res. **17**:5057–5069.
- Brewer, B. J. 1990. Replication and the transcriptional organization of the *Escherichia coli* chromosome, p. 61–84. In K. Drlica and M. Riley (ed.), The bacterial chromosome. American Society for Microbiology, Washington, D.C.
- Canard, B., and S. T. Cole. 1989. Genome organization of the anaerobic pathogen *Clostridium perfringens*. Proc. Natl. Acad. Sci. USA **86**:6676–6680.
- Canard, B., and S. T. Cole. 1990. Lysogenic phages of *Clostridium perfringens*: mapping of the chromosomal attachment sites. FEMS Microbiol. Lett. **66**:323–326.
- Chapot-Chartier, M. P., M. Nardi, M. C. Chopin, A. Chopin, and J. C. Gripon. 1993. Cloning and sequencing of *pepC*, a cysteine aminopeptidase gene from *Lactococcus lactis* subsp. *cremoris* AM2. Appl. Environ. Microbiol. **59**:330–333.
- Chiaruttini, C., and M. Milet. 1993. Gene organization, primary structure and RNA processing analysis of a ribosomal RNA operon in *Lactococcus lactis*. J. Mol. Biol. **230**:57–76.
- Chopin, A. 1993. Organization and regulation of genes for amino acid biosynthesis in lactic acid bacteria. FEMS Microbiol. Rev. **12**:21–38.
- 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.
- Christiansen, B., M. G. Johnsen, E. Stenby, F. K. Vogensen, and K. Hammer. 1994. Characterization of the lactococcal temperate phage TP901-1 and its site-specific integration. J. Bacteriol. **176**:1069–1076.
- Cole, S. T., and I. Saint Girons. 1994. Bacterial genomics. FEMS Microbiol. Rev. **14**:139–160.
- Daniels, D. L. 1990. The complete *AvrII* restriction map of the *Escherichia coli* genome and comparisons of several laboratory strains. Nucleic Acids Res. **18**:2649–2651.
- Davies, F. L., H. M. Underwood, and M. J. Gasson. 1981. The value of plasmid profile for strain identification in lactic streptococci and the relationship between *Streptococcus lactis* 712, ML3 and C2. J. Appl. Bacteriol. **51**:325–337.
- Delorme, C., S. D. Ehrlich, and P. Renault. 1992. Histidine biosynthesis genes in *Lactococcus lactis* subsp. *lactis*. J. Bacteriol. **174**:6571–6579.
- Denayrolles, M., M. Aigle, and A. Lonvaud-Funel. 1994. Cloning and sequencing analysis of the gene encoding *Lactococcus lactis* malolactic enzyme: relationships with malic enzymes. FEMS Microbiol. Lett. **116**:79–86.
- de Vos, W. M. 1994. Personal communication.
- Dinsmore, P. K., D. A. Romero, and T. R. Klaenhammer. 1993. Insertional mutagenesis in *Lactococcus lactis* subsp. *lactis* mediated by IS946. FEMS Microbiol. Lett. **107**:43–48.
- Dodd, H. M., N. Horn, and M. J. Gasson. 1990. Analysis of the genetic determinants for production of the peptide antibiotic nisin. J. Gen. Microbiol. **136**:555–566.
- Dodd, H. M., N. Horn, and M. J. Gasson. 1994. Characterization of IS905, a new multicopy insertion sequence identified in lactococci. J. Bacteriol. **176**:3393–3396.
- Duwat, P. 1994. Personal communication.
- Duwat, P., S. D. Ehrlich, and A. Gruss. 1992. Use of degenerate primers for polymerase chain reaction cloning and sequencing of the *Lactococcus lactis* subsp. *lactis* *recA* gene. Appl. Environ. Microbiol. **58**:2674–2678.
- Eaton, T. J., C. A. Shearman, and M. J. Gasson. 1993. Cloning and sequence analysis of the *dnaK* gene region of *Lactococcus lactis* subsp. *lactis*. J. Gen. Microbiol. **139**:3253–3264.
- Gansel, X., A. Hartke, P. Boutibonnes, and Y. Auffray. 1993. Nucleotide sequence of the *Lactococcus lactis* NCDO 763 (ML3) *rpoD* gene. Biochim. Biophys. Acta Gene Struct. Expression **1216**:115–118.
- Garnier, T., B. Canard, and S. T. Cole. 1991. Cloning, mapping, and molecular characterization of the rRNA operons of *Clostridium perfringens*. J. Bacteriol. **173**:5431–5438.
- Gasc, A. M., L. Kauc, P. Barraillé, M. Sicard, and S. H. Goodgal. 1991. Gene localization, size, and physical map of the chromosome of *Streptococcus pneumoniae*. J. Bacteriol. **173**:7361–7367.
- Gasson, M. J. 1983. Plasmid complements of *Streptococcus lactis* NCDO 712 and other lactic streptococci after protoplast-induced curing. J. Bacteriol. **154**:1–9.
- Gasson, M. J. 1994. Personal communication.
- Geller, B. L., R. G. Ivey, J. E. Trempey, and B. Hettinger-Smith. 1993. Cloning of a chromosomal gene required for phage infection of *Lactococcus lactis* subsp. *lactis* C2. J. Bacteriol. **175**:5510–5519.
- Godon, J. J. 1994. Personal communication.
- Godon, J. J., M. C. Chopin, and S. D. Ehrlich. 1992. Branched-chain amino acid biosynthesis genes in *Lactococcus lactis* subsp. *lactis*. J. Bacteriol. **174**:6580–6589.
- Godon, J. J., C. Delorme, S. D. Ehrlich, and P. Renault. 1992. Divergence of genomic sequences between *Lactococcus lactis* subsp. *lactis* and *Lactococcus lactis* subsp. *cremoris*. Appl. Environ. Microbiol. **58**:4045–4047.
- Godon, J. J., K. Jury, C. A. Shearman, and M. J. Gasson. 1994. The *Lactococcus lactis* sex-factor aggregation gene *cluA*. Mol. Microbiol. **12**:655–663.
- Hall, L. M. C. 1994. Are point mutations or DNA rearrangements responsible for the restriction fragment length polymorphisms that are used to type bacteria? Microbiology **140**:197–204.
- Hantman, M. J., S. Sun, P. J. Piggett, and L. Daneo-Moore. 1993. Chromosome organization of *Streptococcus mutans* GS-5. J. Gen. Microbiol. **139**:67–77.
- Hill, C. W., and B. W. Harnish. 1981. Inversions between ribosomal RNA genes of *Escherichia coli*. Proc. Natl. Acad. Sci. USA **78**:7069–7072.
- Huang, D. C., M. Novel, X. F. Huang, and G. Novel. 1992. Nonidentity between plasmid and chromosomal copies of ISS1-like sequences in *Lactococcus lactis* subsp. *lactis* CNRZ270 and their possible role in chromosomal integration of plasmid genes. Gene **118**:39–46.
- Huang, D. C., M. Novel, and G. Novel. 1991. A transposon-like element on the lactose plasmid of *Lactococcus lactis* subsp. *lactis* Z270. FEMS Microbiol. Lett. **77**:101–106.
- Israelsen, H., and E. B. Hansen. 1993. Insertion of transposon Tn917 derivatives into the *Lactococcus lactis* subsp. *lactis* chromosome. Appl. Environ. Microbiol. **59**:21–26.
- Jarvis, A. W., and B. D. W. Jarvis. 1981. Deoxyribonucleic acid homology among lactic streptococci. Appl. Environ. Microbiol. **41**:77–83.
- Klijn, N., A. H. Weerkamp, and W. M. de Vos. 1991. Identification of mesophilic lactic acid bacteria by using polymerase chain reaction-amplified variable regions of 16S rRNA and specific DNA probes. Appl. Environ. Microbiol. **57**:3390–3393.
- Köhler, G., W. Ludwig, and K. H. Schleifer. 1991. Differentiation of lactococci by rRNA gene restriction analysis. FEMS Microbiol. Lett. **84**:307–312.
- Koivula, T., and H. Hemilä. 1991. Nucleotide sequence of a *Lactococcus lactis* gene cluster encoding adenylate kinase, initiation factor 1 and ribosomal proteins. J. Gen. Microbiol. **137**:2595–2600.
- Kok, J., and K. Venema. 1992. Personal communication.
- Komoda, Y., M. Enomoto, and A. Tominaga. 1991. Large inversion in *Escherichia coli* K-12 14851N between inversely oriented IS3 elements near *lac* and *cdd*. Genetics **129**:639–645.
- Ladefoged, S. A., and G. Christiansen. 1992. Physical and genetic mapping of the genomes of five *Mycoplasma hominis* strains by pulsed-field gel electrophoresis. J. Bacteriol. **174**:2199–2207.
- Le Bourgeois, P., M. Lautier, M. Mata, and P. Ritzenthaler. 1992. Physical and genetic map of the chromosome of *Lactococcus lactis* subsp. *lactis* IL1403. J. Bacteriol. **174**:6752–6762.
- Le Bourgeois, P., M. Lautier, M. Mata, and P. Ritzenthaler. 1992. New tools for the physical and genetic mapping of *Lactococcus* strains. Gene **111**:109–114.
- Le Bourgeois, P., M. Lautier, and P. Ritzenthaler. 1993. Chromosome mapping in lactic acid bacteria. FEMS Microbiol. Rev. **12**:109–124.
- Le Bourgeois, P., M. Lautier, L. van den Berghe, N. Guichard, M. Mata, and P. Ritzenthaler. 1993. Comparison of the genome maps of two *Lactococcus lactis* strains IL1403 and MG1363. FEMS Microbiol. Rev. **12**:156. (Abstract.)
- Le Bourgeois, P., M. Mata, and P. Ritzenthaler. 1989. Genome comparison of *Lactococcus* strains by pulsed-field gel electrophoresis. FEMS Microbiol. Lett. **59**:65–70.
- Le Bourgeois, P., M. Mata, and P. Ritzenthaler. 1991. Pulsed-field gel electrophoresis as a tool for studying the phylogeny and genetic history of lactococcal strains, p. 140–145. In G. M. Dunne, P. P. Cleary, and L. L. McKay (ed.), Genetics and molecular biology of streptococci, lactococci, and enterococci. American Society for Microbiology, Washington, D.C.
- Liu, S. L., A. Hessel, and K. E. Sanderson. 1993. The *XbaI*-*BlnI*-*CeuI* genomic cleavage map of *Salmonella enteritidis* shows an inversion relative to *Salmonella typhimurium* LT2. Mol. Microbiol. **10**:655–664.
- Liu, S. L., A. Hessel, and K. E. Sanderson. 1993. Genomic mapping with *I*-*CeuI*, an intron-encoded endonuclease specific for genes for ribosomal RNA, in *Salmonella* spp., *Escherichia coli*, and other bacteria. Proc. Natl. Acad. Sci. USA **90**:6874–6878.
- Louarn, J. M., J. P. Bouché, F. Legendre, J. Louarn, and J. Patte. 1985. Characterization and properties of very large inversions of the *E. coli* chromosome along the origin-to-terminus axis. Mol. Gen. Genet. **201**:467–476.
- Lucey, M., C. Daly, and G. F. Fitzgerald. 1993. Relationship between *Lactococcus lactis* subsp. *lactis* 952, an industrial lactococcal starter culture, and strains 712, ML3 and C2. J. Appl. Bacteriol. **75**:326–335.
- Maguin, E. 1994. Personal communication.
- Maguin, E., P. Duwat, T. Hege, S. D. Ehrlich, and A. Gruss. 1992. New thermosensitive plasmid for gram-positive bacteria. J. Bacteriol. **174**:5633–5638.

61. Maniatis, T., E. F. Fritsch, and J. Sambrook. 1989. Molecular cloning: a laboratory manual, 2nd ed. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y.
62. Marugg, J. D., D. Goelling, U. Stahl, A. M. Ledeboer, M. Y. Toonen, W. M. Verhulst, and C. T. Verrips. 1994. Identification and characterization of the alpha-acetolactate synthase gene from *Lactococcus lactis* subsp. *lactis* biovar diacetylactis. Appl. Environ. Microbiol. **60**:1390–1394.
63. Mayo, B., G. Buist, and J. Kok. 1994. Personal communication.
64. McClelland, M., R. Patel, and M. Nelson. 1987. Restriction endonucleases for pulsed-field mapping of bacterial genomes. Nucleic Acids Res. **15**:5985–6005.
65. 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.
66. Mercenier, A. 1994. Personal communication.
67. Mierau, I., A. J. Haandrikman, O. Velterop, P. S. T. Tan, K. L. Leenhouts, W. N. Konings, G. Venema, and J. Kok. 1994. Tripeptidase gene (*pepT*) of *Lactococcus lactis*: molecular cloning and nucleotide sequencing of *pepT* and construction of a chromosomal deletion mutant. J. Bacteriol. **176**:2854–2861.
68. Muramatsu, S., M. Kato, Y. Kohara, and T. Mizuno. 1988. Insertion sequence IS5 contains a sharply curved DNA structure at its terminus. Mol. Gen. Genet. **214**:433–438.
69. Nardi, M., M. C. Chopin, A. Chopin, M. M. Cals, and J. C. Gripon. 1991. Cloning and DNA sequence analysis of an X-prolyl dipeptidyl aminopeptidase gene from *Lactococcus lactis* subsp. *lactis* NCDO 763. Appl. Environ. Microbiol. **57**:45–50.
70. Ochman, H., and A. C. Wilson. 1987. Evolutionary history of enteric bacteria, p. 1649–1654. In F. C. Neidhardt, J. L. Ingraham, K. B. Low, B. Magasanik, M. Schaechter, and H. E. Umbarger (ed.), *Escherichia coli* and *Salmonella typhimurium*: cellular and molecular biology. American Society for Microbiology, Washington, D.C.
71. Piggot, P. J. 1990. Genetic map of *Bacillus subtilis* 168, p. 107–145. In K. Drlica and M. Riley (ed.), *The bacterial chromosome*. American Society for Microbiology, Washington, D.C.
72. Polzin, K. M., and L. L. McKay. 1991. Identification, DNA sequence, and distribution of IS981, a new, high-copy-number insertion sequence in lactococci. Appl. Environ. Microbiol. **57**:734–743.
73. Polzin, K. M., and M. Shimizu-Kadota. 1987. Identification of a new insertion element, similar to gram-negative IS26, on the lactose plasmid of *Streptococcus lactis* ML3. J. Bacteriol. **169**:5481–5488.
74. Powell, I. B., M. G. Achen, A. J. Hillier, and B. E. Davidson. 1988. A simple and rapid method for genetic transformation of lactic streptococci by electroporation. Appl. Environ. Microbiol. **54**:655–660.
75. Rauch, P. J. G., and W. M. de Vos. 1992. Characterization of the novel nisin-sucrose conjugative transposon Tn5276 and its insertion in *Lactococcus lactis*. J. Bacteriol. **174**:1280–1287.
76. Renault, P. 1994. Personal communication.
77. Renault, P., C. Gaillardin, and H. Heslot. 1989. Product of the *Lactococcus lactis* gene required for malolactic fermentation is homologous to a family of positive regulators. J. Bacteriol. **171**:3108–3114.
78. Riley, M., and K. E. Sanderson. 1990. Comparative genetics of *Escherichia coli* and *Salmonella typhimurium*, p. 85–95. In K. Drlica and M. Riley (ed.), *The bacterial chromosome*. American Society for Microbiology, Washington, D.C.
79. Ross, P., F. O'Gara, and S. Condon. 1990. Cloning and characterization of the thymidylate synthase gene from *Lactococcus lactis* subsp. *lactis*. Appl. Environ. Microbiol. **56**:2156–2163.
80. Salama, M. S., W. E. Sandine, and S. J. Giovannoni. 1991. Development and application of oligonucleotide probes for identification of *Lactococcus lactis* subsp. *cremoris*. Appl. Environ. Microbiol. **57**:1313–1318.
81. Sanders, J. W. 1994. Personal communication.
82. Smeltzer, M. S., M. E. Hart, and J. J. Iandolo. 1994. The effect of lysogeny on the genomic organization of *Staphylococcus aureus*. Gene **138**:51–57.
83. Stroman, P. 1992. Sequence of a gene (*lap*) encoding a 95.3-kDa aminopeptidase from *Lactococcus lactis* subsp. *cremoris* Wg2. Gene **113**:107–112.
84. Tanskanen, E. L., D. L. Tulloch, A. J. Hillier, and B. E. Davidson. 1990. Pulsed-field gel electrophoresis of *SmaI* digest of lactococcal genomic DNA, a novel method of strain identification. Appl. Environ. Microbiol. **56**:3105–3111.
85. Terzaghi, B. E., and W. E. Sandine. 1975. Improved medium for lactic streptococci and their bacteriophages. Appl. Microbiol. **29**:807–813.
86. Tulloch, D. L., L. R. Finch, A. J. Hillier, and B. E. Davidson. 1991. Physical map of the chromosome of *Lactococcus lactis* subsp. *lactis* DL11 and localization of six putative rRNA operons. J. Bacteriol. **173**:2768–2775.
87. Tynkynen, S., G. Buist, E. Kunji, J. Kok, B. Poolman, G. Venema, and A. J. Haandrikman. 1993. Genetic and biochemical characterization of the oligopeptide transport system of *Lactococcus lactis*. J. Bacteriol. **175**:7523–7532.
88. van Asseldonk, M., G. Rutten, M. Otemans, R. J. Siezen, W. M. de Vos, and G. Simons. 1990. Cloning of *usp45*, a gene encoding a secreted protein from *Lactococcus lactis* subsp. *lactis* MG1363. Gene **95**:155–160.
89. van Asseldonk, M., A. Simons, H. Visser, W. M. de Vos, and G. Simons. 1993. Cloning, nucleotide sequence, and regulatory analysis of the *Lactococcus lactis* *dnaJ* gene. J. Bacteriol. **175**:1637–1644.
90. Versalovic, J., T. Koeuth, R. Britton, K. Geszvain, and J. R. Lupski. 1993. Conservation and evolution of the *rpsU-dnaG-rpoD* macromolecular synthesis operon in bacteria. Mol. Microbiol. **8**:343–355.
91. Walker, J. E., N. J. Gay, M. Saraste, and A. N. Eberle. 1984. DNA sequence around the *Escherichia coli* *unc* operon. Biochem. J. **224**:799–815.
92. Ward, A. C., A. J. Hillier, B. E. Davidson, and I. B. Powell. 1993. Stability analysis of the *Lactococcus lactis* DCR1 lactose plasmid using pulsed-field gel electrophoresis. Plasmid **29**:70–73.
93. Ward, L. J. H., J. C. S. Brown, and G. P. Davey. 1993. Insertion sequence analysis of protoplast fused strains of *Lactococcus lactis* ssp. *cremoris*. FEMS Microbiol. Lett. **113**:315–320.
94. Waterbury, P. G., and M. J. Lane. 1987. Generation of lambda concatemers for use as pulsed-field electrophoresis size markers. Nucleic Acids Res. **15**:3930.
95. Wong, K. K., and M. McClelland. 1992. A *BlnI* restriction map of the *Salmonella typhimurium* LT2 genome. J. Bacteriol. **174**:1656–1661.
96. Ziegler, D., and D. Dean. 1990. Orientation of genes in the *Bacillus subtilis* chromosome. Genetics **125**:703–708.
97. Zuerner, R. L., J. L. Herrmann, and I. Saint Girons. 1993. Comparison of genetic maps for two *Leptospira interrogans* serovars provides evidence for two chromosomes and intraspecies heterogeneity. J. Bacteriol. **175**:5445–5451.