HGT, horizontal gene transfer.

INTRODUCTION

Horizontal gene transfer (HGT) accounts for a significant proportion of the genetic variability in bacteria. Plasmids are amongst the best characterized elements facilitating HGT between bacterial cells. Bacterial plasmids can be considered as dispersive elements conveying horizontal transfer of a communal gene pool (Baquero, 2004; Norman et al., 2009). To that communal gene pool belong, among others, those involved in different housekeeping functions that, when incorporated in a new bacterial host, may significantly modify its phenotype, facilitating adaptation of the bacterial host to specific environmental niches. These genes can be grouped in a module, termed the ‘adaptation module’ (Norman et al., 2009). Examples include those genes conferring resistance to antimicrobial compounds, virulence and the ability to degrade different metabolites (Frost et al., 2005). Horizontally transferred DNA (HGT DNA) can usually be identified because of its unusual GC content (Ilatovskiy & Petukhov, 2009). Genes belonging to the plasmid adaptation modules are strongly AT-biased (Koonin et al., 2001).

The challenge for a bacterium that incorporates genes from a plasmid adaptation module is to rapidly integrate them into the cell regulatory networks. In bacteria, precise and complex regulatory networks control and determine the specific gene expression pattern that optimizes the adaptive response of the bacterial cell to a changing environment. In the short term, plasmid-encoded genes may be elusive to the modulatory effect of the bacterial regulatory networks. Uncontrolled expression of plasmid-encoded genes might then result in a fitness cost. In the long term, adaptive mutations might compensate for this or even result in plasmids accounting for an increase in bacterial fitness (Dionisio et al., 2005; Poole et al., 2011). Hence, it appears critical to control unwanted expression of a recently incorporated DNA molecule.

In Gram-negative bacteria, the H-NS protein is a key element that silences unwanted expression of HGT DNA (Navarre et al., 2006; Oshima et al., 2006). H-NS belongs to the superfamily of the bacterial nucleoid-associated proteins (Dillon & Dorman, 2010; Dorman, 2004) and is widely distributed. H-NS contributes to the DNA architecture and, in addition, modulates gene expression. H-NS targets both chromosomal and HGT genes (Dorman, 2007) and binds AT-rich DNA sequences with an intrinsic curvature (Owen-Hughes et al., 1992). Interaction of H-NS with DNA leads to H-NS oligomerization, resulting in transcriptional silencing and repression of the corresponding gene. In recent years, several independent reports have shown that a preferential target for H-NS is HGT DNA (Lucchini et al., 2006; Navarre et al., 2006). Interestingly, H-NS is also encoded in several plasmids (Takeda et al., 2011). With respect to H-NS paralogues of plasmid origin, it has been shown that the main role of plasmid-encoded H-NS proteins is to increase intracellular H-NS concentration (Doyle et al., 2007). This would prevent plasmid AT-rich sequences from titrating out chromosomally encoded H-NS protein, altering the regulation of H-NS-dependent genes in the recipient cell. Furthermore, it has...
also been shown that plasmid-encoded H-NS proteins have evolved to target preferentially HGT DNA but not core genome DNA (Baños et al., 2009). These features should facilitate transmission of several plasmids within bacteria encoding H-NS proteins.

Members of the Enterobacteriaceae encode a group of proteins of about half the molecular mass of H-NS: the Hha/YmoA family (Madrid et al., 2007; Paytubi et al., 2011). These proteins mimic the N-terminal domain of H-NS and interact with the latter protein to modulate expression of several H-NS-targeted genes (Nieto et al., 2002; Paytubi et al., 2004; Vivero et al., 2008). Specifically, HGT genes modulated by H-NS appear to be modulated by Hha (Baños et al., 2009; Vivero et al., 2008). Hha-like proteins are also encoded on several plasmids (Takeda et al., 2011). Interestingly, 55% of those plasmids encoding an H-NS paralogue also encode an Hha paralogue. This further supports the close relationship between H-NS and Hha-like proteins. Plasmid-encoded Hha-like proteins have been less studied, but it is remarkable that they are completely restricted to plasmids isolated from the Enterobacteria.

As mentioned above, it is now well established that H-NS proteins play relevant roles facilitating HGT incorporation into the bacterial cell (Lucchini et al., 2006; Navarre et al., 2006; Oshima et al., 2006) and avoiding fitness costs in cells incorporating plasmids (Doyle et al., 2007). It has also been shown that Hha proteins co-modulate HGT gene expression with H-NS (Baños et al., 2009; Vivero et al., 2008). Hitherto, no experimental evidence has linked Hha proteins to the fitness of bacterial cells that incorporate HGT DNA. In this report we associate Hha expression with an increase in fitness of bacterial cells that incorporate HGT DNA. In this report we associate Hha expression with an increase in fitness of bacterial cells that incorporate HGT DNA. In this report we associate Hha expression with an increase in fitness of bacterial cells that incorporate HGT DNA. In this report we associate Hha expression with an increase in fitness of bacterial cells that incorporate HGT DNA.

**Methods**

**Bacterial strains, plasmids and culture media.** Bacterial strains and plasmids used in this study are listed in Table 1. Cells were grown in Luria–Bertani (LB) medium (per litre: 10 g NaCl, 10 g tryptone, 5 g yeast extract) at 37°C unless otherwise indicated. For R27 mating experiments, strains were incubated in Penassay broth (per litre: 1.5 g meat extract, 1.5 g yeast extract, 5 g peptone, 1 g glucose, 3.5 g NaCl, 1.32 g KH₂PO₄, 4.82 g K₂HPO₄, 3H₂O) at 25°C. The antibiotics used were 15 μg tetracycline ml⁻¹, 25 μg kanamycin ml⁻¹, 50 μg ampicillin ml⁻¹ and 25 μg chloramphenicol ml⁻¹.

**Mating experiments.** R27 plasmid was transferred to the recipient strain SV5015 or its mutant derivatives as described by Taylor & Levine (1980).

Plasmid pHly152 was conjugated to the corresponding recipient strain as follows. Overnight cultures of the donor and recipient cells grown in LB with the corresponding antibiotics were harvested by centrifugation, and resuspended in the same volume of LB to remove the antibiotics. Donor and receptor strains were mixed at a ratio of 10:1, 1:1 or 1:10. The suspension of cells was then passed through a 0.22 μm pore-diameter filter (Millipore) and the filter was placed onto an LB agar plate facing up. Mating took place at 30°C for 6 h. After this period, the filters were immersed in 2 ml LB and shaken vigorously. Dilutions (10⁻¹–10⁻⁴) of the resulting suspension were plated on LB agar supplemented with 5% defibrinated sheep blood (Oxoid) plus the appropriate antibiotics corresponding to the recipient strain. Colonies showing a haemolytic phenotype (shown by a clear halo around the bacterial colony) were selected. As controls, 0.1 ml of both the donor and the recipient cultures were also spread on selective plates.

**Fitness assays.** Overnight LB cultures were washed and diluted in fresh LB medium. Cultures were inoculated with 10⁵ cells ml⁻¹ of each competitor in a final volume of 3 ml, and the mixture was incubated with shaking at 37°C. The ratio of each strain was determined on LB plates containing the appropriate antibiotics at time zero and after 24 h of growth. The percentage of the cost (c) of a given plasmid or mutation was determined as the difference in cell doublings between the two strains being compared, c=(1−W)×100, where W is the relative fitness, W=log(Nf/Ni)/log(Ni/N0) (De Gelder et al., 2007). Student’s t-test among the same population (single strains compared one-to-one) was used to contrast the significance of the percentage of the cost (C>0). A P-value <0.05 is shown in the figures with an asterisk. Student’s t-test (with unilateral alternative) between two populations was used to contrast the significance of the differences between the cost percentages of different groups of strains (a P-value <0.05 is shown on the top of the figures).

**Plasmid curing frequency.** The frequencies of curing of the plasmids under study were determined by successive subculturing at a ratio of 1:100 of the plasmid-carrier strain on LB media without chloramphenicol over five consecutive days. Every 24 h, the cultures were spread on LB agar plates and the isolated colonies were replica printed in duplicate to LB agar with or without chloramphenicol. Plasmid curing frequency was calculated as the ratio between the number of chloramphenicol-sensitive isolates and the total number of colonies replicated on LB agar without chloramphenicol.

**Electrophoresis and Western blotting analysis of proteins.** Protein samples were analysed by SDS-PAGE at 15%. Gels were used to transfer the proteins to PVDF membranes. Western blot analysis was performed with polyclonal antibodies raised against E. coli H-NS protein (1:5000) or monoclonal DnaK antibodies (Enzo Life Sciences) (1:10,000). Horseradish peroxidase-conjugated goat anti-rabbit IgG (1:10,000 (Sigma)) or anti-mouse IgG (1:3000 (Promega)) were used as secondary antibodies, respectively. Detection was performed by enhanced chemiluminescence using the software QuantityOne (Bio-Rad).

**Genetic manipulations and molecular techniques.** Hha and promoter-hlyC mutants were obtained by using the procedure described by Datsenko & Wanner (2000). We used the sequence of each gene to define the corresponding amplification oligonucleotides. Briefly, the antibiotic resistance of the plasmid pKD3 (Cm) or pKD4 (Km) was amplified, using as oligonucleotides the sequences corresponding to P1 and P2 of plasmid pKD3/pKD4, with homology extensions of the gene sequence to be replaced (listed in Table 2). The PCR product was DpnI-digested, purified and used to electroporate strains MG1655 and MG1655pHly152 carrying the plasmid pKD4 grown at 30°C in the presence of 10 mM arabinose, conditions at which expression of Red recombinase was induced. Recombinants were selected at 37°C in LB medium containing chloramphenicol (Cm) or kanamycin (Km) and then tested for the presence of pKD4.
Table 1. Strains and plasmids used in this work

<table>
<thead>
<tr>
<th>Strain/plasmid</th>
<th>Relevant characteristics</th>
<th>Reference or source</th>
</tr>
</thead>
<tbody>
<tr>
<td>S. Typhimurium</td>
<td></td>
<td></td>
</tr>
<tr>
<td>SV5015</td>
<td>SL1344 His+</td>
<td>J. Casadesús</td>
</tr>
<tr>
<td>SV5015H</td>
<td>SV5015 Δhha::Cm</td>
<td>Vivero et al. (2008)</td>
</tr>
<tr>
<td>SV5015HY</td>
<td>SV5015 Δhha::Cm Δydg::Km</td>
<td>Vivero et al. (2008)</td>
</tr>
<tr>
<td>E. coli</td>
<td></td>
<td></td>
</tr>
<tr>
<td>MG1655</td>
<td>F-lambda- ilvG- rfb-50 rph-1</td>
<td>Bachmann (1987)</td>
</tr>
<tr>
<td>MG1655H</td>
<td>F-lambda- ilvG- rfb-50 rph-1 Δhha::Km</td>
<td>M. Gilbert</td>
</tr>
<tr>
<td>MG1655HY</td>
<td>F-lambda- ilvG- rfb-50 rph-1 hha::Tn5 Δydg</td>
<td>Pedró et al. (2011)</td>
</tr>
<tr>
<td>Plasmid</td>
<td></td>
<td></td>
</tr>
<tr>
<td>R27</td>
<td>IncHI1, Tc'</td>
<td>Grindley et al. (1972)</td>
</tr>
<tr>
<td>R27Δhha</td>
<td>R27 hha::mini-Tn5Km1</td>
<td>Forns et al. (2005)</td>
</tr>
<tr>
<td>pHly152</td>
<td>hlyR, hlyABC</td>
<td>Noegel et al. (1981)</td>
</tr>
<tr>
<td>pHlyC::Cm</td>
<td>pHly152 promoter-hlyC::Cm</td>
<td>This work</td>
</tr>
<tr>
<td>pANN202-312R</td>
<td>hlyR hlyCABD cloned in pACYC184, Cm'</td>
<td>Godessart et al. (1988)</td>
</tr>
<tr>
<td>pANN202-312R'</td>
<td>pANN202-312R hlyCABD</td>
<td>Pedró et al. (2011)</td>
</tr>
<tr>
<td>pKD3</td>
<td>Template plasmid, Cm'</td>
<td>Datsenko &amp; Wanner (2000)</td>
</tr>
<tr>
<td>pKD4</td>
<td>Template plasmid, Km'</td>
<td>Datsenko &amp; Wanner (2000)</td>
</tr>
</tbody>
</table>

All the recombinants obtained were checked by PCR with UP and DOWN oligonucleotides (Table 2).

Evaluation of haemolysin production. Haemolysin in the culture supernatants was assayed by measuring the haemolytic activity as described by Godessart et al. (1988).

Sequence of the hns gene. The sequence of the hns gene and its surrounding non-coding regions was PCR-amplified with oligonucleotides SeqH-NS1_for and SeqH-NS1_rev, and SeqH-NS2_for and SeqH-NS2_rev (Table 2). The corresponding PCR products were sequenced with the above-mentioned oligonucleotides. DNA sequencing was performed with the ABI PRISM Big Dye Terminator Cycle Sequencing Ready Reaction kit (Applied Biosystems) in an ABI PRISM 3700 DNA Analyser (Applied Biosystems), according to the manufacturer’s instructions.

Table 2. Oligonucleotides used in this work

<table>
<thead>
<tr>
<th>Name</th>
<th>Sequence</th>
</tr>
</thead>
<tbody>
<tr>
<td>HhaP1</td>
<td>5’-ATGTCCGAAAACCTTTAAGCAGAAAACCGGTTTATTTATGCGTGTGAGGGTGGAGGCTGCTTC-3’</td>
</tr>
<tr>
<td>HhaP2</td>
<td>5’-TTAGCGAATAAATTCTCAGAGAAAGGATCTTGTGATACATAGATATCCTGCTT-3’</td>
</tr>
<tr>
<td>Hhap1UP</td>
<td>5’-CCTCGGAGTTTACCTTGTAG-3’</td>
</tr>
<tr>
<td>HhaP2DOWN</td>
<td>5’-CGGTGGTATTGCGAAACCG-3’</td>
</tr>
<tr>
<td>Hly-P1</td>
<td>5’-GACGAAGTGTGATAACTGGTATACGAGATTAATCTAAAC-3’</td>
</tr>
<tr>
<td>Hly-P2</td>
<td>5’-GGTATCCTGAAATCTGGTCTGACATAGATATCCTCCTT-3’</td>
</tr>
<tr>
<td>HlyUP</td>
<td>5’-GACGAAGTGTGATAACTGGT-3’</td>
</tr>
<tr>
<td>HlyDOWN</td>
<td>5’-CGGAGGAGCTCAGATCTGACATG-3’</td>
</tr>
<tr>
<td>SeqH-NS1_for</td>
<td>5’-GGGTGATGACAAACCTTTATGCGTGTGAGGGTGGAGGCTGCTTC-3’</td>
</tr>
<tr>
<td>SeqH-NS1_rev</td>
<td>5’-CAATACCCGTAGAGGATGACATG-3’</td>
</tr>
<tr>
<td>SeqH-NS2_for</td>
<td>5’-GCTGATGAGGAGGATGACATG-3’</td>
</tr>
</tbody>
</table>

RESULTS

Role of Hha and YdgT proteins in E. coli MG1655(pHly152) fitness

Plasmid pHly152 belongs to the I2 incompatibility group and was isolated from an E. coli clinical strain (Noegel et al., 1981). This 41 MDa plasmid has been studied extensively because its 'adaptation module' is the haemolysin operon (hlyCABD), which encodes the toxin α-haemolysin. Expression of the hlyCABD operon is modulated by H-NS and Hha proteins (Madrid et al., 2002). Plasmids belonging to the IncI group are narrow host-range plasmids, restricted to the family Enterobacteriaceae (Suzuki et al., 2010). We first determined whether plasmid pHly152 had any fitness...
cost in the wild-type strain. No fitness cost was apparent (data not shown). We then decided to use plasmid pHly152 to assess the impact of Hha loss in the fitness of cells that incorporate this plasmid. We initially compared fitness of the plasmid-free E. coli MG1655 strain and its Δhha derivative. Under the conditions tested, no significant differences in fitness were apparent (Fig. 1a). We next conjugated plasmid pHly152 to both strains, and repeated the fitness assay. As expected, the lack of the Hha protein resulted in a significant fitness loss for cells harbouring plasmid pHly152. It has been shown that some enteric bacteria such as E. coli or Salmonella encode a paralogue of Hha, the YdgT protein (as reviewed by Madrid et al., 2007).

Although the specific regulatory role of the YdgT protein is poorly understood, it is well characterized that YdgT is overexpressed in an hha mutant and that this overexpression can compensate, at least partially, for some of the hha-induced phenotypes (Paytubi et al., 2004). To check whether this protein was actually compensating for the lack of Hha with respect to the fitness cost of cells harbouring plasmid pHly152, a double hha ydgT mutant was constructed. First, the effect of the double mutation on fitness in plasmid-free cells was tested. In contrast with the single hha mutant, the double hha ydgT mutant showed a significantly lower fitness than the wild-type cells (Fig. 1a). Plasmid pHly152 was then conjugated to the hha ydgT double mutant, and competition...
assays were performed once more. The combination of both mutations accounts for a significant fitness reduction in cells that incorporated pHly152 (Fig. 1a).

As the ‘adaptation module’ of this plasmid is the hlyCABD operon, which is a target for the Hha protein, we tried to correlate fitness loss with an altered regulation of this operon. To do this, we obtained a deletion mutant of the hlyCABD promoter plus the hlyC gene of plasmid pHly152 by introducing a chloramphenicol cassette by homologous recombination with the upstream sequence of the promoter and the coding sequence of the hlyC gene, as described in Methods. The resulting plasmid pHly152 promoter, hlyC::Cm (pHlyC::Cm), was subsequently conjugated into strains MG1655 and MG1655HY and fitness experiments were performed. Loss of expression of the haemolysin gene resulted in a significant reduction of the cost of fitness of the plasmid in the double hha ydgT mutant. Thus, acquisition of plasmid pHlyC::Cm by strain MG1655HY does not result in a significant fitness cost (Fig. 1a).

Role of Hha and YdgT proteins in fitness of S. Typhimurium harbouring plasmid R27

As a complementary model to that of E. coli and plasmid pHly152, we decided to assess how Hha/YdgT proteins might influence the fitness of S. Typhimurium harbouring the IncHI1 plasmid R27. Although the Enterobacteriaceae are the potential host range of IncHI1 plasmids, most of them have been isolated solely from the genus Salmonella (Suzuki et al., 2010). In fact, IncH1 plasmids account for a significant percentage of the multiple antibiotic resistance phenotypes in this genus (Fica et al., 1997). Plasmid R27 confers to its host resistance to tetracycline. Plasmid R27 also encodes paralogues of H-NS and Hha proteins. We therefore considered that this combination of bacterial host (S. Typhimurium SV5015) and plasmid (R27) would represent a model complementary to E. coli and plasmid pHly152. We first performed a competition assay between the parental strain SV5015 and its corresponding hha derivative. The S. Typhimurium hha mutant did not show

![Fig. 2. Effect of the hha mutation on the maintenance of plasmids (a) pANN202-312R and (b) pANN202-312R'. Plasmid curing frequencies were determined by successive subculturing of the MG1655 and MG1655H strains with the indicated plasmids for 5 days. Every 24 h the curing frequency of the plasmid was determined as the percentage of plasmid-cured cells relative to the total cells. The mean ± SD of three independent experiments is shown.](http://mic.sgmjournals.org)
In this case depletion of both chromosomal and plasmid-encoded Hha significantly reduced the fitness of the double mutant strain (Fig. 1b). We also checked the role of the YdgT protein. Competition experiments between the wild-type strain and the double hha ydgT mutant indicated that simultaneous depletion of Hha and YdgT results in a significant fitness cost. Whereas in E. coli loss of Hha and YdgT resulted in a fitness cost of about 8%, in S. Typhimurium the loss of both proteins represents a 40% fitness cost. Introduction of plasmid R27 into strain SV5015H did not significantly increase the fitness cost despite the depletion of Hha and YdgT proteins (data not shown).

### Role of Hha protein in the maintenance of plasmid pANN202-312R

We also tested whether the loss of Hha protein would also influence the maintenance of a given plasmid. We checked the stability of plasmids pHly152 and R27 in E. coli MG1655 and S. Typhimurium SV5015, respectively. When culturing cells in LB medium at 37 °C, both plasmids appeared to be stable and no curing was observed at a detectable rate (data not shown). We subsequently decided to use the recombinant multicopy plasmid pANN202-312R. This plasmid is the result of cloning into the plasmid pACY184 the haemolytic operon hlyCABD (Godessart et al., 1988). Plasmid pANN202-312R (AhlyCABD) was also used. Both plasmids were transformed into strains E. coli MG1655 and E. coli MG1655H. Cultures of clones harbouring both plasmids were grown in LB containing chloramphenicol to the stationary phase and then subcultured in LB media for 5 days as described in Methods. At defined time intervals, the frequency of curing of the plasmids was determined. With respect to plasmid pANN202-312R, the frequency of curing of this plasmid was shown to be significantly higher in strain MG1655H than in the wild-type strain (Fig. 2a). In contrast, the frequency of curing of plasmid pANN202-312R was similar in both strains (Fig. 2b). Hence, expression of Hha protein facilitates maintenance of plasmid pANN202-312R.

### Overexpression of the H-NS protein compensates for the fitness loss of strain MG1655H(pHly152)

The functional relationship between members of the H-NS and Hha families of proteins is well established. Both genetic/biochemical (Ellison & Miller, 2006; Madrid et al., 2002; Nieto et al., 2000, 2002; Olekhnovich & Kadner, 2006; Paytubi et al., 2004) and bioinformatic data (Takeda et al., 2011) support this relationship. Therefore, we hypothesized whether higher levels of H-NS protein could compensate for the loss of Hha and YdgT. To support this hypothesis, we decided to look for spontaneously arising derivatives of strain MG1655H(pHly152) that showed an increased growth rate, and to determine in these clones the protein levels of H-NS by immunodetection analysis. Upon repeated subculturing of strain MG1655H(pHly152) in
LB medium (five successive cultures, 1:1000 dilution as inoculum for the next culture), colonies with larger size, and hence suggesting an increased growth rate and fitness, were selected. Three such colonies were isolated and used to obtain crude cell extracts. H-NS was detected in these extracts by Western blot analysis. One of them, MG1655HY-11(pHly152), expressed higher levels of H-NS than the parental strain MG1655HY(pHly152) (Fig. 3a). The fitness of this clone was subsequently determined. The results obtained (Fig. 3b) provided evidence that strain MG1655HY-11(pHly152) shows a significant increase in fitness compared with the parental strain MG1655HY(pHly152). Evaluation of haemolytic activity in strains MG1655HY(pHly152) and MG1655HY-11(pHly152) confirmed that this latter strain shows a reduced haemolysin production (Fig. 4). Plasmid pHly152 from strain MG1655HY-11 was conjugated back to strain MG1655HY. Transconjugants from this strain did not show either reduced haemolysin production or increased fitness (data not shown). Hence, alterations in the haemolytic plasmid in strain MG1655HY-11 did not account for these phenotypes. To further understand the reason why strain MG1655HY-11(pHly152) showed an increased expression of the H-NS protein, a 960 bp DNA fragment around the hns gene (including the hns regulatory region) was PCR-amplified and sequenced. Interestingly, a copy of an IS1 element was found to be inserted at position −89 (Fig. 5). The H-NS protein autoregulates its own expression. Moreover, auto-regulation of hns transcription is associated with a 130 bp fragment that contains the hns promoter (Dersch et al., 1993). To repress gene expression, H-NS usually binds to two target sequences located at a certain distance, looping the intervening DNA (Dorman, 2004). Interestingly, the presence of the IS1 element increases the distance between two H-NS target sequences that are close to the hns promoter. Increasing

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**Fig. 4.** Haemolysin production by strains MG1655, MG1655HY and MG1655HY-11 harbouring the haemolytic plasmid pHly152 at an OD<sub>600</sub> of 0.4 and 0.8. The haemolytic activity of the mutant strains is shown as a percentage relative to the wild-type strain. The mean ± SD of three independent experiments is shown.

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**Fig. 5.** Physical map of the promoter region of the hns gene of strains MG1655 and MG1655HY-11. Light and dark grey boxes indicate putative binding sites for FIS and H-NS proteins. The white box indicates the IS1 element.
the distance between these sequences may account for a reduced ability of H-NS to repress its own promoter.

**DISCUSSION**

By using plasmids as a model, we provide here evidence that, in enteric bacteria, proteins of the Hha family are relevant elements that facilitate acquisition of foreign DNA. With both enterobacterial representatives used in this work (*E. coli* and *Salmonella*) we have shown that the loss of Hha-like proteins leads to a significant fitness reduction when conjugative plasmids harbouring adaptation genes are incorporated. With respect to *E. coli* cultured in LB medium at 37 °C, deletion of the Hha protein does not decrease the fitness of plasmid-free cells. Nevertheless, acquisition of the haemolytic plasmid pHly132 results in a significant fitness loss. The impact on fitness is much higher if, in addition to Hha, cells are also depleted of the YdgT protein. Complementation of the hha mutant phenotype by the YdgT protein is well documented (Paytubi et al., 2004). The highly significant effect of the double hha ydgT mutation on the fitness of cells harbouring plasmid pHly152 might be correlated with the ‘adaptation module’ of this plasmid, the haemolysin operon. Switching off its expression completely reverses the effect of the double hha ydgT mutation on fitness. Plasmid pHly152 not expressing the hlyCABD genes may not have a cost for any of the strains. Accordingly, neither Hha nor YdgT play a role on the fitness of these cells.

Results obtained for *E. coli* MG1655(pHly152) were also corroborated for *S. Typhimurium*(R27). In this experimental model, *Salmonella* cells incorporate a plasmid that, in addition to adaptive genes, also carries a gene encoding an Hha parologue. When considering the effect of the hha mutation in strain SV5015, a significant effect on fitness can only be seen when, in addition to the chromosomal hha allele, the plasmid-encoded hha allele is also deleted. This highlights the role of these plasmid-encoded paralogues of global modulators such as H-NS or Hha. The impact of the double hha ydgT mutation on fitness of plasmid-free SV5015 cells is much higher than in *E. coli* MG1655 cells. A likely explanation for this is the presence in the chromosome of, among others, genetic determinants of the *Salmonella* pathogenicity islands. Many of these genes are regulated by Hha/YdgT (Coombes et al., 2005; Fahlen et al., 2001; Silphaduang et al., 2007). This might explain why incorporation of R27 does not further increase the fitness cost on an hha ydgT strain.

Loss of Hha function not only affects the fitness of cells that incorporate HGT DNA, but also favours the selection of clones that are spontaneously cured from the corresponding plasmid. This can be shown by using the medium-copy-number recombinant plasmid pANN202-312R. In the absence of the Hha protein, loss of the plasmid occurs at a higher frequency than in the wild-type cells. The use of plasmid replicons containing or lacking the hlyCABD operon shows that selection of cured clones in hha mutants is dependent on the presence of the ‘adaptation module’. Hence, the presence of Hha-target genes in HGT DNA requires the host cells to express the Hha protein to avoid a fitness cost or spontaneous selection of clones that lose the acquired foreign DNA. Mourino et al. (1998) showed that some environmental conditions may slightly modify Hha levels. Nevertheless, conditions repressing Hha expression were not found. It is therefore likely that under several natural conditions, these proteins are expressed and play a key role facilitating incorporation of HGT DNA. The presence of hha paralogues in several conjugative plasmids (Takeda et al., 2011) further supports this assumption. It is hence remarkable that enterobacteria have evolved to potentiate H-NS silencing of xenogeneic DNA by expressing Hha-like proteins.

The interplay between Hha-like and H-NS-like proteins modulating HGT DNA (Baños et al., 2009; Vivero et al., 2008) is further supported by the data obtained in this work. The fitness reduction associated with the loss of Hha and YdgT in strain MG1655(pHly152) can in some instances be compensated by increasing the expression of the H-NS protein. Strain MG1655HY-11 is an interesting example of how IS-mediated spontaneous genetic rearrangements may modify H-NS expression and hence compensate for alterations in expression of other nucleoid-associated proteins such as Hha or YdgT.

**ACKNOWLEDGEMENTS**

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