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LETTER TO THE EDITOR

The AI-2-dependent regulator LsrR has a limited regulon in *Salmonella* Typhimurium

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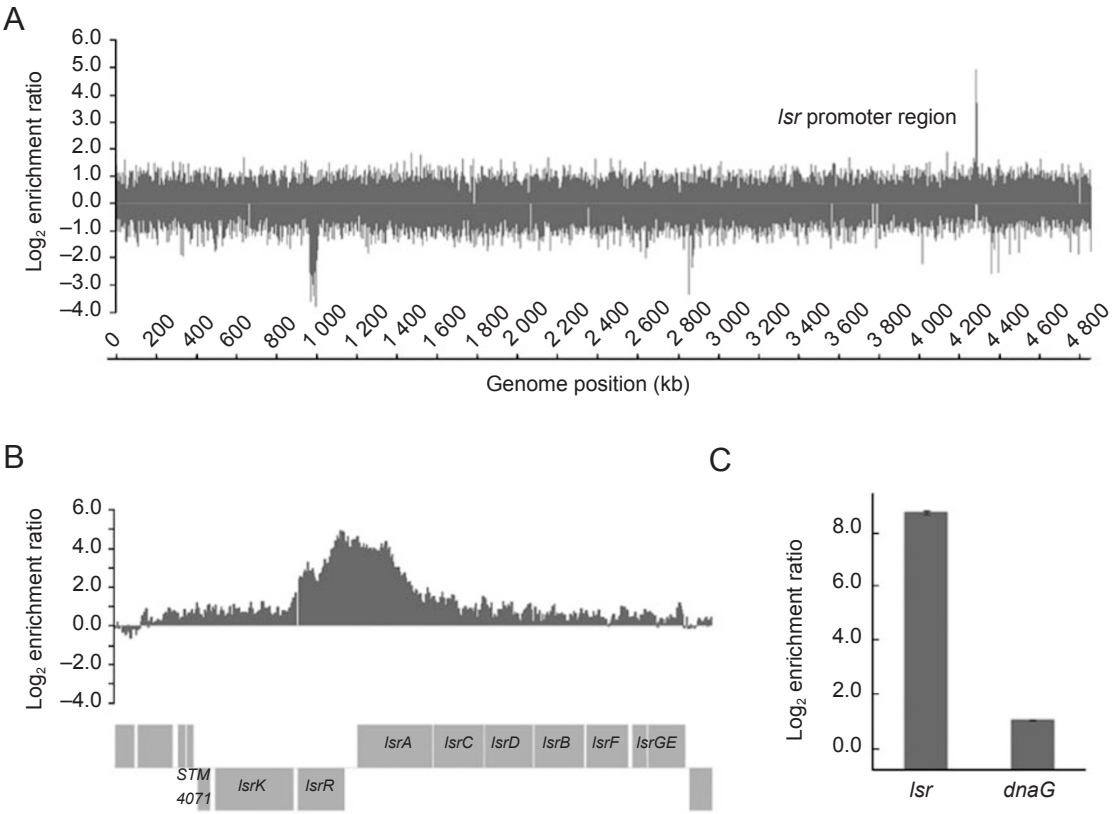
Dear Editor,

Bacteria are able to communicate with each other through small, diffusible molecules. In some species, this type of communication has been linked to virulence. Therefore, bacterial cell-cell communication pathways have recently been put forward as promising targets for new anti-microbial therapeutics [1]. Several different communication systems have been identified in bacteria, both for intraspecies and for interspecies communication. The AI-2 signal is hypothesized to be involved in interspecies bacterial communication, since it is produced by an enzyme, LuxS, which is widely conserved among Gram-negatives and Gram-positives [2]. *Salmonella enterica* serovar Typhimurium (*S.* Typhimurium), an important food pathogen that causes diseases ranging from self-limiting gastroenteritis to life-threatening systemic infections, also produces AI-2 through LuxS. In *S.* Typhimurium, however, the role of AI-2 as a signaling molecule is still controversial, including the possible role in its pathogenicity. Most studies unraveling the possible role of AI-2 have focused their efforts on studying the effects of a *luxS* mutation [3]. However, LuxS also functions in the pathway for metabolism of *S*-adenosylmethionine (SAM), the major cellular methyl donor [4]. As a result, the important metabolic role of LuxS makes interpretation of data reflecting either cell-cell signaling or metabolic effects very difficult [5]. In this study, we therefore aimed at identifying the direct transcriptional targets of LsrR, the sole transcriptional regulator that has been associated with AI-2 in *S.* Typhimurium [6]. A better knowledge of the *Salmonella* LsrR regulon may further clarify the role of AI-2. By using a combination of high-throughput technologies, we show that the LsrR regulator has a limited regulon in *S.* Typhimurium.

Till now, the only AI-2-dependent genes known in *S.* Typhimurium, as identified in a genetic screen for *luxS*-regulated genes [6], are in the *lsr* operon, which is repressed by LsrR when no AI-2 is present in the cell. The *lsr* operon consists of various genes for AI-2 transport and processing. *lsrACD* encode an ATP binding cassette (ABC) transporter, which is highly similar to the ribose transport system of *E. coli* and *S.* Typhimurium, that internalizes extracellular AI-2 [6]. LsrB is a periplasmic binding protein component that directly binds AI-2 and is required for AI-2 transport into the cell [7]. LsrF and LsrG are involved in further processing of the internalized AI-2 [8, 9]. No function for the *lsrE* gene, which is homologous to genes encoding sugar epimerases, has yet been identified. Encoded in the opposite direction of the *lsr* operon and also shown to be repressed by LsrR in *E. coli* [10] are *lsrR* and *lsrK*. LsrK is a kinase that phosphorylates AI-2 upon its entry into the cell [8]. Phospho-AI-2 then directly binds the repressor LsrR, thus relieving the repression of the *lsr* operon by LsrR [8, 10]. In *S.* Typhimurium, AI-2 production is stimulated in conditions of low pH and high osmolarity, conditions that are prevalent in the intestine [11]. Therefore, AI-2 has been hypothesized to play a role in host-associated phenotypes and even in the pathogenicity of *S.* Typhimurium, yet contradicting observations are reported [12].

Recently, the LsrR binding site in the promoter region of both *lsrR* and *lsrA* has been determined *in vitro* in *E. coli* [10]. Additionally, the effect of an *lsrR* deletion on genome-wide expression has been studied in *E. coli* [13]. However, no distinction between the direct targets of LsrR (constituting the LsrR regulon) and indirect targets – affected by an *lsrR* mutation modulating the rate at which AI-2 is imported and processed – has been made. Based on these observations, LsrR – being a specific AI-2-related regulator with a broad impact – has been put forward as a promising gateway towards further understanding AI-2-based communication and, possibly, towards establishing a foundation for antimicrobial therapies targeting LsrR [1]. In this study, we therefore aimed at mapping the full LsrR regulon of *S.* Typhimurium, in order to better understand its role by identifying its direct target genes. To this end, we combined chromatin immunoprecipitation (ChIP) coupled with *S.* Typhimurium whole-genome tiling arrays (ChIP-chip) and transcriptomics comparing gene expression in an *lsrR* deletion mutant and wild-type *S.* Typhimurium.



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Gene number[a]	Gene name[a]	Gene function[a]	Log_2 ratio gene expression Δ*lsrR*/wt[b]
Upregulated in lsrR *mutant versus wild-type*			
STM4071[c]		Putative mannose-6-phosphate isomerase	2.82
STM4072	*lsrK*	Kinase	1.94
STM4074[c]	*lsrA*	ABC-type transport system	3.99
STM4075	*lsrC*	ABC-type transport system	4.10
STM4076	*lsrD*	ABC-type transport system	4.07
STM4077	*lsrB*	ABC-type transport system	4.56
STM4078	*lsrF*	Putative fructose-1,6-bisphosphate aldolase	5.19
STM4079	*lsrG*	Processing of phospho-AI-2	4.23
STM4080	*lsrE*	Putative ribulose-5-phosphate 3-epimerase	4.04
Downregulated in lsrR *mutant versus wild-type*			
STM4459[c]	*pyrI*	Aspartate carbamoyltransferase, regulatory subunit	−2.25

Figure 1 Genome-wide **(A)** and *lsr* region view **(B)** of LsrR ChIP-chip data. Each bar represents the log_2 enrichment ratio, or difference between normalized ChIP and mock ChIP log_2 ratios, for a particular probe. The peak indicating binding of LsrR in the *lsr* operon upstream intergenic region is indicated in **(A)** and a detailed view of the *lsr* region is given in **(B)**. In the latter, the lower strip represents the annotated genome; genes encoded on the direct strand are represented by bars above the horizontal line, genes encoded on the complementary strand below. The negative peaks in **(A)** are an artefact, caused by the absence of Fels-1 and Fels-2 in *S.* Typhimurium SL1344 (used for the ChIP-chip analysis) as compared to *S.* Typhimurium LT2 (genome sequence used for the tiling array design, Supplementary information, Data S1). The validation of the ChIP-chip data by ChIP-qPCR is shown in **(C)**; *dnaG* is a negative control (Supplementary information, Data S1). Values shown are log_2 enrichment ratios of ChIP over mock ChIP samples and averages of triplicate qPCRs; standard deviations are indicated. Values are representative for three biological repeats. **(D)** Significantly differentially expressed genes in an *lsrR* mutant versus wild-type *S.* Typhimurium. [a]STM numbers, gene names and gene functions are taken from NCBI Refseq NC_003197 [16] and adapted according to recent literature. Results are sorted according to STM number. [b]Differentially expressed genes were detected by *t*-test with multiple testing correction; see Supplementary information, Data S1.[c]Differential expression of STM4071, *lsrA* and *pyrI* was validated by qRT-PCR (Supplementary information, Data S1). qRT-PCR log_2 ratios of expression in the *lsrR* mutant versus the wild-type *S.* Typhimurium were 3.74 (± 0.59) for STM4071, 4.84 (± 0.23) for *lsrA* and −0.70 (± 0.13) for *pyrI*.

ChIP-chip was performed as previously described ([14] and Supplementary information, Data S1) on *S.* Typhimurium whole genome tiling arrays after 4 h of growth (1.5E9 CFU/ml) under aerobic conditions. We chose a time point at which the extracellular AI-2 level is higher for the wild-type than for the *lsrR* mutant (data not shown), indicating that at this time point LsrR is still repressing the *lsr* operon and, possibly, its other yet unidentified target genes. For LsrR, the intergenic region between *lsrR* and the *lsr* operon was the only *in vivo* bound target that could be identified (Figure 1A and 1B). Although this direct control has already been shown *in vitro* for *E. coli* [10], here we show that LsrR also binds the *lsr* operon promoter *in vivo* in *S.* Typhimurium. More importantly, however, no other targets bound by LsrR *in vivo* could be detected. To ensure that we did not fail to identify other LsrR target genes, bound by the regulator at another time point, ChIP-chip was repeated after 10 h of growth (5E9 CFU/ml) but no additional peaks could be observed (data not shown). ChIP-chip data were validated by performing ChIP and assessing the enrichment of the *lsr* promoter by quantitative PCR (Figure 1C).

To complement the ChIP-chip experiment – ChIP-chip data give information on the location of a regulator, not on its functioning – we performed a transcriptomics study comparing mRNA levels in the wild-type and the *lsrR* mutant strain CMPG5608 at the same time point (4 h) as for the ChIP-chip experiment (Supplementary information, Data S1). As expected, genes of the *lsr* operon were significantly upregulated in the *lsrR* mutant versus the wild-type, confirming the repressor function of LsrR (Figure 1D). Divergently transcribed from the *lsr* operon, *lsrK* and further downstream-located STM4071 (Figure 1B) were also upregulated in the *lsrR* mutant (Figure 1D). STM4071 has not been associated with the *lsr* genes before. STM4071 encodes a protein with similarity to polyketide synthesis domain proteins and was previously annotated as a putative mannose-6-phosphate isomerase; STM4071 has no homologue in *E. coli*. Significantly downregulated in the *lsrR* mutant versus the wild-type was *pyrI*, encoding the allosteric regulatory subunit of the aspartate carbamoyltransferase, involved in pyrimidine biosynthesis. Just below the analysis threshold were the differential expression values of *pyrB*, encoding the catalytic subunit of the aspartate carbamoyltransferase, and *carAB*, encoding the glutamine-hydrolyzing small and large subunits of the carbamoyl phosphate synthase; carbamoylphosphate is a precursor in pyrimidine biosynthesis. Differential expression of these genes may be an indirect effect of the *lsrR* mutation. Pyrimidine biosynthesis has been associated with AI-2 before [15]. Through deleting *lsrR*, uptake and processing of AI-2 molecules is increased when compared to the wild-type. Possibly, the altered balance in AI-2-derived metabolites affects other (metabolic) pathways in *S.* Typhimurium, although suggesting a direct link between LsrR/AI-2 and pyrimidine biosynthesis is not evident. As a validation of the microarray results, we confirmed STM4071, *lsrA* and *pyrI* differential expression by quantitative real-time PCR (qRT-PCR; Figure 1D and Supplementary information, Data S1). These differential expression results agree with a transcriptomics study comparing gene expression in an *lsrR* mutant and that in wild-type *E. coli* [13]: the *lsr* genes and a number of nucleotide biosynthesis genes were differentially expressed in both the *E. coli* and *S.* Typhimurium *lsrR* mutants versus the wild-type. The study in *E. coli* identified a much higher number of differentially expressed genes, but no distinction was made between the direct (LsrR-bound) and indirect target genes of LsrR [13]. Furthermore, the study in *E. coli* sampled RNA at a different time point (early stationary phase at OD_{600} 2.4 for *E. coli*, versus late exponential phase at OD_{600} 1.3 for this study). However, our *S.* Typhimurium ChIP-chip experiment after 10 h of growth (stationary phase, OD_{600} 2.8) did not show additional direct LsrR targets. Whereas the experimental conditions used in this study have been shown to be representative for studying AI-2 in *S.* Typhimurium [6], it cannot be excluded that LsrR might control additional genes under other experimental conditions. It is also plausible that AI-2 and LsrR can have different functions in *E. coli* versus *S.* Typhimurium. The *lsr* operons are different in *E. coli* and *S.* Typhimurium, for instance. As mentioned above, the STM4071 gene encoded downstream of *lsrK* and transcribed in the same direction has no homologue in *E. coli*, as is the case for *lsrE* located downstream of *lsrG* in *S.* Typhimurium.

In conclusion, by performing ChIP-chip and transcriptomics to delineate the AI-2-dependent LsrR regulon in *S.* Typhimurium, we show that LsrR controls *lsr* gene expression by binding the *lsr* promoter region *in vivo*. The absence of other LsrR-bound target genes shows that LsrR, under experimental conditions that have been shown to be relevant for the AI-2 phenotype, is a specialized regulator with a limited regulon in *S.* Typhimurium. This may limit the suitability of LsrR as a target to combat *S.* Typhimurium infections, although the function of LsrR as a regulator of AI-2 uptake may have broader indirect implications. Based on these results, we therefore suggest that research focusing on the AI-2 molecule itself – including its breakdown products – is highly relevant to unravel the role and impact of putative AI-2 signaling in *S.* Typhimurium.

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Inge M Thijs[1], Hui Zhao[1], Ami De Weerdt[1], Kristof Engelen[1], David De Coster[1], Geert Schoofs[1], Michael McClelland[2], Jos Vanderleyden[1], Kathleen Marchal[1], Sigrid CJ De Keersmaecker[1]

[1]Centre of Microbial and Plant Genetics, KU Leuven, Kasteelpark Arenberg 20, 3001 Leuven, Belgium; [2]Vaccine Research Institute of San Diego, 10835 Road to the Cure, San Diego, CA 92121, USA

Correspondence: Sigrid CJ De Keersmaecker
Tel: +32-16-321631; Fax: +32-16-321966
E-mail: sigrid.dekeersmaecker@biw.kuleuven.be

References

1 Byrd CM, Bentley WE. Quieting cross talk - the quorum sensing regulator LsrR as a possible target for fighting bacterial infections. *Cell Res* 2009; **19**:1229-1230.
2 Surette MG, Miller MB, Bassler BL. Quorum sensing in *Escherichia coli*, *Salmonella typhimurium*, and *Vibrio harveyi*: a new family of genes responsible for autoinducer production. *Proc Natl Acad Sci USA* 1999; **96**:1639-1644.
3 Vendeville A, Winzer K, Heurlier K, Tang CM, Hardie KR. Making 'sense' of metabolism: autoinducer-2, LuxS and pathogenic bacteria. *Nat Rev Microbiol* 2005; **3**:383-396.
4 Winzer K, Hardie KR, Burgess N, *et al*. LuxS: its role in central metabolism and the *in vitro* synthesis of 4-hydroxy-5-methyl-3(2H)-furanone. *Microbiology* 2002; **148**:909-922.
5 De Keersmaecker SC, Sonck K, Vanderleyden J. Let LuxS speak up in AI-2 signaling. *Trends Microbiol* 2006; **14**:114-119.
6 Taga ME, Semmelhack JL, Bassler BL. The LuxS-dependent autoinducer AI-2 controls the expression of an ABC transporter that functions in AI-2 uptake in *Salmonella typhimurium*. *Mol Microbiol* 2001; **42**:777-793.
7 Miller ST, Xavier KB, Campagna SR, *et al. Salmonella typhimurium* recognizes a chemically distinct form of the bacterial quorum-sensing signal AI-2. *Mol Cell* 2004; **15**:677-687.
8 Xavier KB, Miller ST, Lu W, *et al.* Phosphorylation and processing of the quorum-sensing molecule autoinducer-2 in enteric bacteria. *ACS Chem Biol* 2007; **20**:128-136.
9 Diaz Z, Xavier KB, Miller ST. The crystal structure of the *Escherichia coli* autoinducer-2 processing protein LsrF. *PLoS ONE* 2009; **4**:e6820.
10 Xue T, Zhao L, Sun H, Zhou X, Sun B. LsrR-binding site recognition and regulatory characteristics in *Escherichia coli* AI-2 quorum sensing. *Cell Res* 2009; **19**:1258-1268.
11 Surette MG, Bassler BL. Regulation of autoinducer production in *Salmonella typhimurium*. *Mol Microbiol* 1999; **31**:585-595.
12 Perrett CA, Karavolos MH, Humphrey S, *et al.* LuxS-based quorum sensing does not affect the ability of *Salmonella enterica* serovar Typhimurium to express the SPI-1 type 3 secretion system, induce membrane ruffles, or invade epithelial cells. *J Bacteriol* 2009; **191**:7253-7259.
13 Li J, Attila C, Wang L, *et al.* Quorum sensing in *Escherichia coli* is signaled by AI-2/LsrR: effects on small RNA and biofilm architecture. *J Bacteriol* 2007; **189**:6011-6020.
14 Thijs IM, De Keersmaecker SC, Fadda A, *et al.* Delineation of the *Salmonella enterica* serovar Typhimurium HilA regulon through genome-wide location and transcript analysis. *J Bacteriol* 2007; **189**:4587-4596.
15 Lee J, Zhang XS, Hegde M, *et al.* Indole cell signaling occurs primarily at low temperatures in *Escherichia coli. ISME J* 2008; **2**:1007-1023.
16 McClelland M, Sanderson KE, Spieth J, *et al.* Complete genome sequence of *Salmonella enterica* serovar Typhimurium LT2. *Nature* 2001; **413**:852-856.

(**Supplementary information** is linked to the online version of the paper on *Cell Research* website.)