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NOTES

Spontaneous Deletion Mutants of the *Lactococcus lactis* Temperate Bacteriophage BK5-T and Localization of the BK5-T *attP* Site

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Spontaneous deletion mutants of the temperate lactococcal bacteriophage BK5-T were obtained when the phage was grown vegetatively on the indicator strain *Lactococcus lactis* subsp. *cremoris* H2. One deletion mutant was unable to form stable lysogens, and analysis of this mutant led to the identification of the BK5-T *attP* site and the integrase gene (*int*). The core sequences of the BK5-T *attP* and host *attB* regions are conserved in a number of lactococcal phages and *L. lactis* strains.

BK5-T is a temperate lactococcal bacteriophage that can be induced from the lysogen *Lactococcus lactis* subsp. *cremoris* BK5 by mitomycin treatment (9). Under certain propagation conditions, BK5-T spontaneously loses the ability to lysogenize, possibly as a result of one or more deletion events (7). The availability of detailed information concerning the BK5-T genome (3, 11) prompted us to seek deletion mutants of BK5-T that had lost the ability to form stable lysogens as a strategy for identifying genes essential for the establishment and/or maintenance of lysogeny. Characterization of one of these mutants enabled us to identify and sequence the phage and host attachment sites, *attP* and *attB*, respectively. Deletion of a 536-codon open reading frame (ORF) and tandemly repeated segments within a 1,904-codon ORF in BK5-T did not affect the frequency of lysogeny, thereby eliminating the possibility that these gene products are required for lysogeny.

Deletions within the BK5-T genome during lytic propagation. The indicator strain *L. lactis* H2 (9) was infected with BK5-T.H2L (BK5-T isolated after induction of the lysogen *L. lactis* H2L [3]) and incubated until the culture lysed. The culture was centrifuged (5,000 × *g*) for 10 min, and the cell-free lysate was added to an appropriate volume of uninfected cells. This lysis/infection cycle was repeated 20 times, and the resulting phage was designated BK5-T.H2cyc20. Analysis of *Eco*RI-*Pst*I digests of BK5-T.H2L DNA and BK5-T.H2cyc20 DNA, together with hybridization studies with *Eco*RI-a and *Eco*RI-b (11), showed that at least nine fragments (in addition to the fragments containing *cos*) in the BK5-T.H2cyc20 DNA digest were present in submolar amounts (Fig. 1). Five of these fragments were produced by specific deletions within *Eco*RI-b (11) (Fig. 1B, lane 2). *Eco*RI-b contains four perfect tandem repeats of 468 bp and a fifth incomplete tandem repeat within the large ORF1904 (3). The 7.4-kbp fragment that hybridized to *Eco*RI-b was the full-length *Eco*RI-b(P1) fragment (11), while the sizes of four other submolar fragments (6.9, 6.4, 6.0, and 5.5 kbp) were consistent with their being produced by the loss of one, two, three, or four of the 468-bp tandem repeats, respectively. The other four submolar fragments (5.2, 4.3, 3.2, and 2.1 kbp in size) resulted from deletions within *Eco*RI-a (11) (Fig. 1C, lane 2).

Characterization of BK5-T deletion mutants. The presence of many submolar fragments in restriction digests of BK5-T.H2cyc20 DNA suggested that the BK5-T.H2cyc20 preparation comprised a mixed population. To characterize the deletions more precisely, three deletion mutants of BK5-T (BK5-T.H2Δ8, BK5-T.H2Δ10, and BK5-T.H2Δ11) were randomly selected from single plaques of BK5-T.H2cyc20. Deletions within BK5-T.H2Δ8, BK5-T.H2Δ10, and BK5-T.H2Δ11 DNA were located by restriction mapping, Southern hybridization (Fig. 1), and DNA sequencing. Each mutant contained a deletion within the tandem repeat region of ORF1904 (Fig. 2) (3), while BK5-T.H2Δ8 and BK5-T.H2Δ10 also contained deletions within *Eco*RI-a. The deletion within ORF1904 could be explained by a single crossover recombination event between two 5-bp "core sequences" of ACGGA situated at 4198 and 6070 bp (3) in BK5-T.H2Δ11 DNA or between two 8-bp homologous regions situated at 4222 and 6094 bp (3) in BK5-T.H2Δ8 DNA (Fig. 2). Each of these deletions removed 1,872 bp of DNA, equivalent to four of the 468-bp tandem repeats identified previously (3). These deletions shortened ORF1904 by 624 codons without changing the reading phase. BK5-T.H2Δ10 phage comprised a mixed population of phages carrying deletions within ORF1904 corresponding to the loss of two, three, or four 468-bp repeats. The precise endpoints of these deletions could not be determined. Each phage deletion mutant was able to propagate vegetatively on *L. lactis* H2, yielding slightly larger plaques (1 to 1.5 mm) than did BK5-T.H2L (0.5 to 1 mm).

FASTA (15) comparison of the ORF1904 amino acid sequence with all protein sequences in GenBank showed significant homology with a number of proteins from the collagen family. This homology was centered around the repeated Gly-X-Y motif, found 64 times in ORF1904. FASTA analysis also indicated similarity between ORF1904 and an unidentified ORF (ORF35) from the lactococcal bacteriophage bIL67 (19) and less similarity to the lactococcal lytic bacteriophage US3

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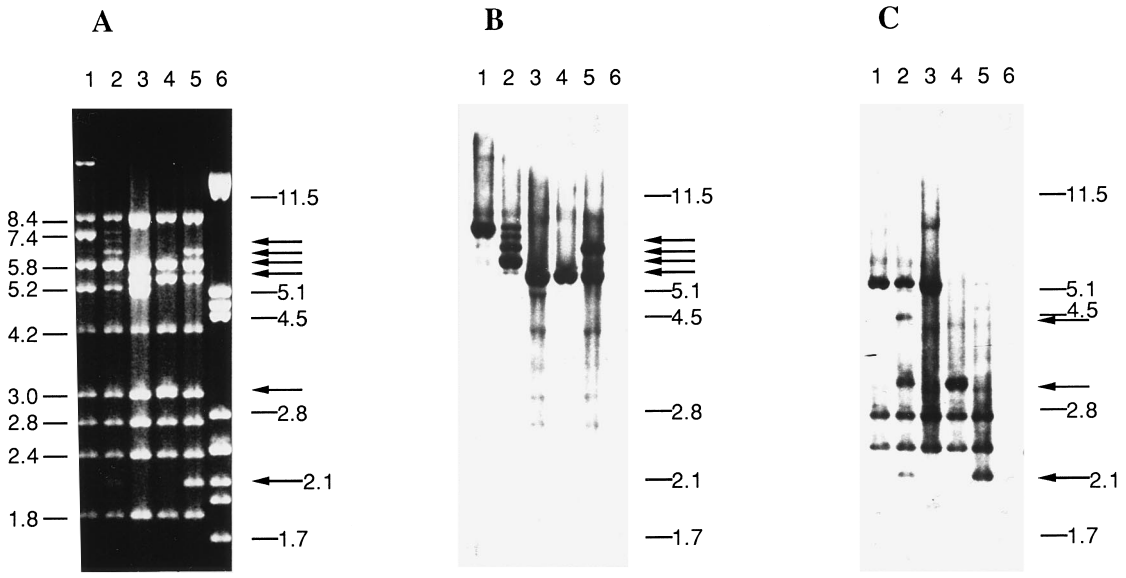


FIG. 1. Electrophoretic separation of *Eco*RI-*Pst*I restriction digests of DNA from BK5-T.H2L and deletion mutants. (A) BK5-T DNA was digested with both *Eco*RI and *Pst*I, and the resulting fragments were electrophoresed through a 0.8% (wt/vol) agarose gel and stained with ethidium bromide. The DNA was from BK5-T.H2L (lane 1), BK5-T.H2cyc20 (lane 2), BK5-T.H2Δ11 (lane 3), BK5-T.H2Δ8 (lane 4), BK5-T.H2Δ10 (lane 5), and λ DNA digested with *Pst*I (lane 6). (B) Southern blot of the gel shown in panel A after hybridization with ^{32}P-labeled *Eco*RI-b (11). (C) Southern blot of the gel shown in panel A after hybridization with ^{32}P-labeled *Eco*RI-a (11). Numbers on the right of each panel indicate the sizes (in kilobase pairs) of fragments from a *Pst*I digest of λ DNA, and arrows show positions of fragments present in the digests of mutant phages which were not present in the digest of BK5-T.H2L. Numbers on the left of panel A indicate the sizes (in kilobase pairs) of BK5-T.H2L fragments.

lytic enzyme (16) and the *Bacillus subtilis* xylose isomerase (22). Analysis by COMPARE (18) indicated similarity between ORF1904 and numerous proteins involved in binding and/or degradation of cell wall glycoproteins. These sequence similarities and the observation that a number of cell wall-lytic enzymes contain repeated sequence motifs (5, 8, 10, 12) suggest that ORF1904 may be involved in cell lysis during the lytic cycle of BK5-T or in cell wall hydrolysis to enable phage DNA injection.

The genomes of BK5-T.H2Δ8 and BK5-T.H2Δ10 also con-

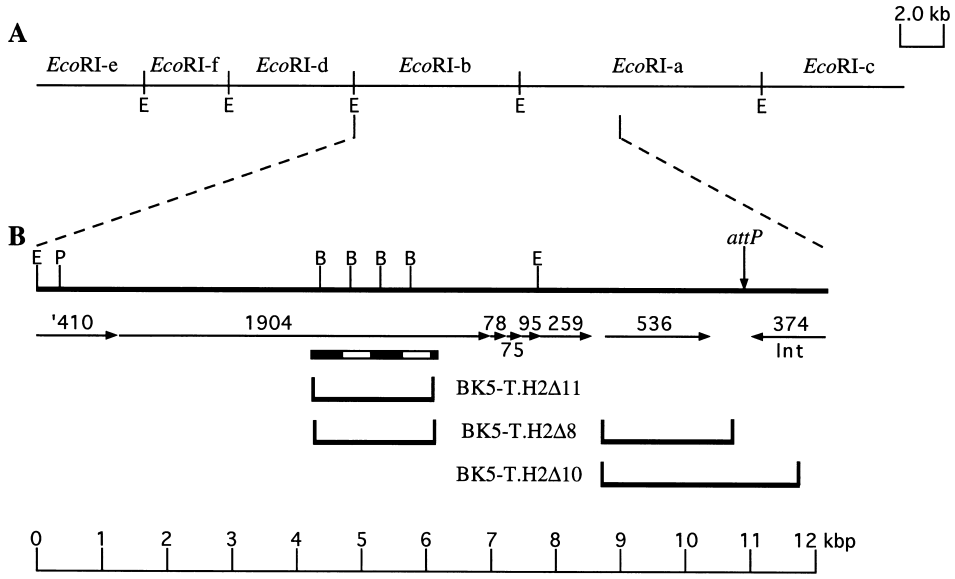
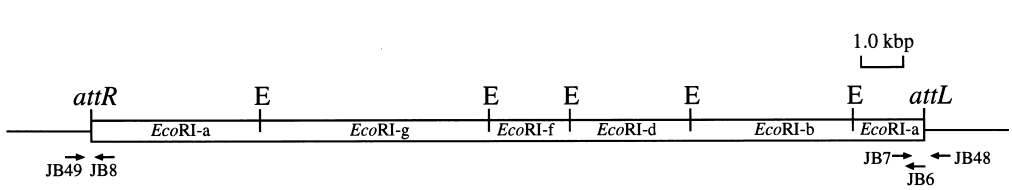


FIG. 2. Physical map of the BK5-T genome. (A) *Eco*RI (E) restriction map of the BK5-T genome. Restriction fragments are named as described previously (11). The ends of the map correspond to the *cos* ends of the phage DNA (3). (B) Physical map of *Eco*RI-b and part of *Eco*RI-a showing the locations of all *Eco*RI (E), *Pst*I (P), and *Bgl*I (B) sites and the positions of relevant ORFs as determined by nucleotide sequence analysis (3). Horizontal arrows indicate the orientation of each ORF, and adjacent numbers show the number of codons in each ORF from the first ATG codon to the first stop codon. ORF′410 is open at the 5′ end. The positions of the tandem duplications are indicated by solid and open rectangles. *attP* is the phage attachment site. The positions of the deletions within the genomes of BK5-T.H2Δ11, BK5-T.H2Δ8 (two separate deletions), and BK5-T.H2Δ10 phage are bracketed. Since BK5-T.H2Δ10 was a mixed population of phages carrying various deletions within *Eco*RI-b, only the deletion within *Eco*RI-a has been shown.



B

BK5-T *attP*	CATCTATCCTTTACTATGTAG**TTCTTCATG**AACGATAATAAACAATAATAG
L. lactis* H2L *attR	CATCTATCCTTTACTATGTAG**TTCTTCATG**GGAGCTCAAGAAGAATAAAAG
L. lactis* H2L *attL	AAAGAATTGCAAACCTTGTTA**TTCTTCATG**AACGATAATAAACAATAATAG
L. lactis* H2 *attB	AAAGAATTGCAAACCTTGTTA**TTCTTCATG**GGAGCTCAAGAAGAATAAAAG

FIG. 3. Nucleotide sequence of the *att* regions of BK5-T, *L. lactis* H2, and *L. lactis* H2L. (A) Physical map of the BK5-T prophage. BK5-T is designated by the open rectangle, while the *L. lactis* chromosomal DNA is indicated by the horizontal line. The positions of oligonucleotides used to amplify the *attL* and *attR* regions are shown by arrows, with the arrowheads designating the 3′ end of each oligonucleotide. (B) Nucleotide sequences of *attP*, *attR*, *attL*, and *attB*. The designation of *attL* and *attR* was in accordance with the system of Lillehaug and Birkeland (13). The nucleotide sequence of each region was determined as described in the text. The 9-nucleotide core sequence 5′-TTCTTCATG-3′ present in all *att* regions is shown in boldface type. The sequence of the *attL*, *attR*, and *attB* regions was determined in only one strand.

tained deletions within *Eco*RI-a (Fig. 2). These deletions resulted from a single-crossover recombination event between two 11-bp core sequences of TTTTTTTGTTT situated at 8677 and 10701 bp in BK5-T.H2Δ8 DNA (3) (Fig. 2) or between two 6-bp core sequences of GTGTTT situated at 8674 and 11737 bp in BK5-T.H2Δ10 DNA (3) (Fig. 2). Both of these deletions removed ORF536, while the BK5-T.H2Δ10 deletion also removed the ORF374-to-ORF536 intergenic region and 256 codons from the C-terminal end of ORF374 (Fig. 2).

The phage deletion mutants were tested for their ability to form lysogens on *L. lactis* H2. BK5-T.H2Δ11 and BK5-T.H2Δ8 remained lysogenic, but BK5-T.H2Δ10 was not. This indicated that neither the full-length ORF1904 nor ORF536 (Fig. 2) is essential for vegetative growth or the formation and maintenance of stable lysogens. Coliphage λ also contains genes that encode products that are not essential for normal propagation (6). The function of the ORF536 protein is unknown, and its amino acid sequence exhibits no significant homology with any prokaryotic proteins in the GenBank database. The inability of BK5-T.H2Δ10 to form lysogens indicates that ORF374 and/or the ORF536-to-ORF374 intergenic DNA is essential for the establishment and/or maintenance of lysogeny.

Localization of the BK5-T attachment site. Since BK5-T.H2Δ10 contained deletions within the *Eco*RI a fragment, previously shown to contain *attP* (11), it was decided to locate *attP* more precisely to determine whether loss of this feature was related to the nonlysogenic nature of BK5-T.H2Δ10. Subfragments of BK5-T *Eco*RI-a were used to probe Southern blots of *Eco*RI digests of chromosomal DNA from the BK5-T lysogens *L. lactis* BK5 and H2L (data not shown). BK5-T subfragments spanning *attP* would hybridize to two chromosomal fragments, whereas subfragments which did not contain *attP* would hybridize to only one fragment. By using this approach, *attP* was localized between 10769 and 10999 bp (3) and the phage/host junctions were shown to be in chromosomal *Xba*I fragments of 90 and 18 kbp in *L. lactis* BK5 and H2L (data not shown).

The sequences of these phage/host junctions (*attL* and *attR*) in *L. lactis* H2L were then determined (Fig. 3B). For *attL*, this was done by sequencing a 900-bp PCR fragment, obtained by inverse PCR (14) with JB6 and JB7 as primers and a ligated *Ssp*I digest of the gel-purified 90-kbp *Xba*I chromosomal fragment as the template. The obtained sequence revealed 100% sequence identity between the 21 bp of host DNA adjacent to *attL* in BK5-T and in ϕLC3, another *L. lactis* temperate phage (13). The existence of this identity and of the 97% identity between the 1,627 bp of DNA surrounding *attP* of BK5-T and ϕLC3 (13) enabled us to use the nucleotide sequence surrounding *attB* in the ϕLC3 lysogen *L. lactis* IMN-C18 (13) to design PCR primers that amplified *L. lactis* H2 *attB* (JB48 and JB49 [Table 1]) and *L. lactis* H2L *attR* (JB8 and JB49 [Table 1]) and provided DNA for sequencing.

Comparison of the DNA sequences of the *attP*, *attL*, *attR*, and *attB* regions (Fig. 3B) identified a common 9-bp core sequence, 5′-TTCTTCATG-3′ (bases 10882 to 10874) (3), within which recombination between BK5-T and the host genome is likely to occur. Thus, BK5-T *attP* is located in a region of the BK5-T genome that was deleted in BK5-T.H2Δ10 but not in BK5-T.H2Δ11 or BK5-T.H2Δ8 (Fig. 2). The deletion of *attP* in BK5-T.H2Δ10 provides an explanation for the inability of this phage to form stable lysogens. This observation is of particular interest in an industrial context, since to our knowledge it is the first demonstration of spontaneous mutations

TABLE 1. Oligonucleotides used in this investigation[a]

Number	Oligonucleotide sequence (5′-3′)	Region of BK5-T[b]
JB6	GATCATTAGGAATACTCCCC	10012–9994
JB7	GATCGACATGGGAGAAGGTAAAGG	10259–10281
JB8	CACACAGCAAACCTATATCC	11051–11032
JB48	TGTTAAAGCAGGAATCAAAGG	Comp[c]
JB49	AATACCTAAGCACACGAAGGTT	Comp

[a] Oligonucleotides were synthesized in an Applied Biosystems model 381 DNA synthesizer.

[b] Sequence numbers refer to the nucleotide sequence of BK5-T as determined previously (3).

[c] Comp, complementary to *L. lactis* IMN-C18 genomic DNA (13).

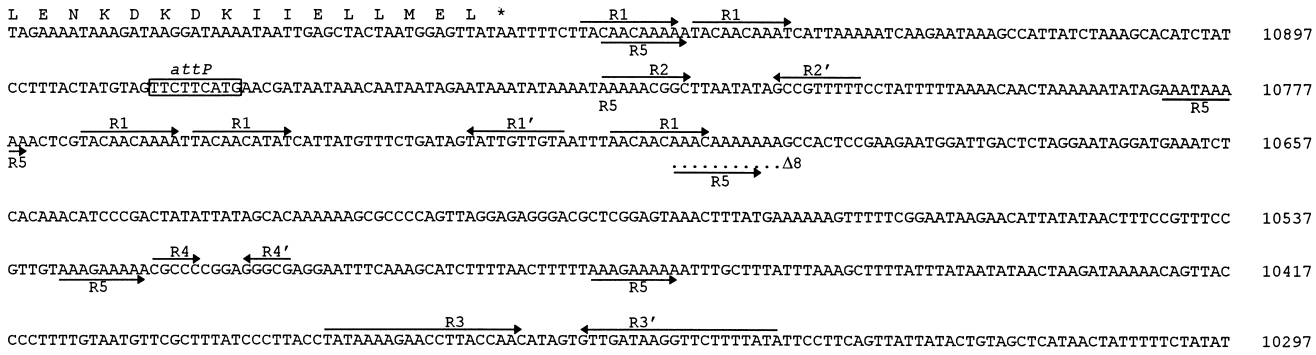


FIG. 4. Nucleotide sequence surrounding BK5-T *attP*. The core *attP* sequence is boxed, and repeated sequences (R1, R5) and inverted repeats (R2, R3, and R4) surrounding *attP*, which may be involved in protein binding necessary for phage integration, are identified by arrows above the sequence with arrowheads to designate polarity. The 16 C-terminal amino acids of ORF374 (Int) are shown in single-letter code above the sequence, with * designating the stop codon of the ORF. The core sequence involved in the deletion within the phage genome BK5-T.H2Δ8 is indicated by periods and labeled Δ8. Sequence numbers are as presented previously (3); however, the complementary strand is shown to allow easy comparison with the sequences surrounding the φLC3 and Tuc2009 *attP* regions (13, 20).

resulting in a lytic phenotype in a temperate lactococcal bacteriophage. These lytic phages are not virulent, because they cannot infect BK5-T lysogens and thus would grow only on strains which did not express the BK5-T repressor protein or a functional equivalent.

BK5-T *attP* is identical to *attP* of the lactococcal bacteriophages φLC3 and Tuc2009 (13, 20). Moreover, *L. lactis* H2 *attB* and the 21 bases on either side are identical to the corresponding bases in the *L. lactis* indicator strains IMN-C18 (φLC3), UC509 (Tuc2009), and UC506 (Tuc2009) and differ by 1 base from that of MG1363 (Tuc2009) (13, 20). Five (TTCTT) of the nine bases of the BK5-T *attP* core sequence are identical to bases present in the 16-bp core sequence of the *Lactobacillus gasseri* phage φadh *attP* (17). There was no apparent homology between BK5-T *attP* and *attP* of the temperate lactococcal phage TP901-1 (4). This indicates that there are at least two classes of integration system in temperate lactococcal phages.

The nucleotide sequence surrounding the BK5-T *attP* core sequence contained a number of repeated and/or palindromic sequences (Fig. 4). These regions of DNA may be important in binding integrase or an *L. lactis* IHF homolog. Identical sequences also surround the φLC3 and Tuc2009 *attP* sites (13, 20). Because the deletion mutant BK5-T.H2Δ8 was able to form stable lysogens, only the repeated sequences at positions >10701 bp (Fig. 4) can be essential for phage integration.

Deduced amino acid sequence of the BK5-T integrase. FASTA comparison of the deduced amino acid sequence of ORF374, which is adjacent to *attP* (Fig. 2), with all GenBank proteins revealed significant homology with a number of site-specific recombinases. In particular, ORF374 shared 99.4 and 98.7% identity with the deduced amino acid sequences of the integrase proteins from the Tuc2009 and φLC3 phages, respectively (13, 20). All of these lactococcal phage integrase proteins contain the highly conserved residues of site-specific recombinases of the λ integrase family (1).

Overall homology between BK5-T, φLC3, and Tuc2009. Comparison of the nucleotide sequence surrounding *int* and *attP* in BK5-T (bases 12212 to 10341) and Tuc2009 (bases 1 to 1872 [20]) identified a region of 1,632 bp including *int* and *attP* that showed 97% identity at the nucleotide level. Preceding this homologous region, the nucleotide sequences diverge and the deduced amino acid sequence of ORF536, the adjacent gene in BK5-T, shows no homology with the protein encoded by the adjacent gene in Tuc2009. It is not possible at this stage to compare sequences at the other end of the 1,632-bp homologous region. However, a similar pattern of homologous and divergent regions is seen within the putative *c*I genes of the two phages (2, 21). This is suggestive of a common cassette containing the integration region (*attP* and *int*) and possibly *c*I in BK5-T, Tuc2009, and φLC3. Despite these similarities in the integration region, DNA from φLC3 hybridizes with the type phage P335 whereas BK5-T DNA does not (4). Moreover, phage TP901-1, which contains an integration system different from those of BK5-T, φLC3, and Tuc2009, also belongs to the P335 group of phages (4). Two different types of DNA-packaging systems have also been identified in temperate lactococcal bacteriophages. The genomes of BK5-T and φLC3 contains cohesive ends, whereas Tuc2009 and TP901-1 contain a *pac* site and package DNA into the phage heads by a headful mechanism. Further comparison of nucleotide sequence data from these phages is necessary to clarify the evolutionary and functional relationships between them.

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REFERENCES

1. **Argos, P., A. Landy, K. Abremski, J. B. Egan, E. Haggard-Ljungquist, R. H. Hoess, M. L. Kahn, B. Kalionis, S. V. L. Narayana, L. S. Pierson III, N. Sternberg, and J. M. Leong.** 1986. The integrase family of site-specific recombinases: regional similarities and global diversity. EMBO J. **5:**433–440.
2. **Boyce, J. D., B. E. Davidson, and A. J. Hillier.** 1995. Identification of prophage genes expressed in lysogens of the *Lactococcus lactis* bacteriophage BK5-T. Appl. Environ. Microbiol. **61:**4099–4104.
3. **Boyce, J. D., B. E. Davidson, and A. J. Hillier.** 1995. Sequence analysis of the *Lactococcus lactis* temperate bacteriophage BK5-T and demonstration that the phage DNA has cohesive ends. Appl. Environ. Microbiol. **61:**4089–4098.
4. **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.
5. **Chu, C., R. Kariyama, L. Daneo-Moore, and G. D. Shockman.** 1992. Cloning and sequence analysis of the muramidase-2 gene from *Enterococcus hirae*. J. Bacteriol. **174:**1619–1625.
6. **Court, D., and A. B. Oppenheim.** 1983. Phage lambda's accessory genes, p. 251–277. *In* R. W. Hendrix, J. W. Roberts, F. W. Stahl, and R. A. Weisberg (ed.), Lambda II. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
7. **Davidson, B. E., I. B. Powell, and A. J. Hillier.** 1990. Temperate bacteriophages and lysogeny in lactic acid bacteria. FEMS Microbiol. Rev. **87:**79–90.
8. **Garcia, E., J. L. Garcia, P. Garcia, A. Arraras, J. M. Sanchez-Puelles, and R. Lopez.** 1988. Molecular evolution of lytic enzymes of *Streptococcus pneumoniae* and its bacteriophages. Proc. Natl. Acad. Sci. USA **85:**914–918.

9. **Huggins, A. R., and W. E. Sandine.** 1977. Incidence and properties of temperate bacteriophages induced from lactic streptococci. Appl. Environ. Microbiol. **33:**184–191.
10. **Kawazu, T., Y. Nakanishi, N. Uozumi, T. Sasaki, H. Yamagata, N. Tsukagoshi, and S. Udaka.** 1987. Cloning and nucleotide sequence of the gene coding for enzymatically active fragments of the *Bacillus polymyxa* β-amylase. J. Bacteriol. **169:**1564–1570.
11. **Lakshmidevi, G., B. E. Davidson, and A. J. Hillier.** 1988. Circular permutation of the genome of a temperate bacteriophage from *Streptococcus cremoris* BK5. Appl. Environ. Microbiol. **54:**1039–1045.
12. **Lemaire, M., and P. Beguin.** 1993. Nucleotide sequence of the *celG* gene of *Clostridium thermocellum* and characterization of its product, endoglucanase CelG. J. Bacteriol. **175:**3353–3360.
13. **Lillehaug, D., and N. Birkeland.** 1993. Characterization of genetic elements required for site-specific integration of the temperate lactococcal bacteriophage ϕLC3 and construction of integration-negative ϕLC3 mutants. J. Bacteriol. **175:**1745–1755.
14. **Ochman, H., A. S. Gerber, and D. L. Hartl.** 1988. Genetic applications of an inverse polymerase chain reaction. Genetics **120:**621–623.
15. **Pearson, W. R., and D. J. Lipman.** 1988. Improved tools for biological sequence comparison. Proc. Natl. Acad. Sci. USA **85:**2444–2448.
16. **Platteeuw, C., and W. M. de Vos.** 1992. Location, characterization and expression of lytic enzyme-encoding gene, *lytA*, of *Lactococcus lactis* bacteriophage ϕUS3. Gene **118:**115–120.
17. **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.
18. **Reisner, A. H., and N. H. Westwood.** 1982. Assessment of similarities of pairs and groups of proteins using transformed amino-acid-residue data. J. Mol. Evol. **18:**240–250.
19. **Schouler, C., S. D. Ehrlich, and M. Chopin.** 1994. Sequence and organization of the lactococcal prolate-headed bIL67 phage genome. Microbiology **140:** 3061–3069.
20. **van de Guchte, M., C. Daly, G. F. Fitzgerald, and E. K. Arendt.** 1994. Identification of *int* and *attP* on the genome of lactococcal bacteriophage Tuc2009 and their use for site-specific plasmid integration in the chromosome of Tuc2009-resistant *Lactococcus lactis* MG1363. Appl. Environ. Microbiol. **60:**2324–2329.
21. **van de Guchte, M., C. Daly, G. F. Fitzgerald, and E. K. Arendt.** 1994. Identification of the putative repressor-encoding gene *cI* of the temperate lactococcal bacteriophage Tuc2009. Gene **144:**93–95.
22. **Wilhelm, M., and C. P. Hollenberg.** 1985. Nucleotide sequence of the *Bacillus subtilis* xylose isomerase gene: extensive homology between the *Bacillus* and *Escherichia coli* enzyme. Nucleic Acids Res. **13:**5717–5722.