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Identification of a RecA Homolog ($RecA_{LP}$) on the Conjugative Lactococcal Phage Resistance Plasmid pNP40: Evidence of a Role for Chromosomally Encoded $RecA_{L}$ in Abortive Infection

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The determinants for two bacteriophage resistance mechanisms, AbiE and AbiF, are separated by approximately 3,300 nucleotides on the lactococcal plasmid pNP40 (P. Garvey, G. F. Fitzgerald, and C. Hill, Appl. Environ. Microbiol. 61:4321–4328, 1995). DNA sequence analysis of the intervening region led to the identification of two open reading frames (ORFs) which are transcribed in the opposite direction to the Abi determinants. One of these ORFs encodes a *recA* homolog (designated *$recA_{LP}$*). This is the first report of a *recA*-like determinant located to a plasmid. The second ORF (*orfU*) shares homology with the *umuC* gene of the SOS response. Analysis of a number of lactococcal strains confirmed the presence of *$recA_{LP}$*-like sequences in at least two other lactococcal strains. The proximity of the *recA* and *umuC* homologs suggested a possible role in the phage resistance encoded by the Abi determinants. However, no evidence was obtained to demonstrate a function for either ORF in the expression of either AbiE or AbiF. Nor could the *$recA_{LP}$* gene restore resistance to mitomycin in a *recA*-deficient lactococcal strain, VEL1122. Interestingly, it was shown that the chromosomally encoded *recA* is necessary for complete expression of the AbiF phenotype, confirming a role for RecA in this abortive infection system.

Lactococci typically harbor between 6 and 10 plasmid species, representing up to 10% of the total genetic complement of the cell (4). The determinants for many industrially important traits are located on plasmids, and consequently, a considerable amount of research has been devoted both to the functions they encode and the genes involved in plasmid replication, maintenance, and mobility. Plasmid pNP40 is a 65-kb molecule, originally identified in *Lactococcus lactis* subsp. *lactis* bv. diacetylactis DRC3 (23). This plasmid encodes resistance to the industrially significant antimicrobial agent nisin, in addition to bacteriophage resistance (9). A practical application resulting from the analysis of the nisin resistance determinant has been the generation of a food-grade cloning vector. The identification of the nisin resistance gene and an adjacent origin of plasmid replication allowed Froseth and McKay (10) to exploit this region to generate a vector by using the nisin resistance as a selectable marker.

pNP40 is also conjugative, which has allowed it to be transferred between lactococcal strains (23). The plasmid conferred a high degree of phage resistance when it was introduced into the industrial strain *L. lactis* subsp. *lactis* bv. diacetylactis 425A, which had previously been sensitive to attack (15). This insensitivity has been shown to be due to the presence of at least three distinct phage resistance systems, two of which operate by abortive infection mechanisms (AbiE and AbiF) (11) and the third of which inhibits phage DNA injection (12). pNP40 also carries at least three insertion sequence elements, two copies of ISS*1* and one of IS*981*.

Sequence analysis of the determinants for AbiE and AbiF showed that two open reading frames (ORFs) were required for expression of AbiE whereas a single ORF, 3.3 kb downstream, encoded AbiF (11). This report describes the characterization of two ORFs located between the determinants for AbiE and AbiF. One of these, based on amino acid sequence homology, codes for a RecA homolog (designated $RecA_{LP}$), and the second codes for a protein which shares homology with UmuC-like proteins. In gram-negative bacteria, RecA has been implicated in phage resistance; for example, the *sulA* gene product, which contributes to an abortive infection mechanism in *Vibrio cholerae*, has been reported to be cleaved by RecA (3), and it has been suggested that in *Escherichia coli* LexA may be involved in the regulation of an *abi* gene (14). The possible involvement of $RecA_{LP}$, OrfU, and the chromosomally encoded $RecA_{L}$ in the phenotypic expression of AbiE and AbiF was investigated.

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MATERIALS AND METHODS

Bacterial strains, plasmids, and culture conditions. Lactococcal cultures were grown at 30°C in M17 medium supplemented with 0.5% glucose or lactose as appropriate (32). *E. coli* cultures were propagated in Luria-Bertani broth and incubated at 37°C (29). M13 phage was propagated as outlined by Sambrook et al. (29). Plasmids pPG01, pPG09 (AbiE), pPG23 (AbiF), and pCG1 (AbiF, OrfU, and $RecA_{LP}$) were maintained in lactococci (strains PG001, PG009, PG023, and PG020, respectively [11]) with chloramphenicol at 10 μg/ml.

Plasmid DNA preparation. The lysis procedure of Anderson and McKay (1) was used to isolate plasmid DNA from lactococcal strains. *E. coli* plasmid DNA was obtained by the method of Birnboim and Doly (2), and large-scale preparations were purified by cesium chloride-ethidium bromide density gradient ultracentrifugation in a Beckman VTi65 rotor.

Restriction endonucleases and molecular cloning techniques. Restriction enzymes, the Klenow fragment of DNA polymerase I, and T4 DNA ligase were obtained from Boehringer Corp., Dublin, Ireland. DNA digestions and ligations were performed as outlined by Sambrook et al. (29). DNA fragments were isolated from agarose gels with the Gene Clean Kit II (BIO 101, La Jolla, Calif.).

Electroporation of bacteria. Electroporation of lactococcal strains was performed by the procedure of Holo and Nes (16) with the Gene Pulser apparatus (Bio-Rad Corp., Richmond, Calif.). *E. coli* transformations were performed under the conditions outlined in the Bio-Rad manual.

Nucleotide sequence analysis. Relevant DNA fragments were cloned in M13mp18 and M13mp19 vectors (34). The nucleotide sequence was determined by using both single-stranded M13 clones and alkali-denatured pPG01 and pCG1 (11) templates and the Sequenase 2.0 kit (U.S. Biochemical, Cleveland, Ohio) or the *Taq* Track sequencing system (Promega Corp., Madison, Wis.). Sequencing

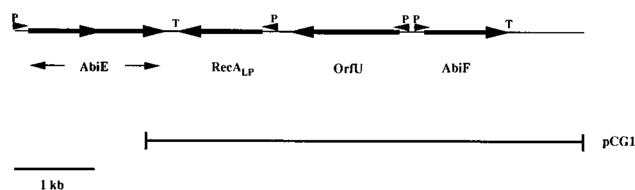

FIG. 1. Genetic organization of the 7.2-kb region of pNP40 showing the determinants for AbiE, AbiF, RecA$_{LP}$, and OrfU. The direction of the arrows denotes the direction of transcription. The locations of putative promoters (P) and terminators (T) are indicated. The region encompassed by the recombinant plasmid pCG1 is depicted below.

was initiated with commercial M13 primers and continued with specific synthetic 17-mer primers prepared with a DNA synthesizer (PCR-MATE; Applied Biosystems, Foster City, Calif.). Each strand of DNA was sequenced at least once. Sequencing gels were run as outlined by Bio-Rad. Sequence data were analyzed with the Gene Jockey, DNAstar database (Apple Computers Inc., Cupertino, Calif.) and Blastp software.

Detection of *recA* genes in lactococcal strains by PCR. Lactococcal cultures were grown for 14 to 16 h in M17 medium supplemented with 0.5% glucose or lactose as appropriate. A 1.5-ml volume of culture was centrifuged at 15,000 × *g* for 5 min and resuspended in Ringer's solution (Merck, Darmstadt, Germany) before the centrifugation was repeated. The cells were finally resuspended in 1 ml of Ringer's solution and subjected to lysis with the 'shake-it-baby' cell disrupter (Biospec Products, Bartleville, Okla.) for 7 min in the presence of glass beads (diameter, 106 μm; Sigma Corp., Poole, United Kingdom). The glass beads were sedimented by centrifugation, and 5 μl of supernatant was used as the template in the PCR. We chose 17-mer primers which were specific for the lactococcal chromosomal *recA*$_L$ gene (6) (forward primer, 5′ CGTGATAAAGCA TTGGC 3′; reverse primer, 5′ AAAGCTGTAGTTTCTTC 3′) and the pNP40-encoded *recA*$_{LP}$ gene (forward primer, 5′ TTAGCTATTCTCAAAGC 3′; reverse primer, 5′ ACTCCAAGTTGAAGTGC 3′). Reactions were performed with the Promega *Taq* polymerase system as specified by the manufacturer. The Hybaid Omnigene PCR system was programmed as follows: template DNA was denatured for 4 min at 94°C followed by 30 cycles of 94°C for 1 min, 50°C for 1 min, and 72°C for 1 min.

RNA isolation and analysis. Total RNA was isolated by the method of Keilhauer et al. (18) from early-exponential-phase cultures of *L. lactis* in M17 medium supplemented with 0.5% glucose. RNA blots were made, as described by Sambrook et al. (29), by filtration of 10 μg of DNase-treated total RNA on a Hybond-N$^+$ nylon membrane (Amersham, Little Chalfont, United Kingdom). Synthetic oligonucleotides specific for the *recA*$_{LP}$, *orfU*, and *abiF* genes were constructed as follows: *recA*$_{LP}$, 5′ TTATTTAAGAACAAGCCACTCATGGTA GAG 3′; *orfU*, 5′ GGTTATGTACCAGGAACGGAAGTTCTTTGG 3′; and *abiF*, 5′ TGTAGGTTTGATTTGGC 3′. The oligonucleotides were end labelled with ^{32}P by using polynucleotide kinase and used to probe the RNA blot in 0.5 M phosphate buffer (pH 7.0) containing 5% sodium dodecyl sulfate at 55°C.

Nucleotide sequence accession number. The sequences reported in this manuscript have been deposited with GenBank and are available under accession number U36837.

RESULTS

DNA sequence analysis of the region between *abiF* and *abiE*. A 7.2-kb region of pNP40 was previously shown to encode two distinct abortive infection mechanisms, AbiE and AbiF (11). In the present study, the DNA sequence of the 3.3 kb of DNA located between *abiE* and *abiF* was determined, revealing the presence of two ORFs transcribed in reverse orientation relative to the phage resistance genes (Fig. 1). One of these ORFs was found to code for a *recA* homolog based on DNA sequence homology. It is 1,023 bp long and has the capacity to encode a protein of 341 amino acids (aa) with a predicted molecular mass of 37.2 kDa (Fig. 2). This corresponds favorably to the sizes of known RecA proteins (24). A putative ribosome-binding site (RBS) (AAAGGAG) with a ΔG value of -16.2 kcal/mol complementary to the 16S rRNA of gram-positive and gram-negative bacteria was found 6 bp upstream of the ATG start codon. A putative -10 sequence (AATAAT) was identified 99 bp upstream of the RBS and was separated by 19 bp from a -35 sequence (TTGTAG), each of which partially resembled the -10 and -35 sequences (TATAAT and TTG ACA, respectively) of the consensus lactococcal promoter (33). However, no TG dinucleotide was located upstream of the -10 sequence as has been identified in the promoters of many lactococcal genes (5). A 14-bp inverted repeat with a ΔG value of -25.2 kcal/mol, 1 bp downstream of the ochre stop codon, has the potential to form a hairpin loop structure and thus could serve as a transcriptional terminator. The overall G+C content is 36%, which is comparable to the 37% average for lactococcal genes (33). This plasmid-encoded *recA* was designated *recA*$_{LP}$ (for lactococcal plasmid) to distinguish it from the chromosomally located lactococcal *recA* (*recA*$_L$) identified by Duwat et al. (6).

The second, larger ORF, designated *orfU* (Fig. 2), is located 400 bp upstream of the ATG start codon of *recA*$_{LP}$. It is 1,464 bp long and has the capacity to encode a protein with a predicted molecular mass of 55.9 kDa. A putative RBS (GGAGG) with a ΔG value of -14.4 kcal/mol was found 5 bp upstream of the ATG start codon. A consensus -10 sequence (TATAAT) was identified 37 bp upstream of the RBS and was separated by 17 bp from a -35 sequence (TTGATT). In this case, a TG dinucleotide was observed immediately preceding the -10 sequence. No obvious transcriptional terminator was identified after the opal stop codon. The overall G+C content of this ORF is 35%, which is marginally lower than the 37% average for lactococcal genes.

Amino acid sequence analysis. Comparison of the deduced amino acid sequence of RecA$_{LP}$ with those of the RecA proteins of *E. coli* and four gram-positive bacteria demonstrated 40 to 46% identity and up to 89% conservation overall (Fig. 3). The RecAs of *Streptococcus pneumoniae* (22) and *Bacillus subtilis* (21) showed the highest level of identity and conservation, respectively, with RecA$_{LP}$. Homology was as low as 18% at the termini, increasing to 60% in the protein core. In contrast to the complete amino acid sequence identity shared by the three lactococcal RecAs identified by Duwat et al. (6), RecA$_{LP}$ showed only 45% identity and 86% conservation with these proteins.

In recent years, the RecA proteins of more than 50 bacteria, both gram positive and gram negative, have been analyzed at the DNA sequence level. Comparison of their deduced amino acid sequences has revealed regions which are highly conserved and thus are believed to be functionally important. All sequences examined to date have contained a 9-aa RecA signature motif. A nonapeptide (A-L-K-F-Y-S-S-V-R) which conforms to this consensus sequence (A-L-K-F-F/Y-S/T/A-S/T/A-V-R) is located from aa 225 to 233 on RecA$_{LP}$ (Fig. 3). In addition, a P-loop motif (G-A-E-S-S-G-K-T) conforming to the consensus ATP-binding motif (G/A-x-x-x-x-G-K-T/S) found in all RecAs is located from aa 80 to 87.

Studies by Story et al. (31) on the RecA protein of *E. coli* have suggested a model for its structure in vivo which identifies two motifs or loops (L1 and L2) believed to be responsible for double-stranded and single-stranded DNA binding, respectively. L1 extends from aa 157 to 164 and L2 extends from aa 195 to 209 in the *E. coli* RecA. While L1 and L2, as expected, are conserved in RecA$_L$, these regions show considerable disparity in RecA$_{LP}$. In region L1, there appears to be a 3-aa deletion in the plasmid-encoded version, while only 7 of the 15 amino acids in L2 are identical to the consensus sequence (Fig. 3).

A comparison of the deduced amino acid sequence of OrfU with known sequences in the Swiss and PIR and translated protein databases by using the DNAstar (DNAstar Inc., Madison, Wis.) and Blastp software indicated that it displays 22% identity and up to 62% conservation with respect to UmuC-like proteins of *E. coli* and *Salmonella typhimurium*. UmuC is one of two proteins encoded by the *umuDC* operon, which forms part of the SOS regulon. These operons are LexA reg-

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   1                                      AGTAACCTTTACTACACTATTTTATTGTAAAAAAAAAGTCTTTC
  45 CATGATGGCGCTTGTTTTAAAAACAGAGGCGTGGGAAGTACTGATATATATATTAAAACATATTTTAGTTTGAAATTCA
 124 AGAGATTTTAATTAAAAATAGTTTTTTTTACTGGAAAAGTTTAAATTCCAAACGTTTTACTATTTATTCTGATATTAT
 203 ATATTGATTTTAGGACTTTTTCAATGTATAATATCATTTAAACAGATATTTTGTGTTTGATATACTACTGGAGGTGCAA
         -35                *  -10                                           RBS
     Met Gly Ile Gln Ile Leu Asn Asn Gln Phe Asp Tyr Ser Leu Glu Pro Arg Arg Ala Ile     20
 282 ATG GGA ATA CAA ATA CTA AAT AAC CAA TTT GAC TAT TCA CTT GAA CCT CGT CGA GCT ATC
     Phe Phe Glu Asp Val Lys Ser Asn Tyr Ala Ser Ile Glu Cys Ile Glu Arg Gly Leu Asn     40
 343 TTT TTT GAA GAT GTT AAA TCT AAT TAC GCT TCA ATT GAA TGT ATT GAA CGT GGG TTA AAT
     Pro Leu Thr Thr Ser Leu Cys Val Met Ser Arg Ala Asp Asn Ser Asn Gly Leu Thr Leu     60
 403 CCC CTG ACT ACT TCT CTT TGT GTA ATG AGT AGA GCT GAT AAT TCA AAT GGC TTA ACA CTT
     Ala Ala Ser Pro Thr Phe Lys Lys Val Phe Gly Met Ser Asn Val Ser His Ser Lys Glu     80
 463 GCT GCT AGT CCA ACT TTC AAG AAA GTA TTT GGA ATG TCT AAT GTT AGT CAT TCC AAA GAA
     Leu Pro Phe Leu Val His Asn Arg Lys Phe Asn Tyr Arg Leu Trp Tyr Lys Lys His Thr    100
 523 CTT CCG TTC CTG GTA CAT AAC CGT AAA TTT AAC TAT CGG CTA TGG TAC AAA AAA CAT ACA
     Asp Ile Phe Gly Gln Thr Val Glu Pro Asp Pro Lys Tyr Ile Ser Glu Val Glu Arg Trp    120
 583 GAT ATT TTT GGA CAG ACT GTA GAA CCT GAT CCA AAA TAT ATT TCT GAA GTT GAA CGT TGG
     Ala Arg Gln Thr Tyr Ile Val Pro Pro Gln Met Leu Leu Tyr Ile Lys Lys Asn Leu Glu    140
 643 GCA AGA CAA ACT TAT ATT GTT CCT CCT CAA ATG CTG CTA TAT ATC AAA AAA AAT TTA GAA
     Val Ile Asn Ile Leu Arg Glu Ile Thr Ser Ile Asp Glu Ile His Ala Tyr Ser Ile Asp    160
 703 GTA ATC AAT ATT TTG AGA GAA ATT ACC TCT ATA GAT GAA ATC CAT GCT TAC TCT ATA GAT
     Glu Ser Cys Leu Asp Val Thr Glu Ser Leu Asp Phe Phe Phe Pro Glu Ile Thr Asn Thr    180
 763 GAA TCC TGT TTA GAT GTT ACC GAA TCT TTG GAC TTC TTC TTT CCT GAA ATT ACT AAT ACA
     Tyr Glu Gln Met Asp Lys Leu Ala Gln Met Leu Gln Arg Lys Ile Tyr His Lys Thr Gly    200
 823 TAC GAA CAA ATG GAT AAG TTA GCT CAA ATG CTG CAG CGT AAA ATT TAT CAT AAA ACT GGC
     Leu Tyr Val Thr Ile Gly Met Gly Asp Asn Pro Leu Leu Ala Lys Leu Ala Met Asp Asn    220
 883 TTA TAT GTG ACA ATT GGA ATG GGA GAC AAT CCA CTT CTC GCA AAA CTT GCA ATG GAT AAT
     Tyr Ala Lys His Asn Thr Asn Met Arg Ala Leu Ile Arg Tyr Glu Asp Val Pro Ser Lys    240
 943 TAT GCT AAA CAT AAT ACC AAC ATG AGA GCC TTG ATT CGC TAT GAA GAT GTT CCC TCT AAG
     Val Trp Ser Ile Ser Asp Met Thr Asp Phe Trp Gly Ile Asn Val Arg Thr Glu Ala Arg    260
1003 GTG TGG TCA ATC TCT GAT ATG ACT GAC TTT TGG GGT ATT AAT GTA AGA ACT GAA GCA CGT
     Leu Asn Lys Leu Gly Ile His Ser Ile Lys Glu Leu Ala His Ala Asp Pro Asp Met Leu    280
1063 TTA AAT AAG TTG GGG ATT CAT TCA ATA AAA GAG CTT GCT CAT GCT GAC CCT GAT ATG TTA
     Lys Arg Glu Leu Gly Val Ile Gly Leu Gln Gln Phe Phe His Ala Asn Gly Ile Asp Glu    300
1123 AAG CGT GAA TTA GGA GTG ATT GGG CTT CAA CAA TTT TTT CAT GCT AAC GGA ATT GAT GAA
     Thr Arg Leu Thr Asp Lys Tyr Lys Arg Lys Ser Val Ser Phe Ser Asn Ser Gln Thr Leu    320
1183 ACA CGT TTA ACT GAC AAG TAT AAA AGG AAA TCT GTC AGT TTC TCA AAT AGT CAA ACC CTA
     Pro Arg Asp Tyr Thr Arg Lys Ser Glu Ile Gly Leu Ile Ile Asn Glu Met Ala Glu Gln    340
1243 CCT AGA GAT TAT ACA CGT AAA TCG GAA ATA GGA TTG ATA ATA AAT GAA ATG GCT GAA CAA
     Val Ala Val Arg Leu Arg Lys Ser Lys Lys Lys Ala Thr Asn Phe Ser Leu Phe Val Gly    360
1303 GTT GCT GTG AGA CTA AGA AAA TCA AAG AAA AAA GCG ACA AAT TTT TCG CTC TTT GTG GGA
     Phe Ser Met Ala Asp Tyr Lys Lys Ser Leu Ser Val Ser Arg Lys Ile Glu Pro Thr Ser    380
1363 TTT TCA ATG GCT GAT TAT AAA AAG TCA CTC TCA GTT TCT AGA AAG ATT GAA CCA ACT AGC
     Ser Thr Lys Asp Leu Gln Glu Ile Ala Thr Arg Leu Phe Asn Glu Lys Tyr Asp Glu Gly    400
1423 TCT ACT AAA GAT TTA CAA GAA ATT GCT ACC AGA CTA TTT AAT GAA AAA TAT GAT GAA GGC
     Ala Val Arg Arg Ile Gly Val Ser Ala Asn Asn Leu Ile Asp Glu Pro Tyr Gln Leu Ile    420
1483 GCA GTT AGA CGG ATT GGG GTT AGT GCC AAT AAT CTG ATA GAC GAA CCT TAT CAA CTT ATT
     Ser Leu Phe Asp Ser Asp Glu Glu Asn Glu Glu Thr Ile Lys Gln Lys Lys Asp Glu Ala    440
1543 TCA CTC TTT GAT TCT GAT GAA GAA AAC GAA GAA ACA ATT AAA CAA AAA AAG GAC GAA GCT
     Val Gln Glu Ala Leu Asp Ser Ile Arg Gln Lys Tyr His Phe Val Ser Val Gln Lys Ala    460
1603 GTG CAA GAA GCA CTT GAC TCG ATT CGT CAA AAA TAT CAC TTT GTT TCT GTT CAA AAA GCA
     Thr Val Leu Lys Lys Gly Ser Arg Ala Val Ala Arg Ser Lys Met Val Gly Gly His Ser    480
1663 ACT GTT CTT AAG AAA GGG TCA CGT GCA GTT GCA AGA AGC AAA ATG GTA GGA GGA CAC TCT
     Ala Gly Gly Leu Glu Gly Leu Asn Stop                                               488
1723 GCA GGT GGA TTG GAG GGC TTG AAT TGA GTAGTGTTGACAGGTCTTATAGTAAATATGAATCAATAAGAAC
1793 GTATGAAGATAGAGGGAAAATGAAATGAATCCTTTTGCGACTCCGAACTTCTTCAGCTCATCGTGATTATCATAAAGAA
1872 TTTACCTTTGAAGAGCCAGATTTTTCTTTGGAACAAGATGAGATTCTTACCATGATTTCTTTCGCAAAGAGCCTCAGCT
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FIG. 2. DNA sequence of the 3.3-kb region of pNP40. The amino acid sequences of OrfU and $RecA_{LP}$ are specified by the three-letter code designation. Putative RBSs and −10 and −35 sequences are underlined. Arrows indicate the putative transcriptional terminator. The asterisk denotes the start of the putative LexA-binding site.

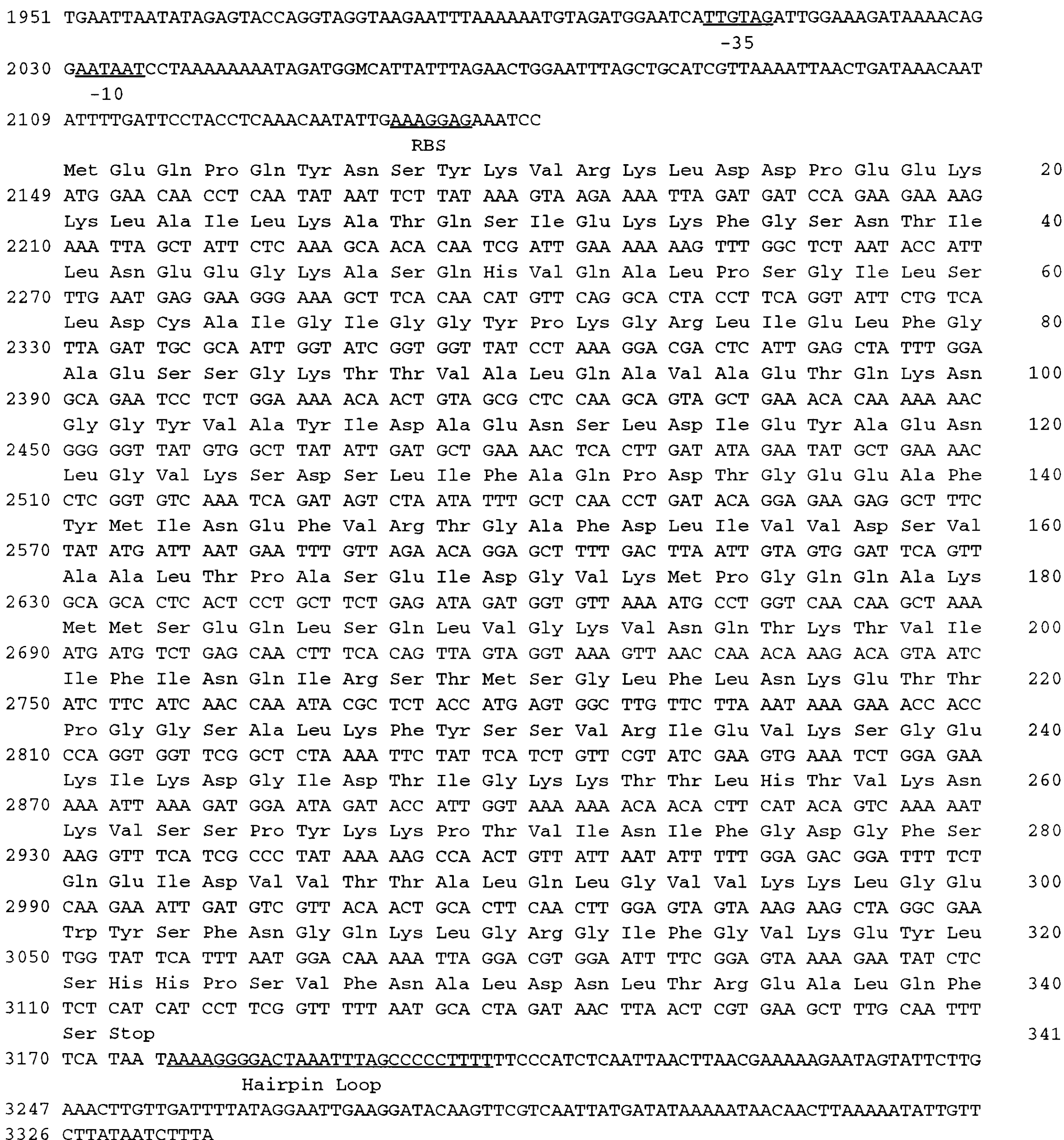

FIG. 2—*Continued.*

ulated and are always preceded by SOS boxes for LexA binding. A search of the promoter regions of *recA*$_{LP}$ and *orfU* for sequences resembling the consensus *E. coli* LexA binding site (t a C T G T a t a t a a a a a C A G t a) was performed. A sequence, which partially agrees with this motif, is located within the putative promoter of *orfU* (Fig. 2).

Complementation studies. *L. lactis* subsp. *lactis* VEL1122 is a RecA-deficient derivative of MG1363 generated by Duwat and Gruss (7) by replacement recombination. To assess if RecA$_{LP}$ had the ability to complement the RecA mutation, a recombinant plasmid, pCG1, containing a fragment of pNP40 encoding the *recA*$_{LP}$ gene (Fig. 1) was introduced into VEL1122, generating the PG030. The activity of RecA can be assessed in a number of ways; the coprotease function, for example, can be investigated by inducing DNA damage by exposure to mitomycin. As expected, VEL1122 failed to produce colonies when plated on GM17 containing 50 ng of mitomycin per ml, whereas the Rec$^+$ strain MG1363 grew as normal. Although evidence which confirmed that the *recA*$_{LP}$ and *orfU* genes are transcribed from pCG1 was obtained (Fig. 4), strain PG030 also failed to produce colonies on GM17 containing 50 ng of mitomycin per ml, demonstrating that RecA$_{LP}$ was unable to induce the SOS response and was therefore deficient in coprotease activity (coprotease activity refers to the ability of activated RecA to facilitate autoproteolysis of a number of proteins, leading to the SOS response).

RecA plays a role in the AbiF phenotype. The proximity of *recA*$_{LP}$ to the phage resistance determinants on pNP40 and the

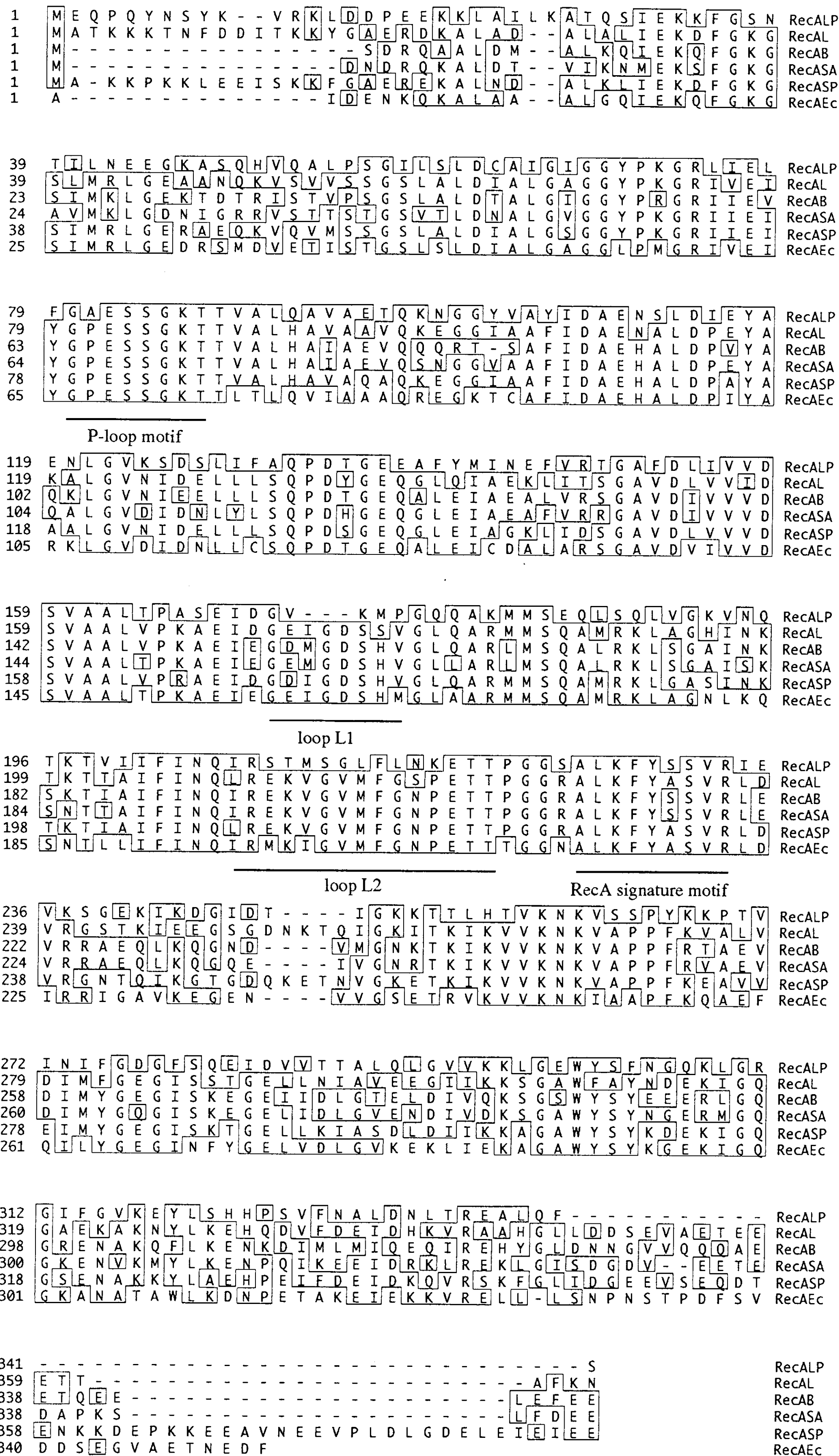

FIG. 3. Amino acid sequence comparison of $RecA_{LP}$ with RecA from *Lactococcus lactis* ($RecA_L$), *Bacillus subtilis* ($RecA_B$), *Staphylococcus aureus* ($RecA_{SA}$), *Streptococcus pneumoniae* ($RecA_{SP}$), and *E. coli* ($RecA_{Ec}$). Boxed areas indicated complete amino acid sequence identity. Regions involved in ATP binding (P-loop), double-stranded DNA binding (loop L1), and single-stranded DNA binding (loop L2) and the RecA signature motif are indicated.

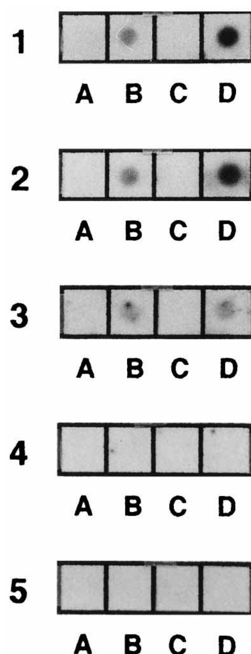

FIG. 4. Evidence of $recA_{LP}$ and *orfU* transcription in MG1614 ($RecA^+$) and VEL1122 ($RecA^-$). Spots represent DNase-treated total RNA from MG1614 harboring either the vector pAM401 (A) or pCG1 (B) and VEL1122 harboring either pAM401 (C) or pCG1 (D). The blots were probed with ^{32}P-end-labelled oligonucleotides specific for the $recA_{LP}$ (panel 1), *orfU* (panel 2), or *abiF* (panel 3) genes. Negative controls consist of RNase-treated total RNA hybridized with the $recA_{LP}$ (panel 4) and *abiF* (panel 5) probes.

involvement of RecA in abortive infection in other bacteria prompted an assessment of the phenotypic expression of AbiE and AbiF in the RecA-deficient host. AbiE was previously shown to confer resistance to the small isometric-headed phage φ712, while AbiF exhibits resistance to both φ712 and the prolate-headed phage φc2. Following introduction of the phage resistance genes into VEL1122, the efficiencies of plaque formation of phages φc2 and φ712 were evaluated. The results presented in Table 1 show that while the expression of AbiE is independent of $RecA_L$, the resistance conferred by AbiF against φc2 and φ712 is significantly reduced in VEL1122 relative to that conferred in MG1363. Interestingly, the pNP40-encoded $recA_{LP}$ gene was unable to complement the function performed by $RecA_L$ in phage resistance.

Distribution of $recA_{LP}$ in lactococci. The $recA_{LP}$ gene has not been previously described in lactococci, and thus it was of interest to determine whether this particular determinant is also present in other strains. Two sets of primers which would specifically amplify either $recA_L$ or $recA_{LP}$ sequences were designed and used in a PCR assay against total DNA from *L. lactis* subsp. *lactis* bv. diacetylactis DRC3 (the parent strain harboring pNP40) and nine other wild-type lactococcal strains (Table 2). Three strains, including DRC3, gave rise to products of the expected size with the $recA_{LP}$-specific primers. Interestingly, no PCR products were obtained for these three strains with the $recA_L$ primer pair. Conversely, PCR products corresponding to the $recA_L$ gene were obtained as expected for five strains, including *L. lactis* subsp. *lactis* ML3 (the strain from which $recA_L$ was originally sequenced) and its closely related strains 712, 952, and C2 (20). The strains positive for the $recA_L$ sequence were invariably negative for $recA_{LP}$. Furthermore, two additional strains (*L. lactis* subsp. *lactis* HO2 and *L. lactis* subsp. *lactis* bv. diacetylactis 18-16) were negative for both the

TABLE 1. Efficiency of plaque formation and plaque sizes of phages φc2 and φ712 on Rec^+ and Rec^- *L. lactis* subsp. *lactis* MG1363 strains expressing AbiE and AbiF phenotypes[a]

Resistance	Rec^+		Rec^-	
	EOP	Plaque size (mm)	EOP	Plaque size (mm)
φc2				
None	1.0	2–3	1.0	2–3
AbiE (pPG09)	1.0	2–3	1.0	2–3
AbiF (pPG23)	3.0×10^{-4}	0.3–1	1.1×10^{-1}	0.3–1
AbiF + $RecA_{LP}$ + OrfU (pCG1)	3.0×10^{-4}	0.3–1	1.1×10^{-1}	0.3–1
pNP40	NP		ND	
φ712				
None	1.0	1	1.0	1
AbiE (pPG09)	3.0×10^{-4}	0.5–1	7.0×10^{-4}	0.5–1
AbiF (pPG23)	$<10^{-9}$		1.0×10^{-5}	0.5
AbiF + $RecA_{LP}$ + OrfU (pCG1)	$<10^{-9}$		2.0×10^{-5}	0.5
pNP40	$<10^{-9}$		ND	

[a] EOP, efficiency of plaque formation; NP, no plaques; ND, not done; Rec^+ host, MG1363 (13); Rec^- host, VEL1122, a derivative of MG1363 generated by Duwat and Gruss (7).

$recA_L$- and $recA_{LP}$-based primers. This suggests that strains possess either $recA_L$ or $recA_{LP}$ but not both. It also suggests that strains HO2 and 18-16 possess yet another *recA* determinant. However, it is important to consider that relatively small changes in DNA sequence could result in a negative response in a PCR-based assay due to primer mismatching, and these preliminary findings await further analysis.

DISCUSSION

The determinants for many important traits have been localized to plasmids in *Lactococcus*. One of the most extensively studied class of plasmids contains those encoding phage resistance, as represented by the conjugative plasmid pNP40. The continued analysis of this plasmid described in this report has led to the identification of a plasmid-encoded *recA* homolog (designated $recA_{LP}$). Despite its proximity to two abortive infection genes, no role in phage resistance could be assigned to the $recA_{LP}$ gene, since neither the AbiF nor the AbiE phenotype was affected by its presence or absence. The function of this determinant remains unclear, given that it is unable to

TABLE 2. Detection of $recA_L$ and $recA_{LP}$ in *L. lactis* strains by PCR

Strain[a]	Detection of:	
	$recA_L$	$recA_{LP}$
LD DRC3	−	+
LL UC317	−	+
LL UC503	−	+
LC UC653	+	−
LL C2	+	−
LL 712	+	−
LL ML3	+	−
LL 952	+	−
LD 18-16	−	−
LL HO2	−	−

[a] LL, *L. lactis* subsp. *lactis*; LC, *L. lactis* subsp. *cremoris*; LD, *L. lactis* subsp. *lactis* bv. diacetylactis.

restore mitomycin resistance in a RecA-deficient host. Nonetheless, it is interesting that the chromosomally encoded RecA does play a role in the AbiF phenotype. This represents the first description in lactococci of the involvement of a generalized host function in abortive phage infection.

RecA is a multifunctional enzyme which plays a pivotal role in the SOS response in *E. coli* and in homologous recombination (for reviews, see references 24 and 28). It has the ability to bind single-stranded and double-stranded DNA simultaneously, which promotes recombination between homologous DNAs with concomitant hydrolysis of ATP. Furthermore, in response to DNA damage, it acts as a coprotease in the cleavage of the LexA repressor, which results in derepression of the determinants for a group of DNA repair proteins known as the SOS regulon. RecA is also required for prophage induction in several bacteria; for example, the *c*I gene product of the λ prophage, which acts as a repressor of prophage induction, is cleaved in the presence of activated *E. coli* RecA. More recently, Duwat and Gruss (7) have proposed additional functions for RecA in response to oxygen and thermal stress in lactococci, suggesting that it plays a general role in the regulation of genes associated with different types of stress.

Until recently, comparatively little was known about RecA in gram-positive bacteria. However, the DNA sequences of *recA* genes from several gram-positive species have now been elucidated, including two from *L. lactis* subsp. *lactis* and one from *L. lactis* subsp. *cremoris* (6). The amino acid sequences of all three lactococcal RecAs were identical, despite minor differences at the DNA level, and were 61 and 56% identical to the RecAs of *Bacillus subtilis* and *E. coli*, respectively. This study represents the first instance in which a *recA* homolog has been located to an extrachromosomal element. In addition, two other lactococcal strains were found to contain $recA_{LP}$-like sequences, although a plasmid location has yet to be confirmed for these. The initial observation, reported here, that PCR assays failed to detect the $recA_{L}$ determinant in a number of lactococcal strains (including those positive for the $recA_{LP}$ sequence) may be the result of an unfortunate choice of primers or may genuinely reflect the absence of this particular gene in these strains. This will be the subject of further investigations in our laboratory.

In the present study, it was found that the chromosomally encoded RecA was essential for full phenotypic expression of AbiF. This is an interesting finding, particularly in the light of previous indications of RecA involvement in phage resistance mechanisms in gram-negative bacteria. There have been suggestions of RecA involvement in phage abortive infection mechanisms in both *V. cholerae* and *E. coli*. RecA has been implicated in phage resistance in *V. cholerae* biotype El Tor hosts, for which it has been suggested that RecA cleaves the *sulA* gene product (3). *sulA* is one of two genes which together are responsible for the abortive response of El Tor hosts to phage infection. There has also been a suggestion that LexA may be involved in the regulation of an *abi* gene encoded by the *E. coli* plasmid Col1b (14).

The homology detected between OrfU and UmuC-like proteins is also interesting. The *umuDC* operon is a component of the error-prone DNA repair pathway in *E. coli* and *S. typhimurium*. It is believed that an activated UmuDC complex helps DNA polymerase III holoenzyme to synthesize past UV- or chemical-induced lesions in DNA, increasing both the mutability and survival of cells following exposure to UV irradiation (27). RecA is intimately involved in this pathway: first, the *umuDC* operon is regulated by LexA and is thus derepressed following RecA-mediated cleavage of LexA (26); second, UmuD is posttranslationally processed to an active form, UmuD* (26); and, finally, RecA is believed to be responsible for targeting the UmuD*C protein complex to DNA, indicating a direct role for RecA in mutagenesis (8). The proximity of $recA_{LP}$ to *orfU* and their similar transcriptional yields in all strains tested (Fig. 4) could be of consequence in the light of the extensive involvement of RecA in the expression and activity of the UmuDC-like proteins.

The *umuDC* operon has been cloned and sequenced from the chromosome of both *E. coli* (27) and *S. typhimurium* (30). In addition, the determinants for three UmuDC homologs, MucAB (27), SamAB (25), and ImpCAB (19), have been identified on three distinct conjugative plasmids in *S. typhimurium*. These molecules show a high degree of homology to the UmuDC proteins at the amino acid sequence level but may have subtly different roles from that of UmuDC in the cell (17). Thus, OrfU may represent a lactococcal homolog of SamB, MucB, and ImpB on the conjugative plasmid pNP40.

The identification of these two new ORFs, $recA_{LP}$ and *orfU*, on pNP40 raises interesting questions about their functionality, particularly whether they play a role in phage resistance which is not evident from our experiments. In any event, the presence of the RecA homolog on the plasmid has led us to the conclusion that chromosomal RecA is involved in the AbiF phenotype. This result has interesting resonances with abortive infection systems in gram-negative bacteria and will be the subject of continued analysis.

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REFERENCES

1. **Anderson, D. G., and L. L. McKay.** 1983. Simple and rapid method for isolating large plasmid DNA from lactic streptococci. Appl. Environ. Microbiol. **46:**549–552.
2. **Birnboim, H. C., and J. Doly.** 1979. A rapid alkaline extraction procedure for screening recombinant plasmid DNA. Nucleic Acids Res. **7:**1513–1523.
3. **Biswas, S. K., R. Chowdury, and J. Das.** 1992. A 14-kilodalton inner membrane protein of *Vibrio cholerae* biotype *el tor* confers resistance to group IV choleraphage infection to classical vibrios. J. Bacteriol. **174:**6221–6229.
4. **Chopin, A.** 1993. Organisation and regulation of genes for amino acid biosynthesis in lactic acid bacteria. FEMS Microbiol. Rev. **12:**21–37.
5. **de Vos, W. M.** 1987. Gene cloning and expression in lactic streptococci. FEMS Microbiol. Rev. **46:**281–295.
6. **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.
7. **Duwat, P., and A. Gruss.** 1994. The *recA* gene of *Lactococcus lactis*: characterization and involvement in oxidative and thermal stress. Mol. Microbiol. **17:**1121–1131.
8. **Frank, E. G., J. Hauser, A. S. Levine, and R. Moodgate.** 1993. Targetting of the UmuD, UmuD′, and MucA′ mutagenesis proteins to DNA by the RecA protein. Proc. Natl. Acad. Sci. USA **90:**8169–8173.
9. **Froseth, B. R., R. E. Herman, and L. L. McKay.** 1988. Cloning of nisin resistance determinant and replication origin on a 7.6-kilobase *Eco*RI fragment of pNP40 from *Streptococcus lactis* ssp. *diacetylactis* DRC3. Appl. Environ. Microbiol. **54:**2136–2139.
10. **Froseth, B. R., and L. L. McKay.** 1991. Development and application of pFM011 as a possible food-grade cloning vector. J. Dairy Sci. **74:**1445–1453.
11. **Garvey, P., G. F. Fitzgerald, and C. Hill.** 1995. Cloning and DNA sequence analysis of two abortive infection phage resistance determinants from the lactococcal plasmid pNP40. Appl. Environ. Microbiol. **61:**4321–4328.
12. **Garvey, P., C. Hill, and G. F. Fitzgerald.** 1996. The lactococcal plasmid pNP40 encodes a third bacteriophage resistance mechanism, one which affects phage DNA penetration. Appl. Environ. Microbiol. **62:**676–679.
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. **Gupta, S. K., and D. J. McCorquodale.** 1988. Nucleotide sequence of a DNA fragment that contains the *abi* gene of the ColIb plasmid. Plasmid **20:**194–206.
15. **Harrington, A., and C. Hill.** 1991. Construction of a bacteriophage-resistant derivative of *Lactococcus lactis* subsp. *lactis* 425A by using the conjugal plasmid pNP40. Appl. Environ. Microbiol. **57:**3405–3409.
16. **Holo, H., and I. F. Nes.** 1989. High frequency transformation by electroporation of *Lactococcus lactis* subsp. *cremoris* strains grown with glycine in osmotically stable media. Appl. Environ. Microbiol. **55:**3119–3123.
17. **Janel-Bintz, R., G. Maenhaut-Michel, and R. P. P. Fuchs.** 1994. MucAB but not UmuDC proteins enhance -2 frameshift mutagenesis induced by N-2-acetylaminofluorene at alternating GC sequences. Mol. Gen. Genet. **245:** 279–285.
18. **Keilhauer, C., L. Eggeling, and H. Sahm.** 1993. Isoleucine synthesis in *Corynebacterium glutamicum*: molecular analysis of the *ilvB-ilvN-ilvC* operon. J. Bacteriol. **175:**5595–5603.
19. **Lodwick, D., D. Owen, and P. Strike.** 1990. DNA sequence analysis of the *imp* UV protection and mutagenesis operon of the plasmid TP110: identification of a third gene. Nucleic Acids Res. **18:**5045–5050.
20. **Lucey, M., C. Daly, and G. F. Fitzgerald.** 1993. Relationship between *Lactococcus lactis* ssp. *lactis* 952, an industrial lactococcal starter culture and strains 712, ML3 and C2. J. Appl. Bacteriol. **75:**326–335.
21. **Marrero, R., and R. E. Yasbin.** 1988. Cloning of the *Bacillus subtilis* $recE^+$ gene and functional expression of $recE^+$ in *B. subtilis*. J. Bacteriol. **170:**335–344.
22. **Martin, B., P. Garcia, M.-P. Castanie, B. Glise, and J.-P. Claverys.** 1994. The *recA* gene of *Streptococcus pneumoniae* is part of a competence-induced operon and controls a SOS regulon, abstr. M51. *In* ASM Conference on Streptococcal Genetics. American Society for Microbiology, Washington, D.C.
23. **McKay, L. L., and K. A. Baldwin.** 1984. Conjugative 40-megadalton plasmid in *Streptococcus lactis* subsp. *diacetylactis* DRC3 is associated with resistance to nisin and bacteriophage. Appl. Environ. Microbiol. **47:**68–74.
24. **Miller, R. V., and T. A. Kokjohn.** 1990. General microbiology of *recA*: environmental and evolutionary significance. Annu. Rev. Microbiol. **44:**365–394.
25. **Nohmi, T., A. Hakura, Y. Nakai, M. Watanabe, S. Y. Murayama, and T. Sofuni.** 1991. *Salmonella typhimurium* has two homologous but different *umuDC* operons: cloning of a new *umuDC*-like operon (*samAB*) present in a 60-megadalton cryptic plasmid of *S. typhimurium*. J. Bacteriol. **173:**1051–1063.
26. **Nohmi, T., J. R. Battista, L. A. Dodson, and G. C. Walker.** 1988. RecA-mediated cleavage activates UmuD for mutagenesis: mechanistic relationship between transcriptional derepression and posttranslational activation. Proc. Natl. Acad. Sci. USA **85:**1816–1820.
27. **Perry, K. L., S. J. Elledge, B. B. Mitchell, L. Marsh, and G. C. Walker.** 1985. *umuDC* and *mucAB* operons whose products are required for UV light- and chemical-induced mutagenesis: UmuD, MucA, and LexA proteins share homology. Proc. Natl. Acad. Sci. USA **82:**4331–4335.
28. **Radding, C. M.** 1991. Helical interactions in homologous pairing and strand exchange driven by RecA protein. J. Biol. Chem. **266:**5355–5358.
29. **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.
30. **Smith, C. M., W. H. Koch, S. B. Franklin, P. L. Foster, T. A. Cebula, and E. Eisenstadt.** 1990. Sequence analysis and mapping of the *Salmonella typhimurium* LT2 *umuDC* operon. J. Bacteriol. **172:**4964–4978.
31. **Story, R. M., I. T. Weber, and T. A. Steitz.** 1992. The structure of the *E. coli* RecA protein monomer and polymer. Nature **355:**318–325.
32. **Terzaghi, B. E., and W. E. Sandine.** 1975. Improved medium for lactic streptococci and their bacteriophages. Appl. Microbiol. **29:**807–813.
33. **van de Guchte, M., J. Kok, and G. Venema.** 1992. Gene expression in *Lactococcus lactis*. FEMS Microbiol. Rev. **88:**73–92.
34. **Yanisch-Perron, C., J. Vieira, and J. Messing.** 1985. Improved M13 phage cloning vectors and host strains: nucleotide sequence analysis of the M13mp18 and pUC19 vectors. Gene **33:**103–119.