Strong mutator phenotype drives faster adaptation from growth on glucose to growth on acetate in Salmonella

Hervé Le Bars,1,2 Martine Bonnaure-Mallet,1,2 Frédérique Barloy-Hubler,3,4 Anne Jolivet-Gougeon1,2 and Latifa Bousarghin1

Correspondence
Latifa Bousarghin
latifa.bousarghin@univ-rennes1.fr

Received 21 March 2014
Accepted 14 July 2014

1Equipe Microbiologie, EA 1254, SFR BIOSIT, Université Européenne de Bretagne, Université de Rennes I, 2 Avenue du Professeur Léon Bernard, 35043 Rennes, France
2CHU, 2 rue Henri Le Guilloux, 35033 Rennes cedex 9, France
3CNRS UMR 6290, IGDR, Equipe Sp@rte, Université de Rennes 1, Campus de Beaulieu, Rennes, France
4Amadeus Platform, SFR BIOSIT, Rennes, France

The metabolic adaptation of strong mutator strains was studied to better understand the link between the strong mutator phenotype and virulence. Analysis of the growth curves of isogenic strains of Salmonella, which were previously grown in M63 glucose media, revealed that the exponential phase of growth was reached earlier in an M63 acetate medium with strong mutator strains (mutated in mutS or in mutL) than with normomutator strains (P<0.05). Complemented strains confirmed the direct role of the strong mutator phenotype in this faster metabolic adaptation to the assimilation of acetate. In a mixed cell population, proliferation of strong mutators over normomutators was observed when the carbon source was switched from glucose to acetate. These results add to the sparse body of knowledge about strong mutators and highlight the selective advantage conferred by the strong mutator phenotype to adapt to a switch of carbon source in the environment. This work may provide clinically useful information given that there is a high prevalence of strong mutators among pathogenic strains of Salmonella and that acetate is the principal short chain fatty acid of the human terminal ileum and colon where Salmonella infection is localized.

INTRODUCTION

Strong mutator bacteria are characterized by an enhanced frequency of mutation (LeClerc et al., 1996; Matic et al., 1997; Baquero et al., 2004). They are detected by enumeration of antibiotic-resistant mutants on culture media containing rifampicin, fosfomycin, nalidixic acid, streptomycin or spectinomycin (LeClerc et al., 1996; Matic et al., 1997). In most cases, the mutator phenotype is due to a defective methyl mismatch repair (MMR) system (LeClerc et al., 1996). The MMR system plays a key role in the correction of base-base mismatches and insertion/deletion mispairs that appear during DNA replication. Mutations in either of two genes required for MMR (mutS and mutL) have been described as the main cause of hypermutability (LeClerc et al., 1996; Feliziani et al., 2010).

LeClerc et al. (1996) identified five strong mutators among 137 strains of Salmonella from the US Food and Drug Administration’s collection, demonstrating a relatively high frequency of strong mutators (3.6%) among Salmonella pathogens. Since then, several studies revealed that the prevalence of strong mutators ranges from 1% among pathogenic strains of Escherichia coli (Baquero et al., 2004) to more than 30% among strains of Pseudomonas aeruginosa isolated from cystic fibrosis (CF) patients (Oliver et al., 2000). The role of the strong mutator phenotype in pathogenic bacteria has already been discussed (Jolivet-Gougeon et al., 2011), but the link between this phenotype and virulence or adaptation to varied environments is not yet well understood.

As expected from computer simulations of clonal bacterial populations adapting to a new environment (Taddei et al., 1997), a strong mutator genotype generates favourable mutations and drives adaptive radiation (Rainey & Travisano, 1998) to hostile conditions (Taddei et al., 1997). Results of in vitro experiments have demonstrated how antibiotic selection can increase the percentage of
strong mutators in bacterial populations (Mao et al., 1997). Beyond antibiotic resistance, the strong mutator phenotype may additionally have important effects on genetic adaptation, which promote the colonization of host tissues such as airways of CF patients (Mena et al., 2008). Studies on bacterial strains isolated from CF patients favour a role of the strong mutator phenotype in the metabolic adaptation of the strains (Hoboth et al., 2009; Oliver & Mena, 2010). Among the metabolic pathways activated in these strains are the glyoxylate shunt and the tricarboxylic acid cycle (Hoboth et al., 2009). Compared with growth in the presence of glucose, these two pathways are activated during growth on acetate as the sole carbon source (Wolfe, 2005).

Salmonella enterica subsp. enterica are transmissible from animals to humans (Vaillant et al., 2005) and many serovars, including S. Heidelberg, are responsible for food-related illnesses causing localized gastroenteritis. Strains of Salmonella may therefore benefit from adaptive mutations to survive in unfamiliar environmental conditions such as in the human gut, which may be of clinical significance as colonization of the terminal ileum and the colon is one of the first steps of the gastroenteritis induced by Salmonella (Darwin & Miller, 1999). As acetate is the principal short chain fatty acid of the human terminal ileum and large intestine, activation of the metabolic pathways that enable utilization of this toxic metabolite as the source of carbon and energy is likely to be important for the survival and the pathogenicity of food-borne Salmonella in the human gut.

As metabolic selection may be a cause of the increase in the proportion of strong mutators in bacterial populations, the adaptation of normomutator and strong mutant isogenic strains of S. Heidelberg for growth on acetate as the sole carbon source was studied to better understand the link between the strong mutator phenotype and virulence.

The adaptation to a change of carbon source drove the selection of strong mutators, mutated in mutS or in mutL. Taken together, our results show that the time to reach the exponential growth phase in medium containing acetate as the sole source of carbon is shorter with strong mutators. Finally, study of the growth of mixed normomutator and strong mutator populations clearly shows the overgrowth of strong mutators.

The study of strong mutator strains, such as those described in this work, might help expand our knowledge and provide clinically useful information given that there is a high prevalence of strong mutators among strains isolated from clinical specimens (Oliver et al., 2000; Jolivet-Gougeon et al., 2011).

### METHODS

**Bacterial strains, plasmids and strain construction.** The Salmonella strains used in this study are listed in Table 1. Two previously isolated strong mutator strains, one with a deletion of 12 bp in mutS (Le Bars et al., 2013) and one with an insertion of 6 bp in mutL (Le Bars et al., 2012a, 2013) were used. Isogenic mutants of the normomutator strains of S. Heidelberg wt (deletion of 12 bp in mutS) and SL486 (insertion of 6 bp in mutL) were produced by allelic exchange as described previously (Philippe et al., 2004). The cloning steps were performed in SM10 ?pir strains (LMBP 3889, BCCM/ LMBP plasmid collections, Gent, Belgium) to allow replication of the pPDS132 plasmid. The primers mutS–Sact, mutS–Spht, mutL–Sact and mutL–Spht (Le Bars et al., 2012a, 2013) were used. Strains were stored on cryobeads (MAST CRYOBANK) at −80 °C.

**Growth conditions.** Bacteria were grown at 37 °C in Luria–Bertani (LB) medium [per litre: 10 g tryptone (pancreatic digest of casein), 5 g yeast extract, 10 g NaCl] and in M63 medium [0.1 M KH2PO4, 15 mM (NH4)2SO4, 1.7 mM MgSO4, 1.8 μM FeSO4.7H2O; pH adjusted to 7.2 with KOH] (Pardee et al., 1958) containing either 0.4% glucose or 0.4% potassium acetate as the sole carbon source (components used in M63 medium were from Sigma Aldrich).

**Mutation frequencies.** Rifampicin resistance mutation frequencies were determined as described previously (LeClerc et al., 1996). Briefly, a single colony of the bacterial strain was suspended in 10 ml LB broth (AES Laboratoires) and incubated at 37 °C for 24 h. Then, 100 μl of this culture was spread onto LB agar plates with and without

### Table 1. Bacterial strains used in this study

<table>
<thead>
<tr>
<th>Salmonella strain*</th>
<th>Genotype(s)</th>
<th>Phenotypes</th>
<th>Reference/source</th>
</tr>
</thead>
<tbody>
<tr>
<td>wt</td>
<td>Wild-type mutS and mutL†</td>
<td>MutS+, MutL+</td>
<td>Le Gall et al. (2009)</td>
</tr>
<tr>
<td>(ΔmutS), isogenic strain of wt</td>
<td>bla inserted in mutS</td>
<td>MutS–, MutL–</td>
<td>Le Bars et al. (2012a)</td>
</tr>
<tr>
<td>wt (Δ12bpmutS), isogenic strain of wt</td>
<td>Δ12bpmutS</td>
<td>MutS–, MutL–</td>
<td>Le Bars et al. (2012a)</td>
</tr>
<tr>
<td>wt (Δ12bpmutS complemented), isogenic strain of wt (Δ12bpmutS)</td>
<td>Wild-type mutS and mutL†</td>
<td>MutS–, MutL–</td>
<td>This study</td>
</tr>
<tr>
<td>wt (6bpinsmutL), isogenic strain of wt</td>
<td>6 bp inserted in mutL</td>
<td>MutS+, MutL–</td>
<td>Le Bars et al. (2013)</td>
</tr>
<tr>
<td>STM HS20, non-isogenic strain of wt</td>
<td>6 bp inserted in mutL</td>
<td>MutS+, MutL–</td>
<td>Le Bars et al. (2013)</td>
</tr>
<tr>
<td>SL486, non-isogenic strain of wt</td>
<td>Wild-type mutS and mutL†</td>
<td>MutS–, MutL–</td>
<td>SGSC‡</td>
</tr>
<tr>
<td>SL486 (6bpinsmutL), isogenic strain of SL486</td>
<td>6 bp inserted in mutL</td>
<td>MutS+, MutL–</td>
<td>This study</td>
</tr>
<tr>
<td>B182, non-isogenic strain of wt</td>
<td>Δ12bpmutS†</td>
<td>MutS–, MutL+</td>
<td>Le Bars et al. (2012b)</td>
</tr>
</tbody>
</table>

*All strains except STM HS20 are S. Heidelberg. STM HS20 is S. Typhimurium.
†Versus NZ ABEL00000000: accession number of the genome of the normomutator strain of S. Heidelberg SL486.
‡Salmonella genetic stock centre (University of Calgary, Canada).
rifampicin (Sigma Aldrich) at 100 mg l⁻¹. The mutation frequencies were reported as the number of antibiotic-resistant colony-forming units per milliliter (c.f.u. ml⁻¹) to the number of c.f.u. ml⁻¹ on LB agar plates lacking antibiotics that were plated in parallel. For each strain, mutation frequencies were determined in triplicate.

Adaptation for growth on acetate as the sole carbon source. Bacteria from cryobeads were first cultured on LB agar incubated at 37 °C for 24 h to verify the purity of the strain. An LB broth was then inoculated with one colony of each strain and incubated at 37 °C for 24 h. Cultures were centrifuged for 5 min at 4500 g at 4 °C. The supernatants were discarded. The bacterial pellets were washed by resuspending in sterile physiological water followed by a centrifugation step for 5 min at 4500 g at 4 °C. This washing step was repeated twice and bacteria were finally resuspended in sterile physiological water to inoculate a prewarmed 50 ml LB broth or M63 broth containing 0.4 % of glucose as the sole carbon source. Inoculum volumes were calculated to reach an OD₆₀₀ of 0.001. Media were incubated at 37 °C with shaking. The bacterial growth curves were determined by bacterial viable cell count after plating on LB agar (incubated at 37 °C) and OD₆₀₀ measurements every hour until the stationary phase had been reached. Stationary phase cultures in M63 broth containing 0.4 % glucose were then washed, as previously described, to inoculate prewarmed 50 ml M63 broth containing 0.4 % (or 0.04 %) potassium acetate as the sole carbon source. The inoculum volumes were calculated to reach an OD₆₀₀ of 0.001. Media were inoculated at 37 °C with shaking. The bacterial growth curves were determined by bacterial viable cell count after plating on LB agar (incubated at 37 °C) until the stationary phase had been reached. Two consecutive growth periods in M63 broth containing 0.4 % (or 0.04 %) potassium acetate, separated by a washing step as previously described, were repeated. To study the growth of a 1:1 mixture of a mixed population of normomutator and strong mutator isogenic strains, M63 broth media containing 0.4 % (or 0.04 %) potassium acetate were inoculated with volumes calculated to reach an OD₆₀₀ of 0.001 for each of the two strains previously grown in M63 broth containing 0.4 % glucose until the stationary phase had been reached and washed, as previously described.

The experiments were performed in triplicate and repeated at least three times. The time to reach the exponential phase of growth was determined as the intersection of the tangent to the growth curve in the exponential phase of growth with the abscissa. The exponential phase of growth was considered as the phase characterized by the shorter generation time (G). The generation time (in minutes) was calculated using the following equation: \( G = \frac{3.3 \log (B/B)}{t} \), where \( B \) was the number of viable bacterial cells at time \( T1 \), \( b \) the number of viable bacterial cells at time \( T2 \) and \( t = T2 - T1 \).

**Table 2.** Rifampicin resistance mutation frequencies and mutator phenotypes of strains used in this study, according to the criteria of Baquero et al. (2004)

<table>
<thead>
<tr>
<th>Strain</th>
<th>Rifampicin resistance mutation frequency</th>
<th>Phenotype</th>
</tr>
</thead>
<tbody>
<tr>
<td>wt</td>
<td>( 4 \times 10^{-8} \pm 2 \times 10^{-8} )</td>
<td>Normomutator</td>
</tr>
<tr>
<td>wt (ΔmutS)</td>
<td>( 1.2 \times 10^{-5} \pm 4 \times 10^{-6} )</td>
<td>Strong mutator</td>
</tr>
<tr>
<td>wt (Δ12bpmutS)</td>
<td>( 6 \times 10^{-6} \pm 2 \times 10^{-6} )</td>
<td>Strong mutator</td>
</tr>
<tr>
<td>wt (Δ12bpmutS complemented)</td>
<td>( 2 \times 10^{-8} \pm 2 \times 10^{-8} )</td>
<td>Normomutator</td>
</tr>
<tr>
<td>wt (6bpinsmutL)</td>
<td>( 9.2 \times 10^{-6} \pm 1 \times 10^{-6} )</td>
<td>Strong mutator</td>
</tr>
<tr>
<td>STM HS20</td>
<td>( 4.8 \times 10^{-6} \pm 1 \times 10^{-6} )</td>
<td>Strong mutator</td>
</tr>
<tr>
<td>SL486</td>
<td>( 2.2 \times 10^{-8} \pm 1 \times 10^{-8} )</td>
<td>Normomutator</td>
</tr>
<tr>
<td>SL486 (6bpinsmutL)</td>
<td>( 8 \times 10^{-6} \pm 2 \times 10^{-6} )</td>
<td>Strong mutator</td>
</tr>
<tr>
<td>B182</td>
<td>( 4 \times 10^{-6} \pm 1 \times 10^{-6} )</td>
<td>Strong mutator</td>
</tr>
</tbody>
</table>

**Statistical analysis.** Statistical analysis was performed using the Mann–Whitney U-test at a 5% significance level.

**RESULTS**

Strains with mutation in mutS or in mutL showed a strong mutator phenotype

Rifampicin resistance mutation frequencies (fr) of the strains of Salmonella and their isogenic mutants are reported in Table 2. Strains were classified using the system developed for *E. coli* by Baquero et al. (2004). Strains wt and SL486 were normomutators (fr=4.8×10⁻⁸±2×10⁻⁸ and 2.2×10⁻⁸±1×10⁻⁸, respectively) whereas their isogenic mutants in either mutS or mutL were strong mutators (fr=6×10⁻⁶±2×10⁻⁶ and 8×10⁻⁶±2×10⁻⁶, respectively). Complementations of these strains resulted in the suppression of the strong mutator phenotype (fr=2×10⁻⁸±2×10⁻⁸).

Adaptation of strong mutator strains to growth on acetate is faster

The growth curves in M63 broth containing 0.4 % glucose as the sole carbon source were identical for the normomutator wt and the isogenic strong mutator strains wt (ΔmutS), wt (Δ12bpmutS) and wt (6bpinsmutL) (Fig. 1a). The generation time was 27 min. The stationary phase was reached after 11 h.

Comparison of the growth curves in M63 broth containing 0.4 % potassium acetate revealed that the time to reach the exponential phase of growth was shorter for the strong mutator strains (ΔmutS), wt (Δ12bpmutS) and wt (6bpinsmutL) (1.88 days) than for their isogenic normomutator strain wt (2.56 days) (Fig. 1b). The stationary phase was reached after 2.5 days for the strong mutator strains whereas it was reached after 3.5 days for the normomutator wt. The generation times were 177 min for the strong mutator strains and 209 min for the normomutator wt. Addition of glucose or acetate to M63 broth was required for growth, as shown by the absence
of growth in M63 broth lacking any carbon source (Fig. 1b).

This experiment was repeated and the time to reach the exponential phase of growth in M63 broth containing 0.4 % potassium acetate was always shorter with the strong mutator strains than with wt, as was shown for strain wt (Δ12bpμtS) (Table 3). The differences between strong mutators and wt were significant at the 5 % significance level (Mann–Whitney U-test). Complementation of the deleted mutS gene in wt (Δ12bpμtS) restored the normomutator phenotype (Table 2) and the delayed time to reach the exponential phase of growth (Fig. 2a).

There was no difference between normomutator wt and strong mutators in a second consecutive growth experiment in M63 broth containing 0.4 % potassium acetate (Fig. 2b).

**In a mixed cell population, the strong mutator grew faster than normomutator**

The growth curve of a 1 : 1 mixture of the normomutator wt and the strong mutator wt (Δ12bpμtS) was the same

![Graph](http://mic.sgmjournals.org)

**Table 3.** Time to reach the exponential phase of growth in M63 broth containing 0.4 % potassium acetate (over seven different experiments)

<table>
<thead>
<tr>
<th>Strain</th>
<th>Time to reach exponential growth phase (days)</th>
</tr>
</thead>
<tbody>
<tr>
<td>wt</td>
<td>2.56 2.95 3.11 2.95 2.65 2.95 2.77</td>
</tr>
<tr>
<td>wt (Δ12bpμtS)</td>
<td>1.88 0.95 1.94 0.55 1.35 0.95 0.95</td>
</tr>
</tbody>
</table>

(Fig. 1. Strong mutator strains grow faster in medium with acetate as the sole carbon source. (a) Growth of strains in M63 medium broth containing 0.4 % glucose. (b) Growth of strains in M63 medium broth containing 0.4 % potassium acetate as the single carbon source. Each experiment was performed in triplicate and values are shown as means (± SD).
as that obtained with wt (Δ12bpmutS) alone (Fig. 3). The frequencies of mutation on rifampicin of 100 randomly selected clones during the stationary phase of growth of this 1:1 mixture of wt and wt (Δ12bpmutS) showed that the selected clones were all strong mutators and that therefore the wt strain was fully displaced.

**Strains SL486 with mutation in mutL also have faster adaptation to acetate switch**

Comparison of the normomutator strain SL486 and its isogenic strong mutator SL486 (6bpinsmutL) showed that the time to reach the exponential phase of growth was shorter for the strong mutator in M63 broth containing 0.04% potassium acetate (Fig. 4). There was no difference between SL486 and SL486 (6bpinsmutL) in M63 broth containing 0.4% potassium acetate (data not shown).

**DISCUSSION**

Identification of the environmental stresses which lead to the proliferation of strong mutators is crucial to understand the pathogenic properties of these strains, which are found at high frequencies among *E. coli* and *Salmonella* pathogens (LeClerc et al., 1996) and among *P. aeruginosa* isolates from chronically colonized CF patients (Oliver et al., 2000). In the present study, strong mutator strains of *Salmonella* previously cultured in M63 broth containing glucose as the sole carbon source grew earlier than isogenic normomutator strains in M63 broth containing potassium acetate as the sole carbon source. This result is not due to the use of a deficient strain of *Salmonella* as was observed with the two distinct strains of *Salmonella*, wt and SL486 (Figs 1b and 4). The role of the strong mutator phenotype in this difference of growth in M63 broth containing potassium acetate was strongly suspected as the assays...
showed that it was obtained with strains with defects in either mutS or mutL alleles. The direct role of MMR system inactivation was confirmed by the use of a complemented strain, which grew as the normomutator wt in M63 broth containing potassium acetate (Fig. 2a). This result is of primary importance since it highlights the selective advantage conferred by the strong mutator phenotype, as was shown with cultures of mixed populations (Fig. 3). Mao et al. (1997) showed the proliferation of strong mutators in a cell population treated with antibiotics. The peculiarity of this work lies in the fact that the environmental pressure studied is the change of the carbon source in the culture medium and not the antibiotic stress. The glucose of the first growth medium was replaced by acetate as the sole carbon source in the second growth medium. *Salmonella* were able to assimilate acetate and to grow with acetate as the sole carbon source. The assimilation of acetate required the activation of metabolic pathways such as the tricarboxylic acid cycle and the glyoxylate shunt, which were repressed during growth on glucose (Oh et al., 2002). The ‘acetate switch’ occurs when bacteria deplete their environment in glucose and begin to rely on their ability to assimilate acetate (Wolfe, 2005). Here, strong mutators were characterized by a faster metabolic adaptation to the ‘acetate switch’. A recent study showed that the tricarboxylic acid cycle and the glyoxylate shunt were both activated in strains isolated from the airways of CF patients (Hoboth et al., 2009). This suggests

---

**Fig. 3.** In a mixed cell population, strong mutators grow faster than normomutators: growth of the normomutator wt, the isogenic strong mutator wt (Δ12bpmutS) and a 1 : 1 mixture of those two strains [wt + wt (Δ12bpmutS)] in M63 broth containing 0.4 % potassium acetate as the single carbon source. Results of the determination of the frequencies of mutation on rifampicin of 100 randomly selected clones at day 0 and during the stationary phase of growth of this 1 : 1 mixture are presented as the ratio w/w (Δ12bpmutS). Each experiment was performed in triplicate and values are shown as means (+SD).

---

**Fig. 4.** Strong mutator strains SL486 with mutation in *mutL* showed faster adaptation to the change of carbon source. Growth of the normomutator SL486 and its isogenic strong mutator strain SL486 (6bpinsmutL) in M63 broth containing 0.04 % potassium acetate as the single carbon source.
that the ‘acetate switch’ occurred during the adaptation of bacteria to this environment. As acetate is the main short chain fatty acid in the human terminal ileum and colon, particularly as a by-product of bacterial metabolism (Wolfe, 2005), this faster adaptation may promote colonization by strong mutator strains of Salmonella and explain their high frequency among the strains isolated from infected patients (LeClerc et al., 1996). This faster adaptation to the assimilation of acetate may also favour strong mutator strains of Salmonella to cause infections, as the glyoxylate shunt appears to play a critical role in the ability of Salmonella and other intracellular pathogens to persist in mammalian hosts (Fang et al., 2005). Although the strong mutator phenotype has been shown to be a key factor in the development of antibiotic resistance (Maciá et al., 2005), its role in genetic and metabolic adaptation during infectious diseases such as salmonellosis must be suspected.

The growth curves of the strong mutator and normomutator strains were identical in a second consecutive growth experiment on acetate. This confirms that the growth difference between those strains was due to a faster adaptation to the change of carbon source. It may be due to the selection of suitable acetate-adapted mutants whose random production was boosted by the strong mutator phenotype during growth in M63 glucose broth. Such acetate-adapted mutants may also be present in the normomutator M63 glucose broth but in smaller numbers than in the strong mutator broth, leading to a difference of the acetate-adapted inoculum between strong mutator and normomutator and therefore to a prolonged lag phase with the normomutator strain. Accordingly, the observed difference between the strong mutator and the normomutator strains reflects the greater diversity of the strong mutator population. This is in agreement with the observation that the wild-type strain was able to grow equally well as the strong mutator strain in acetate media once pre-grown in acetate (Fig. 2b), which may lead to an acetate-adapted inoculum. This raises the question of the infectious dose, which may consequently be lower for the strong mutator strains.

The use of high-throughput sequencing technologies would be helpful to search for such suitable mutants and to measure the diversity of the strong mutator population as several operons are involved in energy homeostasis during growth on acetate (Wolfe, 2005; Chan et al., 2011). Searching for mutations in the aceBAK operon, which is involved in the glyoxylate shunt and in the icaR gene encoding its repressor, would be of great interest as mutations have already been found in those genes in strains of E. coli cultured in a mixture of glucose and acetate (Spencer et al., 2007). Whole genome sequencing of multiple clones will be necessary to fully describe the mutations as many parallel and some unique genetic changes may occur (Herron & Doebeli, 2013).

The study of strong mutator strains such as described in this work allows us to expand our knowledge and provide clinically useful information, given that there is a high prevalence of strong mutators among strains, not only in constructed mutants but also in pathogenic clinical specimens.

ACKNOWLEDGEMENTS

This study was supported by Rennes Métropole, the Conseil Régional de Bretagne, grants from the Langlois Foundation and funding from FEDER. We thank the LMBP 3889 and BCCM/LMBP plasmid collections (Gent, Belgium) for the SM10 εpir strains, and D. Schneider at the Université Joseph Fourier for pDS132. We thank the Salmonella genetic stock centre (University of Calgary, Canada) for S. Heidelberg SL486.

REFERENCES


Edited by: A. van Vliet