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The small regulatory RNA molecule MicA is involved in *Salmonella enterica* serovar Typhimurium biofilm formation

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Abstract

Background: LuxS is the synthase enzyme of the quorum sensing signal AI-2. In *Salmonella* Typhimurium, it was previously shown that a *luxS* deletion mutant is impaired in biofilm formation. However, this phenotype could not be complemented by extracellular addition of quorum sensing signal molecules.

Results: Analysis of additional *S. Typhimurium luxS* mutants indicated that the LuxS enzyme itself is not a prerequisite for a wild type mature biofilm. However, in close proximity of the *luxS* coding sequence, a small RNA molecule, MicA, is encoded on the opposite DNA strand. Interference with the MicA expression level showed that a balanced MicA level is essential for mature *Salmonella* biofilm formation. Several MicA targets known to date have previously been reported to be implicated in biofilm formation in *Salmonella* or in other bacterial species. Additionally, we showed by RT-qPCR analysis that MicA levels are indeed altered in some *luxS* mutants, corresponding to their biofilm formation phenotype.

Conclusions: We show that the *S. Typhimurium* biofilm formation phenotype of a *luxS* mutant in which the complete coding region is deleted, is dependent on the sRNA molecule MicA, encoded in the *luxS* adjacent genomic region, rather than on LuxS itself. Future studies are required to fully elucidate the role of MicA in *Salmonella* biofilm formation.

Background

Salmonella enterica serovar Typhimurium (*S. Typhimurium*) is an important pathogen causing gastroenteritis in humans [1]. *Salmonella* is able to form biofilms on both biotic and abiotic surfaces. Growth in such biofilm structures increases the resistance against antibacterial treatments and enhances their spread and persistence outside the host [2]. Also, contamination of processed foods in industrial plants is often due to biofilm formation on both food and food-contact surfaces [3].

In some bacterial species, it has been reported that biofilm formation is partially regulated by a communication system called quorum sensing, more specifically depending on the quorum sensing synthase enzyme LuxS and the signaling molecule autoinducer-2 (AI-2) produced by LuxS [4-9]. In the case of *Salmonella* Typhimurium, it has been reported that biofilm

formation is affected by mutating the *luxS* gene [10-12]. However, De Keersmaecker *et al.* [10] showed that, although genetic complementation could be accomplished, the biofilm forming phenotype could not be rescued by the addition of synthetic DPD, which non-catalytically is converted to AI-2. This suggested that AI-2 is not the actual signal involved in the formation of a *Salmonella* Typhimurium biofilm. Similarly, Karavolos *et al.* [13] reported altered flagellar phase variation in a *S. Typhimurium luxS* deletion mutant independent of quorum sensing signals.

In order to further reveal the exact role of the *luxS* region in *S. Typhimurium* biofilm formation, we analyzed additional *S. Typhimurium luxS* mutants for their biofilm phenotype. We show that the *S. Typhimurium* biofilm formation phenotype is dependent on the sRNA molecule MicA, encoded in the *luxS* adjacent genomic region, rather than on LuxS itself.

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Results

Phenotypic analysis of different *luxS* mutants

Previously, we reported that a *S. Typhimurium* SL1344 *luxS* mutant lacking the entire LuxS coding sequence - from start to stopcodon - (CMPG5602) is unable to form a mature biofilm [10]. This phenotype could be complemented by introduction of the *luxS* gene under control of its own promoter but not by expressing LuxS from a constitutive *nptII* promoter [10]. To further elaborate on this observation, we tested the biofilm formation capacity of other defined *S. Typhimurium luxS* mutants. Figure 1 depicts the genomic *luxS* region in *S. Typhimurium* and indicates the genotype differences among the *luxS* mutants discussed in this study. A *S. Typhimurium luxS::Km* insertion mutant (CMPG5702, [14]) carrying a kanamycin resistance cassette chromosomally inserted in a *Clal* restriction site in the *luxS* coding sequence is unable to form AI-2. This is in agreement with the lack of AI-2 production in the deletion mutant CMPG5602 [10,14] and is as expected since both mutants, CMPG5702 and CMPG5602, are unable to form the AI-2 synthase enzyme LuxS, confirmed by western blot analysis with anti-LuxS antibody (data not shown). However, the insertion mutant still makes wildtype biofilm (Figure 2). To eliminate possible polar effects due to the presence of the kanamycin resistance cassette, a second *luxS* deletion mutant was constructed, using the same procedure as for the first deletion mutant CMPG5602. Yet, this second mutant (CMPG5630) only lacks the 3' part of the *luxS* coding sequence starting from the *Clal* restriction site where the kanamycin cassette was inserted in CMPG5702 (Figure 1). Western blot analysis and AI-2 tests showed that this mutant is unable to form LuxS protein and AI-2 (data not shown). Nevertheless, similarly to the *luxS* insertion mutant, strain CMPG5630 is still able to form a mature wildtype biofilm (Figure 2).

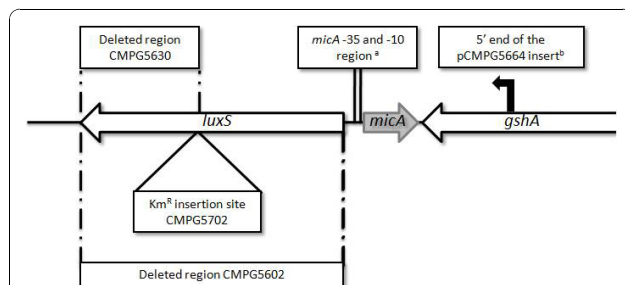
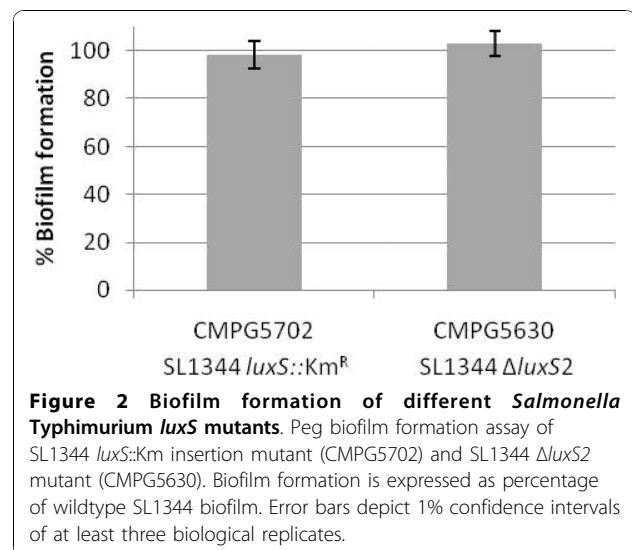


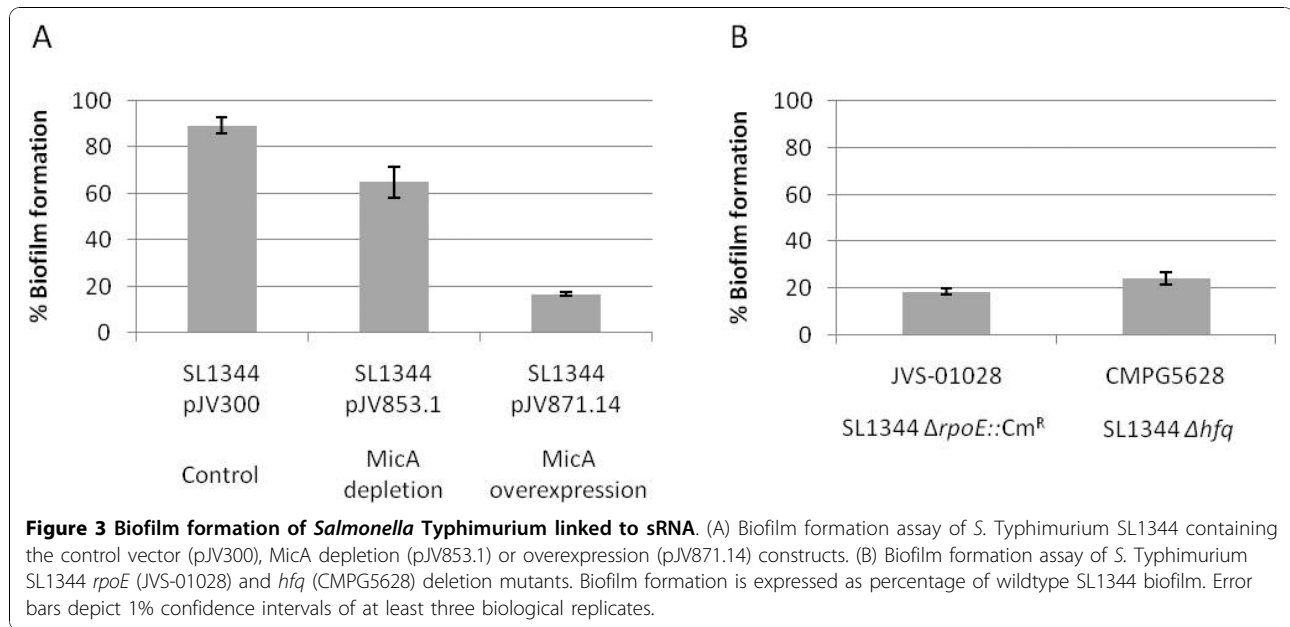
Figure 1 Genomic organization of the *luxS* region in *Salmonella Typhimurium*. Coding sequences are depicted with arrows. Mutated regions in different *luxS* mutants are indicated. The figure is drawn to scale. ^aThe putative -10 and -35 regions of *MicA* as reported by Udekwu et al. [17]. ^b5' end of the *luxS* fragment with own promoter for the construction of the complementation construct pCMPG5664 as reported by De Keersmaecker et al. [10].



The question then rises which features of the *luxS* genomic region can explain the differences in biofilm formation phenotype between strain CMPG5602 - lacking the entire *luxS* coding sequence - on the one hand and both CMPG5702 and CMPG5630 on the other hand. In *Salmonella Typhimurium*, as in *E. coli*, a small non-coding RNA molecule, termed *MicA*, is encoded in the opposite strand of *luxS* (Figure 1) [15]. The close proximity of both genes could imply interference with *MicA* expression when the *luxS* genomic region is mutated. We therefore investigated the possibility that the defect of biofilm formation by CMPG5602 could be due to interference of the *luxS* deletion with *MicA* expression.

MicA has an effect on *Salmonella* biofilm formation

To assess the effect of *MicA* on biofilm formation, two different plasmids were used. The first plasmid, pJV853.1, encodes a *MicA* antisense sequence, thereby leading to partial depletion of *MicA* in the cell due to formation of unstable double stranded RNA. The second plasmid, pJV871.14, is a *MicA* overexpression construct, constitutively expressing *MicA* from a strong P_{LacO} promoter. The ampicillin resistant pJV300 plasmid used for both constructs, was included as a negative control. All plasmids were electroporated to wildtype *S. Typhimurium* SL1344 and the resulting strains were tested for biofilm formation using the peg system quantifying the formed biofilms with crystal violet [10]. The results are shown in Figure 3A. Interestingly, the presence of either the overexpression or the depletion construct had an impact on the biofilm forming capacity of *S. Typhimurium* although not to the same extent. Biofilm formation was almost completely abolished in the *MicA* overexpression strain while only slightly, but significantly



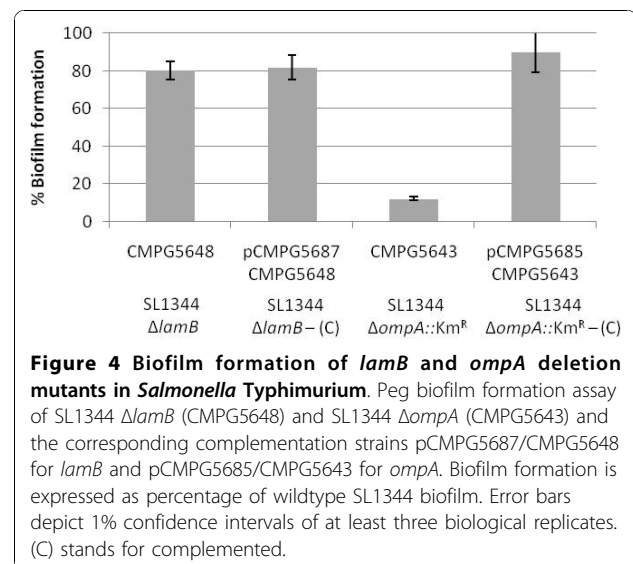
decreased in the MicA depletion strain. This indicates that a tightly regulated balance of MicA expression is essential for proper biofilm formation in *Salmonella* Typhimurium. Note that all strains with the above plasmid constructs produce wildtype AI-2 levels (data not shown).

Further indirect evidence of small RNA molecules being involved in the regulation of biofilm formation was provided by the analysis of both *hfq* and *rpoE* mutants. Hfq is a prerequisite for the binding of many sRNAs to their *trans*-encoded targets [16,17], while sigmaE, encoded by *rpoE*, has been shown to be involved in the transcription of several small RNAs, including MicA [18-20]. In the peg biofilm assay, neither of these strains were able to form mature biofilms (Figure 3B). The phenotype could genetically be complemented by introducing the corresponding gene *in trans* on a plasmid carrying a constitutive promoter (data not shown).

MicA targets involved in *Salmonella* biofilm formation

Most likely, the impact of MicA on biofilm formation in *Salmonella* is through one of its *Salmonella* targets. To date, four *trans* encoded targets, all negatively regulated by MicA, have already been reported in *Escherichia coli*, i.e. the outer membrane porins OmpA [17,21] and OmpX [22], the maltoporin LamB [23] and recently the PhoPQ two-component system [24]. Two of these targets, PhoPQ and OmpA, were previously shown to be involved in biofilm formation [25-27], i.e. Prouty and Gunn [25] demonstrated that a *S. Typhimurium phoP* null mutant has an enhanced biofilm forming capacity, while a PhoP constitutive mutant is unable to develop a

mature biofilm. OmpA was shown to be involved in *E. coli* biofilm formation [26,27]. To assess whether OmpA is also implicated in biofilm formation in *Salmonella*, we constructed an *ompA* deletion mutant in *S. Typhimurium* SL1344 and tested this strain with the peg biofilm assay. As in *E. coli*, a *S. Typhimurium ompA* mutant is unable to form biofilm, and this phenotype can be complemented by introducing *ompA in trans* (Figure 4). As no information is yet reported on the role of LamB in biofilm formation, we also constructed a *lamB* deletion mutant. The results in Figure 4 indicate that this mutant is not significantly affected in its biofilm forming capacity, confirming that not all MicA



targets known to date are implicated in biofilm formation. Note that both the *S. Typhimurium lamB* and *ompA* deletion mutant are still capable of forming AI-2 (data not shown).

Analysis of MicA levels in *S. Typhimurium luxS* mutants

From the results described in the previous paragraphs, it can be concluded that the sRNA MicA is indeed implicated in the regulation of biofilm formation in *S. Typhimurium*. The question remains however, whether different MicA levels occur in wildtype and the *luxS* deletion mutant (CMPG5602), thereby explaining the biofilm formation phenotype of the latter. Using RT-qPCR, the amount of MicA was quantitatively assessed in wildtype SL1344, the *luxS* deletion mutant CMPG5602 -unable to form a mature biofilm - and the *luxS* insertion mutant CMPG5702 and partial deletion mutant CMPG5630 - forming a wildtype biofilm, all strains grown under biofilm forming conditions. The entire *luxS* CDS deletion strain CMPG5602 contains significantly less MicA compared to wildtype SL1344. Conversely, both CMPG5702 and CMPG5630, still capable of making biofilm, have a MicA expression level comparable to the wildtype strain (Figure 5). To rule out the possibility that these differential expression levels are due to the difference between biofilm cells (in wildtype) and planktonic cells (in the *luxS* deletion mutant), we performed the experiment also using planktonic wildtype cells from the medium above the biofilm, sampled similarly as for the *luxS* deletion mutant cells (*cf.* Methods section). The relative difference in MicA expression level was similar in this experimental setup, i.e. a significantly lower MicA expression level in the

luxS deletion as compared to wildtype *S. Typhimurium* (data not shown). Overall, these results confirm that mutating the *luxS* genomic region can have a significant impact on MicA sRNA levels, consequently affecting the MicA regulated biofilm phenotype, independently of quorum sensing.

Discussion

In several bacteria, biofilm formation capacity has been linked to *luxS* based quorum sensing, mediated by AI-2 signaling molecules [4-9]. In *Salmonella Typhimurium*, it was previously reported that a deletion mutant of the AI-2 synthase enzyme *luxS* has an impaired biofilm formation capacity [10]. However, this phenotype could not be chemically complemented by extracellular addition of synthetic DPD, nor by expressing *luxS* from a constitutive promoter on a plasmid. On the other hand, introduction of *luxS* with its native promoter did complement the biofilm phenotype [10]. In this study, we showed that both a *luxS::Km* insertion mutant and a deletion mutant of the 3' end of the *luxS* coding sequence are still able to form a mature biofilm, despite the fact that these strains are unable to form the type-2 quorum sensing signaling molecule AI-2.

Adjacent to the *luxS* coding sequence, a small non-coding RNA molecule named MicA is encoded in the opposite strand [15]. Using MicA depletion and overexpression constructs, respectively, we showed that a tightly balanced MicA concentration is essential for proper biofilm formation in *S. Typhimurium*. This suggests that the final impact of MicA regulation on biofilm formation is based on a complex interplay of several of its targets, a fine-tuning process in which timing is also likely to play a role. It is interesting to note that the MicA depletion strain does not completely abolish the biofilm formation capacity. This could be explained by an incomplete silencing of MicA in this strain or by the fact that other sRNA molecules take over the role of MicA. It is not uncommon that mRNA targets are redundantly regulated by multiple sRNA molecules fine-tuning their expression in a complex way [28,29]. The fact that deletion of both *rpoE* or *hfq* fully inhibited biofilm formation supports the hypothesis that other sRNA molecules are implicated in regulation of biofilm formation.

In literature, two MicA targets known to date were previously linked to biofilm formation. An *E. coli ompA* mutant is unable to form a mature biofilm on plastic substrates [27]. We showed that also in *Salmonella Typhimurium*, *OmpA* is involved in biofilm formation as an *ompA* deletion mutant is unable to form a mature biofilm. Furthermore, the two-component system PhoPQ, previously shown to be implicated in regulation of *Salmonella* biofilm formation [25], was recently

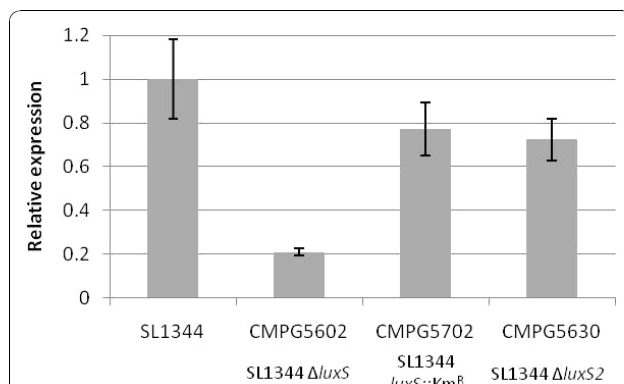


Figure 5 RT-qPCR analysis of different *S. Typhimurium luxS* mutants with MicA primers. MicA sRNA expression levels were measured with RT-qPCR as described in the Methods section. Representative means and standard deviations of three RT-qPCRs are shown. Gene expression is expressed relative to the wildtype SL1344 level. CMPG5602: SL1344 $\Delta luxS$ deletion mutant; CMPG5702: SL1344 $luxS::Km^R$ insertion mutant; CMPG5630: SL1344 $\Delta luxS2$ deletion mutant.

described as a target of MicA in *E. coli* [24], implying indirect regulation of the entire PhoPQ regulon by MicA. At this moment, it cannot be excluded that other, yet uncharacterized targets of MicA exist which are related to biofilm formation. Nevertheless, it is already clear that MicA regulation comprises a complex network of interactions influencing a broad range of genes either directly or indirectly.

Using RT-qPCR analyses, we were able to confirm that the levels of MicA in the *luxS* CDS deletion mutant CMPG5602 compared to wildtype and the insertion mutant CMPG5702 differ. This supports our formulated hypothesis that an impaired biofilm formation phenotype in a *Salmonella* Typhimurium *luxS* deletion mutant is due to an imbalanced MicA level, rather than to the absence of LuxS itself. Remark that complementation of the CMPG5602 phenotype requiring expression of *luxS* from its native promoter [10] also corroborates with this model (Figure 1). Indeed, MicA is encoded in this promoter region and hence, the biofilm phenotype can only be complemented by reintroduction of MicA.

Presently, it is still unclear how deletion of the *luxS* CDS influences MicA expression. The putative -10 and -35 regions of MicA as reported by Udekwu *et al.* [17] do not overlap with the coding region of *luxS* (Figure 1). However, this coding region might include other regulatory elements interfering with MicA expression. Further studies of both *luxS* and *micA* promoter regions and transcription are required to elucidate the mechanism of interference between both genetic loci.

Conclusions

In this study, we showed by analyzing different *S. Typhimurium* mutants that biofilm formation is influenced by the sRNA molecule MicA. This sRNA is encoded in close proximity of the quorum sensing synthase *luxS* and mutating this region can therefore mutually affect both genetic loci. Given the evolutionary conservation of MicA in several *Enterobacteriaceae*, this regulatory mechanism of biofilm formation might also apply to bacterial species other than *Salmonella*.

Methods

Bacterial strains and growth conditions

The parental strains and plasmids that were used in this study are listed in Table 1. *Salmonella* Typhimurium SL1344 is the wildtype strain [30]. The *Salmonella* Typhimurium Δhfq (CMPG5628), *S. Typhimurium* $\Delta luxS2$ (CMPG5630) and $\Delta lamB$ (CMPG5648) mutants were constructed using the procedure of Datsenko and Wanner [31], with pKD3 as a template plasmid (all primers used in this study are listed in Table 2). All strains were verified by PCR and sequencing. For the OmpA and LamB complementation constructs, *ompA* and *lamB* were amplified

with PCR using primers PRO-0101/PRO-0102 and PRO-0474/PRO-0475, respectively, and cloned as an *XbaI/PstI* fragment into pFAJ1708 [32]. Both plasmids were verified by PCR and sequencing and finally electroporated to the corresponding SL1344 mutant background.

When appropriate, antibiotics were applied at the following concentrations: 25 $\mu\text{g/ml}$ chloramphenicol and 100 $\mu\text{g/ml}$ ampicillin. Strains were grown as a biofilm using the peg system as previously described [10]. For accurate comparison of data between peg plates, wildtype *S. Typhimurium* SL1344 was included in every plate as a control and data analysis was performed relative to the wildtype SL1344 values. In all figures, results are shown as a percentage of biofilm compared to wildtype SL1344 (100%). Error bars depict 1% confidence intervals of at least three biological replicates and each biological replicate is the average biofilm formation of eight technical replicates.

AI-2 measurement

To measure AI-2 production of specific *S. Typhimurium* strains, the reporter plasmid pCMPG5638 was electroporated to the strains of interest. This plasmid contains a transcriptional fusion of the *lsrA* promoter region to the *luxCDABE* luminescence reporter gene operon of *Photobacterium luminescens* [10]. In *S. Typhimurium*, the expression of the *lsr* operon is regulated by AI-2 levels, and therefore luminescence of strains carrying the reporter plasmid is a measure for AI-2 production. Overnight cultures of strains of interest, were diluted 1:100 in fresh LB medium and grown for approximately 4 h, shaking at 37°C. Then, luminescence was measured together with the optical density at 600 nm. Wildtype SL1344 and CMPG5602 - *luxS* deletion mutant - were used as positive and negative control strains, respectively.

RT-qPCR analysis

For RNA isolation, strains were grown as a biofilm in round petridishes. An overnight preculture in 5 ml Luria-Bertani broth (LB) medium, was diluted 1:100 in 20 ml 1:20 diluted TSB medium (Bacto™ Tryptic Soy Broth from BD Biosciences, 30 g/l) (resulting in approximately 10^7 cfu/ml) and poured carefully into a round petridish. These petridishes were incubated non-shaking at 16°C for 24 h. After the medium was removed, cells from the biofilm were scraped from the plate in a mixture of 1 ml 1:20 TSB and 200 μl ice-cold phenol:ethanol (5:95) and transferred to a microcentrifuge tube which was immediately frozen in liquid nitrogen and stored at -80°C. For strain CMPG5602, which is unable to form a mature biofilm, cells were incubated under the same conditions, but removed from the medium by centrifugation. Subsequent steps were identical for all strains. Total RNA was isolated from the cells using the SV Total RNA Isolation kit

Table 1 Bacterial strains and plasmids

Strains	Description	Source or reference
<i>Escherichia coli</i>		
DH5 α	F' ϕ 80 Δ lacZM15 Δ (lacZYAargF)U169 deoP recA1 endA1 hsdR17 (r _k ⁻ m _k ⁻)	Gibco BRL
TOP10 ^F	F' {lacIq Tn10(TetR)} mcrA Δ (mrr-hsdRMS-mcrBC) ϕ 80lacZ Δ M15 Δ lacX74 deoR recA1 araD139 Δ (ara-leu)7697 galU galK rpsL (Str ^R) endA1 nupG	Invitrogen
<i>Salmonella enterica</i> serovar Typhimurium		
SL1344	xyl hisG rpsL; virulent; Sm ^R	[30]
CMPG5602	SL1344 Δ luxS - deletion of the entire luxS CDS	[10]
CMPG5702	SL1344 luxS::Km ^R	[14]
CMPG5630	SL1344 Δ luxS2 - deletion of the 3' end of luxS CDS	This study
CMPG5628	SL1344 Δ hfq	This study
JVS-01028	SL1344 Δ rpoE::Cm ^R	[34]
CMPG5643	SL1344 Δ ompA::Km ^R	Phage lysate of J. Vogel
CMPG5648	SL1344 Δ lamB	This study
Plasmids		
pJV300	pZE12-luc based plasmid; P _{LacO} -rrnB terminator; short nonsense transcript; control plasmid; Amp ^R	[35]
pJV871.14	pZE12-luc based plasmid; LT2 MicA overexpression construct; MicA transcription driven from constitutive P _{LacO} , starting precisely at G ₋₁ ; Amp ^R	[36]
pJV853.1	pZE12-luc based plasmid; LT2 anti-MicA expression construct; anti-MicA transcription driven from constitutive P _{LacO} ; Amp ^R	J. Vogel, unpublished data
pKD3	Template for mutant construction; carries chloramphenicol-resistance cassette; oriRy origin; Amp ^R	[31]
pKD46	P _{araB} - γ - β -exo; temperature-sensitive lambda-red recombinase expression plasmid; oriR101 origin; Amp ^R	[31]
pCP20	Temperature-sensitive FLP recombinase expression plasmid; oriR101 origin; Amp ^R , Cm ^R	[31]
pFAJ1708	Derivative of RK-2; Amp ^R ; Tc ^R ; contains nptII promoter of pUC18-2	[32]
pCMPG5685	pFAJ1708 OmpA complementation construct	This study
pCMPG5687	pFAJ1708 LamB complementation construct	This study
pCMPG5638	pCS26-Pac plasmid carrying a transcriptional reporter fusion between the promoter of the <i>IsrACDBFGE</i> operon and luxCDABE	[10]

Table 2 Primers used in this study

Primer	Sequence	Purpose ^a
PRO-483	TTTCAGAATCGAAAGGTTCAAAGTACAATAAGCATATAAGGAAAAGAGAGTGTAGGCTGGAGCTGCTTC	FW CMPG5628
PRO-484	AGCGGGGGCGATTATCCGACGCCCCCGACATGGATAAACAGCGCGTGAACCATATGAATATCCTCCTT	RV CMPG5628
PRO-487	TTTGCTGGCTTTATGCGCGACCACCTCAACGGTAACGGCGTTGAGATTATGTGTAGGCTGGAGCTGCTTC	FW CMPG5630
PRO-229	CGGCCATAAACCGGGGTTAATTTAAATACTGGAACCGCTTACAATAAGACATATGAATATCCTCCTTA	RV CMPG5630
PRO-0472	TGATGTTTTCCGAGGGGCTTGCGCCCTCGTTACGTCAGATGACCATCGTACATATGAATATCCTCCTTA	FW CMPG5648
PRO-0473	CCATTCGCAGTTTTAGAAGGTGGCAGCGTTTTAAAGAAAAGCAATGATCTCGTGTAGGCTGGAGCTGCTTC	RV CMPG5648
PRO-0101	ATTCTAGACTTTACATCGCCAGGGGTGCTCAG	FW pCMPG5685
PRO-0102	ATCTGCAGCGCTGAAAGGCGTTGTCATCCAG	RV pCMPG5685
PRO-0474	ATTCTAGACCATTGCGAGTTTTAGAAGG	FW pCMPG5687
PRO-0475	ATCTGCAGTCATCAGACCTGATGTTCC	RV pCMPG5687
PRO-2993	CTCACGGAGTGGCCAAAATT	FW RT-qPCR MicA
PRO-2994	GACGCGCATTTGTTATCATCAT	RV RT-qPCR MicA
PRO-1150	AAACGCGGGCAACTTCAG	FW RT-qPCR rfaH
PRO-1151	GTCAGGCAACTTACCGCTTGT	RV RT-qPCR rfaH

^aFW: Forward primer; RV: Reverse primer.

(Promega). This kit also allows extraction of small RNA molecules. RNA isolation was performed according to the manufacturer's instructions except for the DNase treatment, which was separately performed using the TURBO DNA-free Kit (Ambion) according to the manufacturer's instructions. DNA contamination of the RNA samples was checked by PCR. RT-qPCR analysis was essentially performed as previously described [33] with some minor modifications. 1.5 µg of RNA was reverse transcribed using the RevertAid H Minus First strand cDNA Synthesis Kit (Fermentas). After dilution of cDNA, 5 µl of cDNA (2 ng/µl), 0.9 µl of each specific primer (20 µM) and 3.2 µl of RT-qPCR grade water (Ambion) were mixed with 10 µl of Power SYBR Green PCR Master Mix. *rfaH* showed an invariant expression between the strains tested and was used as a reference gene [34]. Wildtype SL1344 samples were routinely used as reference sample.

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Authors' contributions

GK participated in the design of the study and drafted the manuscript. DDC carried out part of the experimental work. KM participated in the design of the study. JV and SCJDK conceived the study, participated in its design and coordination and helped to draft the manuscript. SCJDK also performed part of the experimental work. All authors read and approved the final manuscript.

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References

1. Raffatellu M, Tükel C, Chessa D, Wilson RP, Bäumlner AJ: **The intestinal phase of *Salmonella* infections.** In *Salmonella. Molecular biology and pathogenesis*. Edited by: Rhen M, Maskell DJ, Mastroeni P, Thelfall J. Wyomondham, Norfolk, United Kingdom: Horizon Bioscience; 2007:31-52.
2. Olson ME, Ceri H, Morck DW, Buret AG, Read RR: **Biofilm bacteria: formation and comparative susceptibility to antibiotics.** *Canadian Journal of Veterinary Research-Revue Canadienne de Recherche Veterinaire* 2002, **66**:86-92.
3. Kumar CG, Anand SK: **Significance of microbial biofilms in food industry: a review.** *International Journal of Food Microbiology* 1998, **42**:9-27.
4. Balestrino D, Haagensen JAJ, Rich C, Forestier C: **Characterization of type 2 quorum sensing in *Klebsiella pneumoniae* and relationship with biofilm formation.** *J Bacteriol* 2005, **187**:2870-2880.
5. Cole SP, Harwood J, Lee R, She R, Guiney DG: **Characterization of monospecies biofilm formation by *Helicobacter pylori*.** *J Bacteriol* 2004, **186**:3124-3132.
6. Daines DA, Bothwell M, Furrer J, Unrath W, Nelson K, Jarisch J, Melrose N, Greiner L, Apicella M, Smith AL: ***Haemophilus influenzae luxS* mutants form a biofilm and have increased virulence.** *Microbial Pathogenesis* 2005, **39**:87-96.
7. Merritt J, Qi FX, Goodman SD, Anderson MH, Shi WY: **Mutation of *luxS* affects biofilm formation in *Streptococcus mutans*.** *Infect Immun* 2003, **71**:1972-1979.
8. Shao H, Lamont RJ, Demuth DR: **Autoinducer 2 is required for Biofilm growth of *Aggregatibacter (Actinobacillus) actinomycetemcomitans*.** *Infect Immun* 2007, **75**:4211-4218.
9. Hardie KR, Heurlier K: **Establishing bacterial communities by 'word of mouth': *LuxS* and autoinducer 2 in biofilm development.** *Nat Rev Microbiol* 2008, **6**:635-643.
10. De Keersmaecker SCJ, Varszegi C, van Boxel N, Habel LW, Metzger K, Daniels R, Marchal K, De Vos D, Vanderleyden J: **Chemical synthesis of (S)-4,5-dihydroxy-2,3-pentanedione, a bacterial signal molecule precursor, and validation of its activity in *Salmonella typhimurium*.** *J Biol Chem* 2005, **280**:19563-19568.
11. Prouty AM, Schwesinger WH, Gunn JS: **Biofilm formation and interaction with the surfaces of gallstones by *Salmonella* spp.** *Infect Immun* 2002, **70**:2640-2649.
12. Jesudhasan PR, Cepeda ML, Widmer K, Dowd SE, Soni KA, Hume ME, Zhu J, Pillai SD: **Transcriptome analysis of genes controlled by *luxS*/autoinducer-2 in *Salmonella enterica* serovar Typhimurium.** *Foodborne Pathog Dis* 2010, **7**:399-410.
13. Karavolos MH, Bulmer DM, Winzer K, Wilson M, Mastroeni P, Williams P, Khan CMA: ***LuxS* affects flagellar phase variation independently of quorum sensing in *Salmonella enterica* serovar Typhimurium.** *J Bacteriol* 2008, **190**:769-771.
14. Kint G, Sonck KAJ, Schoofs G, De Coster D, Vanderleyden J, De Keersmaecker SCJ: **2D Proteome Analysis initiates new Insights on the *Salmonella* Typhimurium *LuxS* Protein.** *BMC Microbiol* 2009, **9**:198.
15. Argaman L, Hershberg R, Vogel J, Bejerano G, Wagner EGH, Margalit H, Altuvia S: **Novel small RNA-encoding genes in the intergenic regions of *Escherichia coli*.** *Current Biology* 2001, **11**:941-950.
16. Valentin-Hansen P, Eriksen M, Udesen C: **The bacterial Sm-like protein Hfq: a key player in RNA transactions.** *Mol Microbiol* 2004, **51**:1525-1533.
17. Udekwi KI, Darfeuille F, Vogel J, Reimegard J, Holmqvist E, Wagner EGH: **Hfq-dependent regulation of *OmpA* synthesis is mediated by an antisense RNA.** *Genes Dev* 2005, **19**:2355-2366.
18. Udekwi KI, Wagner EGH: **Sigma E controls biogenesis of the antisense RNA *MicA*.** *Nucleic Acids Res* 2007, **35**:1279-1288.
19. Figueroa-Bossi N, Lemire S, Malorid D, Balbontin R, Casades J, Bossi L: **Loss of Hfq activates the sigma(E)-dependent envelope stress response in *Salmonella enterica*.** *Mol Microbiol* 2006, **62**:838-852.
20. Johansen J, Rasmussen AA, Overgaard M, Valentin-Hansen P: **Conserved small non-coding RNAs that belong to the sigma(E) regulon: Role in down-regulation of outer membrane proteins.** *J Mol Biol* 2006, **364**:1-8.
21. Rasmussen AA, Eriksen M, Gilany K, Udesen C, Franch T, Petersen C, Valentin-Hansen P: **Regulation of *ompA* mRNA stability: the role of a small regulatory RNA in growth phase-dependent control.** *Mol Microbiol* 2005, **58**:1421-1429.
22. Johansen J, Eriksen M, Kallipolitis B, Valentin-Hansen P: **Down-regulation of Outer Membrane Proteins by Noncoding RNAs: Unraveling the cAMP-CRP- and sigma(E)-Dependent *CyaR-ompX* Regulatory Case.** *J Mol Biol* 2008, **383**:1-9.
23. Bossi L, Figueroa-Bossi N: **A small RNA downregulates LamB maltoporin in *Salmonella*.** *Mol Microbiol* 2007, **65**:799-810.
24. Coornaert A, Lu A, Mandin P, Springer M, Gottesman S, Guillier M: ***MicA* sRNA links the PhoP regulon to cell envelope stress.** *Mol Microbiol* 2010, **76**:467-479.
25. Prouty AM, Gunn JS: **Comparative analysis of *Salmonella enterica* serovar Typhimurium biofilm formation on gallstones and on glass.** *Infect Immun* 2003, **71**:7154-7158.
26. Barrios AFG, Zuo RJ, Ren DC, Wood TK: **Hha, YbaJ, and *OmpA* regulate *Escherichia coli* K12 biofilm formation and conjugation plasmids abolish motility.** *Biotechnology and Bioengineering* 2006, **93**:188-200.
27. Ma Q, Wood TK: ***OmpA* influences *Escherichia coli* biofilm formation by repressing cellulose production through the CpxRA two-component system.** *Environmental Microbiology* 2009, **11**:2735-2746.
28. Vogel J: **A rough guide to the non-coding RNA world of *Salmonella*.** *Mol Microbiol* 2009, **71**:1-11.
29. Waters LS, Storz G: **Regulatory RNAs in Bacteria.** *Cell* 2009, **136**:615-628.

30. Hoiseth SK, Stocker BAD: **Aromatic-Dependent *Salmonella* Typhimurium Are Non-Virulent and Effective As Live Vaccines.** *Nature* 1981, **291**:238-239.
31. Datsenko KA, Wanner BL: **One-step inactivation of chromosomal genes in *Escherichia coli* K-12 using PCR products.** *Proc Natl Acad Sci USA* 2000, **97**:6640-6645.
32. Dombrecht B, Vanderleyden J, Michiels J: **Stable RK2-derived cloning vectors for the analysis of gene expression and gene function in Gram-negative bacteria.** *Mol Plant Microbe Interact* 2001, **14**:426-430.
33. Vercruyse M, Fauvart M, Cloots L, Engelen K, Thijs IM, Marchal K, Michiels J: **Genome-wide detection of predicted non-coding RNAs in *Rhizobium etli* expressed during free-living and host-associated growth using a high-resolution tiling array.** *BMC Genomics* 2010, **11**:53.
34. Pappenfort K, Pfeiffer V, Mika F, Lucchini S, Hinton JCD, Vogel J: **sigma(E)-dependent small RNAs of *Salmonella* respond to membrane stress by accelerating global omp mRNA decay.** *Mol Microbiol* 2006, **62**:1674-1688.
35. Sittka A, Pfeiffer V, Tedin K, Vogel J: **The RNA chaperone Hfq is essential for the virulence of *Salmonella typhimurium*.** *Mol Microbiol* 2007, **63**:193-217.
36. Bouvier M, Sharma CM, Mika F, Nierhaus KH, Vogel J: **Small RNA Binding to 5' mRNA Coding Region Inhibits Translational Initiation.** *Mol Cell* 2008, **32**:827-837.

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