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Impact of a mutator phenotype on motility and cell adherence in *Salmonella* Heidelberg

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ABSTRACT

In this study, we investigated adherence and motility of the hypermutator *Salmonella enterica* Heidelberg B182 bovine strain related to a 12 bp deletion in *mutS*. This mutator phenotype was associated with increased adherence to epithelial cells and with high expression of *fimA* as shown by real-time RT-PCR. Motility studies showed that *fliC* were up-regulated in the B182 strain, while *fljA* and *fljB* were down-regulated.

In order to determine if mutated *mutS* is implicated in this genes expression, isogenic strains, derived from a WT strain, containing the 12 bp deletion in *mutS* (Δ*12bpmutS*) or an inactivated *mutS* (Δ*mutS*) were generated. Δ*12bpmutS* and Δ*mutS* strains showed a spontaneous mutation rate similar to the environmental strain B182, but exhibited lower adherence capacity and *fimA* expression. In contrast to the fimbriae genes, in Δ*12bpmutS*, *fliC* expression was up-regulated, but *fljA* and *fljB* expression were decreased, as in the B182 strain. Only *fljB* expression was increased in Δ*mutS* mutants.

Taken together, our data suggest that *mutS* alteration does not influence fimbriae expression but can impact flagella genes.

1. Introduction

Among natural *Salmonella* population some strains display a hypermutator phenotype by harboring mutations in Methyl Mismatch Repair system (MMR) and exhibiting increased mutation rates (Giraud et al., 2003). A mutation in *mutS* is predominantly responsible for the hypermutator phenotype (Oliver et al., 2000, 2002).

A relationship between mutator phenotypes and virulence has previously been demonstrated in several bacterial pathogens (Merino et al., 2002; Mena et al., 2007). The mechanisms used by *Salmonella* to establish infection in a human host include motility, adherence, invasion, and growth within the host cells. Adherence to tissue culture cells is mediated by mannose-sensitive type 1 fimbriae, such as FimA (Rossolini et al., 1994), FimH (Boddicker et al., 2002) and FimI (Rossolini et al., 1993). Extensive in vivo and in vitro studies have investigated the role of the flagella, complex surface-associated structures used by bacteria to move forward, in *S.* Typhimurium invasiveness (Jones et al., 1992). *Salmonella* has two distinct flagellar antigens, FliC (flagellin phase type 1/H1) and FljB (flagellin phase type 2/H2), that are coordinately regulated so that only one flagellar antigen is expressed at any time (Silverman, 1980). *fljB* constitutes an operon together with *fljA*, which encodes a negative regulator for *fliC* expression (Smith and Selander, 1991).

The main purpose of this study was to investigate the relationship between the acquisition of the hypermutator

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Table 1
Bacterial strains used in this study.

Salmonella Heidelberg Strains	*mutS* genotype	References or sources
B182	Deletion of 12 bp in *mutS*	Le Gall et al. (2009)
B182/pGEMT*mutS*	B182 strain complemented with wild type *mutS* encoded by pGEMT plasmid	Le Gall et al. (2009)
WT	Wild type *mutS*	Le Gall et al. (2009)
Δ12bpmutS	Wild type *mutS* modified by the deletion of 12 bp in *mutS*	In this study
ΔmutS	Wild type *mutS* fully inactivated	In this study

phenotype and the expression of virulence factors implicated in adherence and motility. We previously identified a bovine strain of *Salmonella enterica* serovar Heidelberg (*S.* Heidelberg) with a hypermutator phenotype caused by a 12 bp deletion in *mutS* (Le Gall et al., 2009). The aim of this work was to compare the adherence and motility of a normal phenotype strain of *S.* Heidelberg (WT) counterpart serovar, without mutations in *mutS*, to two isogenic strains carrying *mutS* mutations (*Δ12bpmutS* and *ΔmutS*).

2. Materials and methods

2.1. Bacterial strains and culture conditions

The *Salmonella* strains used in this study are listed in Table 1. Fresh bacteria were grown overnight at 37 °C and subcultured by dilution in 100 ml fresh Luria-Bertani (LB) medium (AES Laboratory) followed by incubation for 90 min at 37 °C. Ten milliliters of each culture were then centrifuged at 3000 × *g* for 15 min. The pellets were resuspended in 1 ml of Dulbecco's minimal essential medium (DMEM) and used to infect HeLa cells.

2.2. Generation of the *Δ12bpmutS* and *ΔmutS* mutants

The *Δ12bpmutS* mutant was constructed by homologous recombination of the *mutS* gene containing the same 12 bp deletion described in the B182 strain (Le Gall et al., 2009). The *mutS* gene was PCR amplified using the B182 strain chromosomal DNA as template, the primers (Table 2) *mutS-SacI* and *mutS-SphI* containing *SacI* and *SphI* restriction sites, and Phusion high-fidelity DNA polymerase (Finnzymes) according to the manufacturer's recommendations. After digestion with *SacI* and *SphI*, the PCR product was cloned into the *SacI* and *SphI* sites of the vector pDS132 (Philippe et al., 2004). Sequencing of the insert was carried out with primers *mutS*8, *mutS*9, *mutS-SacI* and *mutS-SphI* using the Big Dye Terminator Kit version 3.1 (Applied Biosystems) (Table 2). The cloning steps were performed in SM10 λpir strain (LMBP 3889, BCCM/LMBP plasmid collections, Gent, Belgium) to allow plasmid replication. Plasmids carrying the *Δ12bpmutS* gene were introduced in the *Escherichia coli* SM10 λpir strain and then transferred to WT by conjugation. Allelic exchange was performed as previously described (Philippe et al., 2004).

Table 2
Primers used in this study.

Gene amplified	Primers	Primer sequences (5′–3′)
Primers used for PCR amplification in the construction of mutS mutants		
mutS for *Δ12bpmutS* strain construction	*mutS-SacI*	GTAAAGAGCTCATGAATGAGTCATTTGATA
	mutS-SphI	TCTGGCATGCTTACACCAGACTTTTCAG
	*mutS*8	GGTAGCCGAATGCTTAAACG
	*mutS*9	TTTGACCACGGCTAATCTGA
mutS for *ΔmutS* strain construction	*mutS-SacI-ΔmutS*	CCCCCTGAGCTCGTCCAGGAATGATGAGAA
	mutS-XbaI-ΔmutS	CCAGCTCTAGAGCATTCGGCTACCCATTG
	mutS-AscI-ΔmutS	CCAGCGGCGCGCCGCATTCGGCTACCCATTG
	mutS-SphI-ΔmutS	AAACGGCATGCACAGGGAAAGAGGAGGACGGA
bla for *ΔmutS* strain construction	*βLac-XbaI*	AATGTGCTCTAGACCCCTATTTGTTTATTTTTCTA
	*βLac-Asc*I	AAGCCAGGCGCGCCGGAAAAAGAGTTGGTAGCTC
Primers used for real-time RT-PCR		
fimA	*1*	ACG GCG ATT GGT AAT ACG AC
	2	GGT GTT ATC TGC CTG ACC A
fimH	*1*	TCG AAA CCG ATG AAG GAA AC
	2	GGG TAG GCT TGC AAT GTG AT
fimI	*1*	GCT TCA TTA CAC GCC CAT TT
	2	GGG TAA ACC AGC GTA AAC CA
fliC	*1*	GAG ACA TGT TGG AAA CTTC GG
	2	TTG ACA CGT TAC GTT CTG ACC
fljA	*1*	GAT GTC CTC ACA CCA ACC TG
	2	GCT GAC GGA TTT AAT CTT CAG C
fljB	*1*	TGA TGT GAA AGA TAC AGC AG
	2	GTC AAA TTT AAC CGC ACC AC
16S rRNA	*1*	AGG CCT TCG GGT TGT AAA GT
	2	GAC TCA AGC CTG CCA GTT C