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Functional Characterization of Excision Repair and RecA-Dependent Recombinational DNA Repair in *Campylobacter jejuni*▽

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The presence and functionality of DNA repair mechanisms in *Campylobacter jejuni* are largely unknown. In silico analysis of the complete translated genome of *C. jejuni* NCTC 11168 suggests the presence of genes involved in methyl-directed mismatch repair (MMR), nucleotide excision repair, base excision repair (BER), and recombinational repair. To assess the functionality of these putative repair mechanisms in *C. jejuni*, *mutS*, *uvrB*, *ung*, and *recA* knockout mutants were constructed and analyzed for their ability to repair spontaneous point mutations, UV irradiation-induced DNA damage, and nicked DNA. Inactivation of the different putative DNA repair genes did not alter the spontaneous mutation frequency. Disruption of the UvrB and RecA orthologues, but not the putative MutS or Ung proteins, resulted in a significant reduction in viability after exposure to UV irradiation. Assays performed with uracil-containing plasmid DNA showed that the putative uracil-DNA glycosylase (Ung) protein, important for initiation of the BER pathway, is also functional in *C. jejuni*. Inactivation of *recA* also resulted in a loss of natural transformation. Overall, the data indicate that *C. jejuni* has multiple functional DNA repair systems that may protect against DNA damage and limit the generation of genetic diversity. On the other hand, the apparent absence of a functional MMR pathway may enhance the frequency of on-and-off switching of phase variable genes typical for *C. jejuni* and may contribute to the genetic heterogeneity of the *C. jejuni* population.

The gram-negative, microaerophilic bacterium *Campylobacter jejuni* is one of the most frequent causes of human bacterial gastroenteritis worldwide (7). Infections with *C. jejuni* are also associated with the development of a paralyzing neuropathy, the Guillain-Barré syndrome (64). *C. jejuni* can be isolated from various sources, including the chicken intestine and surface water (38). At the population level, *C. jejuni* is genetically highly diverse (11, 60, 62), which may facilitate bacterial environmental adaptation. Genetic diversity in *C. jejuni* is generated via horizontal gene transfer (9, 10, 51), intragenomic rearrangements (9), and the presence of numerous stretches of nucleotide repeats that are prone to mispairing during DNA replication (26, 41, 42, 46). In addition, the genomic DNA is probably subject to various types of damage caused by a range of endogenous and environmental factors which may cause single- or double-strand breaks, nucleotide modifications, abasic sites, bulky adducts, and mismatches (14). Virtually all bacteria have evolved more or less sophisticated DNA repair mechanisms to limit the detrimental effects of DNA damage and to maintain the structure and genetic integrity of their DNA (16). The importance of DNA repair for the survival and genetic diversity of *C. jejuni*, however, is still largely unknown.

Bacterial DNA repair mechanisms can be divided into three classes, namely, direct repair, excision repair, and recombinational repair (14). Direct repair involves the reversal of the mutagenic event without the need for synthesis of a new phosphodiester bond. During excision repair, DNA abnormalities are removed and repaired using the intact strand as a template. Recombinational repair involves the reversal of DNA abnormalities via homologous recombination. In contrast to direct repair, DNA repair by excision and recombination does require synthesis of new phosphodiester bonds (56). The focus of the current work is on the presence of the latter two repair mechanisms in *C. jejuni*.

Most knowledge of excision and recombinational DNA repair processes comes from studies of *Escherichia coli*. In *E. coli*, methyl-directed mismatch repair (MMR) is operating at the level of excision repair. MMR repairs replication errors that arise from misincorporations (mismatches) and strand slippage (frameshift errors). In addition, MMR inhibits recombination between homologous sequences (47). During MMR, MutS recognizes and binds to replication errors and, together with MutL, activates MutH. This protein cleaves the unmethylated daughter strand at hemimethylated GATC sequences. Part of the daughter strand with the mutation is excised by single-strand nucleases, and the gap is repaired (25, 37). A second excision repair mechanism of *E. coli* is nucleotide excision repair (NER). NER detects and repairs conformational

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TABLE 1. Bacterial strains used in this study

Bacterial strain	Relevant genotype or phenotype[a]	Source or reference
C. jejuni parental strains		
NCTC 11168	Sequenced strain; naturally competent	Sanger Institute
NCTC 11168-O	Original clinical isolate; naturally competent	18
2412	Chicken isolate; naturally competent	29
2535	Chicken isolate; naturally competent	29
C. jejuni mutant strains		
11168*mutS*::*cat*	*cat* inserted in *mutS*; Cm^r	This study
11168*uvrB*::*cat*	*cat* inserted in *uvrB*; Cm^r	This study
11168*ung*::*cat*	*cat* inserted in *ung*; Cm^r	This study
11168*recA*::*cat*	*cat* inserted in *recA*; Cm^r	This study
2412*mutS*::*cat*	*cat* inserted in *mutS*; Cm^r	This study
2412*uvrB*::*cat*	*cat* inserted in *uvrB*; Cm^r	This study
2412*ung*::*cat*	*cat* inserted in *ung*; Cm^r	This study
2535*mutS*::*cat*	*cat* inserted in *mutS*; Cm^r	This study
2535*uvrB*::*cat*	*cat* inserted in *uvrB*; Cm^r	This study
2535*ung*::*cat*	*cat* inserted in *ung*; Cm^r	This study
2535*recA*::*cat*	*cat* inserted in *recA*; Cm^r	This study
2535*htrA*::*aphA-3*	*aphA-3* inserted in *htrA*; Km^r	9
E. coli strains		
DH5α	F^- ϕ80*lacZ*ΔM15 Δ(*lacZYA-argF*)*U169 recA1 endA1 hsdR17*(r_K^- m_K^+) *phoA supE44* λ^- *thi-1 gyrA96 relA1*	Invitrogen, Breda, The Netherlands
CJ236	FΔ(HindIII)::*cat* (Tra^+ Pil^+ Cam^r)/*ung-1 relA1 dut-1 thi-1 spoT1 mcrA*	New England Biolabs, Ipswich, MA
Top10F′	F′{*lacI*^{q} Tn*10* (Tet^r)} *mcrA* Δ(*mrr-hsdRMS-mcrBC*) ϕ80*lacZ*ΔM15 Δ*lacX74 recA1 araD139* Δ(*ara-leu*)*7697 galU galK rpsL endA1 nupG*	Invitrogen
XL-1 Blue	*recA1 endA1 gyrA96 thi-1 hsdR17 supE44 relA1 lac* [F′ *proAB lacI*^{q}*ZΔM15* Tn*10* (Tet^r)]	Stratagene, La Jolla, CA

[a] Cm^r, chloramphenicol resistance; Km^r, kanamycin resistance; Tet^r, tetracycline resistance.

changes present in DNA. Major components of the NER pathway are the UvrABC proteins. The UvrA and UvrB proteins form the damage recognition complex. After binding to the DNA, UvrB forms a stable complex with the damaged DNA (UvrB-DNA) and UvrA dissociates. UvrC binds to the UvrB-DNA complex, and incisions are made, thereby excising the damaged DNA as a 12- or 13-nucleotide-long oligomer. The resulting gap is repaired using the undamaged strand as a template (55). The third excision repair mechanism of *E. coli* is base excision repair (BER). This system detects and repairs modified bases. Different glycosylases, such as the uracil-DNA glycosylase Ung, are involved in the recognition of specific DNA alterations. These enzymes remove damaged bases from the DNA by cleavage of *N*-glycosylic bonds, leaving an apurinic or apyrimidinic site (AP site). An AP endonuclease (XthA) is necessary for cleavage of the phosphodiester bond, and the remaining deoxyribose phosphate moiety is removed by a deoxyribose phosphodiesterase (RecJ) after which the gap in the DNA is repaired (49). The recombinational repair mechanism of *E. coli* is involved in the repair of stalled or collapsed replication forks caused by conformational changes resulting from unrepaired mutations (8). When nicks or other lesions are present in the DNA, *E. coli* RecA binds to the damaged DNA and catalyzes recombinational repair via double-strand break repair or daughter strand gap repair (35).

The subset and specificity of DNA repair mechanisms differ between species (1). The goal of this study was to decipher the presence and functionality of three excision repair mechanisms (MMR, NER, and BER) and RecA-dependent recombinational repair in *C. jejuni*. Using a set of genetically defined mutants, we present evidence that recombinational repair and the NER system, but not the MMR pathway, are functional in *C. jejuni*. In addition, proof was obtained that *C. jejuni* has a functional Ung protein involved in the BER pathway.

MATERIALS AND METHODS

In silico analysis of the *C. jejuni* genome. The published genome sequence of *C. jejuni* NCTC 11168 (22, 42) was searched for orthologues of proteins involved in excision repair (MMR, NER, and BER) and RecA-dependent recombinational DNA repair of *E. coli* using the Blastp algorithm (protein-protein BLAST; http://blast.ncbi.nlm.nih.gov/Blast.cgi). Genes were assumed not to be present in *C. jejuni* when the probability values of the first hit obtained were ≥1. The overall similarities were determined by performing pairwise alignments with EMBOSS-Align (http://www.ebi.ac.uk/Tools/emboss/align/) covering the full-length sequences.

Bacterial strains and growth conditions. The bacterial strains used in this study are listed in Table 1. *C. jejuni* was routinely grown on heart infusion sheep blood agar plates (HIS plates) or on blood agar base no. 2 medium (Oxoid) supplemented with 4% sheep blood lysed with 0.7% saponin (saponin plates). Bacteria were grown at 37°C for 48 h under microaerobic conditions (6% O_2, 7% CO_2, 7% H_2, and 80% N_2) created with an Anoxomat system (Mart Microbiology B.V., Drachten, The Netherlands). *E. coli* was grown on Luria-Bertani (LB) agar or in LB broth at 37°C for 24 h under aerobic conditions. When appropriate, media were supplemented with ampicillin (100 μg ml^{-1}), chloramphenicol (12.5 μg ml^{-1}), kanamycin (30 μg ml^{-1}), nalidixic acid (100 μg ml^{-1}), or tetracycline (12 μg ml^{-1}).

Electrotransformation of *C. jejuni*. Electrotransformation of *C. jejuni* was essentially performed as described previously (61). In short, *C. jejuni* was harvested from 25 HIS plates after 16 h of growth, suspended in distilled water containing 15% glycerol and 272 mM sucrose (2 ml per plate; 4°C), and then centrifuged (3,500 × *g*, 8 min, 4°C). The bacteria were rinsed three times with glycerol-sucrose (with 20 ml the first time, 10 ml the second time, and 1 ml the third time), and after each wash, the bacteria were collected by centrifugation. Finally, the bacteria were suspended in glycerol-sucrose at approximately 10^{11}

TABLE 2. Primers used in this study

Primer[a]	Sequence (5′–3′)[b]
MutS-F	TTTTTGCAAGAGATAAGGAGATTT
MutS-R	GTCGGTTTTGCCACACTAA
UvrB-F	CAGCGAATTTAAACCCAGTCC
UvrB-R	CTTTCGCTAGCAGGCATTTT
Ung-F	GGAAGAAATTACAATAAACATAG
Ung-R	CCAATCTATAGGAATTTTACC
RecA-F	TTGGAAAAGGCACTATT
RecA-R	TATTCTTCTCCTTCGTCAT
UngSacII	CATATGCCGCGGCGGAGATTTAAGCTCTTGGG
UngMfeI	GTCGACCAATTGCTTTTTGCTGGAGTGATG
Cat-F	CACAACGCCGGAAACAAG
Cat-R	CCGCAGGACGCACTACTCT
CatSacII	CATATGCCGCGGCACAACGCCGGAAACAAG
CatMfeI	GTCGACCAATTGCCGCAGGACGCACTACTCT

[a] The forward (F) and reverse (R) primers are indicated by the suffix at the end of the primer name.
[b] The endonuclease restriction sites introduced are shown underlined.

bacteria ml^{-1} (optical density at 600 nm of 80). Aliquots (50 μl) were stored at −80°C, and for electrotransformation, 5 μl of the solution containing isolated plasmid DNA was added to the electrocompetent bacteria. The mixture was transferred into a 0.2-cm Gene Pulser cuvette and pulsed (2.48 kV, 25 μF, 600 Ω) with a Gene Pulser (Bio-Rad Hercules, CA). After recovery on nonselective saponin plates (5 h, 37°C, microaerobic conditions), the bacteria were harvested and plated onto selective saponin plates.

Construction of *C. jejuni* knockout mutants. For gene inactivation, the (putative) *mutS* (Cj1052c), *uvrB* (Cj0680c), *ung* (Cj0086c), and *recA* (Cj1673c) orthologues of *C. jejuni* NCTC 11168 were amplified by PCR using the primers listed in Table 2; the four fragments obtained from the four genes (1,893 bp, 1,847 bp, 681 bp, and 972 bp, respectively) were cloned into pCR2.1-TOPO (*mutS uvrB recA*) or pCR2.1 (*ung*), resulting in pCRMutS, pUB, pCRRecA, and pUng, respectively. Next, *mutS* and *recA* from pCRMutS and pCRRecA were subcloned as EcoRI fragments into pBluescript, resulting in pBM and pBR, respectively (Table 3). Inactivation of the genes was achieved by insertion of the chloramphenicol acetyltransferase gene (*cat*; chloramphenicol resistance [Cm^r]) from plasmid pUOA23 (Table 3) of *Campylobacter coli*. In this study, the constructs pBM, pUB, and pBR were then linearized using BclI, BsgI, and BglII, respectively. Overhanging ends were made blunt using Klenow fragment (BclI and BglII) or T4 DNA polymerase (BsgI). In order to insert *cat* into *ung*, the complete construct pUng was amplified using primers UngSacII and UngMfeI, thereby introducing restriction sites for SacII and MfeI in the *ung* coding sequence. The *cat* gene was amplified from pUOA23 with primers Cat-F and Cat-R or CatSacII and CatMfeI, yielding a fragment of 1.0 kb. Ligation of the linearized blunt-ended constructs (pBM, pUB, and pBr) with the *cat* fragment, amplified with Cat-F and Cat-R, resulted in the pBM::cat, pUB::cat, and pBR::cat plasmids, respectively. To disrupt *ung* with *cat*, the PCR products of pUng, amplified with primers UngSacII and UngMfeI, and *cat*, amplified with primers CatSacII and CatMfeI, were digested with SacII and MfeI upon which the *cat* fragment was ligated with pUng, yielding pUng::cat. Finally, the vectors pBM::cat, pUB::cat, pBR::cat, and pUng::cat, which do not replicate in *C. jejuni*, were introduced in the *C. jejuni* strains NCTC 11168-O, 2412, and 2535 by electrotransformation (Table 1). Homologous allelic exchange was verified by PCR using chromosomal DNA as the template with primers complementary to sequences of the disrupted genes upstream and downstream of the *cat* insert.

Determination of nalidixic acid resistance. The frequency of nalidixic acid resistance (Nal^r) was determined by suspending *C. jejuni*, after 16 h of growth on HIS plates, in 1 ml of heart infusion broth (HI broth). The optical density (at 600 nm) was measured and adjusted to the lowest value measured for the parental and corresponding mutant strains. The number of CFU ml^{-1} of the bacterial suspension was determined by spotting 10-μl amounts of serial dilutions (10^{-5} to 10^{-9}) onto nonselective HIS plates (track dilution [30]). To determine the number of Nal^r bacteria ml^{-1}, a total of 500 μl of the undiluted bacterial suspension was plated onto three selective HIS plates containing nalidixic acid (100 μg ml^{-1}). The number of bacterial colonies was counted after 72 h of incubation, and the spontaneous mutation frequency was defined as the ratio of CFU on the plates containing nalidixic acid versus those on the nonselective media. All tests were performed in triplicate. Student's *t* test was used for statistical analysis.

To assess the influence of preexisting mutations on the Nal^r frequencies, Nal^r frequencies were determined in triplicate for nalidixic acid-sensitive single colonies of *C. jejuni* strain NCTC 11168-O and the *mutS* knockout mutant. In comparison with frequencies obtained for the whole population, no significant differences in resistance frequencies were found ($P > 0.1$), excluding a bias in our experiments by preexisting nalidixic acid-resistant mutants.

UV resistance assay. To subject the bacteria to UV stress, *C. jejuni* bacteria grown on a HIS plate (16 h) were suspended in HI broth to an optical density (at 600 nm) of 0.12. Two hundred microliters of this suspension in a 24-well cell culture plate was exposed to UV light of 312 nm created with an UV cross-linker (BioLink BLX-312; Vilber Lourma, France). All strains were irradiated with a dose of 0.12 J cm^{-2}. In a separate experiment, the *recA* mutants and their parental strains were irradiated with a dose of 1.0 J cm^{-2}. Of the UV-exposed and non-UV-exposed bacterial suspensions, 10-μl portions of serial dilutions (10^0 to 10^{-7}) were spotted onto HIS plates. After 72 h of growth, the number of CFU ml^{-1} was determined, and the reduction in CFU ml^{-1} after UV exposure was expressed as $\log_{10}$ values. All tests were performed in triplicate. Student's *t* test was used for statistical analysis.

Determination of the natural transformation frequency. For natural transformation of *C. jejuni*, the biphasic method was used (59). *C. jejuni* bacteria grown on HIS plates (16 h) were suspended in HI broth to an optical density (at 600 nm) of 1.0. Two hundred microliters of this suspension was added to a 5-ml polystyrene tube containing 2 ml of HI agar. After 3 h of incubation at 37°C under microaerobic conditions, 2 μg of chromosomal DNA was added. The chromosomal DNA used was isolated from *C. jejuni* strain 2535*htrA*::*aphA-3* (Table 1), containing a kanamycin resistance (Km^r) gene (*aphA-3*) in *htrA* (Cj1228c, serine protease), using the Puregene DNA isolation kit (Gentra Sys-

TABLE 3. Plasmids used in this study

Plasmid	Relevant characteristic(s)[a]	Source or reference
pUOA23	*E. coli*/*C. jejuni* shuttle vector containing *cat* of *C. coli*; Cm^r	52
pHipO::tet	*C. jejuni* suicide vector with *tet* inserted in *hipO*; Tet^r	P. de Boer
pCR2.1 TOPO	TA cloning vector; Amp^r Km^r; *lacZ′*	Invitrogen
pCR2.1	TA cloning vector; Amp^r Km^r; *lacZ′*	Invitrogen
pBluescript	Phagemid vector; Amp^r; *lacZ′*	Stratagene
pCRMutS	pCR2.1 TOPO containing part of *mutS* of *C. jejuni* strain NCTC 11168	This study
pUB	pCR2.1 TOPO containing part of *uvrB* of *C. jejuni* strain NCTC 11168	This study
pCRRecA	pCR2.1 TOPO containing part of *recA* of *C. jejuni* strain NCTC 11168	This study
pUng	pCR2.1 containing part of *ung* of *C. jejuni* strain NCTC 11168	This study
pBM	pBluescript containing EcoRI fragment of pCRMutS	This study
pBR	pBluescript containing EcoRI fragment of pCRRecA	This study
pBM::cat	pBM with *cat* inserted in *mutS*; Cm^r	This study
pUB::cat	pUB with *cat* inserted in *uvrB*; Cm^r	This study
pBR::cat	pBR with *cat* inserted in *recA*; Cm^r	This study
pUng::cat	pUng with *cat* inserted in *ung*; Cm^r	This study

[a] Cm^r, chloramphenicol resistance; Tet^r, tetracycline resistance; Amp^r, ampicillin resistance; Km^r, kanamycin resistance.

TABLE 4. Putative orthologues in *C. jejuni* NCTC 11168 of genes that are essential in excision repair (MMR, NER, and BER) and RecA-dependent recombinational DNA repair in *E. coli* K-12

Repair	*E. coli* K-12[a]		*C. jejuni* NCTC 11168[a]		Similarity (%)[b]
	Gene	Locus tag	Gene	Locus tag	
Excision repair					
MMR	*mutS*	b2733	*mutS*	Cj1052c	21.9
	mutL	b4170			
	mutH	b2831			
NER	*uvrA*	b4058	*uvrA*	Cj0342c	67.6
	uvrB	b0779	*uvrB*	Cj0680c	69.2
	uvrC	b1913	*uvrC*	Cj1246c	49.1
BER	*ung*	b2580	*ung*	Cj0086c	61.7
	xthA	b1749	*exoA*	Cj0255c	48.9
	recJ	b2892	*recJ*	Cj0028	49.2
Recombinational repair	*recA*	b2699	*recA*	Cj1673c	76.0

[a] The NCBI reference sequence numbers of the genome sequences of *E. coli* K-12 and *C. jejuni* NCTC 11168 used are NC_000913 and NC_002163, respectively.

[b] The overall similarities were determined by performing pairwise alignments with EMBOSS-Align (http://www.ebi.ac.uk/Tools/emboss/align/) covering full-length sequences.

tems, BIOzymTC, Landgraaf, The Netherlands) according to the manufacturer's protocol for gram-negative bacteria. After a second incubation (3 h, 37°C, microaerobic conditions), the bacteria were collected by centrifugation (3,300 × *g*, 8 min), and resuspended in 400 μl of HI broth. To determine the number of transformants ml^{-1}, 200-μl portions of the appropriate serial dilutions (10^{-2} to 10^{-4}) were plated onto selective saponin plates. The CFU ml^{-1} was quantified using track dilution (30), whereby 10-μl portions of serial dilutions (10^{-4} to 10^{-8}) were spotted onto nonselective saponin plates. The natural transformation frequency was determined as the ratio of the number of transformants ml^{-1} grown on the selective plates versus the number of CFU ml^{-1} grown on the nonselective plates. All tests were performed in triplicate.

Ung activity assay. For the determination of Ung activity, *C. jejuni* bacteria grown in 50 ml of HI broth (16 h, 37°C, 160 rpm, microaerobic conditions) were collected by centrifugation (2,328 × *g*, 20 min, 4°C), rinsed once with 25 ml of extraction buffer (50 mM Tris-HCl, 300 mM NaCl, 5 mM dithiothreitol, 2 mM EDTA, 5% glycerol [pH 7.8]), resuspended in 300 μl of extraction buffer, and sonicated (three cycles, with one cycle consisting of 30 seconds on and 30 seconds off at 200 W) using a Bioruptor UCD-200 (Diagenode SA, Liège, Belgium) to release the cell content. Cell debris was removed by centrifugation (10,000 × *g*, 30 min, 4°C). The protein concentration of the supernatant was determined with Coomassie Plus protein assay reagent (Pierce, Rockford, IL) according to the manufacturer's protocol. The supernatant was assayed for Ung activity in 30 μl of extraction buffer by mixing 10 μg of protein of the supernatant with 130 ng of the uracil-containing plasmid pHipO::tet(+U) or the control (uracil-negative) plasmid pHipO::tet(−U) isolated from *E. coli* strain CJ236 or DH5α, respectively. After 30 min of incubation at 37°C, 5 μl of 6× blue and orange loading dye (Promega Benelux BV, Leiden, The Netherlands) was added, and Ung activity was analyzed by assessing the degradation of DNA on 0.8% agarose gels.

RESULTS

Identification of putative excision and recombinational repair genes in *C. jejuni*. In silico analysis of the *C. jejuni* genome using the Blastp algorithm was applied to identify proteins potentially involved in excision repair (MMR, NER, and BER) and RecA-dependent recombinational DNA repair in *C. jejuni*. In *E. coli*, MutS, MutL, and MutH are essential for MMR (25, 37). Inspection of the *C. jejuni* genome for these genes yielded a putative MutS orthologue (Cj1052c) but no potential *mutL* and *mutH* genes (Table 4). On the basis of the results of structural and phylogenetic analyses, the prokaryotic MutS family can be divided into the MutS1 and MutS2 subfamilies (13, 31). Amino acid alignments and phylogenetic analyses indicated that the MutS protein found in *C. jejuni* likely belongs to the family of MutS2 proteins (31), which are generally not involved in MMR, in contrast to the MutS1 proteins (13). Together with the apparent absence of MutL, which is essential in the MMR process of other species (14), these results suggest that *C. jejuni* may not have a functional MMR system.

Inspection of the *C. jejuni* genome for orthologues of the *E. coli* UvrA, UvrB, and UvrC proteins involved in the NER pathway (55) identified Cj0342c, Cj0680c, and Cj1246c of *C. jejuni* NCTC 11168 as genes likely to encode *C. jejuni* UvrA, UvrB, and UvrC, respectively (Table 4). The presence of these genes suggests that *C. jejuni* may have a functional NER system.

In *E. coli*, the BER system depends on several glycosylases (such as the uracil-DNA glycosylase Ung), an AP endonuclease (XthA; exonuclease III), and a deoxyribose phosphodiesterase (RecJ; single-stranded-DNA exonuclease) (49). A search for Ung, XthA, and RecJ orthologues in *C. jejuni* indicated Cj0086c, Cj0255c, and Cj0028 as genes likely to encode *C. jejuni* Ung, XthA (ExoA), and RecJ, respectively (Table 4), suggesting that *C. jejuni* may have a functional BER system.

In the recombinational repair of *E. coli*, RecA plays a central role. This protein has previously been identified in *C. jejuni* strain 81-176 and was shown to be essential for DNA recombination (21). An intact *recA* gene is also present in strain NCTC 11168 (Cj1673c) (Table 4).

Inactivation of putative DNA repair pathways in *C. jejuni*. To be able to assess the function of the various DNA repair systems in *C. jejuni*, genetically defined mutations were constructed in a set of genes essential for recognition of DNA damage for each system in *E. coli*. Gene inactivation was established by allelic replacement with a defective gene copy containing an inserted chloramphenicol resistance gene (*cat*). Genes selected for inactivation were *uvrB* (NER), *ung* (BER), and *recA* (recombinational repair). Although the in silico data suggested that the MMR system of *C. jejuni* NCTC 11168 was nonfunctional, we also constructed a *mutS* mutant for further analysis. The four genes were disrupted in the virulent version of the sequenced *C. jejuni* strain NCTC 11168, namely, strain NCTC 11168-O, and in two chicken isolates (strains 2412 and 2535; Table 1). For strain 2412, a *recA*::*cat* mutant could not be obtained, most likely due to inefficient transformation of this *C. jejuni* strain with (heterologous) *E. coli* DNA.

Repair of point mutations in *C. jejuni*. To investigate the function of the putative excision and recombinational DNA repair systems in the repair of point mutations, we determined the frequency of spontaneous nalidixic acid resistance in both the parental and mutant *C. jejuni* strains. The development of nalidixic acid resistance is based on point mutations within the *C. jejuni gyrA* gene (58) and can be used as an indication of the spontaneous mutation rate (44, 63). For all three *C. jejuni* parental strains, equivalent spontaneous nalidixic acid resistance frequencies of $\sim 10^{-8}$ were obtained. Similar frequencies of nalidixic acid resistance were obtained for each of the knockout mutants (Fig. 1). There was no clear evidence that disruption of a particular gene consistently caused an increase

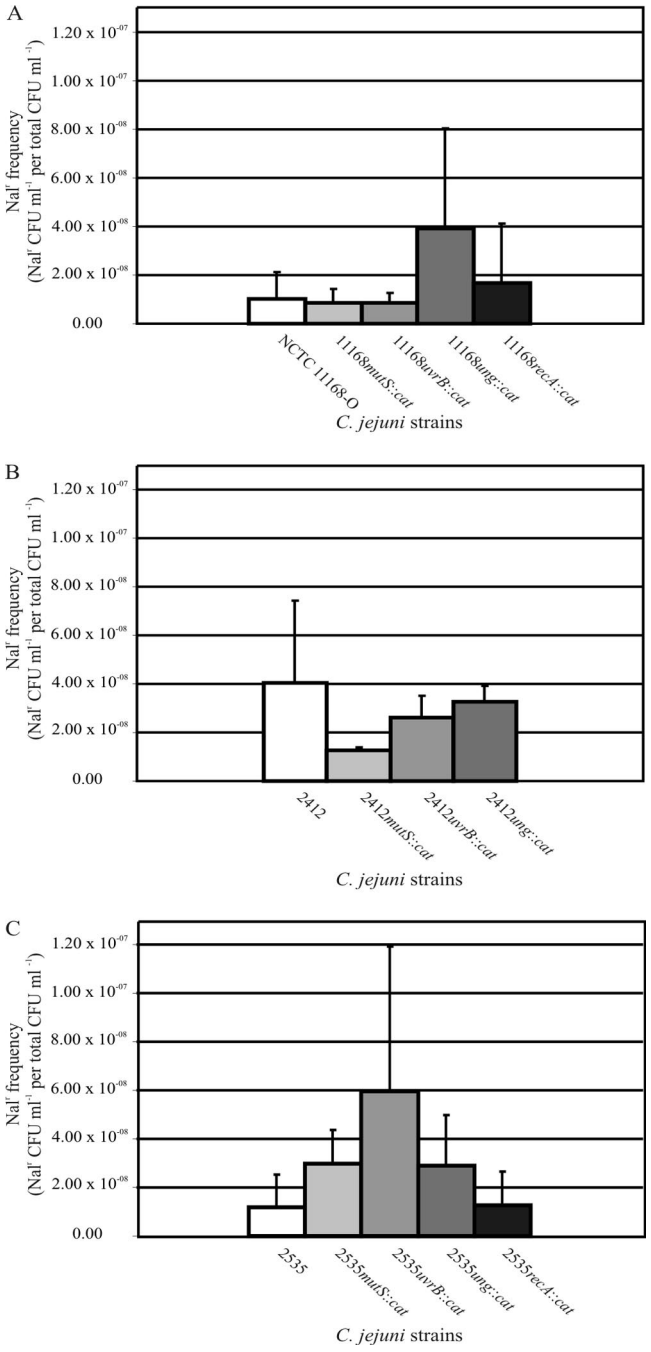

FIG. 1. Average nalidixic acid resistance frequencies of *C. jejuni* parental strains and *mutS*, *uvrB*, *ung*, and *recA* knockout mutants. *C. jejuni* strains NCTC 11168-O (A), 2412 (B), and 2535 (C) were examined. The resistance frequency was determined by dividing the number of Nal^r CFU ml^{-1} by the total number of CFU ml^{-1} and represents the average for three experiments.

in the Nal^r frequency in any of the three *C. jejuni* strains investigated ($P > 0.1$). These results suggest that none of the tested putative excision and recombinational DNA repair genes of *C. jejuni* affects repair of point mutations.

Repair of UV-damaged DNA in *C. jejuni*. To assess the roles of the putative *C. jejuni* excision and recombinational DNA repair genes in repair of structural DNA damage, all three *C. jejuni* parental strains and knockout mutants were exposed to UV irradiation. UV irradiation induces DNA damage by the formation of pyrimidine dimers and 6-4 photoproducts (19), which inhibit replication and transcription (4). The reduction in viability of *C. jejuni* after exposure to UV light was determined by enumeration of viable bacteria in UV-exposed and non-UV-exposed suspensions. For the *uvrB* knockout mutants, UV exposure (0.12 J cm^{-2}) resulted in a 1,000- to 10,000-fold reduction in viability compared to the viability of their respective parental strains (Fig. 2A, B, and C). UV exposure of the other knockout mutants (*mutS*, *ung*, and *recA* mutants) yielded bacterial viability levels similar to those of the parental strains (Fig. 2A, B, and C). As previous reports showed that *recA* mutants of *E. coli*, *H. pylori*, and *C. jejuni* are sensitive to UV exposure (21, 27, 48), the *recA* mutants and their parental strains were also exposed to a higher dose of UV irradiation (1.0 J cm^{-2}; Fig. 2D). For the *recA* mutants, this resulted in a 10-fold reduction in viability compared to their corresponding parental strains. Higher UV doses did not further increase the differences in viability between *recA* mutants and parental strains; a dose of 5.0 J cm^{-2} resulted in complete loss of viability. These results indicate that in *C. jejuni*, UvrB and RecA, but not MutS or Ung, are involved in the repair of UV-induced DNA damage.

Functional characterization of Ung. In *E. coli*, Ung detects and removes uracil from DNA, leaving an apyrimidinic site and rendering the DNA sensitive to degradation (32, 43). As a role for Ung in repair of point mutations or structurally damaged DNA could not be demonstrated with the assays employed, we tested the activity of Ung in removing uracil from DNA directly. In this study, an assay was developed in which cell-free lysates of *C. jejuni* were incubated with the uracil-containing plasmid pHipO::tet(+U), isolated from the *dut ung E. coli* mutant strain CJ236, and the uracil negative-control plasmid pHipO::tet(−U), isolated from *E. coli* DH5α. Incubation of the plasmids with lysates derived from *C. jejuni* parental strains caused degradation of uracil-containing plasmid DNA [pHipO::tet(+U); Fig. 3, lanes 3, 9, and 15], but not of the control plasmid lacking uracil [pHipO::tet(−U); Fig. 3, lanes 2, 8, and 14]. In contrast, cell-free lysates from *ung*-deficient strains 11168*ung*::*cat*, 2412*ung*::*cat*, and 2535*ung*::*cat* failed to degrade the uracil-containing plasmid (Fig. 3, lanes 6, 12, and 18). These results strongly suggest that the *C. jejuni* Ung protein is active and is likely to be involved in the repair of uracil-containing DNA during base excision repair.

Homologous recombination in *C. jejuni*. Homologous recombination is involved in the repair of nicked DNA (40) and plays a role during natural transformation of chromosomal DNA (23). Since the exchange of DNA strands occurs in both mechanisms, the role of the putative DNA repair genes in recombinational DNA repair of *C. jejuni* was investigated through determination of natural transformation frequencies for the parental strains and all knockout mutants. In this study, natural transformation experiments were performed using chromosomal DNA isolated from the kanamycin-resistant *C. jejuni* strain 2535*htrA*::*aphA-3* as donor DNA. This DNA could be successfully transferred via natural transformation to strain 2535 and, at lower frequencies, to strains NCTC 11168-O and 2412 (Table 5). As expected, inactivation of *recA* in strain NCTC 11168-O and strain 2535 abolished this process, con-

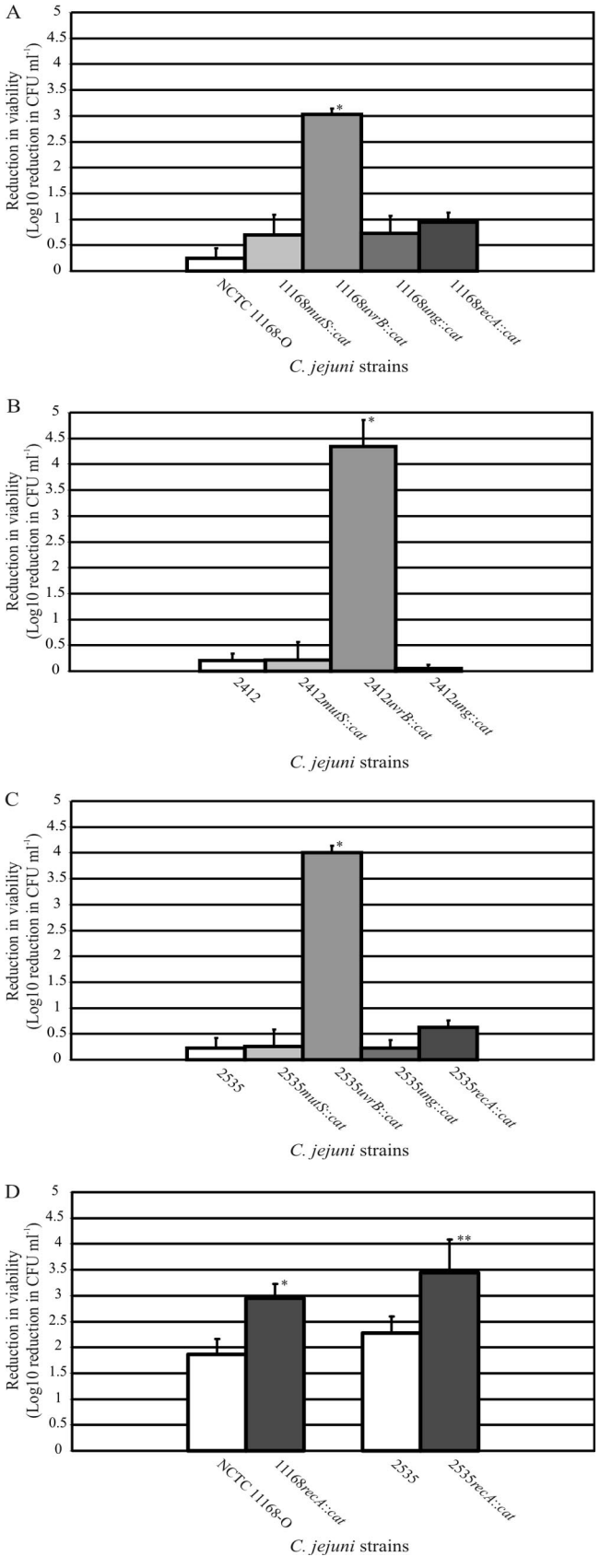

FIG. 2. Average reduction in viability of *C. jejuni* parental strains and *mutS*, *uvrB*, *ung*, and *recA* knockout mutants after UV irradiation with 0.12 J cm^{-2} (A, B, and C), and 1.0 J cm^{-2} (D). *C. jejuni* strains NCTC 11168-O (A and D), 2412 (B), and 2535 (C and D) were examined. The reduction in viability of each strain was determined in triplicate and expressed as $\log_{10}$ reduction in total CFU ml^{-1}. Values that were significantly different from the value for the parental strain are indicated by asterisks as follows: *, $P < 0.01$; **, $P < 0.1$.

firming the key role of RecA in natural transformation (21). The *C. jejuni* mutants with defects in *mutS*, *uvrB*, and *ung* showed natural transformation frequencies similar to those of their respective parental strains (Table 5). These results demonstrate that in *C. jejuni*, RecA, but not MutS, UvrB, or Ung, is involved in homologous recombination.

DISCUSSION

DNA repair is important for bacterial survival and can influence the generation of genetic diversity in bacterial populations. In the present study, we investigated the presence and function of three DNA excision repair systems (MMR, NER, and BER) and RecA-dependent recombinational DNA repair in *C. jejuni*. Our results indicate that *C. jejuni* has at least three DNA repair mechanisms, namely, NER, BER, and RecA-dependent recombinational repair. No evidence was obtained for the presence of a functional MMR system.

The basis of the identification of the DNA repair mechanisms in *C. jejuni* was in silico analysis of the complete translated genome of *C. jejuni* NCTC 11168 (22, 42) for orthologues of genes essential for excision and recombinational DNA repair in *E. coli*. This revealed genes potentially involved in three (NER, BER, and recombinational repair) out of the four DNA repair mechanisms investigated (Table 4). For the remaining repair system (MMR), only one potential component (MutS) was found in *C. jejuni*. This protein shares characteristics with the MutS2 subfamily of MutS proteins (31), which thus far have not been implicated in MMR (13). On the basis of the presence of *mutS2* and the apparent absence of *mutL* and *mutH* homologues in the *C. jejuni* genome and the seemingly parental behavior of the *C. jejuni mutS* mutant strains in DNA repair, we conclude that *C. jejuni* most likely lacks a functional MMR system. The absence of this system may contribute to the genetic heterogeneity of the *C. jejuni* population, as this species contains a large number of genes with homopolymeric nucleotide repeats that are prone to slippage (2, 20, 54). In other species, MMR repairs slipped strand mispairing (5, 53). An increased frequency of phase variation may enhance the adaptive abilities of the *C. jejuni* population.

A close homologue (59.7% similarity) of *C. jejuni* MutS is the MutS2 protein found in *Helicobacter pylori*. This protein inhibits homologous recombination and homeologous recombination (genetic exchanges between DNA partners) (31, 45). *H. pylori* MutS2 mutant strains show an 3- to 13-fold increase in their transformation frequency when chromosomal DNA is used and an 16- to 520-fold increase when a plasmid or linear DNA is used (31, 45). In the present study, natural transformation frequencies were determined by using chromosomal DNA as a donor. For the *C. jejuni mutS* mutant strains 11168*mutS*::*cat* and 2412*mutS*::*cat*, a threefold increase in nat-

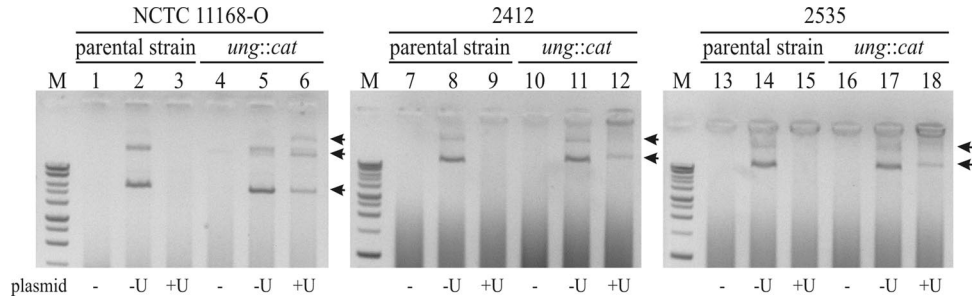

FIG. 3. Determination of the Ung activity of *C. jejuni* parental strains and *ung* knockout mutants. Plasmid DNA (130 ng) without uracil isolated from *E. coli* DH5α [pHipO::tet(−U)] or with uracil isolated from *E. coli* CJ236 [pHipO::tet(+U)] was incubated with cell-free lysates from *C. jejuni* parental strains (NCTC 11168-O, 2412, and 2535) and *ung* knockout mutants (11168*ung*::*cat*, 2412*ung*::*cat*, and 2535*ung*::*cat*) for 30 min at 37°C and analyzed by agarose gel electrophoresis. The presence of plasmid DNA with uracil (+U) or without uracil (−U) or the absence of plasmid DNA (−) is indicated below the three gels. The position of plasmid DNA is indicated by the black arrows to the right of the gels. Lanes: M, 1-kb DNA ladder; 1, lysate of strain NCTC 11168-O without plasmid DNA; 2, lysate of NCTC 11168-O with pHipO::tet(−U); 3, lysate of NCTC 11168-O with pHipO::tet(+U); 4, lysate of strain 11168*ung*::*cat* without plasmid DNA; 5, lysate of 11168*ung*::*cat* with pHipO::tet(−U); 6, lysate of 11168*ung*::*cat* with pHipO::tet(+U); 7, lysate of strain 2412 without plasmid DNA; 8, lysate of 2412 with pHipO::tet(−U); 9, lysate of 2412 with pHipO::tet(+U); 10, lysate of 2412*ung*::*cat* without plasmid DNA; 11, lysate of 2412*ung*::*cat* with pHipO::tet(−U); 12, lysate of 2412*ung*::*cat* with pHipO::tet(+U); 13, lysate of strain 2535 without plasmid DNA; 14, lysate of 2535 with pHipO::tet(−U); 15, lysate of 2535 with pHipO::tet(+U); 16, lysate of 2535*ung*::*cat* without plasmid DNA; 17, lysate of 2535*ung*::*cat* with pHipO::tet(−U); 18, lysate of 2535*ung*::*cat* with pHipO::tet(+U).

ural transformation frequencies was seen compared to the frequencies of the parental strains, whereas a fourfold reduction was detected for strain 2535*mutS*::*cat* (Table 5). Thus, whether the MutS protein found in *C. jejuni* has the same function as the MutS2 protein found in *H. pylori* is not evident. In *H. pylori*, MutS2 also influences the restoration of oxidative DNA damage induced by the inflammatory oxidative stress response during infection (57). In similar assays, we observed no difference in H_2O_2 or oxygen sensitivity between *C. jejuni* parental strains and *mutS* knockout mutants (data not shown). MutS2 proteins typically contain a conserved C-terminal sequence termed the small MutS-related (Smr) domain (36, 39).

TABLE 5. Natural transformation frequencies of *C. jejuni* parental strains and putative DNA repair knockout mutants[a]

C. jejuni strain	Transformation frequency[b]
NCTC 11168-O	$(4.9 \pm 4.3) \times 10^{-5}$
11168*mutS*::*cat*	$(1.7 \pm 1.7) \times 10^{-4}$
11168*uvrB*::*cat*	$(1.4 \pm 1.9) \times 10^{-4}$
11168*ung*::*cat*	$(7.8 \pm 7.2) \times 10^{-5}$
11168*recA*::*cat*	−
2412[c]	$(4.9 \pm 7.0) \times 10^{-4}$
2412*mutS*::*cat*	$(1.6 \pm 2.4) \times 10^{-3}$
2412*uvrB*::*cat*	$(4.2 \pm 6.9) \times 10^{-4}$
2412*ung*::*cat*	$(6.2 \pm 9.7) \times 10^{-4}$
2535	$(0.8 \pm 1.3) \times 10^{-3}$
2535*mutS*::*cat*	$(2.0 \pm 2.5) \times 10^{-4}$
2535*uvrB*::*cat*	$(2.9 \pm 4.7) \times 10^{-4}$
2535*ung*::*cat*	$(0.8 \pm 1.2) \times 10^{-4}$
2535*recA*::*cat*	−

[a] Chromosomal DNA of the kanamycin-resistant *C. jejuni* strain 2535*htrA*::*aphA-3* was used as donor DNA.

[b] The transformation frequency is determined by dividing the number of transformants ml^{-1} by the total number of CFU ml^{-1} and represents the average ± standard deviation from three experiments. Symbol: −, below detection limit [$(1.4 \pm 0.4) \times 10^{-9}$ for strain 11168*recA*::*cat* and $(1.2 \pm 0.8) \times 10^{-9}$ for strain 2535*recA*::*cat*]. This is based on a minimum of one colony per plate, adapted to the volumes used in the procedure.

[c] For strain 2412, a *recA*::*cat* mutant was not obtained.

Biochemical characterization of the MutS2 protein found in *Thermus thermophilus* indicates that this domain displays endonuclease activity (17). *C. jejuni* MutS contains the characteristic Smr domain; however, cell-free lysates of *C. jejuni* did not degrade the pHipO::tet(−U) plasmid (Fig. 3). Thus, at present, no function can be assigned to the MutS protein from *C. jejuni*.

Inactivation of the putative *C. jejuni uvrB* gene caused a strong (1,000- to 10,000-fold) reduction in viability after UV exposure of all three *C. jejuni* strains tested (Fig. 2A, B, and C). UV exposure of DNA generally results in the formation of pyrimidine dimers or 6-4 photoproducts (19), which in *E. coli* are repaired by the NER system (55). This mechanism requires the UvrA, UvrB, and UvrC proteins. The *C. jejuni* genome contains homologues of these proteins (Table 4). The presence of these genes in conjunction with the drastic reduction in survival upon UV exposure of the UvrB knockout strains strongly suggests that the NER pathway is functional in *C. jejuni*. The *C. jejuni uvrB* mutant strains displayed unaltered spontaneous nalidixic acid resistance and natural transformation frequencies compared to the parental strains. This suggests that, similar to NER in *E. coli*, the *C. jejuni* NER pathway is involved in the repair of DNA upon conformational changes, but not in the repair of point mutations or in recombinatorial repair.

The BER pathway plays an important role in the repair of modified nucleotides in prokaryotes and eukaryotes (33). The first step in this repair pathway is the recognition of altered bases by specific glycosylases. One of these enzymes is the uracil-DNA glycosylase Ung. This protein is involved in the recognition of G · U mismatches caused by deamination of cytosine and A · U mismatches resulting from the incorporation of dUTP instead of dTTP (32, 34). Inspection of the *C. jejuni* genome revealed the presence of several potential components of the BER pathway, including Ung (Table 4). Although a direct effect of disruption of Ung on DNA repair in *C. jejuni* was not evident, our assay with cell-free lysates demonstrated that *C. jejuni* Ung is enzymatically active and leads to

degradation of uracil-containing plasmids by cell-free lysates of the parental strains, but not by lysates of Ung mutants (Fig. 3). Ung removes only the uracil nucleobase and leaves the DNA backbone intact. The observed degradation of uracil-containing DNA is probably caused by BER proteins downstream of Ung, similar to that observed in in vitro assays of *E. coli* (11, 50). This suggests that more components of the BER pathway may be functionally active in *C. jejuni*. The apparent absence of an effect of Ung inactivation in intact *C. jejuni* may indicate a relatively low level of spontaneous deamination of cytosine in the bacteria under the conditions employed. The unaltered nalidixic acid resistance frequencies of the *ung* mutants are an unexpected result, as *ung* mutants of *E. coli* display increased spontaneous mutation frequencies (12). In *H. pylori*, inactivation of *ung* results in a small (fourfold) increase of the spontaneous mutation rate. In this species, other BER components are important, such as the adenine DNA glycosylase MutY (28), for which a homologue was found in *C. jejuni* NCTC 11168 (Cj1620c; 63.6% similarity). Thus, the contributions of the different components of the BER pathway to DNA repair may differ between species. The unaltered viability upon UV damage and unaffected natural transformation frequencies of *C. jejuni ung* mutants suggest that Ung from *C. jejuni* does not function in the repair of structural DNA damage or in recombinational repair as has been reported for *H. pylori* Ung.

Recombinational repair is involved in the repair of gaps in double-stranded DNA and in the repair of double-strand breaks (8). RecA is an important protein for this type of repair and is regulated by a network of proteins in bacteria (9). DNA replication of UV-exposed DNA leads to gaps in the DNA; the majority of the gaps are located opposite pyrimidine dimers (3). First, these gaps have to be filled by RecA-dependent sister strand exchange providing an intact complementary strand for the NER system (50). In our experiments, inactivation of *recA* resulted in a small reduction (10-fold) in viability after UV exposure (1.0 J cm^{-2}) of the two *C. jejuni* strains analyzed (Fig. 2D). This indicates that in *C. jejuni* RecA is involved in the repair of structurally damaged DNA, which is in agreement with the results obtained for *recA* mutants of *E. coli*, *H. pylori*, and *C. jejuni* (21, 27, 48). In *E. coli*, RecA is also involved in inactivation of the LexA transcriptional repressor, which results in the SOS response that includes induction of DNA repair (6). The apparent absence of a LexA homologue in the *C. jejuni* genome indicates that no classical SOS response exists in *C. jejuni* (15, 42). Recently, Han et al. (24) showed that upon conditions that evoke an SOS response in other organisms, *C. jejuni* responds with an upregulation of *mfd*, which encodes a transcription repair coupling factor involved in DNA repair.

To investigate the role of *C. jejuni* RecA in repair of double-strand breaks, we determined natural transformation frequencies for all parental strains and *recA* mutants (Table 5). A phenotypic change from naturally transformable into nonnaturally transformable was seen for the *recA* mutants. These results are in agreement with the results obtained for *recA* mutants of *E. coli*, *H. pylori*, and *C. jejuni* (21, 27, 48) and demonstrate that RecA is important for the repair of double-strand breaks in *C. jejuni*.

In conclusion, this study for the first time demonstrates the presence of several excision and recombinational DNA repair mechanisms in *C. jejuni* that may protect against DNA damage. These systems may limit the generation of genetic diversity; on the other hand, the constellation of repair systems may contribute to the heterogeneity of the species. The absence in *C. jejuni* of a RecA-induced SOS response that induces DNA repair systems, as well as the apparent absence of a functional MMR system, may enhance the genetic diversity and adaptive abilities of *C. jejuni*.

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REFERENCES

1. **Aravind, L., D. R. Walker, and E. V. Koonin.** 1999. Conserved domains in DNA repair proteins and evolution of repair systems. Nucleic Acids Res. **27:**1223–1242.
2. **Bacon, D. J., C. M. Szymanski, D. H. Burr, R. P. Silver, R. A. Alm, and P. Guerry.** 2001. A phase-variable capsule is involved in virulence of *Campylobacter jejuni* 81-176. Mol. Microbiol. **40:**769–777.
3. **Bridges, B. A., and S. G. Sedgwick.** 1974. Effect of photoreactivation on the filling of gaps in deoxyribonucleic acid synthesized after exposure of *Escherichia coli* to ultraviolet light. J. Bacteriol. **117:**1077–1081.
4. **Britt, A. B.** 1995. Repair of DNA damage induced by ultraviolet radiation. Plant Physiol. **108:**891–896.
5. **Bucci, C., A. Lavitola, P. Salvatore, L. Del Giudice, D. R. Massardo, C. B. Bruni, and P. Alifano.** 1999. Hypermutation in pathogenic bacteria: frequent phase variation in meningococci is a phenotypic trait of a specialized mutator biotype. Mol. Cell **3:**435–445.
6. **Butala, M., D. Žgur-Bertok, and S. J. W. Busby.** 2009. The bacterial LexA transcriptional repressor. Cell. Mol. Life Sci. **66:**82–93.
7. **Butzler, J. P.** 2004. *Campylobacter*, from obscurity to celebrity. Clin. Microbiol. Infect. **10:**868–876.
8. **Cox, M. M.** 2001. Recombinational DNA repair of damaged replication forks in *Escherichia coli*: questions. Annu. Rev. Genet. **35:**53–82.
9. **de Boer, P., J. A. Wagenaar, R. P. Achterberg, J. P. M. van Putten, L. M. Schouls, and B. Duim.** 2002. Generation of *Campylobacter jejuni* genetic diversity *in vivo*. Mol. Microbiol. **44:**351–359.
10. **Dingle, K. E., F. M. Colles, D. R. A. Wareing, R. Ure, A. J. Fox, F. E. Bolton, H. J. Bootsma, R. J. L. Willems, R. Urwin, and M. C. J. Maiden.** 2001. Multilocus sequence typing system for *Campylobacter jejuni*. J. Clin. Microbiol. **39:**14–23.
11. **Dorrell, N., J. A. Mangan, K. G. Laing, J. Hinds, D. Linton, H. Al-Ghusein, B. G. Barrell, J. Parkhill, N. G. Stoker, A. V. Karlyshev, P. D. Butcher, and B. W. Wren.** 2001. Whole genome comparison of *Campylobacter jejuni* human isolates using a low-cost microarray reveals extensive genetic diversity. Genome Res. **11:**1706–1715.
12. **Duncan, B. K., and B. Weiss.** 1982. Specific mutator effects of *ung* (uracil-DNA glycosylase) mutations in *Escherichia coli*. J. Bacteriol. **151:**750–755.
13. **Eisen, J. A.** 1998. A phylogenomic study of the MutS family of proteins. Nucleic Acids Res. **26:**4291–4300.
14. **Eisen, J. A., and P. C. Hanawalt.** 1999. A phylogenomic study of DNA repair genes, proteins, and processes. Mutat. Res. **435:**171–213.
15. **Erill, I., S. Campoy, and J. Barbé.** 2007. Aeons of distress: an evolutionary perspective on the bacterial SOS response. FEMS Microbiol. Rev. **31:**637–656.
16. **Friedberg, E. C.** 2008. A brief history of the DNA repair field. Cell Res. **18:**3–7.
17. **Fukui, K., H. Kosaka, S. Kuramitsu, and R. Masui.** 2007. Nuclease activity of the MutS homologue MutS2 from *Thermus thermophilus* is confined to the Smr domain. Nucleic Acids Res. **35:**850–860.
18. **Gaynor, E. C., S. Cawthraw, G. Manning, J. K. MacKichan, S. Falkow, and D. G. Newell.** 2004. The genome-sequenced variant of *Campylobacter jejuni* NCTC 11168 and the original clonal clinical isolate differ markedly in colonization, gene expression, and virulence-associated phenotypes. J. Bacteriol. **186:**503–517.
19. **Goodsell, D. S.** 2001. The molecular perspective: ultraviolet light and pyrimidine dimers. Oncologist **6:**298–299.
20. **Grant, C. C. R., M. E. Konkel, W. Cieplak, Jr., and L. S. Tompkins.** 1993. Role of flagella in adherence, internalization, and translocation of *Campylobacter jejuni* in nonpolarized and polarized epithelial cell cultures. Infect. Immun. **61:**1764–1771.
21. **Guerry, P., P. M. Pope, D. H. Burr, J. Leifer, S. W. Joseph, and A. L. Bourgeois.** 1994. Development and characterization of *recA* mutants of *Campylobacter jejuni* for inclusion in attenuated vaccines. Infect. Immun. **62:**426–432.

22. **Gundogdu, O., S. D. Bentley, M. T. Holden, J. Parkhill, N. Dorrell, and B. W. Wren.** 2007. Re-annotation and re-analysis of the *Campylobacter jejuni* NCTC11168 genome sequence. BMC Genomics **8:**162.
23. **Hamilton, H. L., and J. P. Dillard.** 2006. Natural transformation of *Neisseria gonorrhoeae*: from DNA donation to homologous recombination. Mol. Microbiol. **59:**376–385.
24. **Han, J., O. Sahin, Y. W. Barton, and Q. Zhang.** 2008. Key role of Mfd in the development of fluoroquinolone resistance in *Campylobacter jejuni*. PLoS Pathog. **4:**e1000083. doi:10.1371/journal.ppat.1000083.
25. **Harfe, B. D., and S. Jinks-Robertson.** 2000. DNA mismatch repair and genetic instability. Annu. Rev. Genet. **34:**359–399.
26. **Hofreuter, D., J. Tsai, R. O. Watson, V. Novik, B. Altman, M. Benitez, C. Clark, C. Perbost, T. Jarvie, L. Du, and J. E. Galán.** 2006. Unique features of a highly pathogenic *Campylobacter jejuni* strain. Infect. Immun. **74:**4694–4707.
27. **Howard-Flanders, P., L. Theriot, and J. B. Stedeford.** 1969. Some properties of excision-defective recombination-deficient mutants of *Escherichia coli* K-12. J. Bacteriol. **97:**1134–1141.
28. **Huang, S., J. Kang, and M. J. Blaser.** 2006. Antimutator role of the DNA glycosylase *mutY* gene in *Helicobacter pylori*. J. Bacteriol. **188:**6224–6234.
29. **Jacobs-Reitsma, W. F., N. M. Bolder, and R. W. A. W. Mulder.** 1994. Cecal carriage of *Campylobacter* and *Salmonella* in Dutch broiler flocks at slaughter: a one-year study. Poult. Sci. **73:**1260–1266.
30. **Jett, B. D., K. L. Hatter, M. M. Huycke, and M. S. Gilmore.** 1997. Simplified agar plate method for quantifying viable bacteria. BioTechniques **23:**648–650.
31. **Kang, J., S. Huang, and M. J. Blaser.** 2005. Structural and functional divergence of MutS2 from bacterial MutS1 and eukaryotic MSH4-MSH5 homologs. J. Bacteriol. **187:**3528–3537.
32. **Lari, S. U., C. Y. Chen, B. G. Vertéssy, J. Morré, and S. E. Bennett.** 2006. Quantitative determination of uracil residues in *Escherichia coli* DNA: contribution of *ung*, *dug*, and *dut* genes to uracil avoidance. DNA Repair (Amsterdam) **5:**1407–1420.
33. **Lindahl, T.** 1993. Instability and decay of the primary structure of DNA. Nature **362:**709–715.
34. **Lindahl, T.** 1974. An *N*-glycosidase from *Escherichia coli* that releases free uracil from DNA containing deaminated cytosine residues. Proc. Natl. Acad. Sci. USA **71:**3649–3653.
35. **Lusetti, S. L., and M. M. Cox.** 2002. The bacterial RecA protein and the recombinational DNA repair of stalled replication forks. Annu. Rev. Biochem. **71:**71–100.
36. **Malik, H. S., and S. Henikoff.** 2000. Dual recognition-incision enzymes might be involved in mismatch repair and meiosis. Trends Biochem. Sci. **25:**414–418.
37. **Modrich, P., and R. Lahue.** 1996. Mismatch repair in replication fidelity, genetic recombination, and cancer biology. Annu. Rev. Biochem. **65:**101–133.
38. **Moore, J. E., D. Corcoran, J. S. G. Dooley, S. Fanning, B. Lucey, M. Matsuda, D. A. McDowell, F. Mégraud, B. C. Millar, R. O'Mahony, L. O'Riordan, M. O'Rourke, J. R. Rao, P. J. Rooney, A. Sails, and P. Whyte.** 2005. *Campylobacter*. Vet. Res. **36:**351–382.
39. **Moreira, D., and H. Philippe.** 1999. Smr: a bacterial and eukaryotic homologue of the C-terminal region of the MutS2 family. Trends Biochem. Sci. **24:**298–300.
40. **Nowosielska, A.** 2007. Bacterial DNA repair genes and their eukaryotic homologues. 5. The role of recombination in DNA repair and genome stability. Acta Biochim. Pol. **54:**483–494.
41. **Parker, C. T., B. Quiñones, W. G. Miller, S. T. Horn, and R. E. Mandrell.** 2006. Comparative genomic analysis of *Campylobacter jejuni* strains reveals diversity due to genomic elements similar to those present in *C. jejuni* strain RM1221. J. Clin. Microbiol. **44:**4125–4135.
42. **Parkhill, J., B. W. Wren, K. Mungall, J. M. Ketley, C. Churcher, D. Basham, T. Chillingworth, R. M. Davies, T. Feltwell, S. Holroyd, K. Jagels, A. V. Karlyshev, S. Moule, M. J. Pallen, C. W. Penn, M. A. Quail, M.-A. Rajandream, K. M. Rutherford, A. H. M. van Vliet, S. Whitehead, and B. G. Barrell.** 2000. The genome sequence of the food-borne pathogen *Campylobacter jejuni* reveals hypervariable sequences. Nature **403:**665–668.
43. **Pearl, L. H.** 2000. Structure and function in the uracil-DNA glycosylase superfamily. Mutat. Res. **460:**165–181.
44. **Pérez-Vázquez, M., F. Román, B. Aracil, R. Cantón, and J. Campos.** 2004. Laboratory detection of *Haemophilus influenzae* with decreased susceptibility to nalidixic acid, ciprofloxacin, levofloxacin, and moxifloxacin due to *gyrA* and *parC* mutations. J. Clin. Microbiol. **42:**1185–1191.
45. **Pinto, A. V., A. Mathieu, S. Marsin, X. Veaute, L. Ielpi, A. Labigne, and J. P. Radicella.** 2005. Suppression of homologous and homeologous recombination by the bacterial MutS2 protein. Mol. Cell **17:**113–120.
46. **Poly, F., T. Read, D. R. Tribble, S. Baqar, M. Lorenzo, and P. Guerry.** 2007. Genome sequence of a clinical isolate of *Campylobacter jejuni* from Thailand. Infect. Immun. **75:**3425–3433.
47. **Rayssiguier, C., D. S. Thaler, and M. Radman.** 1989. The barrier to recombination between *Escherichia coli* and *Salmonella typhimurium* is disrupted in mismatch-repair mutants. Nature **342:**396–401.
48. **Schmitt, W., S. Odenbreit, D. Heuermann, and R. Haas.** 1995. Cloning of the *Helicobacter pylori recA* gene and functional characterization of its product. Mol. Gen. Genet. **248:**563–572.
49. **Seeberg, E., L. Eide, and M. Bjørås.** 1995. The base excision repair pathway. Trends Biochem. Sci. **20:**391–397.
50. **Smith, K. C., and T.-C. V. Wang.** 1989. *recA*-dependent DNA repair processes. Bioessays **10:**12–16.
51. **Suerbaum, S., M. Lohrengel, A. Sonnevend, F. Ruberg, and M. Kist.** 2001. Allelic diversity and recombination in *Campylobacter jejuni*. J. Bacteriol. **183:**2553–2559.
52. **Taylor, D. E.** 1992. Genetics of *Campylobacter* and *Helicobacter*. Annu. Rev. Microbiol. **46:**35–64.
53. **van den Broek, D., G. V. Bloemberg, and B. Lugtenberg.** 2005. The role of phenotypic variation in rhizosphere *Pseudomonas* bacteria. Environ. Microbiol. **7:**1686–1697.
54. **van der Woude, M. W., and A. J. Bäumler.** 2004. Phase and antigenic variation in bacteria. Clin. Microbiol. Rev. **17:**581–611.
55. **Van Houten, B., D. L. Croteau, M. J. DellaVecchia, H. Wang, and C. Kisker.** 2005. 'Close-fitting sleeves': DNA damage recognition by the UvrABC nuclease system. Mutat. Res. **577:**92–117.
56. **Walker, G. C., L. Marsh, and L. A. Dodson.** 1985. Genetic analyses of DNA repair: inference and extrapolation. Annu. Rev. Genet. **19:**103–126.
57. **Wang, G., P. Alamuri, M. Z. Humayun, D. E. Taylor, and R. J. Maier.** 2005. The *Helicobacter pylori* MutS protein confers protection from oxidative DNA damage. Mol. Microbiol. **58:**166–176.
58. **Wang, Y., W. M. Huang, and D. E. Taylor.** 1993. Cloning and nucleotide sequence of the *Campylobacter jejuni gyrA* gene and characterization of quinolone resistance mutations. Antimicrob. Agents Chemother. **37:**457–463.
59. **Wang, Y., and D. E. Taylor.** 1990. Natural transformation in *Campylobacter* species. J. Bacteriol. **172:**949–955.
60. **Wareing, D. R. A., F. J. Bolton, A. J. Fox, P. A. Wright, and D. L. A. Greenway.** 2002. Phenotypic diversity of *Campylobacter* isolates from sporadic cases of human enteritis in the UK. J. Appl. Microbiol. **92:**502–509.
61. **Wassenaar, T. M., B. N. Fry, and B. A. M. van der Zeijst.** 1993. Genetic manipulation of *Campylobacter*: evaluation of natural transformation and electro-transformation. Gene **132:**131–135.
62. **Wassenaar, T. M., and D. G. Newell.** 2000. Genotyping of *Campylobacter* spp. Appl. Environ. Microbiol. **66:**1–9.
63. **Watson, M. E., Jr., J. L. Burns, and A. L. Smith.** 2004. Hypermutable *Haemophilus influenzae* with mutations in *mutS* are found in cystic fibrosis sputum. Microbiology **150:**2947–2958.
64. **Yuki, N., and M. Koga.** 2006. Bacterial infections in Guillain-Barré and Fisher syndromes. Curr. Opin. Neurol. **19:**451–457.