

Characterization of *Lactococcus lactis* UV-Sensitive Mutants Obtained by *ISS1* Transposition

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Received 13 February 1997/Accepted 5 May 1997

Studies of cellular responses to DNA-damaging agents, mostly in *Escherichia coli*, have revealed numerous genes and pathways involved in DNA repair. However, other species, particularly those which exist under different environmental conditions than does *E. coli*, may have rather different responses. Here, we identify and characterize genes involved in DNA repair in a gram-positive plant and dairy bacterium, *Lactococcus lactis*. Lactococcal strain MG1363 was mutagenized with transposition vector pG⁺host9::ISS1, and 18 mutants sensitive to mitomycin and UV were isolated at 37°C. DNA sequence analyses allowed the identification of 11 loci and showed that insertions are within genes implicated in DNA metabolism (*polA*, *hexB*, and *deoB*), cell envelope formation (*gerC* and *dltD*), various metabolic pathways (*arcD*, *hglA*, *gidA*, *hgrP*, *metB*, and *proA*), and, for seven mutants, nonidentified open reading frames. Seven mutants were chosen for further characterization. They were shown to be UV sensitive at 30°C (the optimal growth temperature of *L. lactis*); three (*gidA*, *polA*, and *uvs-75*) were affected in their capacity to mediate homologous recombination. Our results indicate that UV resistance of the lactococcal strain can be attributed in part to DNA repair but also suggest that other factors, such as cell envelope composition, may be important in mediating resistance to mutagenic stress.

Exposure of bacteria to DNA-damaging agents such as UV light and oxygen or drugs such as mitomycin (MC) may have deleterious effects on the bacterial cell, particularly on chromosomal structure and replication. In response, bacteria have evolved a diverse array of enzymatic pathways which are specialized in the removal of damaged DNA. Since the current model for prokaryotic DNA repair is based on DNA metabolism of enteric bacteria (principally *Escherichia coli*), further studies of nonenteric microbes will be useful in determining the extent to which repair mechanisms are conserved.

Although numerous DNA repair processes identified in *E. coli* are common to other bacteria, differences have also been noted. Photoreactivation systems are lacking in many bacteria such as *Haemophilus influenzae*, *Streptococcus pneumoniae*, *Bacillus subtilis*, *Deinococcus radiodurans*, and *Neisseria gonorrhoeae* (11, 27). In some bacteria, including enteric bacteria closely related to *E. coli*, an inducible mutagenesis DNA repair response may be lacking (22, 58, 59). The species *D. radiodurans* presents a remarkably high UV resistance, which is attributed to the presence of two UvrABC-like excision repair systems (48). In contrast, no system similar to excision repair has been identified in *Pasteurella haemolytica* (42). Methyl-directed mismatch repair (49) and very-short-patch repair systems may also be different among organisms, since neither adenine nor cytosine methylation is universal (25). These few examples illustrate the considerable variations that exist among DNA repair systems of different bacterial species.

Little is known about DNA repair in *Lactococcus lactis*, a gram-positive plant and dairy microorganism. It was reported that unlike *E. coli*, *L. lactis* has no inducible error-free repair system for alkylation damage (2). Only three functionally conserved genes directly involved in DNA repair have been characterized in *L. lactis*: (i) the *fpg* gene codes for an N-glycosylase, which releases the 8-oxoguanine from oxidatively

damaged DNA (19); (ii) the *rexAB* genes code for the major exonuclease of the cell, which is the functional equivalent of the *E. coli* RecBCD or *B. subtilis* AddAB enzymes (20); and (iii) the *recA* gene encodes a highly conserved protein, which is central to DNA repair pathways (17). RecA is a recombinase (for a review, see reference 54) and, as shown for *E. coli* and many other organisms, a regulator of a set of genes which are principally implicated in DNA repair (for a review, see reference 65). The *recA* gene of *L. lactis* encodes a protein that is 56% identical to the *E. coli* RecA protein (16). As expected, the lactococcal *recA* gene is necessary for DNA recombination, repair after mutagenic stress (17), and induction of lysogenic phage (7). It has also been implicated in the function of an abortive phage infection mechanism (21, 29). We recently found that in *L. lactis*, *recA* is also needed for a full response to oxidative and thermal stress conditions (18). Implication of the *recA* gene in heat shock response has not been reported for other microorganisms and may be an example of species particularity of regulation.

To further characterize the role of *recA* and to identify other genes involved in DNA repair in *L. lactis*, we isolated and characterized DNA damage-sensitive insertional mutants; the affected genes are called *uvs* (for UV sensitive). Seven strains mutated in identified genes (*polA*, *hexB*, *deoB*, *dltD*, *gerC*, and *gidA*) or in a non-identified gene (*uvs-75*) were characterized and are described here.

MATERIALS AND METHODS

Bacteria, cell culture, and plasmids. *E. coli* TG1 (57) [*supE hsd-5 thi Δ(lac-proAB) F' (traD6 proAB⁺ lacI⁹ lacZΔM15)*] and TG1rep (TG1 containing a chromosomal copy of the pWV01 *repA* gene [kindly provided by K. Leenhouts]), and *L. lactis* MG1363 (a derivative of strain ML3 cured of plasmids and prophages [23]), VEL1122 (a *recA* derivative of MG1363 containing a tetracycline resistance [Tc^r] marker [18]), and CL56-5 (erythromycin resistance [Em^r] marker chromosomally integrated in MG1363 [41]) were used in this study. *E. coli* cultures were grown on Luria-Bertani medium (47) at 30 or 37°C as needed, and erythromycin (150 μg/ml) was added as required. *L. lactis* cultures were grown at 30 or 37°C, as specified, in M17glu (M17 medium [62] to which 1% [wt/vol] glucose was added after the medium was autoclaved). Erythromycin (3 μg/ml), streptomycin (150 μg/ml), rifampin (50 μg/ml), chloramphenicol (5 μg/ml), and tetracycline (5 μg/ml) were added as needed for *L. lactis*. Adenine, cytosine,

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guanine, and thymine (15 µg/ml per base) were added, when indicated, for growth of the wild-type (wt) and *deoB* strains in liquid or solid M17glu medium. The plasmids used were (i) theta replicating plasmids pIL253 (Em^r) (60) and pIL205 (Cm^r) (40); (ii) thermosensitive (ts) plasmids pG⁺host5 (Em^r) (44), and pG⁺host9:ISS1 (Em^r) (45), referred to as pGh:IS below; (iii) pHist-IL (Em^r; pG⁺host5 containing a 1.7-kb *EcoRI* fragment of the IL1403 histidine operon) and pHist-MG (Em^r; pG⁺host5 containing the corresponding PCR-amplified 1.7-kb fragment of the MG1363 histidine operon cloned into the *EcoRI* site).

DNA manipulations. Cloning, plasmid and chromosomal DNA extraction, restriction analyses, DNA transformation and Southern hybridizations were performed by published methods (16, 31, 39, 53, 57).

Synthetic oligonucleotides and PCR. Single-stranded DNA oligonucleotide primers complementary to DNA sequences in the histidine operon of IL1403 (13) were synthesized in our laboratory on a Beckman synthesizer. The forward primer was 5'-TGATGAGGATTATTTTAGAATTCTTAGAC-3' (corresponding to published DNA sequence positions 3972 through 4000), and the reverse primer was 5'-CGTAAAAATGACGAATCCAAAT-3' (corresponding to published DNA sequence positions 5721 through 5699). These primers were used to PCR amplify (56) a fragment of the histidine operon of the MG1363 chromosome. Reactions (15 s at 94°C, 15 s at 55°C, and 30 s at 72°C for 30 cycles) were performed on a Perkin-Elmer Cetus 2400 apparatus with the Boehringer *Taq* DNA polymerase as recommended by the manufacturer.

Insertional mutagenesis and isolation of MC- and UV-sensitive mutants. Mutagenesis with pGh:IS was performed as previously described (45). The clones selected on M17glu plus erythromycin at 37°C were picked on M17glu plates and on M17glu plates containing 500 ng of MC per ml. After 2 days of growth, the clones scored as MC sensitive were grown in liquid medium and then streaked on solid medium to test for UV sensitivity.

Characterization of MC- and UV-sensitive mutants. Recovery of chromosomal targets of transposon insertion in the MC- and UV-sensitive clones was performed as described previously (45). Transposon insertion results in integration of the entire plasmid, flanked by duplicate copies of ISS1 (41). The ISS1 flanking sequences were recovered on replicative plasmids by digesting total chromosomal DNA with unique restriction enzymes (*HindIII* and *EcoRI*) and transforming ligated DNA in the TG1 or TG1rep strain (45). The ends of ISS1 flanking sequences were sequenced with DyeTerminator and DyePrimer kits (Applied BioSystems). Sequences were analyzed with BLAST programs (1) at the BLAST server (National Center of Biological Information, Bethesda, Md.). Primers were synthesized as necessary to extend sequences or to sequence two strands of chromosomal DNA fragments recovered by cloning the integrated plasmids. Sequencing reactions were performed with the DyeTerminator kits from Applied BioSystems. The *polA* and *deoB* genes were totally sequenced with cloned chromosomal ISS1 flanking sequences. The sequence of *deoB* was completed by using independently cloned chromosomal ISS1 flanking sequences from insertions in the same gene obtained through an unrelated mutagenesis experiment (19a). Nearly complete gene sequences were determined for *hexB* and *gidA* by sequencing the cloned chromosomal fragments. For all other genes, at least 250 bp on either side of the transposon integration sites was sequenced on both strands. To isolate stable ISS1 mutants, the integrated vector was excised as described previously (45); stable mutants were confirmed by Southern hybridization.

Determination of UV sensitivity and recombination frequencies. For precise determination of UV sensitivity, saturated cultures were diluted at least 1,000-fold. For plate tests, exponentially growing cells (optical density at 600 nm [OD₆₀₀] = 0.3 [maximum]) of MG1363, VEL1122, and mutant strains were plated at several dilutions as needed on M17glu. After being dried for 15 min, the plates were UV irradiated (at 254 nm) with a UV Stratalinker 2400 (Stratagene) at doses ranging from 0 to 50 J/m² (as indicated in Fig. 1 and 3) in a dark room. For the saline test, early-exponential-phase cell cultures (OD₆₀₀ ≈ 0.1) were centrifuged to obtain the equivalent of 1 ml at an OD₆₀₀ of 0.1. Pellets were washed with 1 ml of Ringer's solution, resuspended into 0.5 ml of Ringer's solution, and transferred into 24-well microtiter plates. Irradiation was performed with the UV Stratalinker 2400 at the same doses as indicated in Fig. 1. Sequential dilutions of irradiated cells were plated onto M17glu. For both UV tests, the plates were wrapped and incubated at 30°C. After at least 2 days of growth, UV sensitivity was evaluated by colony counting. Recombination experiments were performed as described previously (6, 18); pHist-MG and pHist-IL were used for homologous and homeologous recombination tests, respectively. Both precise determination of UV sensitivity and recombination tests were each performed at least three times.

Determination of conjugation frequencies. Rif^r and Str^r strains were used as recipient cells. Str^r Rif^r derivatives of mutant strains were isolated by first plating cells on medium containing rifampin. The Rif^r clones were then plated on medium containing select Str^r Rif^r double mutants. Surface mating experiments with the pIL205 self-mobilizable plasmid were performed as described previously (40).

Determination of spontaneous mutagenesis frequencies. Rif^r single colonies were resuspended in 5 ml of M17glu, grown at 30°C to an OD₆₀₀ of 0.5 and plated at 30°C onto M17glu or M17glu plus rifampin. Colony counts were determined after 2 days at 30°C to calculate frequencies of mutations to Rif^r.

Nucleotide sequence accession numbers. The sequences described in Results were entered into GenBank. The accession numbers are as follows: Uvs9,

U80409; Uvs27, U81621; Uvs30, U80411; Uvs42, U78972; Uvs56, U79492; Uvs58, U80412; Uvs61, U82105; Uvs62, U78771; Uvs64, U80410; Uvs68, U78972; Uvs72, U79491; Uvs73, U82817; Uvs75, U80599; Uvs76, U81991; Uvs77, U77490.

RESULTS

Isolation of the UV-sensitive mutants. Approximately 5,000 random transposon insertional mutants of the wt MG1363 strain isolated at 37°C were screened for increased sensitivity to 500 ng of MC per ml. A total of 27 clones were selected and further screened for UV sensitivity, giving rise to 19 confirmed UV-sensitive mutants at 37°C. Southern hybridization analyses of 18 mutants confirmed that ISS1 integrated in single copy at distinct locations in the MG1363 chromosome (data not shown).

Cloning of chromosomal sequences flanking ISS1 insertion sites. Replicative transposition leads to integration of the ts plasmid vector between duplicated ISS1 elements (32, 41). The pGh:IS vector contains unique restriction sites adjacent to the ISS1 element, which were used after transposition to clone the ISS1-flanking chromosomal DNA (45). Chromosomal DNA prepared from the insertional mutant strains was digested with *EcoRI* or *HindIII*, religated, and transformed into *E. coli* TG1 or TG1rep. Of the 38 possible ISS1-flanking sequences, 33 were cloned in this way on the excised pGh:IS vector. Five junctions could not be cloned, possibly due to their toxic effects or to DNA instability in *E. coli*. For each clone, a DNA sequence of 300 to 500 bp of chromosomal proximal DNA (adjacent to ISS1) was used to perform DNA and protein homology comparisons in databases by using the BLAST programs. These analyses allowed us to identify 11 of the target genes, although for 3 of them, homology to known genes was revealed with only one of the two ISS1-flanking sequences (Table 1). The other sequences showed no significant homology to sequences present in databases. Mutants with mutations in the putative genes *polA*, *hexB*, *deoB*, *dltD*, *gerC*, *gidA*, and *uvs-75*, were further analyzed. These mutants were stabilized by excision of the transposed vector (45) (see Materials and Methods); the excised strains were used for all further experiments.

UV sensitivity tests. UV survival curves were generated to estimate the relative sensitivity of mutant strains (Fig. 1). The mutants showed varied responses to UV irradiation. None of them was as sensitive as the VEL1122 *recA* strain. Three of the mutants, with mutations in *polA*, *hexB*, and *deoB*, have increased sensitivity at all tested UV doses compared to the wt strain (Fig. 1A); the other four, with mutations in *dltD*, *gerC*, *gidA* and *uvs-75* (Fig. 1B), were UV sensitive only at high irradiation doses (50 J/m²).

As the UV test was performed on rich media, it was possible that toxic compounds were generated during irradiation. To verify that the death of the *gerC*, *dltD*, and *uvs-75* mutants was really due to UV, irradiation was performed in nonreactive medium (Ringer's solution). The results (not shown) were essentially identical to those observed when the mutants were irradiated on the plates. These results indicate that the mutants showing sensitivity to high UV doses are affected by the UV irradiation itself and not by an intermediate toxic product of the medium. We propose that mutants sensitive to both low- and high-dose UV irradiation may play a direct role in DNA metabolism while those sensitive to only high-dose irradiation may affect the permeability of the cell to UV light.

Homologous recombination test. The UV sensitivity of the strains may be due to an incapacity of the mutants to promote recombination. Homologous recombination was therefore examined by measuring the frequencies of plasmid integration into the chromosome; the plasmid used, pHist-MG, is ts and

TABLE 1. Database homologies determined for *uvs* ORFs

UV ^s mutant ^a	Homology to ^b :	Region of homology ^c	% Amino acid identity	Insertion site ^d	Putative ORF function
Uvs9	<i>Bacillus subtilis</i> <i>gidA</i> (628)	260–550	63	360	Unknown
Uvs27	<i>Bacillus subtilis</i> <i>dltD</i> (392)	33–325	43	126	Maturation of teichoic acid
Uvs30	<i>Sorghum bicolor</i> <i>hgrP</i> (283)	132–254	40	132	Unknown
Uvs58	<i>Treponema pallidum</i> <i>proA</i> (410)	156–432	50	332	Proline biosynthesis
Uvs61	<i>Bacillus subtilis</i> <i>gerCA</i> (348) ^e	147–236	25	147	Isoprene biosynthesis
Uvs62	<i>Streptococcus pneumoniae</i> <i>polA</i> (877)	1–877	68	585	DNA synthesis
Uvs64	<i>Bacillus subtilis</i> <i>deoB</i> (396) ^f	1–395	56	70	Pyrimidine salvage
Uvs68	<i>Bacillus subtilis</i> <i>bglA</i> (479)	95–295	48	204	6-Phospho-β-glucosidase
Uvs73	<i>Streptococcus pneumoniae</i> <i>hexB</i> (649)	442–518	50	442	Mismatch repair
Uvs76	<i>Pseudomonas aeruginosa</i> <i>arcD</i> (482)	2–103	42	–45 ^g	Arginine/ornithine antiport
Uvs77	<i>Saccharomyces cerevisiae</i> <i>metB</i> (445)	125–443	46	301	Methionine biosynthesis

^a Results for Uvs21, Uvs42, Uvs30 (*Hind*III cloned junction), Uvs52, Uvs53, Uvs56, Uvs61 (*Eco*RI cloned junction), and Uvs71, Uvs72, and Uvs76 (*Eco*RI cloned junction) are not included in this table, as database searches did not reveal significant homologies (value superior to 10⁻¹⁰) or the chromosomal junction could not be cloned.

^b Organism, gene name and, in parentheses, deduced length (number of amino acids) of the protein sequence giving the highest homology score by the TBLASTN program. A result is given only if the *p*(*N*) probability is lower than 10⁻¹⁰.

^c The numbers correspond to the amino acid position in the full-length polypeptide of the protein with the greatest homology.

^d The numbers correspond to the deduced amino acid position of the *ISS1*-generated interruption in the full-length polypeptides of the protein with the greatest homology.

^e Translation of distal sequences from the *ISS1* insertion site reveals 33% identity with amino acids 20 to 116 of the GerCC protein from *B. subtilis*.

^f Translation of distal sequences from the *ISS1* insertion site reveals 72% identity with amino acids 1 to 126 of the DeoD protein from *Streptococcus thermophilus*.

^g Insertion of the *ISS1* element occurs 45 bp upstream of the coding sequence.

contains a 1.7-kb chromosomal DNA fragment of the MG1363 histidine operon. We observed that efficiency of pHist-MG integration by homologous recombination was reduced 10- to 300-fold in the *L. lactis* *gidA*, *polA* and *uvs-75* mutants compared to the wt strain (Table 2). It is notable that the *gidA* and *uvs-75* mutant strains are only partially UV sensitive yet are significantly reduced in their capacity to promote homologous recombination.

Detailed mutant analyses. Sequence similarities between the interrupted genes and previously described genes (Table 1) allowed us to assign putative functions and/or gene names to the mutated genes under study. Six of these mutants plus one with unknown function were further analyzed to determine their roles in DNA repair in *L. lactis*.

(i) *polA*. The PolI bacterial DNA polymerases are multifunctional enzymes which generally have polymerase and exonuclease activities. A potential transcription initiation site (TT GAGA-17N-TAAAAA) is found 60 bp upstream of the ATG start of the lactococcal *polA* gene. This potential promoter is surrounded by three different sets of direct repeats, which may suggest that the promoter undergoes transcriptional regulation. Sequence analysis shows that a potential open reading frame (ORF) is present 220 bp upstream of and diverging from *polA*. In *E. coli*, the PolI protein is composed of two different functional domains (10, 35). The C-terminal domain, known as the Klenow fragment, has polymerase activity, as well as a 3'-to-5' exonuclease activity. This latter activity is not present in the *S. pneumoniae* PolI protein (14), and similarities between *L. lactis* PolI and its pneumococcal homolog (43) make it likely that the 3'-to-5' exonuclease activity is also absent from the lactococcal protein. The 500-amino-acid N-terminal domain endows the enzyme with 5'-to-3' exonuclease activity. As this activity has been shown in *E. coli* to be necessary for growth in rich medium (34), and as *ISS1* is inserted in the lactococcal *polA* gene at nucleotide 1755 of the coding sequence (equivalent to protein sequence position 585), we think it likely that the truncated gene produces a protein fragment which retains this 5'-to-3' exonuclease activity.

It was shown that the PolI enzyme in *B. subtilis* is necessary for initiation of replication of theta replicating plasmid pAMβ1 (9). To further study the lactococcal *polA* mutant phenotype, we tested whether it affects pAMβ1 replication in *L. lactis*. The transformation efficiency of the pAMβ1 derivative pIL253 was compared with that of the rolling-circle plasmid pG⁺host5 in a wt strain and in the *polA* mutant. We observed comparable transformation efficiencies for the two types of plasmids in the wt strain. However, no transformants were obtained with 1 μg of pIL253 in the *polA* strain despite efficient transformation of the pG⁺host5 (5 × 10³ transformants per μg). These results confirm that *polA* is necessary for pAMβ1 replication in *L. lactis* and indicate that the *polA* mutant is at least affected in its polymerase activity. Given the properties of the *L. lactis* *polA* mutant described above, we believe that PolI, as in other microorganisms, is a multifunctional enzyme involved in DNA repair processes such as excision repair, in chromosomal replication for the processing of Okazaki fragments, and in homologous recombination.

(ii) *hexB*. The general mismatch repair system, called Hex in *S. pneumoniae* (12) and Mut in *E. coli* (49), acts on recombination intermediates as a DNA replication editor, correcting potentially mutagenic mismatches. Hex and Mut eliminate DNA heteroduplexes containing mismatches during homologous recombination in conjugation or transformation. In *S. pneumoniae* or *E. coli*, inactivation of the Hex or Mut system also confers a mutator phenotype. Isolation of the *hexB* mutant of *L. lactis* as significantly UV sensitive was unexpected, because only slight UV sensitivity was reported for a *hexA* mutant of *S. pneumoniae* (3, 37). Recently, however, *E. coli* *mutS* or *mutL* gene products were shown to be required for transcription-coupled nucleotide excision after UV stress, and mutants were reported to confer moderate UV sensitivity (46). The UV-sensitive phenotype of the *hexB* mutant of *L. lactis* may reflect a similar role for the gene.

We examined whether the *hexB* mutant of *L. lactis* shows increased homeologous recombination and spontaneous mutation rates, as is observed for the analog mutants in *E. coli* and

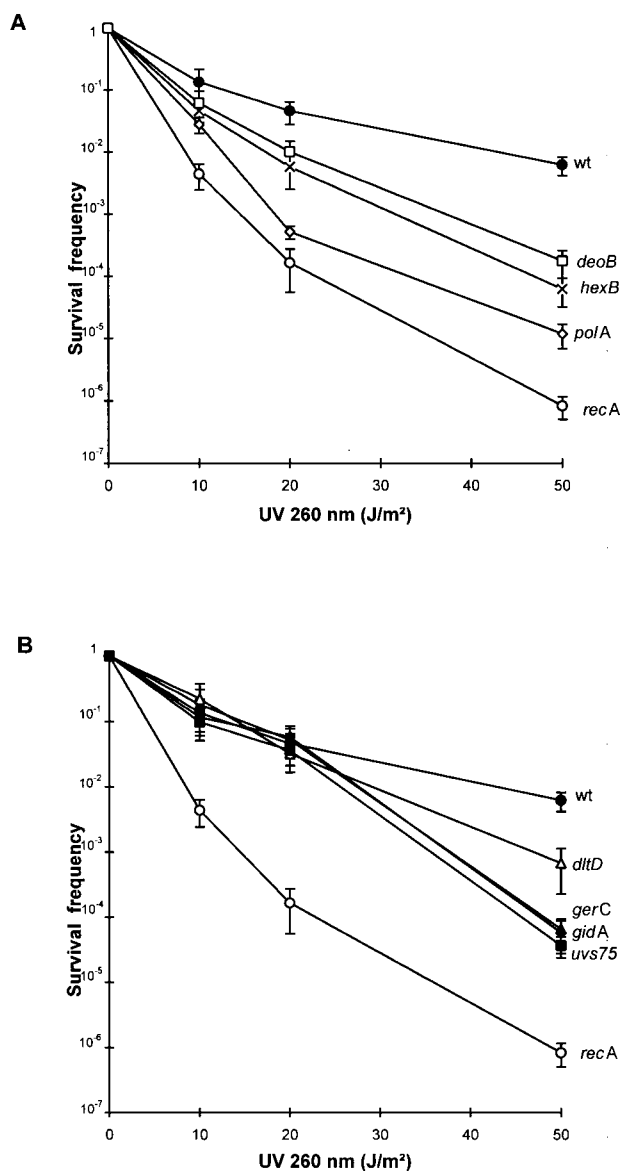


FIG. 1. Resistance to UV irradiation of the MG1363 wt strain, UV-sensitive insertional mutants, and the VEL1122 (*recA*) strain. The results correspond to the mean of at least four independent experiments. Cell cultures were subjected to UV treatments (15) with the indicated doses. (A) ●, wt MG1363; ○, *recA* VEL1122; □, *deoB*; ×, *hexB*; ◇, *polA*. (B) ●, wt MG1363; ○, *recA* VEL1122; ▲, *gidA*; △, *dltD*; ◆, *gerC*; ■, *uvs-75*.

S. pneumoniae. A 1.7-kb fragment of the histidine operon of *L. lactis* subsp. *lactis* IL1403 is 95% identical to the corresponding DNA fragment of MG1363 (data not shown), used above in the plasmid homologous recombination test. Plasmid pHist-IL, containing this DNA fragment, was used to evaluate the effect of mismatches on plasmid integration frequencies in the wt and *hexB* *L. lactis* subsp. *cremoris* strains. Integration of the pHist-MG plasmid occurred at the same frequencies as in the wt strain, indicating that homologous recombination was not affected in the *hexB* strain (Table 3). Homeologous integration of the pHist-IL plasmid is only 100-fold less efficient than homologous integration in the wt strain (Table 3). However, the frequency of integration in the *hexB* strain is about 10 times greater than in the wt strain, indicating that the Hex system in

L. lactis also aborts recombination events between DNA molecules with mismatches. The partial effect of the *hexB* mutation on homeologous recombination may be allele dependent, because ISS1 insertion occurred within the last third of the protein, and may thus result in a truncated peptide with partial activity, as the remaining N-terminal region of HexB presents the highest homology to the MutL protein of *E. coli*. The intermediate phenotype may also be due to the HexA protein, which binds mismatches (33) and may interfere with the strand transfer processes.

The role of HexB in mismatch repair was further confirmed by comparing the spontaneous mutation rates of wt and *hexB* strains, determined by the appearance of Rif^r mutants. The *hexB* mutant exhibits a significant increase in spontaneous mutation frequency (about 130-fold) compared to the wt strain (Table 3).

All these data confirm that a system equivalent to the Hex system of *S. pneumoniae* exists in *L. lactis*. The considerable homeologous recombination that occurs in the wt strain may indicate that, as in *S. pneumoniae*, the Hex system in *L. lactis* can be titrated and hence inactivated if sufficient homeologous DNA is present in the cell.

(iii) *deoB*. The *deo* genes are involved in purine and pyrimidine salvage pathways (50). These genes are necessary for the assimilation of exogenous free bases or nucleosides from the environment and for the reutilization of bases or nucleosides provided by nucleotide turnover. Analysis of the lactococcal sequence allowed us to identify a consensus ribosome binding site (GGAGAGA) and two potential consensus promoters upstream of the translation start of the *deoB* gene. The sequence encoding the C-terminal end of an unidentified protein (67 amino acids), followed by a transcription terminator, is found upstream of *deoB*, and two putative genes, *orfC* and *deoD*, follow *deoB* (Fig. 2). As no terminator and no promoter could be found in the *deoB-orfC* and *orfC-deoD* intergenic regions, we think that *deoB*, *orfC*, and *deoD* are organized in an operon in *L. lactis*. No gene homologous to *orfC* was previously described in the *deoB-deoD* operon of *B. subtilis* or *E. coli*. We believe it likely that transposon insertion in *deoB* leads to inactivation of the entire operon.

deo mutants acquire purine or pyrimidine only by de novo synthesis or by utilization of exogenous bases (26). To test if the UV sensitivity of the *deoB* strain is due to a depletion of the purine and/or pyrimidine pools, we performed UV sensitivity tests with *deoB* cells grown in M17glu medium supplemented with all four bases or not supplemented. Addition of the four bases to the medium restored complete UV resistance to the *deoB* strain (Fig. 3). Based on these observations, we propose that the UV sensitivity of the *deoB* strain is due to a lack of nucleotides and possibly to a diminished capacity to reutilize the bases provided by DNA and RNA degradation following UV stress. It was previously observed that a mutation in a uracil salvage pathway of *B. subtilis* (*upp*) confers a UV-sensitive phenotype (30, 50). We predict that nucleotide addition would also restore UV resistance to that strain and that DNA repair functions are normal in both *B. subtilis upp* and *L. lactis deoB* mutants.

(iv) *dltD*. The DltD enzyme is implicated in cell wall synthesis and is necessary for the esterification of lipoteichoic and cell wall teichoic acids (52). The deduced amino acids sequence from DNA upstream of the putative lactococcal *dltD* gene is highly homologous to the DltB enzyme of *B. subtilis*, encoded by *ipa-4R*. Therefore it is likely that, as in *Lactobacillus casei* and *B. subtilis*, the *dlt* genes of *L. lactis* are organized in an operon.

Inactivation of *dlt* genes in *Lactobacillus casei* affects cell

TABLE 2. Homologous recombination frequencies in *L. lactis* UV-sensitive mutants

Plasmid	Recombination frequency ^a in:								
	wt	<i>recA</i>	<i>deoB</i>	<i>dltD</i>	<i>gerC</i>	<i>hexB</i>	<i>polA</i>	<i>uvs-75</i>	<i>gidA</i>
pG ⁺ host5	7.5×10^{-5}	2.8×10^{-6}	3.5×10^{-5}	4.5×10^{-5}	2.6×10^{-4}	7.3×10^{-5}	2.2×10^{-5}	3.0×10^{-5}	3.1×10^{-5}
pHist-MG	5.6×10^{-2}	3.6×10^{-6}	1.9×10^{-2}	1.0×10^{-2}	4.3×10^{-2}	6.3×10^{-2}	5.5×10^{-3}	1.8×10^{-4}	2.9×10^{-5}

^a Recombination frequencies were measured as the number of Ery^r colonies at the nonpermissive temperature for plasmid replication (37.5°C) divided by the total number of colonies at the permissive temperature (30°C); they correspond to the means of at least three independent experiments. Standard deviations are less than 20% of the given values.

growth and morphology (28). We observed that the *L. lactis* *dltD* mutant grows slowly and forms longer chains than the wt strain (data not shown), suggesting that *dltD* inactivation may affect the organization and structure of the cell wall. The following results support this suggestion: (i) a conjugation test with the self-mobilizable plasmid pIL205 revealed that transfer frequency of pIL205 was 30 times lower in the *dltD* mutant (9.6×10^{-5}) than in the wt strain (2.9×10^{-3}); and (ii) the mutant strain could be made electrocompetent without the usual addition to the medium of glycine, an inhibitor of lactococcal cell wall biosynthesis (data not shown). Thus, phenotypic differences of the *dltD* mutant, which include UV sensitivity, may indicate that the cell wall is altered in this strain and may have a diminished ability to protect the cell from environmental aggressions.

(v) *gerC*. GerC enzymes are involved in the biosynthesis of isoprene, a component of the cell membrane (66). Sequence analysis show that the *ISS1* insertion occurred in an ORF which is homologous to the *B. subtilis* *gerCA* ORF (25% identity and 61% similarity). A second ORF, which is homologous to the *B. subtilis* GerCC protein (33% identity and 55% similarity), was identified 200 bp downstream of the *ISS1* insertion site. The *gerC* locus of *B. subtilis* is organized as an operon containing three *gerC* genes (A, B, and C). We suspect that in *L. lactis*, the *gerC* operon comprises only two genes, *gerCA*, and *gerCC*, because a *gerCB* analog was previously isolated in *L. lactis* (24) on a DNA fragment having no homology overlap with that characterized here. As with *dltD*, we observed that transfer of the self-mobilizable plasmid pIL205 with a *gerC* recipient was less efficient than with a wt recipient (transfer frequency in the *gerC* recipient, 1.3×10^{-4}). Similarly, we explain the UV sensitivity of the *gerC* mutant by an alteration in envelope or membrane organization. To our knowledge, this is the only report of a UV-sensitive phenotype of a *gerC* mutant; this phenotype may be consistent with the capacity of isoprene and isoprene derivatives to absorb UV radiation. It was previously reported that UV radiation affects membrane permeability in *E. coli* (61). Our data suggest that the converse

may also be true. It will also be of interest to determine whether this UV-sensitive phenotype is specific to *L. lactis*.

(vi) *gidA*. *gidA* genes are very well conserved and are generally localized near the chromosomal replication origin (51). However, no clear function has been attributed to their products. In *E. coli*, *gidA* gene inactivation reduces the growth rate and causes filamentation of the cell in medium containing glucose (64). A role for *gidA* in recombination or in UV resistance has not been previously reported. Further characterization, such as complementation experiments, will be necessary to determine whether the observed phenotype is due to the *gidA* mutation itself or to a polar effect of the *ISS1* insertion.

(vii) *uvs-75*. No significant homology could be found between *uvs-75* and known genes or ORFs. However, sequence analysis revealed that the encoded protein contains highly hydrophobic domains (data not shown) and thus is likely to be a membrane protein. In addition, *ISS1*-flanking DNA sequences indicate that *uvs-75* is in an operon (data not shown). Inactivation of *uvs-75* resulted in intermediate UV sensitivity and decreased plasmid recombination compared to those in the wt strain. Analysis of downstream sequences revealed an ORF transcribed in the direction opposite to that of *uvs-75*. The ORF is homologous to the sequences encoding eucaryotic proteins implicated in DNA repair, NucR (63) and Htf9C (8). We consider it possible that the phenotypes of the *uvs-75* mutant are due to a polar effect of the *ISS1* insertion on the expression of the downstream gene.

DISCUSSION

In the present study, we isolated MC- and UV-sensitive insertional mutants of *L. lactis*. All the mutants are independent, and *ISS1* flanking-sequence data allowed us to identify 11 of the 19 target genes. Of the remaining mutated genes, four lack homology with known genes and four were not studied here. Of the seven stabilized mutant strains presented and characterized here, all are sensitive to high doses of UV (50 J/m²) but only three (the *polA*, *hexB*, and *deoB* mutants) are also sensitive to low UV doses. Homologous recombination (as tested by plasmid recombination) is affected only in *polA*, *gidA* and *uvs-75* mutants.

Three classes of mutations are discerned. The first class (*hexB*, *polA*, and *deoB*) corresponds to expected mutations affecting DNA metabolism. The second class (*gerC* and *dltD*

TABLE 3. Effect of the *hexB* mutation on homeologous recombination and spontaneous mutation frequencies

Strain	Recombination frequency ^a in:			Mutagenesis frequency ^b
	pG ⁺ host5	pHist-MG	pHist-IL	
wt	7.5×10^{-5}	5.6×10^{-2}	5.8×10^{-4}	3.2×10^{-7}
<i>hexB</i>	7.3×10^{-5}	6.3×10^{-2}	4.6×10^{-3}	4.3×10^{-5}

^a Recombination frequencies were measured as in Table 2 and correspond to the means of at least three independent experiments. Standard deviations are less than 20% of the given values.

^b Spontaneous mutation frequencies were measured as the number of Rif^r colonies divided by the total number of colonies on nonselective medium and correspond to the means of at least three independent experiments. Standard deviations are less than 35% of the given values.

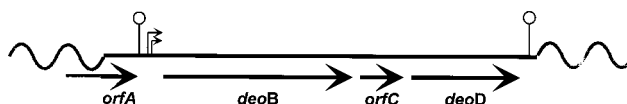


FIG. 2. Structure of the chromosomal region containing the putative *deoB* operon. Arrows correspond to ORFs. Grey lines, unsequenced; black line, sequenced. Potential promoters and terminators are marked as \blacktriangle and \odot , respectively.

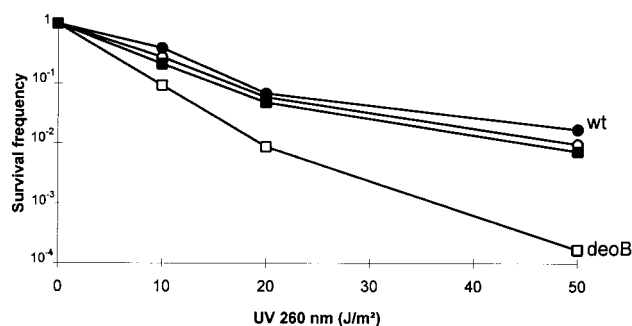


FIG. 3. Effect of nucleoside addition on the UV resistance of MG1363 and the *deoB* mutant. Cell cultures were subjected to UV treatments at the indicated doses. Results correspond to the mean of at least four independent experiments. Standard deviation were not indicated but are less than 70% of the values. Symbols: ○, ●, wt MG1363 strain; □, ■, *deoB* mutant; ○, □, growth in M17glu; ●, ■, growth in M17glu supplemented with the four bases (15 μ g of each base/ml).

mutants) presumably affects cell envelope organization; mutations in this class of genes were not previously described as conferring UV sensitivity. As both of these genes are part of an operon, the UV-sensitive phenotype may also involve downstream genes because of a possible polar effect of the *ISS1* insertion. Our results concerning this class are consistent with previous observations that envelope integrity and DNA repair capacities are somehow related. For example, (i) after SOS induction in *E. coli*, the majority of the RecA protein is found associated with the membrane (36); (ii) chemical modification of membrane lipids may induce the SOS response (4); (iii) in *E. coli* *recA* or *lexA* mutants, membrane permeability is modified (38); and (iv) during the *E. coli* SOS response, septum formation is inhibited, showing that cell division arrest is associated with repair (65). It remains to be tested whether *gerC* and *dltD* mutants of *E. coli* or *B. subtilis* are UV sensitive. A third class, composed of the genes not studied further, including *arcD* (a component of an arginine/ornithine antiporter), *bglA* (encoding a 6-phospho- β -glucosidase), *hgrP* (unknown function), *metB* (coding for a protein involved in methionine metabolism), *proA* (coding for a protein involved in proline metabolism) and *gidA*, corresponds to genes with no known link to UV resistance. It has been observed that in *E. coli*, mutations affecting sugar metabolism or transcription may modify recombination frequencies (5, 55) and that phosphate starvation induces some SOS genes (15). Our data indicate that alterations in general metabolism or the energy level of the cell may change its DNA repair capacities.

Only a few genes directly involved in DNA repair were identified in this mutagenesis. We do not have an explanation for this result. However, stress pathways seem to be more interactive in *L. lactis* than in *E. coli*, so that stresses present during the screening, such as oxygen or temperature, may affect the selection. For example, the UV-sensitive *recA* mutant survives poorly in the presence of either oxygen or elevated temperature (18). This may explain why we did not isolate certain mutants in the present study. It will be interesting to perform the same type of mutagenesis under anaerobic conditions or at 30°C to determine whether more genes directly involved in DNA repair are obtained.

The results presented here reveal elements in the DNA damage response which have not been previously described. It remains to be determined whether the roles of the identified genes in DNA repair are particular to *L. lactis*. Furthermore, characterization of gene promoters (such as that of *polA*) and

of the nonidentified mutants will be important in unraveling the regulation of DNA damage response in *L. lactis*.

ACKNOWLEDGMENTS

We thank J.-P. Claverys, J. Roth, and our team colleagues for stimulating discussions. We especially thank S. Sourice for her invaluable help in DNA sequencing.

This work was supported in part by European Biotech program BIOT-CT94-3055.

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