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Functions of the Mismatch Repair Gene *mutS* from *Acinetobacter* sp. Strain ADP1†

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The genus *Acinetobacter* encompasses a heterogeneous group of bacteria that are ubiquitous in the natural environment due in part to their ability to adapt genetically to novel challenges. *Acinetobacter* sp. strain ADP1 (also known as strain BD413) is naturally transformable and takes up DNA from any source. Donor DNA can be integrated into the chromosome by recombination provided it possesses sufficient levels of nucleotide sequence identity to the recipient's DNA. In other bacteria, the requirement for sequence identity during recombination is partly due to the actions of the mismatch repair system, a key component of which, MutS, recognizes mismatched bases in heteroduplex DNA and, along with MutL, blocks strand exchange. We have cloned *mutS* from strain ADP1 and examined its roles in preventing recombination between divergent DNA and in the repair of spontaneous replication errors. Inactivation of *mutS* resulted in 3- to 17-fold increases in transformation efficiencies with donor sequences that were 8 to 20% divergent relative to the strain ADP1. Strains lacking MutS exhibited increased spontaneous mutation frequencies, and reversion assays demonstrated that MutS preferentially recognized transition mismatches while having little effect on the repair of transversion mismatches. Inactivation of *mutS* also abolished the marker-specific variations in transforming efficiency seen in *mutS*$^+$ recipients where transition and frameshift alleles transformed at eightfold lower frequencies than transversions or large deletions. Comparison of the MutS homologs from five individual *Acinetobacter* strains with those of other gram-negative bacteria revealed that a number of unique indels are conserved among the *Acinetobacter* amino acid sequences.

The ability to maintain the genetic integrity of bacterial cells is balanced by the need to adapt to rapidly changing environments. The mismatch repair system plays a key role in maintaining this balance by recognizing and correcting mismatched bases that arise in duplex DNA as a result of replication error, DNA damage, and recombination between partially divergent, so-called homeologous, DNA (33, 39). A key component of the mismatch repair system is MutS, which initiates the process by recognizing and binding to mismatched bases in double-stranded DNA (33). The importance of MutS in maintaining the stability of the cellular genome is underscored by its ubiquity in the biological world. MutS homologs have been identified in members of all three biological kingdoms, and with the advent of genome sequence analysis, it has become evident that most organisms encode at least one MutS homolog (9).

The genus *Acinetobacter* encompasses a diverse group of gram-negative bacteria whose members are found in most aquatic and terrestrial environments (23). The ubiquity of the group can be attributed to its members' ability to adapt genetically to novel environmental challenges. Documented examples of such adaptation include the ability of clinical *Acinetobacter* isolates to rapidly acquire drug resistance when challenged with antibiotics (48). Such genetic plasticity may also have contributed to the evolution of the diverse nutritional capabilities observed in most *Acinetobacter* species (1, 23).

Acinetobacter sp. strain ADP1 (also known as strain BD413) (24) possesses a natural transformation system that provides an unusual potential for acquiring foreign DNA. Transformation of strain ADP1 does not require uptake sequences to be present in donor DNA, nor does it have any known requirements for extracellular competence factors. Perhaps most importantly, stationary-phase cells become competent in virtually any growth medium following the addition of a fresh carbon source, and cultures remain competent throughout most of the growth cycle (37). DNA from virtually any source is taken up by strain ADP1 and can be incorporated into its chromosome by recombination provided it possesses sequence identity to the recipient's DNA. Although most of the interspecies transformation experiments done with strain ADP1 have used hybrid donor DNA that possessed sequence identity to the recipient chromosome (13, 27), there is evidence that divergent DNA can also be integrated into the chromosome via homeologous recombination (2, 22).

In this report we describe the cloning and characterization of *mutS* from *Acinetobacter* sp. strain ADP1. The *Acinetobacter* MutS protein recognized mismatches that arose during DNA replication and homeologous recombination, preferentially recognizing transition mismatches and 1-bp frameshifts while having virtually no effect on transversion mismatches or large insertions and deletions. *Acinetobacter* strains lacking MutS function exhibited increases in spontaneous mutation frequencies and in the frequency of interspecies transformation. We found that the genetic organization of the *mutS* regions from strain ADP1 and four divergent *Acinetobacter* strains shares similarities with the *mutS* regions from members of other bacterial genera. However, comparison of the MutS amino acid sequences from the *Acinetobacter* strains with those from other

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TABLE 1. Bacterial strains and plasmids used in this study

Bacterial strain or plasmid	Relevant characteristic(s)	Reference or source
Bacterial strains		
Acinetobacter sp. strain ADP1	Wild-type	24
Derivatives of strain ADP1		
ADP1424	*pobR1424*	26
ADP1451	*pobR1451*	26
ADP6205	*pcaH9*	14
ADP6209	Δ*pcaH20*	14
ADP6314	*pcaH5*	14
ADP6407	*pcaH12*	14
ADP6417	Δ*pcaH19 mutS*$^+$	14
ADP7003	*pcaH*$^+$ *pcaG*$^+$ Δ*mutS11*::pGEM3Z(+) (Ap^r)	This study
ADP7118	Δ*pcaH20* Δ*mutS6*::Ω (St^r/Sp^r)	This study
ADP7021	Δ*mutS6*::Ω (St^r/Sp^r)	This study
ADP7037	Δ*pcaH19* Δ*mutS6*::Ω (St^r/Sp^r)	This study
ADP7053	*pcaH5* Δ*mutS6*::Ω (St^r/Sp^r)	This study
ADP7072	*pobR1424* Δ*mutS6*::Ω (St^r/Sp^r)	This study
ADP7073	*pobRI451* Δ*mutS6*::Ω (St^r/Sp^r)	This study
ADP7087	*orf1*::Ω (St^r/Sp^r)	This study
ADP7088	Δ*fdxA*::Ω (St^r/Sp^r)	This study
ADP7098	*pcaH12* Δ*mutS6*::Ω (St^r/Sp^r)	This study
ADP7099	*pcaH9* Δ*mutS6*::Ω (St^r/Sp^r)	This study
Wild-type strains		
Acinetobacter sp. strain 93A2	*pcaH*$^+$ *pcaG*$^+$	1
Acinetobacter sp. strain AD321	*pcaH*$^+$ *pcaG*$^+$	This study
Acinetobacter sp. strain AD532	*pcaH*$^+$ *pcaG*$^+$	This study
Acinetobacter sp. strain 01B0	*pcaH*$^+$ *pcaG*$^+$	1
Acinetobacter sp. strain 48A1	*pcaH*$^+$ *pcaG*$^+$	1
A. haemolyticus 40B4 (ATCC 17906)	*pcaH*$^+$ *pcaG*$^+$	1
A. johnsonii LUH540		L. Dijkshoorn
Acinetobacter sp. strain AC423D		This study
Pseudomonas putida PRS2000	*pcaH*$^+$ *pcaG*$^+$	36
Plasmids		
pGEM 3zf(+)	Cloning vector (Ap^r)	Promega
pHP45Ω	Ω element (Ap^r) (St^r/Sp^r)	38
pUI1638	Ω element (Ap^r) (St^r/Sp^r)	10
pZR2	2,392-bp fragment containing *pcaK′CHG* in pUC19	14
pZR419	Δ*pobSR*::Ω in pUC19 (St^r/Sp^r) (Ap^r)	Tony DiMarco
pZR7000	1942-bp PCR fragment of ADP1 *mutS* inserted in pGEM3Zf(+) (Ap^r)	This study
pZR7006	pZR7000 with the Δ*mutS6*::Ω mutation (Km^r)	This study
pZR7008	pZR7000 with the *mutS8*::Ω mutation (St^r/Sp^r)	This study
pZR7009	5′ end of ADP1 *mutS* and 9 kbp of upstream DNA (Ap^r)	This study
pZR7010	3′ end of ADP1 *mutS* and 8 kbp of downstream DNA (Ap^r)	This study
pZR7072	1,165-bp PCR fragment, containing *orf1*, ligated into pGEM3Zf(+) (Ap^r)	This study
pZR7074	*orf1*::Ω (St^r/Sp^r)	This study
pZR7075	1,532-bp PCR product containing a portion of *fdxA* and downstream DNA, ligated into pGEM3Zf(+) (Ap^r)	This study
pZR7076	Δ*fdxA*::Ω (St^r/Sp^r)	This study

gram-negative bacteria clearly showed that the *Acinetobacter* homologs represent a distinct evolutionary branch within this highly conserved protein family.

MATERIALS AND METHODS

Strains and culture conditions. Strains and plasmids used in this study are listed in Table 1. Unless otherwise indicated, all *Acinetobacter* cultures were grown at 30°C in Luria-Bertani broth (LB) (42) or in mineral medium (44) supplemented with 10 mM succinate or 5 mM *p*-hydroxybenzoate as a sole carbon source. *Escherichia coli* cultures were grown in LB at 37°C. Liquid cultures were incubated while shaking at 180 rpm. Agar plates were prepared by adding Difco agar (1.8% wt/vol) to liquid media prior to autoclaving. When required, growth media were supplemented with ampicillin, streptomycin, spectinomycin, or rifampin at respective concentrations of 100, 10, 40, and 30 μg/ml. For all transformation and mutation assays, selection plates were incubated for 48 h before counting of colonies, and viable cell counts were performed in parallel by plating dilutions of the cultures on LB plates.

PCR amplification of a *mutS* fragment. A segment of *mutS* from *Acinetobacter* sp. strain ADP1 was amplified by PCR using degenerate primers MUTSF2 and MUTSR3 (Table 2 and Fig. 1) in a standard PCR reaction (95°C, 45 s; 50°C, 30 s; 72°C, 2 min; 30 cycles) using *Pfu* polymerase according to the directions of the supplier (Stratagene) and chromosomal DNA as a template.

Cloning and sequencing of the *mutS* region. All DNA manipulations were performed according to standard procedures (42). Recombinant plasmids were

TABLE 2. Primers used for PCR amplification of *Acinetobacter* DNA

Primer	Nucleotide sequence[a]	Annealing site[b]
MUTC	5′-GTCAACTGGGCAGACTTCTAC-3′	3′ strand of *fdxA*
MUTF	5′-CATGGTCGATATTTTGCTGAG-3′	5′ strand of *fdxA*
MUTRR5	5′-GTTTAACGCCGAGACAAG-3′	5′ strand of *mutS*
MUTRR8	5′-CCAGATGAGTGCATTGACTGC-3′	3′ strand of *orf3*
MUTSF2	5′-AYCGXATGGXGAYTTYTAYGARC-3′	5′ strand of *mutS*
MUTSR3	5′-GCXGTYTCXGTCATYTCXAC-3′	3′ strand of *mutS*

[a] Primers MUTSF2 and MUTSR3 are degenerate primers.
[b] Location in the *mutS* chromosomal region of *Acinetobacter* sp. strain ADP1.

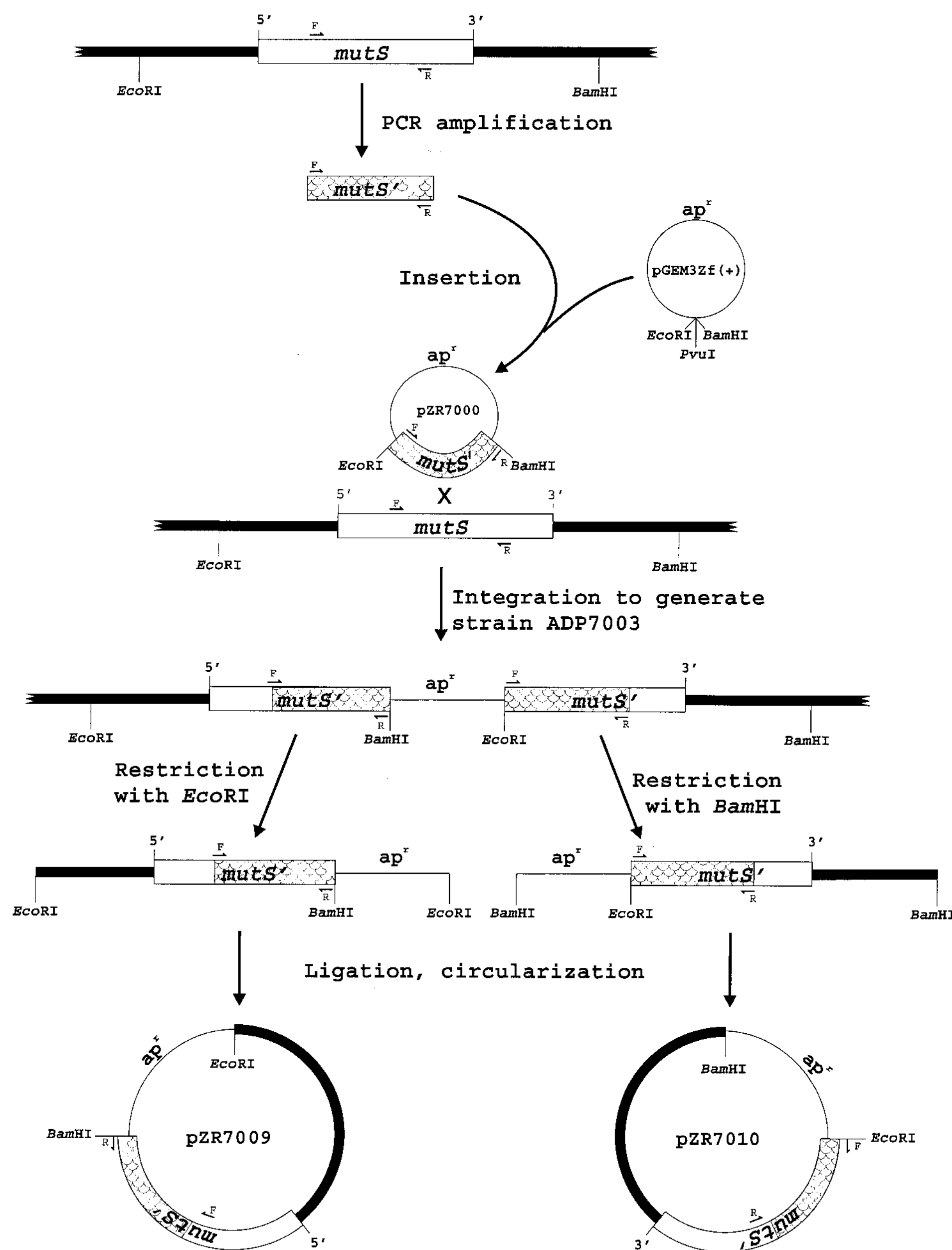

FIG. 1. Cloning of *mutS* and flanking DNA from the chromosome of *Acinetobacter* sp. strain ADP1. Small arrows indicate the degenerate primers MUTSF2 (F) and MUTSR3 (R) used for PCR amplification of a 1.9-kb segment of *mutS*. Shaded regions indicate this portion of *mutS* throughout the figure. Strain ADP7003 was formed by integration of pZR7000 into the chromosome of strain ADP1. pGEM-3Zf(+) does not replicate in strain ADP1, so selection for ampicillin resistance (ap^r), encoded on the vector, demanded strain ADP7003. Digestion of chromosomal DNA from strain ADP7003 with *Eco*RI yielded a fragment containing pGEM-3Zf(+) fused to a segment of upstream DNA that included the 5′ end of *mutS*, and chromosomal DNA extending to the first *Eco*RI site upstream of the gene. Circularization of the restriction fragments by ligation followed by transformation into *E. coli* DH5α and selection for Ap^r resulted in pZR7009. The 3′ end of *mutS* and downstream DNA were cloned in the same manner except that ADP7003 DNA was digested with *Bam*HI rather than *Eco*RI, and the resulting plasmid was designated pZR1010.

isolated by transforming *E. coli* DH5α with the appropriate ligation reaction according to the transformation protocol provided by the supplier (Gibco BRL).

Plasmid pZR7000 was constructed by blunt end ligation of the MUTSF2-MUTSR3 PCR product into the *Sma*I site of pGEM-3Zf(+). Clones containing the 5′ and 3′ ends of *mutS* and the genes flanking it were obtained using the vector integration strategy depicted in Fig. 1 (32). The overlapping nucleotide sequences of pZR7000, pZR7009, and pZR7010 made it possible to assemble a contiguous sequence for *mutS* and flanking DNA from *Acinetobacter* sp. strain ADP1 (Fig. 2).

Construction of Insertion mutations. Plasmid pZR7008 was constructed by ligating the Ω-cassette, cut from pHP45Ω as a *Sma*I fragment, into an *Eco*RV site located in the middle of the pZR7000 insert. Plasmid pZR7006 was constructed by deleting 637 bp of DNA between the *Eco*RV and *Msc*I sites in the pZR7000 insert and inserting a 2-kb *Sma*I fragment containing the Ω element from plasmid pUI1638.

The *orf1*::Ω mutation was constructed using a 1,165-bp PCR product that was amplified in a standard PCR reaction from the chromosome of strain ADP1 with the primers MUTRR5 and MUTC (Table 2). pGEM-3Zf(+) was digested with

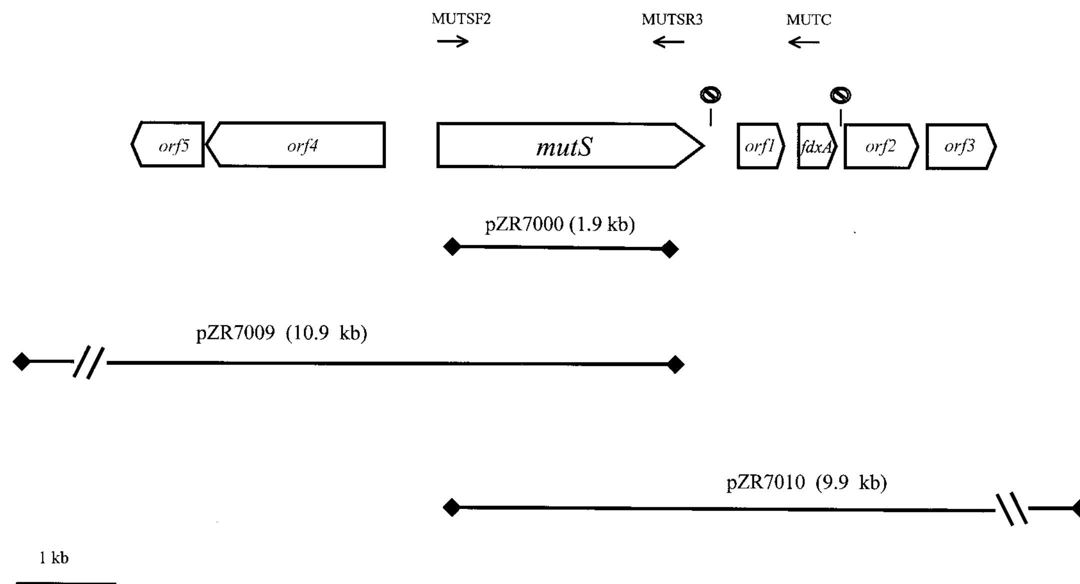

FIG. 2. Genetic organization of the *mutS* region of the *Acinetobacter* sp. strain ADP1 chromosome. The annealing sites of primers MUTSF2, MUTSR3, and MUTC are indicated as horizontal arrows. The symbol ⦸ represents putative transcription terminators downstream from *mutS* (5′-ATAAGTAGCCATCGTGCTACTTAT-3′) and downstream from *fdxA* (5′-AAAAGATCAGCATTAGCTGATCTTTT-3′). Horizontal lines indicate inserts in plasmids containing overlapping portions of *mutS*.

*Sma*I and *Hin*cII, and the PCR product was ligated into the vector, creating pZR7072. Plasmid pZR7074 was created by ligating an *Xba*I fragment, containing the Ω element from pUI1638, into the unique *Xba*I site located in the center of open reading frame 1 (*orf1*) within the pZR7072 insert.

The Δ*fdxA*::Ω mutation was engineered in a 1,532-bp DNA fragment that was PCR amplified from the chromosome of strain ADP1 using the primers MUTRR8 and MUTF (Table 2). The PCR product was digested with *Sph*I, which cut in *orf3* near the end of the gene, and the resulting fragment was ligated into pGEM-3Zf(+) that had been digested with *Hinc*II and *Sph*I, resulting in pZR7075. Plasmid pZR7076 was then constructed by the following steps. First, the pZR7075 insert was excised as a *Bam*HI-*Sph*I fragment. Second, pZR7072 was digested with *Pst*I and *Sph*I, both of which cleave at sites located in the vector, downstream of *fdxA*. Third, the Ω element was cut from pUI1638 as a *Bam*HI-*Pst*I fragment. Finally, the three fragments were ligated together in a forced direction ligation, resulting in pZR7076.

Transformation of *Acinetobacter* strains with engineered mutations. Engineered mutations were integrated into the chromosomes of *Acinetobacter* sp. strain ADP1 and its derivatives by transformation as described previously (8).

DNA sequencing. DNA sequencing was performed as described previously (25), and the PCR primers used in this study were synthesized by the W. M. Keck Foundation Biotechnology Resource Laboratory, Yale University, New Haven, Conn.

Determining mutation frequencies. Mutation frequencies were determined either by selecting for spontaneous rifampin-resistant mutants or by selecting for reversion of various base substitution mutations in *pcaH* (14) or *pobR* (7), genes required for growth with *p*-hydroxybenzoate. For both types of assays, single colonies were used to inoculate 1-ml LB cultures. Following incubation for 24 h at 30°C, a 0.5-ml sample of each culture was pelleted by centrifugation, resuspended in mineral medium, and plated on *p*-hydroxybenzoate plates. At least three experiments were performed for each strain examined, and at least seven individual cultures were assayed for each strain per experiment.

Determining marker replacement frequencies during transformation. Recipients containing *pcaH* mutations were transformed with a 2,392-bp *Hin*dIII-fragment from plasmid pZR2 that contained wild-type *Acinetobacter pcaH* plus flanking DNA. Liquid overnight cultures of each recipient were diluted 1:10 in fresh succinate media and incubated for an additional 2 h to induce competence. Transformation cultures consisted of 0.4 ml of the competent recipient culture plus 0.1 μg of the donor DNA fragment. Negative control cultures to which no donor DNA was added were also set up for each recipient. After incubation for 1 h, 50 μg of DNAse I was added to each culture and 0.2-ml aliquots were removed and plated on *p*-hydroxybenzoate plates.

To control for differences in the competence levels of the recipients, a second set of transformation cultures was set up for each strain in parallel to those above. The control cultures were transformed with a 7.1-kb *Pst*I-*Sac*I fragment, excised from plasmid pZR419, which contained the *pob* genes from strain ADP1 with an Ω element inserted into a *pobS-pobR* deletion. Since large insertions and deletions are not recognized by MutS (3, 33), determining the transformation frequency of this marker for each of the recipients provided a measure of their relative competence levels. This was used to normalize the marker replacement efficiencies obtained for each strain. Transformations with the pZR419 fragment were performed as described above with one exception: following addition of DNAse I, the cultures were allowed to incubate an additional 1.5 h before plating on LB containing spectinomycin and streptomycin. Very little difference in competence was observed between each of the eight recipients.

Isolation and characterization of *mutS-fdxA* DNA from divergent *Acinetobacter* strains. PCR primers MUTSF2 and MUTC (Table 2 and Fig. 2) were used to amplify DNA extending between the 3′ terminus of *mutS* and the 5′ terminus of *fdxA* from different *Acinetobacter* isolates. PCR reactions were performed under standard conditions (95°C, 45 s; 56°C, 45 s; 72°C, 4 min; 30 cycles) using *Taq* polymerase (Boehringer) and chromosomal DNA from each strain as a template. The nucleotide sequences of the PCR products were determined as described above.

Sequence analysis. Multiple sequence alignments were performed using the ClustalW program (18). Phylogenetic trees were constructed from the alignments by the neighbor-joining method (41) with 100 bootstrap trials. National Center for Biotechnology Information (NCBI) database accession numbers for the gram-negative MutS sequences used in the alignments were the following: for *Pseudomonas aeruginosa*, AAF42850; for *E. coli*, CAB43497; for *Azotobacter vinelandii*, AAA16868; for *Salmonella typhimurium*, A28668; for *Vibrio cholerae*, AAF93703; and for *Haemophilus influenzae*, AAC22364. The MutS sequences for *Pseudomonas putida, Pseudomonas syringae*, and *Yersinia pestis* were obtained by performing TBLASTX (1) searches of the NCBI unfinished genome database using the *P. aeruginosa mutS* nucleotide sequence (A220055) as the query sequence.

Determination of interspecies transformation frequencies. Chromosomal DNA from each donor was purified using standard techniques (42). Transformations were performed as they were for determining marker integration frequencies, except that the overnight recipient culture was diluted 1:25, 0.1 μg of chromosomal DNA was used as donor DNA, and the cultures were incubated for 6 h before adding DNAse I. Two negative controls were performed to account for spontaneous reversion of the recipient marker; in one, 0.1 μg of the recipient's own DNA was added as a donor, and in the other, no donor DNA was added. In each experiment, five replicate transformations were performed per donor.

TABLE 3. Frequency of Rif^r mutation for *Acinetobacter* strains containing mutations in the *mutS* region

Strain	Mutation	No. of Rif^r mutants/ viable cell[a]
ADP1	None	4.6×10^{-9}
ADP7021	*mutS*	250.0×10^{-9}
ADP7087	*orf1*	6.6×10^{-9}
ADP7088	*fdxA*	2.8×10^{-9}

[a] Mean of at least 21 replicates, with standard deviation of <0.2 of the mean.

Nucleotide sequence accession numbers. The nucleotide sequences presented in this paper were deposited in the NCBI database under the following accession numbers: for *Acinetobacter* sp. strain ADP1 *mutS* region, AF400582; *Acinetobacter* sp. strain 93A2 *mutS′-fdxA′*, AF400583; *Acinetobacter* sp. strain AD321 *mutS′-fdxA′*, AF400584; *Acinetobacter* sp. strain AC423D *mutS′-fdxA′*, AF400585; and *Acinetobacter johnsonii* LUH540 *mutS′-fdxA′*, AF400586.

RESULTS

Cloning of *mutS* and its surrounding genes from *Acinetobacter* sp. strain ADP1. The high degree of sequence conservation shared among members of the MutS protein family facilitated the design of PCR primers for amplification of a 1.9-kb portion of *mutS* from *Acinetobacter* sp. strain ADP1. Nucleotide sequencing revealed that the *mutS* ORF is 2,646 bp in length and is predicted to encode a 97-kDa product that possesses approximately 50% amino acid sequence identity to the MutS homolog from *E. coli* (43). Sequence analysis of about 3 kb of DNA on both sides of *mutS* disclosed six additional ORFs (Fig. 2). Of particular interest was a 324-bp ORF located downstream of *mutS* that was designated *fdxA* because its predicted product displayed more than 75% amino acid sequence identity with a family of closely related 7Fe ferredoxins (21). *fdxA* homologs have also been reported directly downstream of *mutS* in the chromosomes of *A. vinelandii* (30), *P. aeruginosa* (35), and *P. putida* (28), and a similar gene arrangement is also evident in the unfinished genome sequence of *P. syringae* (see Materials and Methods).

Unlike the *mutS-fdxA* regions of *A. vinelandii* and the three *Pseudomonas* species, where *fdxA* is located immediately downstream of *mutS*, in strain ADP1 the two genes are separated by a 450-bp ORF, designated *orf1* in Fig. 2. The predicted product of *orf1* shares its closest similarity with AppA, a redox regulator involved in photosystem formation in *Rhodobacter sphaeroides* (15). However, functional similarity of the *orf1* product and AppA cannot be inferred, since the amino acid sequence similarity is confined to the N-terminal portions of the proteins, a region containing a novel flavin-binding domain in AppA (15). Of the four remaining ORFs, only *orf3* shared significant sequence similarity with known proteins. The predicted product of *orf3* is a member of the *o*-methyltransferase protein family, and its function in strain ADP1 is unknown.

The effect of *mutS* region mutations on spontaneous mutation frequencies in *Acinetobacter*. Defects in *mutS* have been shown to increase spontaneous mutation frequencies in bacteria (5, 46). To determine if this generalization holds true for *Acinetobacter*, we examined the effect of a knockout mutation in *mutS* on the frequency of rifampin resistance (Rif^r) mutations in strain ADP1. To explore the possibility that the genetically linked genes, *orf1* and *fdxA*, might also contribute to mutation repair, the effects of blocks in these genes were also determined. As shown in Table 3, inactivation of *mutS* increased the frequency of Rif^r mutations 54-fold over that for the wild-type strain. No significant change in mutation frequency was observed in strains defective in *orf1* or *fdxA* (Table 3). Thus, it appears that these genes are not directly involved in mismatch repair under the conditions examined here.

Previous reports demonstrated that transition mutations were more frequent than transversions in *mutS*-deficient bacteria (3). As shown in Table 4, similar results were obtained with *Acinetobacter*, where *mutS* inactivation increased reversion frequencies for strains containing the transition mutations *pcaH5, pcaH12*, and *pobR1451* more than 100-fold to frequencies between 2.2×10^{-8} and 5.5×10^{-8}. Inactivation of *mutS* caused no more than a twofold increase in the frequencies of transversions required for reversion of *pobR1424* and *pcaH9*.

Effect of *mutS* mutations on variations in marker replacement frequencies during transformation. In other naturally transformable bacteria, single mismatches arising during heteroduplex formation are recognized with varying efficiencies by the mismatch repair system, resulting in variable integration efficiencies for the donor fragments (3). We examined whether mismatch repair had a similar effect during transformation of *Acinetobacter*. Recipient strains that contained either a transition, a transversion, a 1-bp frameshift, or a 128-bp deletion in *pcaH* were transformed with a DNA fragment that contained the wild-type *pcaH* allele. As shown in Table 5, in *mutS*$^+$ recipients, transformation of the transversion and the 128-bp deletion alleles occurred at five- to eightfold higher frequencies than transformation of either the transition or 1-bp frameshift. As evidence that the differences in transformation frequencies were due to the activity of the mismatch repair system, strains in which *mutS* had been inactivated exhibited similar frequencies for all four recipient alleles (Table 5). The transformation frequencies for the transversion (*pcaH9*) and large deletion (Δ*pcaH19*) were unaffected by MutS.

Inactivation of *mutS* increases the frequency of interspecies transformation in *Acinetobacter*. Experiments with other bacteria have demonstrated that mutations in *mutS* reduced the requirement for sequence identity between donor and recipient alleles during interspecies recombination following conjugation (39), transduction (39, 50), and transformation (19). To determine whether *mutS* played a similar role in controlling the frequency of interspecies transformation in *Acinetobacter*, recipients containing the *pcaH9* mutation in either a *mutS*$^+$ or

TABLE 4. Reversion frequencies for mutations in *mutS*$^+$ and *mutS* mutant strains

Mutation	Mutation type	No. of revertants/viable cell[a]		*mutS* mutant/ *mutS*$^{+b}$
		mutS$^+$	*mutS* mutant	
pcaH5	Transition (C→T)	2.3×10^{-10}	300.0×10^{-10}	130.0
pcaH12	Transition (C→T)	1.9×10^{-10}	260.0×10^{-10}	137.0
pobR1451	Transition (T→C)	4.8×10^{-10}	550.0×10^{-10}	115.0
pobR1424	Transversion (T→A)	1.9×10^{-10}	2.3×10^{-10}	1.2
pcaH9	Transversion (G→T)	1.5×10^{-10}	3.1×10^{-10}	2.1

[a] Mean of at least 20 replicates with standard deviation of <0.2 of the mean.
[b] Ratio of revertants in *mutS* mutant strains to those in *mutS*$^+$ strains.

TABLE 5. Marker replacement frequencies for *mutS*$^+$ and *mutS* mutant *Acinetobacter* recipients

Recipient mutation	No. of transformants/viable recipient cell[a]	
	mutS$^+$	*mutS* mutant
pcaH12 (C→T transition)	4.6×10^{-4} ($\pm 1.3 \times 10^{-4}$)	40.0×10^{-4} ($\pm 1.3 \times 10^{-3}$)
Δ*pcaH20* (1-bp frameshift)	4.4×10^{-4} ($\pm 7.1 \times 10^{-3}$)	40.0×10^{-4} ($\pm 1.0 \times 10^{-3}$)
pcaH9 (G→T transversion)	36.0×10^{-4} ($\pm 1.1 \times 10^{-3}$)	42.0×10^{-4} ($\pm 1.1 \times 10^{-3}$)
Δ*pcaH19* (128-bp deletion)	24.0×10^{-4} ($\pm 4.3 \times 10^{-4}$)	26.0×10^{-4} ($\pm 9.2 \times 10^{-4}$)

[a] Mean (±standard deviation) of at least 10 replicates.

mutS mutant background were transformed with chromosomal DNA from divergent *Acinetobacter* strains.

Our results indicate that there is not an absolute barrier to interspecies transformation in strain ADP1. All but the most divergent donor DNA yielded *pcaH*$^+$ transformants even when the recipient contained a functional *mutS* gene (Table 6). However, the transformation frequencies for most of the donors were 10^3- to 10^6-fold lower than the frequencies obtained using isogenic donor DNA. Only *Acinetobacter* sp. strain 93A2, which is 1 to 2% divergent relative to strain ADP1 at the nucleotide level, transformed the *pcaH9* mutation as efficiently as strain ADP1.

Although MutS did not completely block interspecies exchange in *Acinetobacter*, its inactivation did significantly increase the transformation frequencies for most of the divergent donors (Table 6). The effect of *mutS* inactivation ranged from 3-fold for strain AD321, whose *pcaH* gene is approximately 12% divergent from that of strain ADP1, to 17-fold for strain 01B0, whose *pcaH* gene is about 20% divergent. Inactivation of *mutS* had no effect on transformation with DNA that was identical (strain ADP1) or nearly identical (strain 93A2) to the recipient. Likewise, MutS did not affect transformation with extremely divergent DNA such as that from *P. putida*, which failed to yield transformants in either the *mutS*$^+$ or *mutS* mutant recipients.

PCR amplification combined with restriction analysis and DNA sequencing was used to examine representative transformants and confirm that the recombinant phenotype was due to replacement of the recipient allele by the divergent donor DNA and not spontaneous reversion (results not shown). These analyses indicated that in most of the transformants, donor DNA had replaced not only the *pcaH* allele but various amounts of flanking DNA as well. In some cases as much as 7 to 8 kb of the recipient's DNA had been replaced.

Close genetic linkage of *mutS* and *fdxA* in *Acinetobacter* strains and other gram-negative bacteria. Our observation that *mutS* and *fdxA* are closely linked in *Acinetobacter* sp. strain ADP1 and in members of the genera *Pseudomonas* and *Azotobacter* made it of interest to determine if such linkage was conserved among other *Acinetobacter* strains. It was also of interest to determine whether the interposition of *orf1* between the two genes, a trait that distinguishes the *mutS-fdxA* region of strain ADP1 from those of *A. vinelandii* and the *Pseudomonas* species, was conserved as well. The PCR primers MUTC and MUTSF2 were used in an attempt to amplify DNA extending from the 5′ end of *mutS* to the 5′ end of *fdxA* in 10 *Acinetobacter* strains (Fig. 2). Eight of the 10 strains examined yielded distinct PCR products that were either 3.6, 3.2, or 2.7 kb in size (data not shown). Sequence analysis of the products from four strains revealed considerable variability. *Acinetobacter* sp. strain 93A2, similar to strain ADP1 in that it is competent for natural transformation (Young and Ornston, unpublished result), possesses 98% nucleotide sequence identity to strain ADP1 throughout the 3.6-kb amplified region which includes a homolog of *orf1*. The 3.2-kb amplified segment from strain AD321 includes a noncoding, 655-bp intergenic region between *mutS* and *fdxA*. In the 2.7-kb amplified region from strains AC423D and LUH540, *fdxA* is about 200 bp downstream from *mutS*, similar to the genetic organization and intergenic spacing observed in the *Pseudomonas* species and *A. vinelandii*.

Conserved and divergent sequences within MutS. Alignment of the *Acinetobacter* sp. strain ADP1 MutS amino acid sequence with the MutS sequences of nine different gram-negative bacteria (see Materials and Methods) revealed substantial sequence conservation (≥43% amino acid similarity), particularly among amino acids comprising the mismatch-binding domain located near the N terminus and those in the ATP-binding and helix-turn-helix domains near the C terminus (Fig. 3) (29, 34). Despite the overall sequence conservation, MutS from strain ADP1 did contain notable signs of divergence. The length of the region spanning from the mismatch binding domain (amino acids 2 to 115 in the *E. coli* MutS sequence [29]) to the helix-turn-helix domain (*E. coli* amino acids 766 to 800) varies by no more than four amino acids in nine other gram-negative MutS homologs, while in the strain

TABLE 6. Interspecies transformation frequencies for *mutS*$^+$ and *mutS* mutant *Acinetobacter* recipients

Donor	% *pcaH* sequence identity[c]	No. of transformants per viable recipient cell[a,b]		*mutS* mutant/*mutS*$^{+d}$
		mutS$^+$	*mutS* mutant	
Acinetobacter sp. strain ADP1	100	2.9×10^{-2}	1.9×10^{-2}	0.65
Acinetobacter sp. strain 93A2	99	2.9×10^{-2}	2.4×10^{-2}	0.83
Acinetobacter sp. strain AD321	88	2.9×10^{-5}	9.9×10^{-5}	3.4
Acinetobacter sp. strain AD532	81	6.5×10^{-7}	76.0×10^{-7}	11.7
Acinetobacter sp. strain 48A1	81	9.3×10^{-8}	110.0×10^{-8}	11.8
A. haemolyticus strain 40B4	80	7.9×10^{-8}	69.0×10^{-8}	8.7
Acinetobacter sp. strain 01B0	80	1.6×10^{-8}	28.0×10^{-8}	17.5
P. putida PRS2000	55	$<1.0 \times 10^{-9}$	$<1.0 \times 10^{-9}$	

[a] Recipients (ADP6205 and ADP7099) contained the *pcaH9* mutation, and transformants were selected on *p*-hydroxybenzoate plates.

[b] Mean frequencies of three experiments, each of which consisted of at least five replicates of each donor/recipient transformation pair. Standard deviations were ≤0.2 of the mean.

[c] *pcaH* sequence identity of each donor relative to that of strain ADP1 is based on partial sequence data. At least 80% of the total *pcaHG* sequence was determined for each donor (Young and Ornston, unpublished results).

[d] Ratio of transformants in *mutS* mutant recipients to those in *mutS*$^+$ recipients.

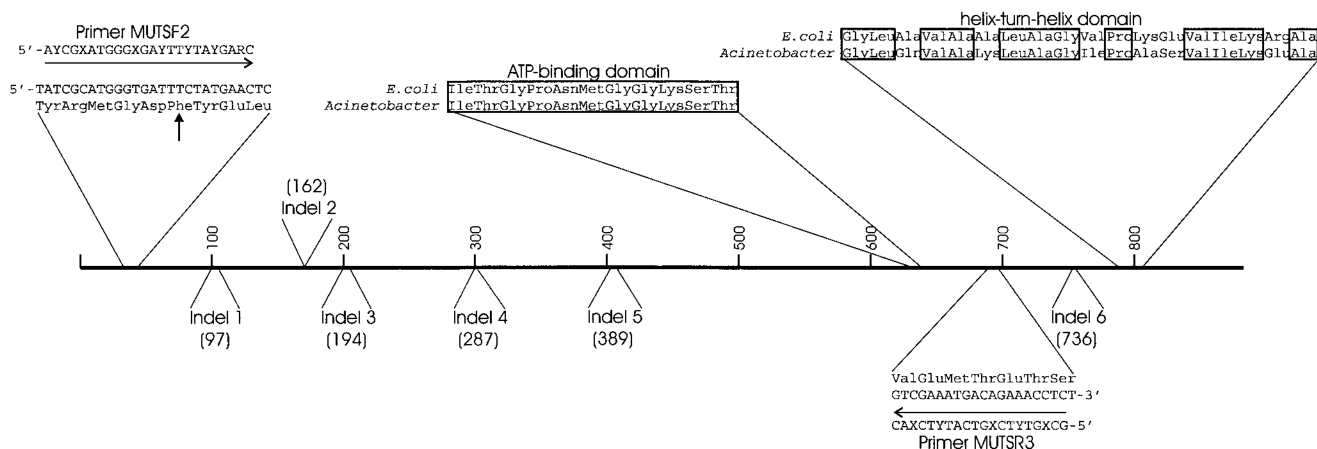

FIG. 3. Conserved and divergent amino acid sequences in *Acinetobacter* MutS. Horizontal arrows indicate degenerate primers originally used to amplify a portion of *mutS* from the chromosome of strain ADP1. Boxes surround amino acid residues conserved in the *Acinetobacter* MutS and in the *E. coli* MutS for which the crystal structure has been determined (29). A vertical arrow indicates a conserved phenylalanine residue that has been shown to be required for mismatch binding in other MutS homologs. Numbers in parentheses indicate positions in the *E. coli* MutS sequence corresponding to the starts of indels that distinguish the primary sequences of the two MutS proteins.

ADP1 homolog, six indels were present that resulted in its being 13 to 17 amino acids longer than the other homologs over the same interval.

Indels represent insertions and deletions that punctuate evolutionary divergence of orthologous proteins (17), and so identification of potential indels in the MutS protein from strain ADP1 warranted determining how conserved the polymorphisms were among other *Acinetobacter* homologs. As shown in Fig. 4, all six indels set representatives of the genus *Acinetobacter* apart from other gram-negative bacteria. Unlike the other indels, indel 1 also separates the *Acinetobacter* strains ADP1, 93A2, and AD321 from strains AC423D and LUH540. The groupings that are based upon the distribution of MutS indels match those found in the phylogenetic tree that is based upon MutS amino acid sequence alignments (Fig. 5). This tree is essentially congruent to one generated from an alignment of the 16S rDNA sequences from these strains (Young and Ornston, unpublished result).

DISCUSSION

Influence of MutS on the frequency of natural transformation in *Acinetobacter* sp. strain ADP1. The efficiency with which MutS binds different mismatches in vivo affects not only the spectrum and frequency of spontaneous mutations in the cell but also the frequency in which mutant alleles are integrated into the chromosome by recombination. Variations in marker transformation frequencies were first reported for *Streptococcus pneumoniae* (16), whose natural transformation system is similar to that of strain ADP1 in many respects (37). As shown

	Indel 1	Indel 2	Indel 3
Acinetobacter ADP1	ICEQVGEGENAGSRCKAPMER	FKVQQHEFKTEQLYIELARLM	ILEQIKKQIECSITKRPNVDF
Acinetobacter 93A2	ICEQVGEGENAGSRGKAPMER	FKVQQHEFKTEQLYIELARLM	ILEQIKKQIECSITKRPNVDF
Acinetobacter AD321	ICEQVGEGESAGSRGKAPMER	FKVQEHDFKTEQLAIELSRLM	ILEQIKKQLDCPITKRPNVDF
Acinetobacter AC423D	ICEQIG-----EVTGKAPVER	FKVQQQEFQLEQLGIELSRLM	IIEHIKKLLDCPVTRRPNVDF
Acinetobacter LUH540	ICEQIG-----EVTGKAPVER	FKVQQQEFQLEQLGIELSRLM	IIEHIKKLLDCPVTRRPNVDF
Pseudomonas aeruginosa	ICEQIG----DPATSKGPVER	FSVQEIKG-WETLLAELERLN	PAEKRR-----GVRRRAPWDF
Azotobacter vinelandii	ICEQIG----DPATSKGPVER	FNVQEIQG-WENLLAELERLN	PLEKRR-----GAHRRAPWDF
Escherichia coli	ICEQIG----DPATSKGPVER	FRLSEPAD-RETMAAELQRTN	LIEGRR-----GLRRRPLWEF
	(97)	(162)	(194)

	Indel 4	Indel 5	Indel 6
Acinetobacter ADP1	LEIIDPLFEHG-TSLFNLIND	ALLPAIQTKSSKLLNELDHEL	YFELTELG-SESAIDNYHVTA
Acinetobacter 93A2	LEIIDPLFEHG-TSLFNLIND	ALLPAIQTKSSKLLNELDHQL	YFELTELG-SESAIDNYHVTA
Acinetobacter AD321	LEIIDPLFEHG-TSLFNLIND	ALLPVIQAQSSALLTDLDQQL	YFELTELG-SEAGIDNYHVTA
Acinetobacter AC423D	LELVEPKFEHG-TSLFQLIND	AIQPVIQSKQSALITQLNEEL	YFELTELA-KETAIDNYHVTA
Acinetobacter LUH540	LELVEPKFEHG-TSLFQLIND	AIQPVIQSKQSALITQLNEEL	YFELTELA-KETAIDNYHVTA
Pseudomonas aeruginosa	LELDINLSGGRENTLQSVVDR	AMTELEAPH----LQALATTI	YFELTCLPESQPAVANVHLNA
Azotobacter vinelandii	LELDVNLSGGRDNTLQSVIDR	ALSPLEAPH----LQALAGNI	YFELTVLAESQPVVANVHLSA
Escherichia coli	LEITQNLAGGAENTLASVLDC	QLETVDSAP----VQALREKM	YFELTQLPEKEMGVANVHLDA
	(287)	(389)	(736)

FIG. 4. Indels that distinguish *Acinetobacter* MutS homologs from those of other gram-negative bacteria. Portions of a multiple sequence alignment depicting *Acinetobacter* MutS indels relative to other gram-negative homologs. Dashes indicate gaps in the aligned sequences. Numerals indicate positions corresponding to amino acids in the *E. coli* MutS primary sequence (29) that are located immediately prior to the start of the indel. Bold type indicates amino acids that are identical to those in *Acinetobacter* sp. strain ADP1.

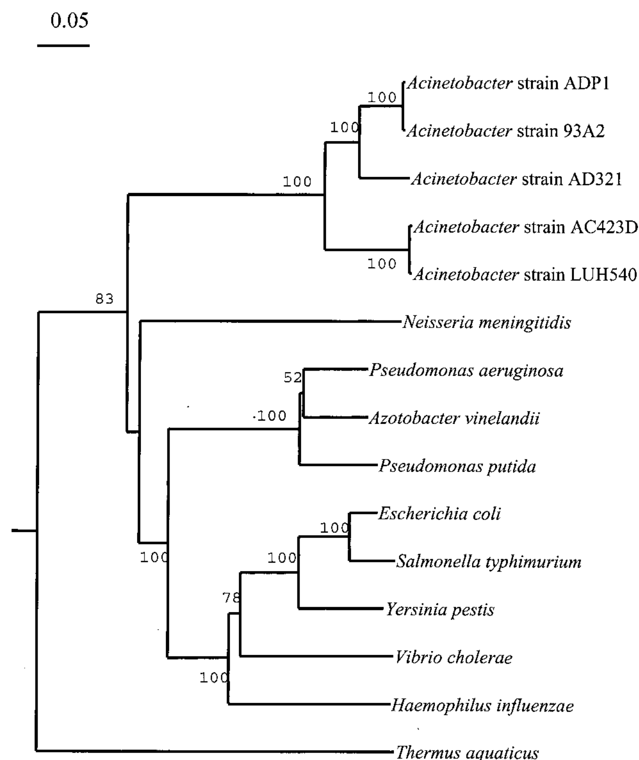

FIG. 5. Phylogenetic tree based on alignments of MutS from *Acinetobacter* with those from nine other gram-negative bacteria. The tree was generated as described in Materials and Methods. Bootstrap values are indicated on tree branches.

in Table 5, the *Acinetobacter* mismatch repair system influenced transformation frequencies in a marker-specific manner as well. The effect of the *Acinetobacter* MutS protein on marker integration frequencies and its specificity towards transition and frameshift mismatches generally parallels the effect reported for HexA, the MutS homolog in *S. pneumoniae* (3, 12). The specificity of MutS was also apparent in the results of spontaneous mutation assays that were used to examine its role in postreplication repair (Table 4).

Although the single-transversion mismatch formed during recombination between the *pcaH9* allele and wild-type DNA was not efficiently recognized by MutS (Table 5), MutS strongly influenced transformation frequencies when *pcaH9* was transformed with heterospecific donor DNA (Table 6), which results in the formation of heteroduplexes containing multiple mismatches. In a wild-type *mutS* background, the frequency of recombinant formation with divergent *Acinetobacter* donors varied more than 10^6-fold (Table 6). The lowest frequencies were observed with donor DNA possessing only 80% nucleotide sequence identity to the recipient, and these frequencies were increased 3- to 17-fold by inactivation of MutS (Table 6).

We have shown that mismatch repair in strain ADP1 is capable of reducing the integration efficiencies of donor fragments containing single transition mismatches (Table 5), yet chromosomal DNA from strain 93A2 transformed the *mutS*$^+$ recipient with approximately the same efficiency as isogenic DNA (Table 6), even though its *pcaH* allele contains multiple transition substitutions in the vicinity of the target mutation. One possible explanation for this result is that the mismatch repair system of strain ADP1 may be susceptible to saturation by high numbers of mismatches, similar to that shown in *S. pneumoniae* (19) and in *E. coli* (31). Saturation results in a transient mutator phenotype as functional MutS homodimers are titrated from the system by binding to numerous mismatches simultaneously. Chromosomal DNA from strain 93A2 could saturate mismatch repair during attempts to undergo homeologous recombination at other sites on the chromosome in addition to the target locus. In *S. pneumoniae*, transformation with highly divergent DNA resulted in saturation even during formation of a single heteroduplex at the target locus (19). Further study is required to determine how much of an effect saturation of mismatch repair has on interspecies transformation frequencies in *Acinetobacter*.

The mutator phenotype of MutS-deficient strains. Inactivation of *Acinetobacter mutS* increased the frequency of Rif^r mutations 54-fold over that for wild-type cells (Table 3). Though a significant value, it is less than the 100- to 1,000-fold effects reported for corresponding mutants of *E. coli* (4) and *P. putida* (28) and more closely resembles the effects reported for mismatch repair-deficient strains of *A. vinelandii* (64-fold) (30) and *S. pneumoniae* (4- to 30-fold) (46, 47). A previous comparison of data reported for the mismatch repair systems of *S. pneumoniae* and *E. coli* indicated that they reduced the frequency of spontaneous mutations at single sites by about 100- and 1,000-fold respectively (3). However, it was pointed out that despite the reduced effect in *S. pneumoniae*, the overall mutation rates for the two bacteria were still within the same range (10^{-8} to 10^{-10} mutations cell^{-1} generation^{-1}), suggesting that the initial fidelity of DNA synthesis may be greater in *S. pneumoniae* (3).

Further investigation is required to determine conclusively whether mismatch repair has a reduced role in maintaining the fidelity of DNA replication in naturally transformable bacteria like *S. pneumoniae* and *Acinetobacter*. Such a situation is not inconceivable, given that attempts to undergo homeologous recombination can result in a transient mutator phenotype due to saturation of the mismatch repair system. Naturally transformable bacteria that are capable of taking up large amounts of heterologous DNA may have evolved mechanisms to increase the fidelity of DNA replication to compensate for frequent mismatch repair saturation. This would allow them to take advantage of the potential for rapid adaptation via interspecies genetic exchange without increasing the frequency of less beneficial spontaneous replication errors.

Chromosomal organization and *mutS* sequence divergence in *Acinetobacter* and other gram-negative bacteria. The observation that *fdxA* homologs are located downstream of *mutS* in members of the genera *Azotobacter* and *Pseudomonas* and in five *Acinetobacter* strains suggested that conservation of this genetic organization might have been selected due to a functional relationship between these genes. However, inactivation of *fdxA* did not result in increased spontaneous mutation frequencies for either *Acinetobacter* (Table 3) or *A. vinelandii* (30), indicating that the genes does not contribute to mutation avoidance when the cells are grown in nutrient broth. Interestingly, the *A. vinelandii fdxA* gene product, *AvFdI*, was recently shown to be a redox sensor involved in gene regulation

under oxidative stress conditions (40). Since MutS is believed to participate in the repair of oxidative DNA damage (45, 51), it will be of interest to see whether inactivation of *fdxA* affects mutation frequencies, or *mutS* expression, when cells are exposed to oxidative stress.

The observation that several indels are conserved among the *Acinetobacter* MutS (Fig. 4) protein sequences raises questions concerning why these polymorphisms have been preserved. In natural populations of *E. coli, mutS* undergoes frequent horizontal transfers as an adaptive mechanism in which mutation and recombination rates are modulated through recurrent losses and reacquisition of *mutS* (6). Reacquisition is often the result of homeologous recombination in which the defective *mutS* allele is replaced by a functional allele from a divergent strain. Frequent horizontal transfer of *mutS* between *Acinetobacter* strains could serve to homogenize its nucleotide sequence and result in conservation of the MutS indels. However, the relatively high levels of sequence divergence exhibited by the *Acinetobacter* MutS sequences, including many amino acid substitutions that are located immediately adjacent to indels (Fig. 4), suggests that this is not the case, since these residues would also be expected to be conserved due to frequent exchange. Frequent horizontal transfer of *mutS* may occur between closely related *Acinetobacter* strains; however, even if such exchange occurs it is unlikely that it would result in the conservation of polymorphisms between members of separate genomic species.

Although none of the *Acinetobacter* indels are located in highly conserved regions of the MutS, there still may be a functional basis for their selection. It has been noted that indels are most likely to occur in loop regions near the surface of a protein's three-dimensional structure (11). There is also evidence that MutS functions as part of multimeric protein complexes, interacting with other mismatch repair enzymes such as MutL and potentially interacting with proteins involved in DNA replication, recombination, and other repair pathways (20). Therefore, although none of the *Acinetobacter* MutS indels are located in regions that have been identified as essential for postreplication repair (49), they may be important for other MutS functions in *Acinetobacter* by facilitating interactions with other proteins.

We have demonstrated that MutS from *Acinetobacter* shares functional similarity with homologs in other bacteria while displaying sequence polymorphisms that appear to set it apart from other gram-negative strains. Continued genetic and biochemical studies of mismatch repair systems in organisms such as *Acinetobacter* allow comparison to be made with paradigmatic systems, such as those of *S. pneumoniae* and *E. coli*. These comparisons will help determine whether individual traits, such as the ability to undergo natural transformation, have affected the evolution of MutS within a particular group of bacteria.

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REFERENCES

1. **Baumann, P., M. Doudoroff, and R. Y. Stanier.** 1968. A study of the *Moraxella* group. II. Oxidative-negative species (genus *Acinetobacter*). J. Bacteriol. **95:**1520–1541.
2. **Brooks, K., and T. Sodeman.** 1974. Clinical studies on a transformation test for identification of *Acinetobacter* (*Mima* and *Herellea*). Appl. Microbiol. **27:**1023–1026.
3. **Claverys, J. P., and S. A. Lacks.** 1986. Heteroduplex deoxyribonucleic acid base mismatch repair in bacteria. Microbiol. Rev. **50:**133–165.
4. **Cox, E. C.** 1976. Bacterial mutator genes and the control of spontaneous mutation. Annu. Rev. Genet. **10:**135–156.
5. **Cox, E. C., G. E. Degnen, and M. L. Scheppe.** 1972. Mutator gene studies in *Escherichia coli*: the *mutS* gene. Genetics **72:**551–567.
6. **Denamur, E., G. Lecointre, P. Darlu, O. Tenaillon, C. Acquaviva, C. Sayada, I. Sunjevaric, R. Rothstein, J. Elion, F. Taddei, M. Radman, and I. Matic.** 2000. Evolutionary implications of the frequent horizontal transfer of mismatch repair genes. Cell **103:**711–721.
7. **DiMarco, A. A., B. Averhoff, and L. N. Ornston.** 1993. Identification of the transcriptional activator *pobR* and characterization of its role in the expression of *pobA*, the structural gene for *p*-hydroxybenzoate hydroxylase in *Acinetobacter calcoaceticus*. J. Bacteriol. **175:**4499–4506.
8. **Doten, R. C., K. L. Ngai, D. J. Mitchell, and L. N. Ornston.** 1987. Cloning and genetic organization of the *pca* gene cluster from *Acinetobacter calcoaceticus*. J. Bacteriol. **169:**3168–3174.
9. **Eisen, J. A.** 1998. A phylogenomic study of the MutS family of proteins. Nucleic Acids Res. **26:**4291–4300.
10. **Eraso, J. M., and S. Kaplan.** 1994. *prrA*, a putative response regulator involved in oxygen regulation of photosynthesis gene expression in *Rhodobacter sphaeroides*. J. Bacteriol. **176:**32–43.
11. **Freimuth, P. I., J. W. Taylor, and E. T. Kaiser.** 1990. Introduction of guest peptides into *Escherichia coli* alkaline phosphatase. Excision and purification of a dynorphin analogue from an active chimeric protein. J. Biol. Chem. **265:**896–901.
12. **Gasc, A. M., A. M. Sicard, and J. P. Claverys.** 1989. Repair of single- and multiple-substitution mismatches during recombination in *Streptococcus pneumoniae*. Genetics **121:**29–36.
13. **Gebhard, F., and K. Smalla.** 1998. Transformation of *Acinetobacter* sp. strain BD413 by transgenic sugar beet DNA. Appl. Environ. Microbiol. **64:**1550–1554.
14. **Gerischer, U., and L. N. Ornston.** 1995. Spontaneous mutations in *pcaH* and *-G*, structural genes for protocatechuate 3,4-dioxygenase in *Acinetobacter calcoaceticus*. J. Bacteriol. **177:**1336–1347.
15. **Gomelsky, M., and S. Kaplan.** 1998. AppA, a redox regulator of photosystem formation in *Rhodobacter sphaeroides* 2.4.1, is a flavoprotein. Identification of a novel fad binding domain. J. Biol. Chem. **273:**35319–35325.
16. **Green, D. M.** 1959. A host-specific variation affecting relative frequency of transformation of two markers in pneumococcus. J. Exp. Cell Res. **16:**466–480.
17. **Gupta, R. S.** 1998. Protein phylogenies and signature sequences: a reappraisal of evolutionary relationships among archaebacteria, eubacteria, and eukaryotes. Microbiol. Mol. Biol. Rev. **62:**1435–1491.
18. **Higgins, D. G., J. D. Thompson, and T. J. Gibson.** 1996. Using CLUSTAL for multiple sequence alignments. Methods Enzymol. **266:**383–402.
19. **Humbert, O., M. Prudhomme, R. Hakenbeck, C. G. Dowson, and J. P. Claverys.** 1995. Homeologous recombination and mismatch repair during transformation in *Streptococcus pneumoniae*: saturation of the Hex mismatch repair system. Proc. Natl. Acad. Sci. USA **92:**9052–9056.
20. **Hsieh, P.** 2001. Molecular mechanisms of DNA mismatch repair. Mutat. Res. **486:**71–87.
21. **Iismaa, S. E., A. E. Vazquez, G. M. Jensen, P. J. Stephens, J. N. Butt, F. A. Armstrong, and B. K. Burgess.** 1991. Site-directed mutagenesis of *Azotobacter vinelandii* ferredoxin I. Changes in [4Fe-4S] cluster reduction potential and reactivity. J. Biol. Chem. **266:**21563–21571.
22. **Juni, E.** 1972. Interspecies transformation of *Acinetobacter*: genetic evidence for a ubiquitous genus. J. Bacteriol. **112:**917–931.
23. **Juni, E.** 1978. Genetics and physiology of *Acinetobacter*. Annu. Rev. Microbiol. **32:**349–371.
24. **Juni, E., and A. Janik.** 1969. Transformation of *Acinetobacter calco aceticus* (*Bacterium anitratum*). J. Bacteriol. **98:**281–288.
25. **Kok, R. G., D. A. D'Argenio, and L. N. Ornston.** 1997. Combining localized PCR mutagenesis and natural transformation in direct genetic analysis of a transcriptional regulator gene, *pobR*. J. Bacteriol. **179:**4270–4276.
26. **Kok, R. G., D. A. D'Argenio, and L. N. Ornston.** 1998. Mutation analysis of PobR and PcaU, closely related transcriptional activators in *Acinetobacter*. J. Bacteriol. **180:**5058–5069.
27. **Kok, R. G., D. M. Young, and L. N. Ornston.** 1999. Phenotypic expression of PCR-generated random mutations in a *Pseudomonas putida* gene after its introduction into an *Acinetobacter* chromosome by natural transformation. Appl. Environ. Microbiol. **65:**1675–1680.
28. **Kurusu, Y., T. Narita, M. Suzuki, and T. Watanabe.** 2000. Genetic analysis

of an incomplete *mutS* gene from *Pseudomonas putida*. J. Bacteriol. **182:**5278–5279.
29. **Lamers, M. H., A. Perrakis, J. H. Enzlin, H. H. Winterwerp, N. de Wind, and T. K. Sixma.** 2000. The crystal structure of DNA mismatch repair protein MutS binding to a G × T mismatch. Nature **407:**711–717.
30. **Le, O., B. Shen, S. E. Iismaa, and B. K. Burgess.** 1993. *Azotobacter vinelandii mutS*: nucleotide sequence and mutant analysis. J. Bacteriol. **175:**7707–7710.
31. **Maas, W. K., C. Wang, T. Lima, A. Hach, and D. Lim.** 1996. Multicopy single-stranded DNA of *Escherichia coli* enhances mutation and recombination frequencies by titrating MutS protein. Mol. Microbiol. **19:**505–509.
32. **Mejean, V., J. P. Claverys, H. Vasseghi, and A. M. Sicard.** 1981. Rapid cloning of specific DNA fragments of *Streptococcus pneumoniae* by vector integration into the chromosome followed by endonucleolytic excision. Gene **15:**289–293.
33. **Modrich, P., and R. Lahue.** 1996. Mismatch repair in replication fidelity, genetic recombination, and cancer biology. Annu. Rev. Biochem. **65:**101–133.
34. **Obmolova, G., C. Ban, P. Hsieh, and W. Yang.** 2000. Crystal structures of mismatch repair protein MutS and its complex with a substrate DNA. Nature **407:**703–710.
35. **Oliver, A., R. Canton, P. Campo, F. Baquero, and J. Blazquez.** 2000. High frequency of hypermutable *Pseudomonas aeruginosa* in cystic fibrosis lung infection. Science **288:**1251–1254.
36. **Ornston, L. N., and R. Y. Stanier.** 1966. The conversion of catechol and protocatechuate to beta-ketoadipate by *Pseudomonas putida*. J. Biol. Chem. **241:**3776–3786.
37. **Palmen, R., and K. J. Hellingwerf.** 1997. Uptake and processing of DNA by *Acinetobacter calcoaceticus*—a review. Gene **192:**179–190.
38. **Prentki, P., and H. M. Krisch.** 1984. In vitro insertional mutagenesis with a selectable DNA fragment. Gene **29:**303–313.
39. **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.
40. **Regnstrom, K., S. Sauge-Merle, K. Chen, and B. K. Burgess.** 1999. In *Azotobacter vinelandii*, the E1 subunit of the pyruvate dehydrogenase complex binds *fpr* promoter region DNA and ferredoxin I. Proc. Natl. Acad. Sci. USA **96:**12389–12393.
41. **Saitou, N., and M. Nei.** 1987. The neighbor-joining method: a new method for reconstructing phylogenetic trees. Mol. Biol. Evol. **4:**406–425.
42. **Sambrook, J., E. F. Fritsch, and T. Maniatis.** 1989. Molecular cloning: a laboratory manual, 2nd edition. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y.
43. **Schlensog, V., and A. Bock.** 1991. The *Escherichia coli fdv* gene probably encodes *mutS* and is located at minute 58.8 adjacent to the *hyc-hyp* gene cluster. J. Bacteriol. **173:**7414–7415.
44. **Shanley, M. S., E. L. Neidle, R. E. Parales, and L. N. Ornston.** 1986. Cloning and expression of *Acinetobacter calcoaceticus catBCDE* genes in *Pseudomonas putida* and *Escherichia coli*. J. Bacteriol. **165:**557–563.
45. **Terato, H., A. Masaoka, M. Kobayashi, S. Fukushima, Y. Ohyama, M. Yoshida, and H. Ide.** 1999. Enzymatic repair of 5-formyluracil. II. Mismatch formation between 5-formyluracil and guanine during DNA replication and its recognition by two proteins involved in base excision repair (AlkA) and mismatch repair (MutS). J. Biol. Chem. **274:**25144–25150.
46. **Tiraby, J. G., and M. S. Fox.** 1973. Marker discrimination in transformation and mutation of pneumococcus. Proc. Natl. Acad. Sci. USA **70:**3541–3545.
47. **Tiraby, G., and M. A. Sicard.** 1973. Integration efficiencies of spontaneous mutant alleles of *amiA* locus in pneumococcal transformation. J. Bacteriol. **116:**1130–1135.
48. **Towner, K. J.** 1997. Clinical importance and antibiotic resistance of *Acinetobacter* spp. J. Med. Microbiol. **46:**721–746.
49. **Wu, T. H., and M. G. Marinus.** 1994. Dominant negative mutator mutations in the *mutS* gene of *Escherichia coli*. J. Bacteriol. **176:**5393–5400.
50. **Zahrt, T. C., G. C. Mora, and S. Maloy.** 1994. Inactivation of mismatch repair overcomes the barrier to transduction between *Salmonella typhimurium* and *Salmonella typhi*. J. Bacteriol. **176:**1527–1529.
51. **Zhao, J., and M. E. Winkler.** 2000. Reduction of GC → TA transversion mutation by overexpression of MutS in *Escherichia coli* K-12. J. Bacteriol. **182:**5025–5028.