Expression profiling of a *spf* deletion mutant suggests biological roles and mRNA targets for Spot 42 in the fish pathogen *Aliivibrio salmonicida*

Geir Åsmund Hansen[1], Rafi Ahmad[1,2], Erik Hjerde[1], Christopher G. Fenton[3], Nils-Peder Willassen[1,2], and Peik Haugen[1,2] *

[1]Department of chemistry, Faculty of science and technology, University of Tromsø, N-9037, Tromsø, Norway.
[2]The Norwegian Structural Biology Centre, University of Tromsø, N-9037, Tromsø, Norway.
[3]Institute of Clinical Medicine, University of Tromsø, N-9037 Tromsø, Norway.

* Corresponding author

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Abbreviations: nc, non-coding; IGR, intergenic region; RACE, rapid amplification of cDNA ends; UTR, untranslated region; PDH, pyruvate dehydrogenase; HHL, hexanoyl-L-homoserine lactone; OHHL, N-3-oxo-hexanoyl-L-homoserine lactone

Abstract

Spot 42 was discovered in *Escherichia coli* nearly 40 years ago as an abundant, small, and unstable RNA. Its biological role has remained obscure until recently, and it is today implicated in having broader roles in the central and secondary metabolism. Spot 42 is encoded by the *spf* gene. The gene is ubiquitous in the *Vibrionaceae* family of gamma-proteobacteria. One member of this family, *Aliivibrio salmonicida*, causes cold-water vibriosis in farmed Atlantic salmon. Its genome encodes Spot 42 with 84 % identity to *E. coli* Spot 42 (*spf*). We generated a *A. salmonicida spf* deletion mutant. We then used microarray and Northern blot analyses to monitor global effects on the transcriptome in order to provide insights into the biological roles of Spot 42 in this bacterium. In the presence of glucose, we found a surprisingly large number of ≥2× differentially expressed genes, and several major cellular processes were affected. A gene encoding a pirin-like protein showed an on/off expression pattern in the presence/absence of Spot 42, which suggests that Spot 42 plays a key regulatory role in the central metabolism by regulating the switch between fermentation and respiration. Interestingly, in a global search, we discovered an sRNA, named VSsrna24, which is encoded immediately downstream of *spf*. This new sRNA has an expression pattern opposite to that of Spot 42, and its expression is highly dependent on glucose. Our hypothesis is that this novel sRNA works in concert with Spot 42 to regulate carbohydrate metabolism and uptake.

Introduction

Bacteria contain a class of regulatory non-coding (nc) RNAs that are transcribed in *trans* from distinct promoters.[1] They are typically between 50 and 200 nt in size[2] and have become known as bacterial small RNAs, or sRNAs. The majority of known sRNA genes are located in intergenic regions, but it is becoming increasingly evident that a relatively large number of RNAs are also being transcribed from the opposite strand of protein coding regions (i.e., anti-sense RNAs). Even though their roles are still mostly unknown, it is likely that anti-sense RNAs also play important roles in gene regulation. *cis*-encoded RNA regulators are typically located in front of protein coding regions as part of the mRNA and change the expression of the corresponding protein by binding small metabolites (i.e., riboswitches).[3]

sRNAs typically bind to the 5' end of mRNAs through short imperfect base-paring and induce degradation of itself and the target.[1] Other mechanisms, like direct interaction with proteins to modulate their activities or increased stability of mRNAs, also occur. Finding the function and/or mechanism of sRNAs can, however, be a daunting task, and the Spot 42 sRNA represents a striking example. It was first described as an unstable RNA species of 109 nt in *Escherichia coli* that accumulated under growth in the presence of glucose (i.e., when cAMP is low).[4] During growth with a non-glucose carbon source (i.e., when cAMP concentrations are high), the Spot 42 concentrations were found to be significantly lower. Later experiments showed that over-expression of Spot 42 (~10 fold increase) resulted in impaired growth and lowered ability to adapt to shifts to richer media or shift from glucose to succinate as the carbon source.[5] Also, deletion of the gene that encodes Spot 42, i.e., *spf* (spot fourty-two) resulted in viable *spf* null mutants, which indicated that the Spot 42 RNA is non-essential.[6] The direct responsiveness of Spot 42 levels to glucose and cAMP is due to repression of *spf* by a cAMP-CRP (cAMP-receptor protein) complex.[7, 8] It was unclear for some years if the function of Spot 42 was mediated through the 109 nt RNA itself or through a 14 amino acids long peptide that is hypothetically encoded from within the sRNA. This controversy was settled by Rice et al.[9] They used a filter binding assay and other methods to show that Spot 42 bound very inefficiently and nonproductive to purified 70S ribosomes and

concluded that Spot 42 function is mediated by the RNA itself. Later, the proximity of *spf* to *polA* lead Dahlberg and co-workers to test whether the products of these genes could influence each other,[7, 8] and they found that reduction in Spot 42 levels, either by deletion of *spf* or by manipulating the growth conditions, both resulted in reduction in DNA pol A activity. The underlying mechanism remains, however, unknown. The first direct Spot 42 target was discovered by Møller et al.[10], who showed that the sRNA can bind specifically by base pairing with a short complementary region of the translation initiation region of *galK*, which is the third gene of the galactose operon (*galETKM*). The Spot 42-binding region overlaps with the *galK* Shine-Dalgarno region, thereby blocking ribosome binding. Spot 42 is therefore responsible for a discoordinate regulation of the *gal* operon. Finally, in a recent work, Beisel and Storz[11] demonstrated with microarray analysis and reporter fusions that Spot 42 plays a broader role in metabolism by regulating at least 14 operons that are dominated by genes involved in uptake and catabolism of non-favored carbon sources. Several of these operons are regulated by both Spot 42 and CRP, and these two regulators can therefore by considered as participating in a feed-forward loop.

Here, we used our model bacteria *Aliivibrio salmonicida*, which belongs to the *Vibrionaceae* family of gamma-proteobacteria, to further address the biological roles of Spot 42. We generated an *A. salmonicida spf* deletion mutant and used microarray and Northern blot analyses to find more clues to its function. Deletion of *spf* has a surprisingly large effect on the transcriptome, with the most dramatic effect on expression of a pirin-like protein gene. In the process, we also discovered a neighboring sRNA, named VSsrna24, which is encoded from the same intergenic region (IGR) and 262 nt downstream of *spf*. Interestingly, this RNA is expressed in a pattern opposite to that of Spot 42, and its expression is highly dependent of glucose.

Results and Discussion

***spf* is highly conserved in *Vibrionaceae* and is located upstream of a previously unrecognized sRNA gene named *VSsrna24*.** Figure 1A shows the genomic location of *spf* and its flanking genes in *A. salmonicida* and *E. coli*. In both genomes, *spf* is flanked by *polA* as the nearest upstream neighbor and *yihA* (or the *engB* homolog) as the nearest downstream protein coding gene. The genomic locus is also home to other important sRNA genes. In *A. salmonicida*, RyhB is encoded from the neighboring and upstream IGR.[12] Interestingly, *spf* is also neighbor to a putative sRNA gene (*VSsRNA24*), which is located in the same IGR as *spf*. In *E. coli*, the IGR downstream of *spf* encodes the CsrC sRNA that interacts directly with and regulates the activity of the global post transcriptional regulator protein CsrA.[13]

We surveyed available genome sequences and found that orthologs of *spf* are also found in virtually all 76 currently available genome sequences from the family *Vibrionaceae*. Available genomes include representatives of the *Vibrio*, *Aliivibrio*, *Photobacterium,* and *Grimontia* genera. Six *V. cholerae* genomes lack a recognizable *spf* gene, but we suspect that this is due to the poor quality of these genomes (i.e., the genome sequences consist of many small unfiltered contigs) or the lack of data. Figure 1B shows an alignment of Spot 42 from selected species of *Vibrionaceae* compared to sequences from *Serratia marcescens* and *E. coli*. A 5' RACE analysis was done to map the 5' end of the *A. salmonicida* Spot 42, and the result showed that the 5' end is the same as for *E. coli* (indicated by vertical arrow in Fig. 1B). Ninety-one of the 109 nt in *E. coli* Spot 42 are invariable when compared to the selected sequences. The highly conserved nature and the wide distribution of *spf* in *Vibrionaceae* and other gamma-proteobacteria (see Rfam database), suggest that, as in *E. coli*, Spot 42 serves important cellular functions that are common to this group of bacteria, regardless if the bacterium is a pathogen or not (although conservation is not always a sign of viability). Since 2002 and until recently, *galK* was the only known target of Spot 42.[10] At the onset of this project, we therefore considered the bacterium as an ideal model for studying roles of Spot 42 other than regulation of *galK* translation. This is still valid even though other targets have been found.[11] In agreement with the lack of a *gal* operon, the bacterium is unable to utilize galactose when grown in a minimal medium optimized for *A.*

salmonicida with 44.4 mM galactose as essentially the only carbon source (Fig. 2). In other words, galactose has little or no effect on growth when compared to a control with no added sugar. In contrast, when 44.4 mM glucose is added as carbon source, the bacterium grows to high densities (i.e, typically to OD_{600nm} >7).

Next, we examined the sequence upstream of *spf* for potential transcription factor binding sites to gain insights into regulation of *spf* transcription. In *E. coli,* expression of *spf* is regulated by a cAMP-CRP complex.[7, 8] Sequences that resemble the *E. coli* CRP binding site are also found upstream of *Vibrionaceae spf* sequences (Fig. 1B). We also predicted -10 and -35 promoter regions (binds sigma 70 transcription factor) using BPROM (http://www.softberry.com). The -10 and -35 promoter regions start 13 and 37 nt upstream of Spot 42, and the distance between the -10 and -35 sites corresponds well with the optimal distance of 17 nt.[14] The putative -10 and -35 regions are moderately conserved.

Expression of Spot 42 is highly dependent on cAMP and glucose and inversely dependent on the neighboring sRNA VSsrna24. It is established that expression of *E. coli* Spot 42 is regulated by the transcription factor CRP and the intracellular levels of cAMP.[7] Expression is also highly dependent on concentrations of available glucose since cAMP adenylyl cyclase (i.e., a cAMP-producing enzyme) is inhibited as a side-effect during glucose transport into the cell. As a result, cAMP levels are low when glucose is rich and is being transported into the cell for consumption, and vice versa.

To test if *A. salmonicida* Spot 42 is expressed similar to that in *E. coli*, the bacterium was cultured in LB medium with 2.5 % NaCl added (*A. salmonicida* requires elevated salt concentrations for efficient growth) under standard lab conditions, and samples were collected throughout the growth cycle. At OD_{600} 0.4, the culture was split and cAMP was added to one half to a final concentration of 1 mM (at early growth phase cAMP levels are expected to be low due to excess amounts of glucose) (Figure 3A). Similarly, the starting culture was also split at OD_{600} 1.2, and glucose was added to one half to a final concentration of 5 mM (i.e., when glucose is expected to be low/exhausted). Cell samples were collected at various time points after the addition of cAMP or glucose to monitor the levels of Spot 42 by Northern blot analysis. Figure 3B

shows that Spot 42 is found at high levels during the early phases of growth but is gradually lost and is below our level of detection at OD_{600} 1.2. The level of Spot 42 is significantly reduced after addition of cAMP and is significantly increased after addition of glucose. These results are in agreement with results from *E. coli*, which suggest that *A. salmonicida* Spot 42 serve roles similar to that in *E. coli*, i.e., in carbohydrate metabolism.

In the same experiment, we also monitored the levels of the putative sRNA named VSsrna24, which is encoded from the same IGR as Spot 42. To our surprise, this sRNA produced a signal corresponding to an RNA of approximately 60 nt in length with an expression pattern opposite to that of Spot 42. Also, expression of VSsrna24 is highly dependent on glucose but independent of cAMP. These observations and the close proximity of VSsrna24 to Spot 42 suggested to us that VSsrna24 might also play roles in carbohydrate metabolism, perhaps in concert with Spot 42.

Finally, in a simple experiment, we subjected *A. salmonicida* to different stress conditions (i.e., low iron conditions, oxidative stress, low/high temperatures, and alcohol) or to one of two quorum sensing signal molecules (hexanoyl-L-homoserine lactone or N-3-oxo-hexanoyl-L-homoserine lactone) and monitored levels of Spot 42 and VSsrna24 by Northern blot analysis (Fig. 3C). This was done to test if expression of Spot 42 and/or VSsrna24 is dependent on external stress factors or communication molecules, which could indicate potential roles in stress response or quorum sensing, respectively. *A. salmonicida* was grown to OD_{600} 0.5 and subjected to the different treatments. Samples were then collected at various time points after treatment. Figure 3C shows that none of the stress conditions dramatically change the expression pattern, maybe except for low iron conditions (addition of 50 uM 2,2'-dipyridyl) that resulted in a moderate reduction in Spot 42 levels. In agreement with the results described above, levels of Spot 42 and VSsrna24 are high and low, respectively, at the early stages of growth, whereas the levels are reversed eight hours after treatment (i.e., during later stages of growth).

To summarize, we found that expression of *A. salmonicida* Spot 42 is highly dependent on glucose and cAMP, whereas expression of a new sRNA named VSsrna24 is dependent on glucose only. Expression of the two sRNAs is also mutually exclusive. We also found that expression of both Spot 42 and VSsrna24 is, in general, not affected by

external stress factors or by the addition of quorum sensing signals. The potential roles of VSsrna24 will be further addressed elsewhere.

Microarray analysis of a *A. salmonicida spf* deletion mutant identifies potential roles and targets. We constructed a *spf* deletion mutant (see Materials and methods for details) to identify potential biological roles and possible mRNA targets for *A. salmonicida* Spot 42. An efficient over-expression system is currently not available for *A. salmonicida* and was in this case not considered. The transcriptome of the *spf* deletion mutant was compared on a global scale to that of the wild-type strain by using microarray analysis. To create growth conditions in which Spot 42 was highly expressed in the wild-type strain, the cells were cultured in ASMM and then stimulated by adding glucose as the main carbon sources. Specifically, in three independent experiments (biological replicates), cells were grown to OD_{600} 0.4 and 44.4 mM glucose was added. Samples were collected 15 min after addition of glucose, total RNA was extracted, and the three independent samples were pooled before cDNA synthesis and hybridization to three (technical replicates) *A. salmonicida* whole genome custom DNA chips (i.e., *Vibrio salmonicida* V1.0.1 AROS). RNA from glucose treated wild-type and *spf* deletion cells were labeled with fluorescent dyes and run on same DNA chips.

Using the LIMMA framework in Bioconductor[15], we considered genes with ≥ 2 fold differential expression and with adjusted p-values ≤0.05. Figure 4 shows a graphical presentation of the functional classes and the number of differentially up- or down-regulated genes in the *spf* deletion mutant 15 min after addition of glucose. Details on some differentially regulated genes are shown in **Tables 1** and **2** (**Supplementary Table 1** contains complete lists of differentially expressed genes). Major findings are (i) that the deletion of *spf* is significantly affecting the expression of a relatively large number of genes (e.g., 385 genes are ≥ 2 fold differentially expressed after addition of glucose) that belong to a wide range of categories, such as “foreign DNA”, “carbohydrate metabolism”, “cell envelope”, “transport proteins”, “motility”, “iron homeostasis”, and “quorum sensing”. Spot 42 therefore has a broad effect on multiple cellular functions. Second, *fruBK* are both on the top 30 list of up-regulated genes and *fruA* is also significantly up-regulated (2.3 fold up-regulated). FruAB constitute the fructose-specific

EII-component of the PTS system. Therefore, the upregulation of *fruABK* supports a similar role for *A. salmonicida* Spot 42 as in *E. coli*, where uptake of less favorable sugars is suppressed by Spot 42 (carbon catabolite repression). Third, a gene encoding a pirin protein is by far the most up-regulated gene (**Table 1**). The $\log_2$ ratio between *spf* deletion mutant and wild- type is 3.97 (equivalent to ~16 fold increase), whereas the same value for the second most up-regulated gene, i.e., VSAL_I2193 that encodes a methyl-accepting chemotaxis protein, is 2.92 (equivalent to ~8 fold increase). The pirin protein (theoretically 283 aa) has 21 and 24 percent identity and 45 and 49 percent similarity to the *E. coli* (231 aa) and *S. marcescens* (313 aa) pirin proteins, respectively, when all positions in the protein sequences are considered (approx. 45 percent identity in conserved regions). Finally, the top 30 list of most up-regulated genes is enriched in genes involved in motility and chemotaxis [*cheV*, *flaA* (subunit E), *flaA* (subunit C), *flaD*, *flaF*, *flgB*, *flgF*, *flgM*, *fliC*, *motX*, VSAL_I0799 and VSAL_I2193] and carbohydrate transport/metabolism [pirin (VSAL_I1200), *fruB*, *fruK*, and a gluconate permease (VSAL_I2593)], whereas the equivalent list of down-regulated genes is dominated by foreign DNA (phage and plasmid DNA) and genes encoding ncRNAs, including a number of predicted sRNA genes. Upregulation of genes with functions in motility and chemotaxis can be explained by that the flagellar biosynthesis sigma factor (sigma F or 28), encoded by *fliA* (VSAL_I2290), the spoIIAA anti-sigma F factor antagonist (VSAL_II0328), and, finally, the anti-sigma 28 factor (VSAL_I2342) are the most up-regulated genes (i.e., 1.7, 1.6, and 1.6 fold, respectively) of all sigma factor related genes in the complete dataset. Flagella-related genes are known from other bacteria to be regulated by the sigma 28 factor (or its orthologs, e.g., sigma F) (see Koo et al 2009). The up-regulated motility and chemotaxis genes are virtually all located in the same gene cluster (approx. VSAL_I2283 to VSAL_I2343).

In summary, our microarray analysis shows that deletion of *spf* has a broad effect on many genes and cellular functions. Interestingly, the most up-regulated gene encodes a putative pirin protein. In *S. marcescens*, pirin has been found to regulate the activity of pyruvat dehydrogenase E1.[16] Pirin is therefore a key regulator in the central metabolism by selecting the destiny of pyruvat, either through fermentation or by respiration through the TCA cycle and electron transport. Given that *A. salmonicida* Spot 42 directly or

indirectly regulates pirin, then Spot 42 can serve as a critical regulatory role in the central metabolism.

Upregulation of pirin and repression of multiple sRNAs are supported by Northern blot analysis. Inspired by findings from the microarray analysis, we next used Northern blot analysis to evaluate results of particular interest. First, we wanted to validate levels of Spot 42 and therefore generated a corresponding [α-^{32}P]-labeled DNA probe. Figure 5A shows the resulting autoradiogram, which confirms that Spot 42 is rich in wild type cells in the presence of glucose and that it is absent in *spf* deletion mutant cells. As expected, Spot 42 is virtually absent in wild type cells grown without any added glucose.

Our microarray data suggested that many predicted sRNAs are significantly down regulated in the *spf* deletion mutant. This observation was tested by designing probes against the neighboring and new VSsrna24 and the RyhB and Qrr sRNAs that are critical components in regulation of iron homeostasis and quorum sensing, respectively. These sRNAs were found to be down regulated approx. 15, 11, and 2 folds, which is in good agreement with the corresponding numbers (approx. 17, 10, and 4 folds, respectively) from the microarray analysis. The underlying mechanism for the apparent reduction in sRNA levels is, however, unclear to us, especially since the levels of Hfq and RNaseE mRNAs are virtually unchanged. Reduction in Hfq and/or upregulation of RNaseE could potentially destabilize sRNAs in general.

We were intrigued by the dramatic effect of *spf* deletion on levels of a putative pirin-encoding mRNA. In agreement with the microarray result, a pirin probe produced no visible band when using total RNA from wild type cells, whereas total RNA from *spf* deletion mutant cell treated with glucose produced a distinct and readily visible band of the expected size. The increase in intensity corresponds to a fold change value of approx. 37 folds (approx. 16 folds in microarray analysis). We next used the RNAhybrid software[17] to test for potential interactions between Spot 42 and the 5' region of the pirin mRNA. This software calculates the most energetically favorable base pairing between a small RNA and a larger target RNA.[17] Figure 6 shows that when using *A. salmonicida* Spot 42 (excluding the terminator) and a sequence extending from -100 nt to +50 relative to the pirin start codon as input sequences to the program, then significant potential for

base-pairing between Spot 42 and the 5' untranslated region (UTR) of the pirin mRNA can be identified. Given that Spot 42 binds directly to this region, then this would be similar to the most common form of sRNA-mRNA interaction, i.e., base pairing within the 5' UTR region.[1] Further analysis to address potential interactions between Spot 42 and pirin mRNA will come from biochemical methods, such as gel mobility shift assay, fusion-reporter assays, structure probing, and site directed mutagenesis.

Finally, we also wanted to test mRNA levels of *aceE*, which encodes enzyme 1 of the pyruvate dehydrogenase (PDH) complex. This was done since the activity of enzyme 1 is regulated by pirin in *S. marcescens*. Northern blot analysis resulted in multiple bands, which is expected since enzyme 1 is encoded from within a multi-gene operon, and it is known from *E. coli* that this operon is expressed from one internal and two major promoters.[18] No differential expression between wild-type and *spf* deletion mutant was detected. This is in agreement with findings from *S. marcescens* where pirin was found to regulate the activity of enzyme 1 at the protein level .[16] Pirin can therefore be regarded as a key regulator for selecting the destiny of pyruvat, either through fermentation or respiration.

Concluding remarks. We have studied potential biological roles of a *A. salmonicida* Spot 42 homolog and discovered a neighboring sRNA, named VSsrna24. The expression of Spot 42 is similar to that in *E. coli*, i.e., expression is highly dependent on the concentration of glucose and cAMP. Expression of VSsrna24 is, in contrast, dependent on glucose only and is apparently opposite to that of Spot 42. A microarray analysis revealed that deletion of *spf* affects a wide range of cellular processes and the expression of a surprisingly large number of genes. After addition of glucose at mid exponential growth phase (i.e., when Spot 42 is highly expressed in the wild type strain), the most differentially up-regulated gene in the *spf* deletion mutant was VSAL_I1200, which encodes a pirin protein with 21 percent identity and 45 percent similarity to the *E. coli* pirin. Other notable results are upregulation of many genes involved in motility and chemotaxis and down regulation of predicted sRNA genes and foreign DNAs. Expression of Spot 42, Vssrna24, Qrr, RyhB, pirin, and *aceE* were validated by Northern blot analysis.

The majority of bacterial regulatory RNAs, like sRNAs, probably remain to be identified. Eighty-seven sRNAs are known in *E. coli*,[2] whereas the corresponding number is much lower for other bacteria. Once identified, finding the function and targets of each sRNA represents a formidable task. Therefore, the current knowledge on sRNA function is in general limited. And although one or a few targets have been identified for a given sRNA, it is likely that many additional targets remain to be found. For example, in *E. coli*, the transcript of the multi-cistronic *gal* operon was for many years the only known Spot 42 target,[10] but a recent microarray analysis in which over-expression of *spf* with an IPTG-inducible promoter was used, suggests many additional targets.[11] The list of targets is enriched in genes involved in sugar transport (*dppB*, *IldP*, *nanC*, *nanT*, *srlA*, and *xylF*) or sugar catabolism (*ebgC*, *fucI*, *fucK*, and *galK*). We have used a knock-out approach to find overexpressed genes that could be potential Spot 42 targets in *A. salmonicida*. Interestingly, the majority of genes that corresponds to mRNA targets in *E. coli* are apparently not present (i.e., they produce no significant hits in BLASTP searches) in the *A. salmonicida* genome (i.e., *IldP*, *nanC*, *srlA*, *ebgC*, *fucI*, *galK*, and *gsp*). Also, apparent homologs of the remaining *E. coli* targets were in general not significantly differentially expressed in our dataset. In summary, these results suggest that there are significant differences in Spot 42 regulation between *E. coli* and *A. salmonicida*.

Perhaps our most intriguing finding is that *A. salmonicida* Spot 42 appears to directly or indirectly regulate the levels of pirin mRNA. This is in agreement with its currently known role in carbohydrate metabolism and that the level of Spot 42 is highly dependent on glucose and cAMP. We are also pursuing potential roles of the neighboring sRNA, named Vssrna24. Its expression is also highly dependent on glucose, and we suspect that this sRNA also could have important roles in carbohydrate metabolism, perhaps in concert with Spot 42.

Materials and Methods

Bacterial strains and growth. *A. salmonicida* LFI1238 and the corresponding *spf* deletion mutant were cultured in LB medium with 2.5 % NaCl added or in *A. salmonicida*-specific minimal media (ASMM) at 12-16 °C in a shaking incubator at 200-230 rpm. The minimal medium contains the following compounds in mM: NaCl, 256.7; KCl, 9.4; $MgCl_2 \cdot 6H_2O$, 50.075, $FeCl_3 \cdot 6H_2O$, 0.0126; NH_4Cl, 18.7, K_2HPO_4, 0.58, Trizma base, 41.3; CaCl2 $\cdot$ $2H_2O$, 0.225; $ZnCl_2$, 0.0085, $CoCl_2 \cdot 6H_2O$, 0.0055, $CuSO_4 \cdot 5\ H_2O$, 0.005; Na_2MoO_4, 0.007, $MnCl_4 \cdot 2H_2O$, 0.011, cycteine, 0.5; isoleucine, 0.5; methionine, 0.5; valine, 2.0; glutamic acid, 40; glucose or galactose (added when indicated), 44.4 mM.

A. salmonicida was subjected to multiple treatments by first growing the bacterium in LB medium with 2.5 % at 16 °C to mid-exponential growth phase (OD_{600} ~0.6). Cultures were then divided into nine equally sized cultures, one for each stimulation, i.e., 50 μM 2,2'-dipyridyl iron chelator, 1 mM hydrogen peroxide, 500 μM paraquat (N,N'-dimethyl-4,4'-bipyridinium dichloride), 0.5 % ethanol, 2 μg/ml hexanoyl-L-homoserine lactone (HHL), 2 μg/ml N-3-oxo-hexanoyl-L-homoserine lactone (OHHL), 4 °C or 20 °C growth temperatures, and one non-stimulated control culture. Ten ml samples were collected 0, 2, 5, 10, and 30 min after stimulation. Cells were harvested by centrifugation at 3,500 × g for 10 min. Pelleted cells were then snap-frozen in a combination of dry-ice and ethanol and kept at -70 °C prior to RNA isolation.

For cAMP and glucose stimulations, *A. salmonicida* was cultured in LB medium at 12 °C and split two times during growth. At OD_{600} 0.4, the culture was split and 1 mM cAMP was added to one half and grown under the same conditions as the untreated control for 240 min. Ten ml samples were collected 5, 30, and 240 min after stimulation. Similarly, at OD_{600} 1.2, the untreated culture was again split and one half was stimulated with 5 mM D(+)-glucose and grown under the same conditions as the untreated control culture for 360 min. Ten ml samples were collected 5, 30, and 360 min after stimulation.

Samples used for Northern blot and microarray analyses were prepared by culturing the *A. salmonicida* wild type and a *spf* deletion mutant strains at 12 °C in

ASMM without any added carbon source to OD_{600} 0.4. At this density, the culture was split into two halves. Glucose to 44.4 mM was added to one half and the other was used as the untreated control. A 15 ml sample was collected 15 min after stimulation and harvested as described above.

Construction of *Spf* knock-out. The construction of the *Spf* knock-out by in frame deletion (allelic exchange) was performed essentially as previously described.[19] A suicide vector (pDM4) containing the regions flanking the *spf* gene was used. The flanking regions were joined by overlap PCR, i.e., two PCR products containing the flanking regions with overlapping ends are extended and then used as template with the outermost primers from the first two PCRs. The final PCR product was cloned into the pDM4 plasmid and introduced into *A. salmonicida* by conjugal mating with the *E. coli* S17-1 λ pir donor strain. Transconjugants were selected for by plating the conjugation mix on LB plates containing 2 μg/ml chloramphenicol at 12 °C. *E. coli* grows very poorly at 12 °C and was therefore selected against. Colonies were next transferred to LB plates with 5 % sucrose to induce expression of the *sacB* suicide gene. The product is lethal to gram-negative bacteria.[20] This step selects against the plasmid, and integrated plasmids (they are most likely to have integrated into chromosomal locations similar to those that were introduced into the plasmid) must therefore be removed from chromosomal positions in a recombination event in order for the bacterium to survive. Resulting chloramphenicol-sensitive colonies were analyzed by PCR to screen for bacteria with the desired chromosomal deletion. PCR products of expected sizes were sequenced to confirm the deletion.

5` rapid amplification of cDNA ends (RACE) for mapping of the 5` end. 5` RACE assays were carried out essentially as previously described[21] using the GeneRacer kit (Invitrogen), 6 μg of total RNA, and a Spot 42-specific oligonucleotide (5'-GCCAAATCCAATAACGTGAAAC-3').

Microarray analyses. Total RNA was extracted from cells using the RNAisol reagent (5 PRIME) followed by a subsequent DNA removing step with DNAfree kit (Applied Biosystems). Salts and leftover traces of DNA were removed by RNeasy Minelute Cleanup kit (Qiagen). Quality of extracted RNA was examined by NanoDrop, and presence of RNase or DNase activity was checked by incubating the RNA with 500 μl

plasmid DNA for 1 h at 37 °C, followed by inspection of degradation on an agarose gel. cDNA was constructed from 15 μg purified RNA using Aminoallyl cDNA labeling kit (Applied Biosystems) and CyDye™ Post-Labeling Reactive Dye Pack (GE Healthcare) for labelling. The labelled samples were hybridized to "*Vibrio salmonicida* V1.0.1 AROS" slides at 42 °C on a TECAN HS4800 hybridisation station. Following the hybridizations, the slides were washed once in 0.1×SSC/0.1 % SDS for 5 min at 42 ºC, once in 0.1×SSC/0.1 % SDS for 10 min at room temperature, and finally four times in 0.1×SSC for 1 min at room temperature. The slides were run in triplets, including one dye swap per triplet. Slides were scanned using a GenePix 4000B scanner (Axon Instruments Inc.) and GenePix Pro v6.1 software. The expression data were analysed using the LIMMA framework in Bioconductor (http://www.bioconductor.org). Microarray data is available at the NCBI Gene Expression Omnibus (GEO) database, with GSE28087 as accession number.

Northern blot analyses. Total RNA was isolated from bacterial cultures using the TRIzol RNA isolation procedure (Invitrogen) and quantified by spectrophotometric methods. Northern blot analysis was done as previously described.[12] Briefly, 10 μg total RNA was separated on 1.2 % formamide agarose gels or 8 M urea/5 % polyacrylamide gels and then transferred to a nylon membrane by upward capillary transfer or electroblotting, respectively. RNA species were detected on membranes using [α-^{32}P]dCTP-labeled double-stranded DNA probes (PCR products), and signals were collected on phosphoimaging screens (Fujifilm) and scanned on a BAS-5000 phosphoimager (Fujifilm). The ImageGauge software v4.0 (Fujifilm) was used to measure the strength of signals and the 5S ribosomal RNA was used to normalize the resulting values.

Promoter and TFBS prediction. The region immediately upstream of *spf* was screened for CRP binding sites using the *E. coli* consensus sequence (5'-AATTGTGATCTAGATCACATTT-3')[22] and the pattern search algorithm Fuzznuc (part of EMBOSS software analysis package)[23]. A maximum of 12 mismatches of the 22-bp consensus were allowed. The CRP-binding site (5'-TTTTGTGATGGCTATTAGAAAT-3') upstream of the *E. coli spf* gene[7, 8] has 10 mismatches to this consensus. In addition, the MEME[24] and Consensus[25] software tools were used to search for any additional

conserved sequence motif in the promoter region of the vibrioVSsrna24 sequences. The BPROM program (from www.softberry.com) was used to predict -10 and -35 promoter sequences.

Computational prediction of Spot42-pirin mRNA base pairing. The program RNAhybrid[17] was used to identify potential base-pair interactions between Spot 42 and the 5' UTR region of the pirin mRNA (VSAL_I1200). The search was restricted to mRNA sequences located 100 nt upstream and 50 nt downstream of the start codon and was performed using default program settings.

Acknowledgements

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References

1. Waters LS, Storz G. Regulatory RNAs in bacteria. Cell 2009; 136:615-28.
2. Huang HY, Chang HY, Chou CH, Tseng CP, Ho SY, Yang CD, et al. sRNAMap: genomic maps for small non-coding RNAs, their regulators and their targets in microbial genomes. Nucleic Acids Res 2009; 37:D150-4.
3. Roth A, Breaker RR. The structural and functional diversity of metabolite-binding riboswitches. Annu Rev Biochem 2009; 78:305-34.
4. Sahagan BG, Dahlberg JE. A small, unstable RNA molecule of Escherichia coli: spot 42 RNA. I. Nucleotide sequence analysis. J Mol Biol 1979; 131:573-92.
5. Rice PW, Dahlberg JE. A gene between polA and glnA retards growth of Escherichia coli when present in multiple copies: physiological effects of the gene for spot 42 RNA. J Bacteriol 1982; 152:1196-210.
6. Hatfull GF, Joyce CM. Deletion of the spf (spot 42 RNA) gene of Escherichia coli. J Bacteriol 1986; 166:746-50.
7. Polayes DA, Rice PW, Garner MM, Dahlberg JE. Cyclic AMP-cyclic AMP receptor protein as a repressor of transcription of the spf gene of Escherichia coli. J Bacteriol 1988; 170:3110-4.
8. Polayes DA, Rice PW, Dahlberg JE. DNA polymerase I activity in Escherichia coli is influenced by spot 42 RNA. J Bacteriol 1988; 170:2083-8.
9. Rice PW, Polayes DA, Dahlberg JE. Spot 42 RNA of Escherichia coli is not an mRNA. J Bacteriol 1987; 169:3850-2.

10. Møller T, Franch T, Udesen C, Gerdes K, Valentin-Hansen P. Spot 42 RNA mediates discoordinate expression of the E. coli galactose operon. Genes Dev 2002; 16:1696-706.
11. Beisel CL, Storz G. The base-pairing RNA spot 42 participates in a multioutput feedforward loop to help enact catabolite repression in Escherichia coli. Mol Cell 2011; 41:286-97.
12. Ahmad R, Hjerde E, Hansen GA, Haugen P, Willassen NP. Prediction and experimental testing of ferric uptake regulator regulons in vibrios. J Mol Microbiol Biotechnol 2009; 16:159-68.
13. Timmermans J, Van Melderen L. Post-transcriptional global regulation by CsrA in bacteria. Cell Mol Life Sci 2010; 67:2897-908.
14. Sinoquet C, Demey S, Braun F. Large-scale computational and statistical analyses of high transcription potentialities in 32 prokaryotic genomes. Nucleic Acids Res 2008; 36:3332-40.
15. Smyth GK. Limma: linear models for microarray data. In: Gentleman R, Carey V, Dudoit S, Irizarry R, Huber W, eds. Bioinformatics and Computational Biology Solutions using R and Bioconductor. New York: Springer, 2005.
16. Soo PC, Horng YT, Lai MJ, Wei JR, Hsieh SC, Chang YL, et al. Pirin regulates pyruvate catabolism by interacting with the pyruvate dehydrogenase E1 subunit and modulating pyruvate dehydrogenase activity. J Bacteriol 2007; 189:109-18.
17. Kruger J, Rehmsmeier M. RNAhybrid: microRNA target prediction easy, fast and flexible. Nucleic Acids Res 2006; 34:W451-4.
18. Quail MA, Haydon DJ, Guest JR. The pdhR-aceEF-lpd operon of Escherichia coli expresses the pyruvate dehydrogenase complex. Mol Microbiol 1994; 12:95-104.
19. Milton DL, O'Toole R, Horstedt P, Wolf-Watz H. Flagellin A is essential for the virulence of Vibrio anguillarum. J Bacteriol 1996; 178:1310-9.
20. Gay P, Le Coq D, Steinmetz M, Berkelman T, Kado CI. Positive selection procedure for entrapment of insertion sequence elements in gram-negative bacteria. J Bacteriol 1985; 164:918-21.
21. Wagner EG, Vogel J. Handbook of RNA Biochemistry. WILEY-VCH, Verlag Gmbh & Co., 2005.

22. Zhang Z, Gosset G, Barabote R, Gonzalez CS, Cuevas WA, Saier MH, Jr. Functional interactions between the carbon and iron utilization regulators, Crp and Fur, in Escherichia coli. J Bacteriol 2005; 187:980-90.
23. Rice P, Longden I, Bleasby A. EMBOSS: the European Molecular Biology Open Software Suite. Trends Genet 2000; 16:276-7.
24. Bailey TL, Elkan C. Fitting a mixture model by expectation maximization to discover motifs in biopolymers. Proc Int Conf Intell Syst Mol Biol 1994; 2:28-36.
25. Hertz GZ, Stormo GD. Identifying DNA and protein patterns with statistically significant alignments of multiple sequences. Bioinformatics 1999; 15:563-77.

Tables

Table 1. Top 30 list of ≥ 2 fold up-regulated genes in *spf* deletion mutant after addition of 44.4 mM glucose.

CDS	Gene	Gene product	Log_2 ratio	Adjusted P-value
VSAL_I1200		putative pirin	3.97	2.49E-04
VSAL_I2193		methyl-accepting chemotaxis protein	2.92	2.97E-04
VSAL_I4139s		VSsrna140 undefined small RNA	2.53	4.98E-04
VSAL_I0799		methyl-accepting chemotaxis protein	2.53	4.02E-04
VSAL_I2318	*flaD*	flagellin subunit D	2.48	4.35E-04
VSAL_II0716		putative exported protein	2.39	3.54E-03
VSAL_I2317	*flaA*	flagellin subunit E	2.33	4.98E-04
VSAL_II0613		putative membrane protein	2.17	8.69E-04
VSAL_I1201		putative IMP dehydrogenase/GMP reductase	2.13	4.44E-04
VSAL_I2319	*flaA*	flagellin subunit C	2.09	5.58E-04
VSAL_I2517	*flaF*	flagellin subunit F	2.07	6.11E-04
VSAL_I2329	*flgM*	flagellar hook-associated protein type 3 FlgM	2.03	4.98E-04
VSAL_I2022	*vcmH*	multidrug efflux pump	1.87	1.20E-03
VSAL_II0587		outer membrane protein, OmpA family	1.87	6.40E-04
VSAL_I2593		gluconate permease	1.81	6.74E-04
VSAL_II0091	*fruK*	1-phosphofructokinase	1.81	9.06E-04

VSAL_II0090	*fruB*	PTS system, fructose-specific IIA/FPR component	1.80	6.11E-04
VSAL_I2578		ABC-type [(GlcNAc)2] transporter, permease protein	1.79	6.11E-04
VSAL_I1857		hypothetical protein	1.78	6.74E-04
VSAL_II0715	*cusB*	putative cation efflux system protein	1.77	8.23E-04
VSAL_I2338	*flgB*	flagellar basal-body rod protein FlgB	1.75	5.13E-04
VSAL_II0231	*cheV*	chemotaxis protein CheV	1.74	8.03E-04
VSAL_I2771	*motX*	sodium-type polar flagellar protein MotX	1.71	7.15E-04
VSAL_II0331		putative exported protein	1.70	6.14E-04
VSAL_I2334	*flgF*	flagellar basal-body rod protein FlgF	1.70	8.55E-04
VSAL_II1080		membrane protein	1.70	7.00E-04
VSAL_I0474	*mshF*	Type IV pilus, mannose-sensitive hemagglutinin protein MshF	1.68	6.11E-04
VSAL_I1401	*tupA*	extracellular tungstate binding protein precursor	1.66	6.86E-04
VSAL_I2327	*fliC*	flagellin subunit A	1.62	7.00E-04
VSAL_II0785		putative exported protein	1.59	6.11E-04
VSAL_I2061		hypothetical protein	1.59	6.11E-04

Table 2. Top 30 list of ≥ 2 fold down-regulated genes in *spf* deletion mutant after addition of 44.4 mM glucose.

Gene CDS	Gene product	Log_2 ratio	Adjusted P-value
VSAL_II0612	HTH-type transcriptional regulator, LysR family (pseudogene)	-5.71	1.24E-04
VSAL_I0768	hypothetical protein, putative phage gene	-5.62	1.24E-04
VSAL_I3103s	VSsrna23 small RNA Spot 42	-5.60	1.24E-03
VSAL_I3178s	VSsrna149 tmRNA	-5.19	1.24E-04
VSAL_I1028	gpN major capsid protein	-5.17	1.46E-04
VSAL_p320_13	putative peptidase, S24-like	-5.16	2.49E-04
VSAL_II2002s	VSAsrna3 undefined small RNA	-5.12	1.46E-04
VSAL_I0985	MrdA penicillin-binding protein 2	-5.06	2.49E-04
VSAL_I1029	gpM phage terminase, endonuclease subunit	-5.02	1.24E-04
VSAL_I4069s	VSsrna70 undefined small RNA	-4.72	4.98E-04
VSAL_I0136	IucC siderophore biosynthesis protein	-4.42	1.97E-04
VSAL_I4155s	VSsrna156 undefined small RNA	-4.20	6.14E-04
VSAL_I1027	gpO phage capsid scaffolding protein	-4.10	3.71E-04
VSAL_I3104s	VSsrna24 small RNA	-4.10	2.97E-04
VSAL_I0135	AlcA siderophore biosynthetis protein	-4.04	3.71E-04
VSAL_p54_02	putative mobilization protein	-3.93	3.71E-04
VSAL_p43_01	replication initiation protein	-3.92	1.24E-04

VSAL_I0134	L-2,4-diaminobutyrate decarboxylase	-3.84	2.49E-04
VSAL_I3073r	5S rRNA 5S rRNA undefined product 93740:93859 forward	-3.82	7.53E-04
VSAL_I1039	probable exported protein, putative phage gene	-3.72	2.49E-04
VSAL_I3157t	tRNA transfer RNA-Ser	-3.64	3.13E-04
VSAL_I0137	TonB-dependent iron-siderophore receptor precursor	-3.58	3.08E-04
VSAL_p54_01	acyltransferase	-3.58	4.98E-04
VSAL_I1751	TonB1 TonB protein (pseudogene)	-3.56	3.71E-04
VSAL_I3144t	tRNA tRNA transfer RNA-Leu 842679:842760 reverse	-3.46	8.25E-04
VSAL_I3072r	23S rRNA 23S rRNA undefined product 90756:93646 forward	-3.37	1.04E-03
VSAL_I1040	hypothetical protein, putative phage gene	-3.33	3.13E-04
VSAL_p320_31	putative phage intergrase	-3.32	3.71E-04
VSAL_p43_02	acetyltransferase	-3.25	1.97E-04
VSAL_I3102s	VSsrna22 small RNA RyhB	-3.23	3.08E-04

Figure legends

Figure 1. Synteny comparison and sequence alignment of *spf*. (A) The order of genes that flank *spf* and other sRNA genes (filled arrows) in *A. salmonicida* and *E. coli* are shown. Question marks indicate genes with unknown function. (B) Sequence alignment of *spf* from selected members of *Vibrionaceae*, *E. coli*, and *Serratia marcescens*. The CRP binding site and -10 and -35 promoter regions in front of *spf* are based on knowledge from *E. coli*.[7, 8] The vertical open arrow denotes the 5' end of Spot 42 based on knowledge from *E. coli* and 5' RACE data on *A. salmonicida* presented in this work. The horizontal arrows denote the area deleted in the *spf* deletion mutant. Numbers in parenthesizes denote numbers of nt to the nearest CDS. Invariable positions are indicated by asterisks.

Figure 2. Growth curve of *A. salmonicida* in a minimal medium with no added sugar (control), 44.4 mM glucose, or 44.4 mM galactose. The bacterium lacks the *gal* operon and is unable to utilize galactose for growth.

Figure 3. Monitoring of Spot 42 and VSsrna24 expression under different treatments or stress conditions with Northern blot analysis. (A) *A. salmonicida* was cultured for approximately 30 hours (h) starting at OD_{600} 0.1 and ending at 1.8 (stationary phase). Samples were collected throughout the growth cycle. The culture was split and 1 mM cAMP was added at OD_{600} 0.4 to one half. Similarly, a second culture was split and 5 mM glucose was added at OD_{600} 1.2. Untreated cells were used as control. (B) Samples collected in A were subjected to Northern blot analysis. From the control, culture samples were collected at OD_{600} 0.2 – 1.7, and after the additions of cAMP or glucose, samples were collected after 5 – 240 min (OD_{600} 0.4 – 0.8) or 5 – 360 (OD_{600} 1.2 – 1.5) min, respectively. Radio-labeled double-stranded DNA probes were used to monitor the levels of Spot 42 and VSsrna24, and 5S rRNA was used to normalize the result. (C) *A. salmonicida* was grown to mid exponential phase (OD_{600} 0.5) and split into nine smaller cultures. These were subjected to hexanoyl-L-homoserine lactone (HHL; 2 μg/ml), N-3-oxo-hexanoyl-L-homoserine lactone, (OHHL; 2 μg/ml), N,N'-dimethyl-4,4'-bipyridinium

dichloride, (paraquat; 500 μM), hydrogen peroxide (H_2O_2; 1mM), iron-chelator (2,2'-dipyridyl; 50 μM), ethanol (EtOH; 0,5 %), 4 °C or 20 °C, or used as control (16 °C). Samples were harvested between 0–30 min and also after 8 and 20 hours (h) for the control culture. RNA samples were run on two gels and transferred to two membranes (Membranes 1 and 2) for practical reasons.

Figure 4. Overview of microarray results with *spf* deletion mutant compared to the wild type strain. Bacteria were grown to OD_{600} 0.4, then the culture was split in two and 44.4 mM glucose was added to one half. Cells were collected after 15 min. CDSs with differential gene expression corresponding to up- or downregulation above or below 2 fold are divided into functional categories as defined by the Sanger Institute Pathogen Sequencing Unit (http://genprotec.mbl.edu/files/MultiFun.html). Numbers in parenthesises indicate in percentage the share of the total number of genes in the genome that each class represents.

Figure 5. Validation of selected microarray results with Northern blot analysis. (A) RNAs from wild type and *spf* deletion mutants (Δ*spf*) were separated on a 5 % denaturating polyacrylamide, transferred to membranes, and tested for presence of the sRNAs Spot 42, RyhB, Qrr, and VSsrna24. 5S rRNA was used as control and to normalize the results. Plus (+) indicates that glucose was added to the culture 15 min prior to sampling, whereas minus indicates that no glucose was added. Numbers to the right of gel pictures indicate the length of RNAs as measured from the gel. (B) The same samples as described above separated on a 1.2 % denaturating formamide gel.

Figure 6. Potential for base-pairing between Spot 42 and the pirin mRNA (VSAL_I1200 was tested using the RNAhybrid software[17].

Fig. 1.

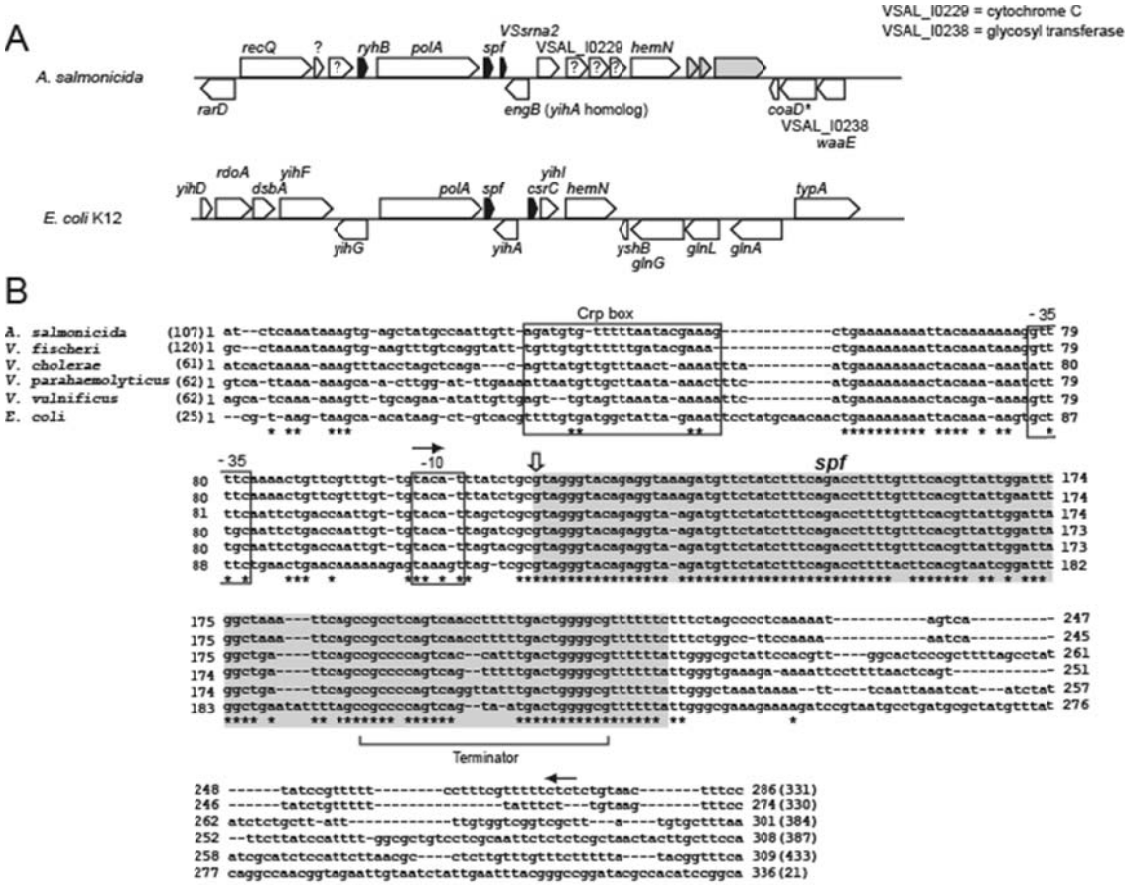

VSAL_I0229 = cytochrome C
VSAL_I0238 = glycosyl transferase
A
A. salmonicida
recQ
?
ryhB
polA
VSsrna2
spf
VSAL_I0229
hemN
rarD
engB (yihA homolog)
coaD*
VSAL_I0238
waaE
E. coli K12
rdoA
yihF
yihD
dsbA
polA
spf
yihI
csrC
hemN
typA
yihG
yihA
yshB
glnG
glnL
glnA
B
Crp box
-35
-10
spf
Terminator

Fig. 2.

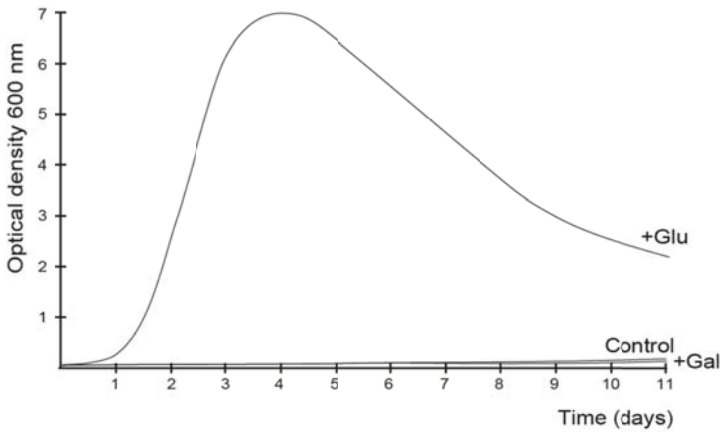

Optical density 600 nm
Time (days)
+Glu
Control
+Gal

Fig. 3.

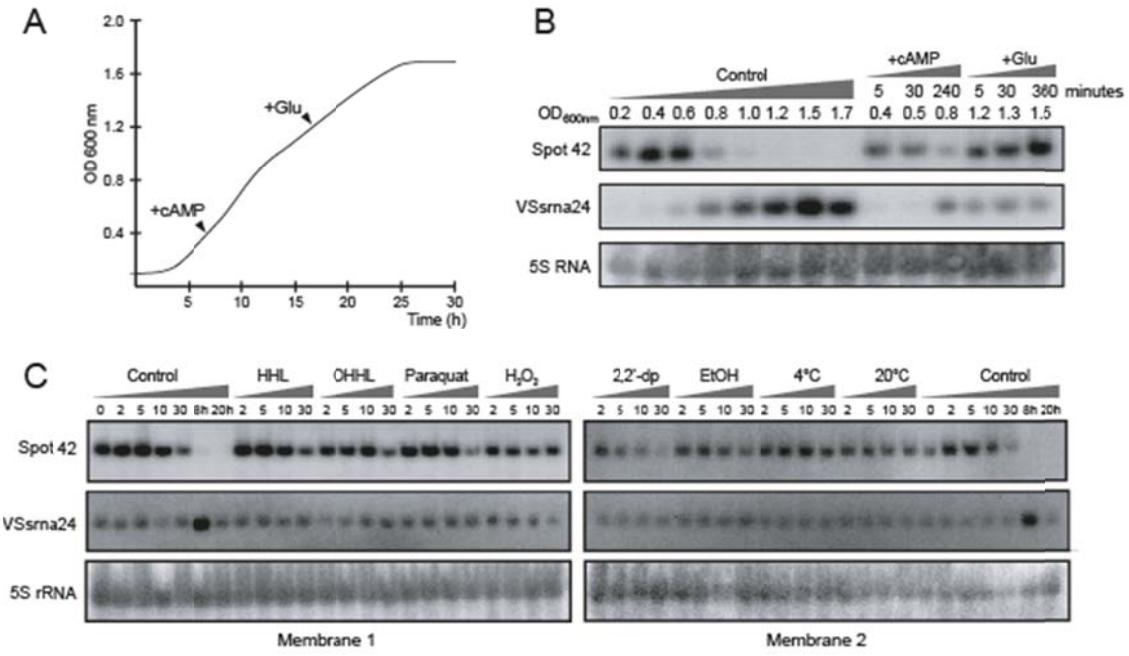

A
OD 600 nm
2.0
1.6
1.2
0.8
0.4
+Glu
+cAMP
5
10
15
20
25
30
Time (h)
B
Control
+cAMP
+Glu
5 30 240 5 30 360 minutes
OD600nm 0.2 0.4 0.6 0.8 1.0 1.2 1.5 1.7 0.4 0.5 0.8 1.2 1.3 1.5
Spot 42
VSsrna24
5S RNA
C
Control
HHL
OHHL
Paraquat
H2O2
2,2'-dp
EtOH
4°C
20°C
Control
0 2 5 10 30 8h 20h 2 5 10 30 2 5 10 30 2 5 10 30 2 5 10 30 2 5 10 30 2 5 10 30 2 5 10 30 2 5 10 30 2 5 10 30 0 2 5 10 30 8h 20h
Spot 42
VSsrna24
5S rRNA
Membrane 1
Membrane 2

Fig. 4.

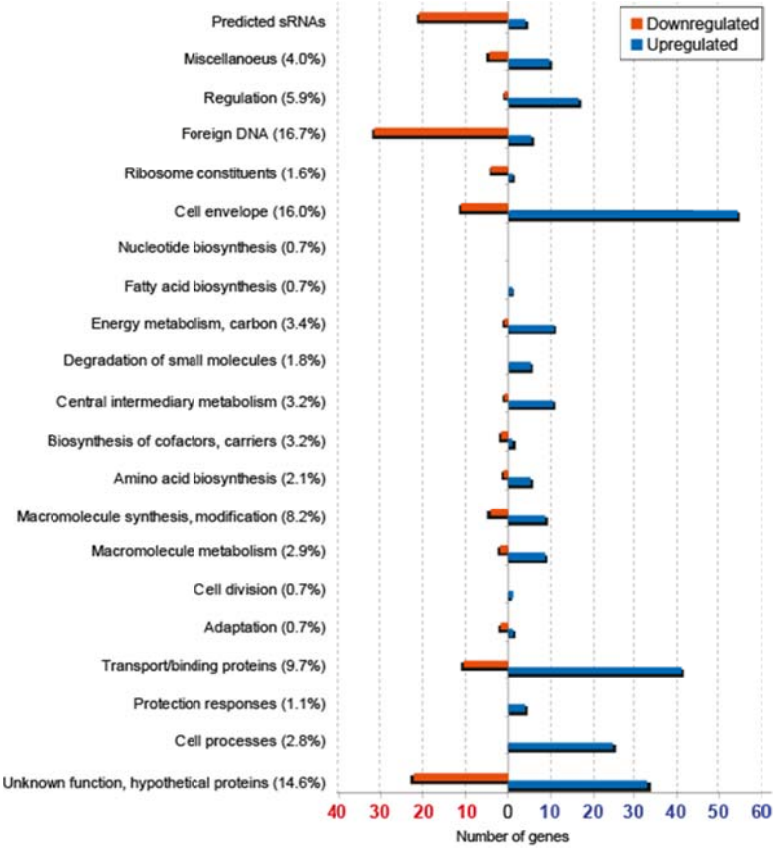

Downregulated
Upregulated
Predicted sRNAs
Miscellanoeus (4.0%)
Regulation (5.9%)
Foreign DNA (16.7%)
Ribosome constituents (1.6%)
Cell envelope (16.0%)
Nucleotide biosynthesis (0.7%)
Fatty acid biosynthesis (0.7%)
Energy metabolism, carbon (3.4%)
Degradation of small molecules (1.8%)
Central intermediary metabolism (3.2%)
Biosynthesis of cofactors, carriers (3.2%)
Amino acid biosynthesis (2.1%)
Macromolecule synthesis, modification (8.2%)
Macromolecule metabolism (2.9%)
Cell division (0.7%)
Adaptation (0.7%)
Transport/binding proteins (9.7%)
Protection responses (1.1%)
Cell processes (2.8%)
Unknown function, hypothetical proteins (14.6%)
40 30 20 10 0 10 20 30 40 50 60
Number of genes

Fig. 5.

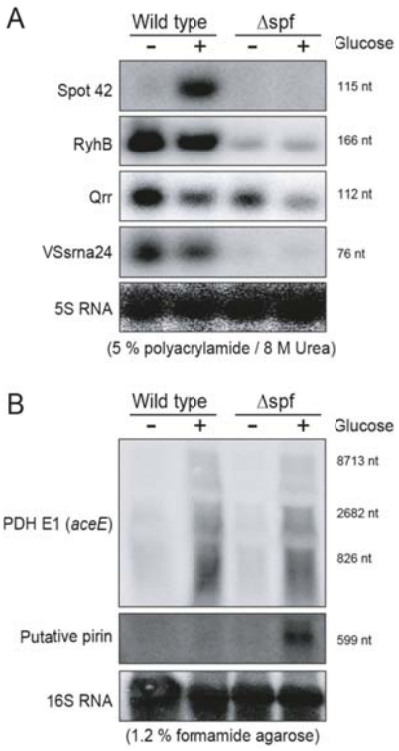
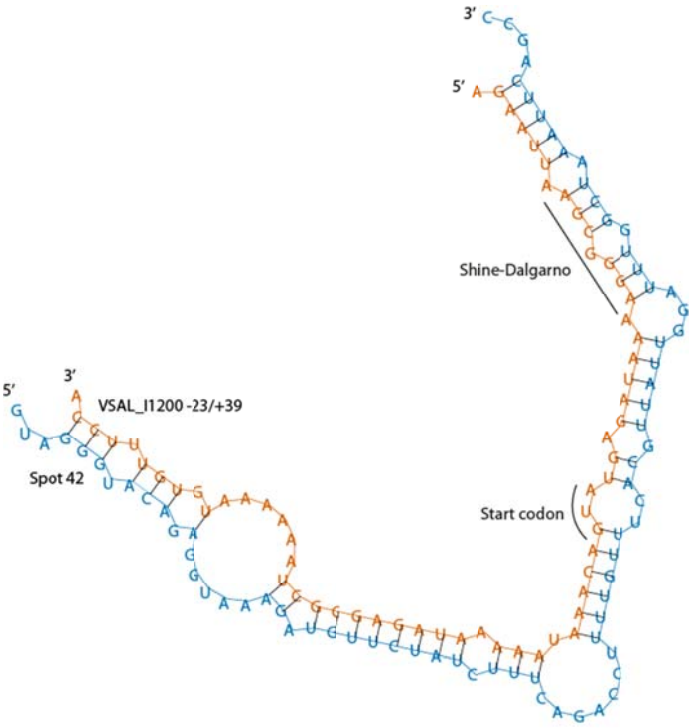

A
Wild type
Δspf
- + - +
Glucose
Spot 42
115 nt
RyhB
166 nt
Qrr
112 nt
VSsrna24
76 nt
5S RNA
(5 % polyacrylamide / 8 M Urea)
B
Wild type
Δspf
- + - +
Glucose
8713 nt
PDH E1 (aceE)
2682 nt
826 nt
Putative pirin
599 nt
16S RNA
(1.2 % formamide agarose)

Fig. 6.


3′
5′
Shine-Dalgarno
5′
3′
VSAL_I1200 -23/+39
Spot 42
Start codon

Supplement file 1 (Table S1)

VSAL ID	Product	log_2 ratio	Adj. p-value
VSAL_I1200	putative pirin	3.97	2.49E-04
VSAL_I2193	methyl-accepting chemotaxis protein	2.92	2.97E-04
VSAL_I4139s	VSsrna140 VSsrna140 undefined product 2463891:2464064 forward	2.53	4.98E-04
VSAL_I0799	methyl-accepting chemotaxis protein	2.53	4.02E-04
VSAL_I2318	flaD flagellin subunit D	2.48	4.35E-04
VSAL_II0716	putative exported protein	2.39	3.54E-03
VSAL_I2317	flaA flagellin subunit E	2.33	4.98E-04
VSAL_II0613	putative membrane protein	2.17	8.69E-04
VSAL_I1201	putative IMP dehydrogenase/GMP reductase	2.13	4.44E-04
VSAL_I2319	flaA flagellin subunit C	2.09	5.58E-04
VSAL_I2517	flaF flagellin subunit F	2.07	6.11E-04
VSAL_I2329	flgM flagellar hook-associated protein type 3 FlgM	2.03	4.98E-04
VSAL_I2022	vcmH multidrug efflux pump	1.87	1.20E-03
VSAL_II0587	outer membrane protein, OmpA family	1.87	6.40E-04
VSAL_I2593	gluconate permease	1.81	6.74E-04
VSAL_II0091	fruK 1-phosphofructokinase	1.81	9.06E-04
VSAL_II0090	fruB PTS system, fructose-specific IIA/FPR component	1.80	6.11E-04
VSAL_I2578	ABC-type [(GlcNAc)2] transporter, permease protein	1.79	6.11E-04
VSAL_I1857	hypothetical protein	1.78	6.74E-04
VSAL_II0715	cusB putative cation efflux system protein	1.77	8.23E-04
VSAL_I2338	flgB flagellar basal-body rod protein FlgB	1.75	5.14E-04
VSAL_II0231	cheV chemotaxis protein CheV	1.74	8.03E-04
VSAL_I2771	motX sodium-type polar flagellar protein MotX	1.71	7.15E-04
VSAL_II0331	putative exported protein	1.70	6.14E-04
VSAL_I2334	flgF flagellar basal-body rod protein FlgF	1.70	8.55E-04
VSAL_II1080	membrane protein	1.70	7.00E-04
VSAL_I0474	mshF type IV pilus, mannose-sensitive hemagglutinin protein MshF	1.68	6.11E-04
VSAL_I1401	tupA extracellular tungstate binding protein precursor	1.66	6.86E-04
VSAL_I2327	fliC flagellin subunit A	1.62	7.00E-04
VSAL_II0785	putative exported protein	1.59	6.11E-04
VSAL_I2061	hypothetical protein	1.59	6.11E-04
VSAL_II0964	luxC acyl-CoA reductase LuxC	1.59	6.86E-04
VSAL_I2315	fliDP polar flagellar hook-associated protein 2 (HAP2) (flagellar cap protein)	1.58	6.14E-04
VSAL_I1851	putative membrane protein	1.58	2.94E-03
VSAL_II0062	membrane protein	1.55	6.14E-04

VSAL_I1260	conserved hypothetical protein	1.54	9.53E-04
VSAL_I2333	flgG flagellar basal-body rod protein FlgG (distal rod protein)	1.53	6.78E-04
VSAL_I0870	yejE inner membrane ABC transporter permease protein	1.53	3.09E-03
VSAL_I2577	ABC-type [(GlcNAc)2] transporter, permease protein	1.53	3.71E-03
VSAL_I1400	tupB permease component of tungstate ABC transporter	1.51	2.24E-03
VSAL_I0899	nanA dihydropicolinate synthase	1.50	1.40E-03
VSAL_I0449	putative membrane protein	1.50	7.45E-04
VSAL_I2788	frdA fumarate reductase complex, flavoprotein subunit	1.49	2.14E-03
VSAL_I2316	flaG polar flagellar protein FlaG (pseudogene)	1.49	6.14E-04
VSAL_II0353	hypothetical protein	1.49	7.05E-04
VSAL_II0240	membrane protein	1.49	1.07E-03
VSAL_I2575	chiS chitin degradation sensor protein	1.49	3.54E-03
VSAL_II0647	hypothetical protein (pseudogene)	1.48	2.96E-03
VSAL_I2156	fadH 2,4-dienoyl-CoA reductase [NADPH]	1.46	5.68E-03
VSAL_I0110	hypothetical protein	1.46	1.49E-02
VSAL_II0720	putative heme-Cu membrane protein (NnrS)	1.44	6.86E-04
VSAL_I2325	flaB flagellin subunit B	1.44	3.13E-03
VSAL_II0249	putative exported protein	1.44	1.07E-03
VSAL_II0182	universal stress protein	1.43	1.18E-03
VSAL_II0051	inner membrane protein	1.43	6.86E-04
VSAL_I0876	membrane dipeptidase	1.43	6.61E-04
VSAL_II1075	putative iron transporter, membrane component	1.43	7.00E-04
VSAL_II1022	methyl-accepting chemotaxis protein	1.42	8.58E-04
VSAL_II0073	astB succinylarginine dihydrolase	1.41	7.15E-04
VSAL_I2069	membrane protein (fragment)	1.40	6.26E-03
VSAL_II0806	hypothetical protein	1.39	1.41E-03
VSAL_I1599	putative polysaccharide deacetylase	1.39	1.72E-03
VSAL_II0398	NAD-dependent formate dehydrogenase alpha subunit	1.38	7.05E-04
VSAL_I1171	vasQ-1 putative type VI secretion protein VasQ-1	1.38	9.10E-04
VSAL_II0792	membrane protein	1.38	9.75E-04
VSAL_I2072	hypothetical protein	1.38	9.55E-04
VSAL_II0109	torC cytochrome C-type protein (pseudogene)	1.37	7.53E-04
VSAL_II0338	MFS transporter	1.37	2.93E-03
VSAL_I2590	glnB nitrogen regulatory protein P-II	1.36	9.75E-04
VSAL_I2006	HTH-type transcriptional regulator, LysR family	1.36	1.16E-03
VSAL_I2202	hypothetical protein	1.35	7.83E-04
VSAL_I2584	chbP chitobiose phosphorylase (glycosyl transferase)	1.35	7.53E-04
VSAL_I0053	ilvD dihydroxy-acid dehydratase	1.35	1.14E-03

VSAL_II0961	luxB alkanal monooxygenase beta chain LuxB (bacterial luciferase beta chain)	1.35	1.50E-03
VSAL_II0943	luxN autoinducer 1 sensor kinase/phosphatase LuxN (pseudogene)	1.35	7.05E-04
VSAL_II0195	putative response regulator (pseudogene)	1.35	7.05E-04
VSAL_I1906	membrane protein	1.35	7.00E-04
VSAL_I0096	binding-protein-dependent transport system, inner membrane component	1.34	1.51E-03
VSAL_I1964	putative multidrug resistance protein (pseudogene)	1.34	2.97E-03
VSAL_II0528	hlyB hemolysin secretion protein	1.33	2.20E-03
VSAL_II0262	membrane protein	1.33	7.45E-04
VSAL_I2729	pabA anthranilate synthase component II (glutamine amido transferase)	1.33	8.25E-04
VSAL_I2829	membrane associated response regulator, histidine kinase	1.32	6.05E-03
VSAL_II0050	chb n,n'-diacetylchitobiase precursor (chitobiase)	1.32	6.19E-03
VSAL_I2330	flgJ peptidoglycan hydrolase FlgJ	1.32	1.34E-03
VSAL_I1271	putative response regulator	1.31	1.26E-03
VSAL_I2346	putative exported protein	1.30	2.18E-03
VSAL_I0536	glsA thermolabile glutaminase	1.30	8.25E-04
VSAL_I1509	putative membrane protein	1.30	1.48E-03
VSAL_I2582	glucosamine kinase	1.29	1.00E-03
VSAL_I2361	putative exported protein	1.29	1.15E-03
VSAL_II0147	membrane associated GGDEF protein	1.29	8.49E-04
VSAL_I0097	binding-protein-dependent transport system, inner membrane component	1.28	1.30E-03
VSAL_I1476	membrane protein	1.28	1.18E-03
VSAL_I1188	putative branched-chain amino acid transport protein	1.28	9.06E-04
VSAL_I2956	eda KHG/KDPG aldolase	1.27	5.53E-03
VSAL_I0936	motA sodium-driven polar flagellar protein MotA	1.27	1.93E-03
VSAL_II0318	putative exported protein	1.27	1.82E-03
VSAL_II0179	conserved hypothetical protein	1.26	9.06E-04
VSAL_II0983	HTH-type transcriptional regulator, LysR-family	1.26	8.69E-04
VSAL_II0708	hutH histidine ammonia-lyase	1.26	8.25E-04
VSAL_I1449	hypothetical protein	1.26	1.61E-03
VSAL_I0686	putative exported protein	1.26	1.03E-03
VSAL_II0238	glgC glucose-1-phosphate adenylyltransferase	1.26	2.20E-03
VSAL_I0625	conserved hypothetical protein	1.26	5.13E-03
VSAL_II0456	conserved hypothetical protein, putative transposase	1.25	9.75E-04
VSAL_I1488	putative exported protein	1.25	9.61E-04
VSAL_I1598	membrane protein	1.25	1.63E-03
VSAL_I2345	putative exported protein	1.25	9.80E-04
VSAL_I0452	putative glycosyl transferase	1.25	2.84E-03
VSAL_II0709	vcmN multidrug efflux pump (pseudogene)	1.25	1.34E-03

VSAL_I0920	nitrate and nitrite sensing methyl-accepting chemotaxis protein	1.24	1.78E-03
VSAL_I1446	mltC membrane-bound lytic murein transglycosylase C	1.24	9.55E-04
VSAL_II0250	putative lipoprotein	1.23	8.67E-04
VSAL_I0380	integral membrane protein, AcrB/AcrD/AcrF family	1.22	8.25E-04
VSAL_II0092	fruA PTS system, fructose-specific IIBC component	1.22	1.10E-03
VSAL_II0194	two-component system sensor protein, histidine kinase	1.22	9.33E-04
VSAL_II0252	putative extracellular protein	1.22	1.41E-03
VSAL_I0937	motB sodium-driven polar flagellar protein MotB	1.22	9.55E-04
VSAL_II0247	nitrate transporter	1.22	2.06E-03
VSAL_I2595	acnB aconitate hydratase 2 (citrate hydro-lyase 2)	1.21	1.24E-03
VSAL_I1876	conserved hypothetical protein	1.21	8.75E-04
VSAL_I2581	putative chitobiase (exoglucosidase)	1.21	2.35E-03
VSAL_I0381	putative exported protein	1.20	1.54E-03
VSAL_I1806	conserved hypothetical protein	1.20	1.39E-03
VSAL_II0224	transcriptional regulator, TetR-family	1.20	3.53E-03
VSAL_I0307	intracellular sulfur oxidation protein DsrE	1.20	9.55E-04
VSAL_II0108	biotin sulfoxide reductase (fragment)	1.20	2.49E-03
VSAL_II0181	ABC-type glycine betaine transport system, substrate binding domain	1.20	8.69E-04
VSAL_I1581	potB spermidine/putrescine transport system permease protein PotB	1.20	6.37E-03
VSAL_II0986	hypothetical protein	1.19	2.42E-03
VSAL_I2078	serC phosphoserine aminotransferase	1.19	1.04E-03
VSAL_II0217	chitinase b	1.19	1.00E-03
VSAL_II0413	putative membrane protein	1.19	1.40E-03
VSAL_I2280	ccmB cytochrome c-type biogenesis protein CcmB	1.18	4.58E-03
VSAL_I2176	yadQ H(+)/Cl(-) exchange transporter	1.18	9.80E-04
VSAL_I1211	putative extracellular solute-binding protein	1.18	1.13E-03
VSAL_I2336	flgD flagellar basal-body rod protein FlgD	1.18	1.34E-03
VSAL_I2340	cheV chemotaxis protein methyltransferase CheV	1.17	8.69E-04
VSAL_II0433	opuD1 transporter, BCCT family	1.17	1.50E-02
VSAL_II0691	hypothetical protein	1.17	1.54E-03
VSAL_I2498	pagO integral membrane protein	1.17	2.94E-03
VSAL_II0410	hypothetical protein, putative phage gene (fragment)	1.17	4.10E-03
VSAL_II0837	tdh L-threonine 3-dehydrogenase	1.17	3.25E-03
VSAL_II0165	membrane protein	1.17	2.90E-03
VSAL_I0762	sodium-dependent transporter	1.16	9.06E-04
VSAL_I4130s	VSsrna131 VSsrna131 undefined product 2278278:2278466 reverse	1.16	4.02E-03
VSAL_II0665	gntP gluconate permease	1.16	2.94E-03
VSAL_II0805	hypothetical protein	1.15	2.06E-03

VSAL_I1905	membrane protein	1.15	7.82E-03
VSAL_II0340	hypothetical protein	1.15	2.12E-03
VSAL_I0760	rimK ribosomal protein S6 modification protein	1.15	9.55E-04
VSAL_I1999	nrfC cytochrome c-type biogenesis protein NrfC	1.15	1.78E-03
VSAL_I1966	putative pyridoxal-phosphate dependent enzyme	1.15	9.06E-04
VSAL_I1790	restriction enzyme	1.15	2.42E-02
VSAL_I2716	pilM fimbrial assembly protein PilM	1.14	1.27E-03
VSAL_I1887	ruvC crossover junction endodeoxyribonuclease RuvC	1.14	1.70E-03
VSAL_I1650	putative membrane protein, putative phage gene	1.14	1.24E-03
VSAL_I1789	conserved hypothetical protein	1.14	9.87E-04
VSAL_I1210	putative NADP or NAD utilising oxidoreductase	1.14	1.88E-03
VSAL_I1143	hypothetical protein	1.13	1.66E-03
VSAL_II0254	speA biosynthetic arginine decarboxylase	1.13	2.76E-03
VSAL_II0086	putative membrane protein	1.13	4.64E-03
VSAL_I1765	putative membrane-associated phosphoesterase	1.13	7.93E-03
VSAL_I1998	nrfB cytochrome c-type protein NrfB precursor	1.12	1.03E-03
VSAL_II0980	putative exported oxidoreductase	1.12	4.21E-03
VSAL_I2790	frdC fumarate reductase complex, membrane anchor subunit C	1.12	3.36E-03
VSAL_I2332	flgH flagellar L-ring protein 1 precursor (basal body L-ring protein 1)	1.12	1.68E-02
VSAL_II0985	membrane transport protein, putative auxin efflux carrier	1.12	1.37E-03
VSAL_II0840	putative Lon protease	1.12	6.19E-03
VSAL_I2828	putative sodium/solute symporter	1.12	1.10E-03
VSAL_II0707	hutU urocanate hydratase	1.11	1.14E-03
VSAL_I0620	deoA thymidine phosphorylase	1.11	1.65E-03
VSAL_II0232	glgX putative alpha amylase	1.11	1.21E-03
VSAL_II1026	trpS2 putative tryptophanyl-tRNA synthetase	1.11	1.90E-03
VSAL_I2789	frdB fumarate reductase complex, iron-sulfur protein	1.11	1.76E-03
VSAL_I2331	flgI flagellar P-ring protein 2 precursor (basal body P-ring protein 2)	1.11	1.14E-03
VSAL_I4086s	VSsrna87 VSsrna87 undefined product 1474432:1474621 forward	1.11	2.93E-03
VSAL_I2453	pstA phosphate import protein, transmembrane component PstA	1.11	9.76E-04
VSAL_II1031	ABC transport system, ATP-binding protein	1.11	4.02E-03
VSAL_II0719	hypothetical protein	1.10	1.24E-03
VSAL_II0222	exonuclease	1.10	3.58E-03
VSAL_I2438	aceA isocitrate lyase	1.10	1.10E-03
VSAL_I2583	exoI beta-hexosaminidase (beta-N-acetylglucosaminidase)	1.10	9.19E-03
VSAL_I1382	norM multidrug resistance protein NorM (sodium/drug antiporter)	1.10	1.97E-03
VSAL_II1055	putative membrane associated protease	1.10	1.27E-03
VSAL_I2576	ABC transporter, periplasmic chitin oligosaccharide [(GlcNAc)n] binding protein (pseudogene)	1.09	1.11E-03

VSAL_I1506	putative phenazine biosynthesis-like protein	1.09	2.42E-03
VSAL_II0180	divalent cation transporter, putative magnesium transporter	1.09	3.88E-03
VSAL_I1525	putative lipoprotein (pseudogene)	1.09	1.06E-03
VSAL_I1172	vasRB-1 putative type VI secretion protein VasRA-1	1.09	9.88E-04
VSAL_I2840	hypothetical protein	1.09	1.45E-03
VSAL_I2054	aapP general L-amino acid transport ATP-binding subunit	1.09	2.83E-03
VSAL_I1070	FAD dependent oxidoreductase	1.09	1.20E-03
VSAL_I1988	membrane protein	1.09	1.41E-03
VSAL_I1807	hypothetical protein	1.08	2.05E-03
VSAL_I1367	rbsR ribose operon repressor	1.08	2.90E-03
VSAL_I1082	response regulator	1.08	1.14E-03
VSAL_I0939	transcriptional regulator, LysR family	1.08	3.35E-03
VSAL_II0781	modB molybdenum transport system permease protein ModB	1.08	1.14E-03
VSAL_I2364	napC cytochrome c-type protein NapC	1.08	2.78E-02
VSAL_I1631	sodium/solute symporter (fragment)	1.07	6.76E-03
VSAL_II0927	malF maltose transport system permease protein MalF (fragment)	1.07	1.02E-03
VSAL_II0953	membrane protein	1.07	1.14E-03
VSAL_I4056s	VSsrna57 VSsrna57 undefined product 795076:795286 forward	1.07	1.99E-03
VSAL_II0845	MFS transporter	1.07	1.21E-03
VSAL_I1406	membrane protein	1.07	2.20E-03
VSAL_I1926	ibrA immunoglobulin-binding regulator (pseudogene)	1.07	1.07E-03
VSAL_II0028	putative membrane protein	1.07	1.15E-03
VSAL_I1706	putative acylphosphatase	1.07	1.25E-03
VSAL_II0400	putative HTH-type transcriptional regulator	1.07	5.75E-03
VSAL_I1427	hypothetical protein	1.07	7.70E-03
VSAL_I1847	gsk inosine-guanosine kinase	1.06	1.06E-03
VSAL_I2579	ABC-type [(GlcNAc)2] transporter, ATP-binding protein	1.06	1.86E-03
VSAL_II0549	hypothetical protein, putative phage gene	1.06	5.60E-03
VSAL_I0962	putative type III restriction enzyme	1.06	1.44E-03
VSAL_I1518	hypothetical protein	1.06	3.73E-03
VSAL_II0142	rhlE putative ATP-dependent RNA helicase (DEAD/DEAH box helicase)	1.06	6.32E-03
VSAL_I1190	putative fumarylacetoacetate hydrolase	1.05	1.20E-03
VSAL_I2589	amtB ammonium transporter	1.05	2.08E-03
VSAL_II0703	hypothetical protein	1.05	1.34E-03
VSAL_I0412	membrane protein	1.05	1.30E-03
VSAL_I1992	hypothetical protein (fragment)	1.05	1.31E-03
VSAL_I1375	membrane protein	1.05	5.43E-03
VSAL_I1392	putative outer membrane protein	1.04	1.52E-03

VSAL_I2852	nudC NADH pyrophosphatase (pseudogene)	1.04	3.25E-03
VSAL_I0606	putative signaling protein	1.04	1.02E-02
VSAL_II0450	5'-nucleotidase	1.04	1.52E-03
VSAL_I0198	hypothetical protein	1.04	1.25E-03
VSAL_I0414	hypothetical protein	1.04	5.21E-03
VSAL_I1805	hypothetical protein	1.04	1.15E-02
VSAL_II0790	nrdG anaerobic ribonucleoside-triphosphate reductase activating protein	1.04	4.70E-03
VSAL_I2372	hypothetical protein	1.03	2.24E-03
VSAL_II0077	HTH-type transcriptional regulator, AraC-family	1.03	5.60E-03
VSAL_I1949	oppD oligopeptide transport ATP-binding protein D	1.03	1.90E-03
VSAL_II0239	glgA glycogen synthase	1.03	2.31E-03
VSAL_II0201	hypothetical protein	1.02	1.57E-03
VSAL_I1304	garR 6-phosphogluconate dehydrogenase	1.02	1.37E-03
VSAL_I2645	ftsW cell division protein FtsW	1.02	3.40E-03
VSAL_I1519	putative membrane protein	1.02	1.37E-02
VSAL_I2594	glxK glycerate kinase	1.02	1.51E-02
VSAL_I0679	putative C4-dicarboxylate/malic acid transport protein	1.02	4.23E-03
VSAL_I1700	hrpA ATP-dependent helicase HrpA	1.02	8.63E-03
VSAL_I0384	putative exported protein	1.01	2.40E-03
VSAL_I2046	putative acetyltransferase (GNAT) family protein	1.01	2.18E-03
VSAL_I1319	membrane receptor, histidine kinase	1.01	1.88E-03
VSAL_I2351	putative lipoprotein	1.01	2.06E-02
VSAL_II0313	putative exported protein	1.01	1.80E-03
VSAL_I2344	putative lipoprotein	1.01	4.85E-03
VSAL_II0168	putative exported protein	1.01	1.44E-03
VSAL_I2436	queA S-adenosylmethionine:tRNA ribosyltransferase-isomerase	1.00	5.75E-03
VSAL_I4151s	VSsrna152 VSsrna152 undefined product 2690352:2690488 forward	-1.01	1.12E-02
VSAL_p840_10	traW conjugative transfer protein TraW	-1.02	2.34E-02
VSAL_I4110s	VSsrna111 VSsrna111 undefined product 1921624:1921875 forward	-1.02	7.93E-03
VSAL_I0577	rpsT 30S ribosomal protein S20	-1.03	1.98E-03
VSAL_I1301	conserved hypothetical protein	-1.03	1.18E-02
VSAL_I2215	infA translation initiation factor IF-1	-1.04	3.15E-03
VSAL_I4107s	VSsrna108 VSsrna108 undefined product 1892040:1892233 forward	-1.04	1.59E-03
VSAL_I1622	putative cell wall lytic enzyme	-1.05	1.81E-03
VSAL_I1712	glpQ glycerophosphoryl diester phosphodiesterase precursor	-1.05	1.28E-03
VSAL_I1019	hypothetical protein, putative phage gene	-1.06	1.41E-02
VSAL_I0051	cspB cold shock protein	-1.07	1.45E-03
VSAL_I2992	MFS transporter	-1.09	4.47E-03

VSAL_I2514	eno enolase	-1.11	1.91E-03
VSAL_I0288	yiiU conserved hypothetical protein	-1.13	1.29E-02
VSAL_I2763	rplI 50S ribosomal subunit protein L9	-1.13	1.96E-03
VSAL_II0112	tolR2 biopolymer transport protein TolR	-1.13	1.35E-03
VSAL_I1014	hypothetical protein, putative phage gene	-1.13	3.18E-03
VSAL_p320_29	iron ion ABC transporter ATP-binding protein	-1.15	1.07E-03
VSAL_I1713	glpT glycerol-3-phosphate transporter	-1.17	8.69E-04
VSAL_I2892	hypothetical protein	-1.17	1.14E-03
VSAL_I3195t	tRNA-Met tRNA-Met undefined product 2893938:2894010 forward	-1.20	2.74E-03
VSAL_I1016	hypothetical protein, putative phage gene	-1.21	9.02E-04
VSAL_p320_30	hypothetical protein	-1.21	5.58E-03
VSAL_p320_08	DNA-binding protein, putative plasmid partition protein	-1.22	1.98E-03
VSAL_I4074s	VSsrna75 VSsrna75 undefined product 1208858:1209241 forward	-1.24	1.49E-03
VSAL_I1018	hypothetical protein, putative phage gene	-1.24	9.04E-03
VSAL_I0590	putative RNA-binding protein	-1.25	9.88E-04
VSAL_I1248	membrane protein	-1.26	2.02E-02
VSAL_p320_25	putative MobA protein (fragment)	-1.27	9.55E-04
VSAL_I0311	rpsG 30S ribosomal protein S7	-1.28	1.14E-03
VSAL_I2450	putative exported protein	-1.29	8.67E-04
VSAL_p840_27	hypothetical protein	-1.30	1.21E-03
VSAL_p840_37	hypothetical protein	-1.31	1.73E-03
VSAL_p320_28	iron ion transport system, inner membrane component	-1.32	7.40E-04
VSAL_II0603	conserved hypothetical protein	-1.35	3.31E-03
VSAL_I3149t	tRNA tRNA transfer RNA-Met 843492:843565 reverse	-1.36	1.60E-03
VSAL_I4006s	VSsrna7 VSsrna7 undefined product 44491:44694 reverse	-1.38	8.25E-04
VSAL_p320_10	hypothetical protein	-1.39	1.14E-03
VSAL_I0345	rplQ 50S ribosomal protein L17	-1.39	7.53E-04
VSAL_I4043s	VSsrna44 VSsrna44 undefined product 638438:638620 forward	-1.43	1.45E-03
VSAL_I3117t	tRNA-Leu tRNA-Leu undefined product 560225:560306 forward	-1.44	2.08E-03
VSAL_I3161t	tRNA-Val tRNA-Val undefined product 1804642:1804714 reverse	-1.44	3.09E-03
VSAL_p320_12	hypothetical protein	-1.53	6.11E-04
VSAL_II0110	TonB dependent receptor	-1.54	8.38E-04
VSAL_p320_01	hypothetical protein	-1.55	1.72E-03
VSAL_p840_25	ardC antirestriction protein ArdC	-1.57	2.49E-03
VSAL_I4076s	VSsrna77 VSsrna77 undefined product 1266633:1266710 forward	-1.57	2.53E-03
VSAL_I1531	hypothetical protein	-1.58	8.49E-04
VSAL_p320_11	putative DNA-damage-inducible protein	-1.60	1.10E-03
VSAL_I3120t	tRNA tRNA transfer RNA-Thr 561497:561569 reverse	-1.61	8.25E-04

VSAL_p840_05	traK conjugative transfer protein TraK	-1.64	1.00E-03
VSAL_p320_27	iron ion ABC transporter, periplasmic component	-1.65	6.11E-04
VSAL_p320_22	hypothetical protein	-1.65	8.25E-04
VSAL_p840_17	traH conjugative transfer protein TraH	-1.66	1.72E-03
VSAL_p320_17	putative exported protein (fragment)	-1.70	9.75E-04
VSAL_I3118t	tRNA tRNA transfer RNA-Asn 561331:561403 reverse	-1.70	2.46E-03
VSAL_I0859	hypothetical protein	-1.71	6.17E-04
VSAL_I1013	probable phage replication protein	-1.71	1.76E-03
VSAL_p320_03	resolvase	-1.71	6.11E-04
VSAL_I3171t	tRNA-Leu tRNA-Leu undefined product 2103371:2103454 forward	-1.73	9.36E-03
VSAL_I3081t	tRNA-Lys tRNA-Lys undefined product 90204:90276 forward	-1.73	1.91E-03
VSAL_I0742	putative endoribonuclease L-PSP	-1.74	3.16E-03
VSAL_II0614	cold shock-like protein	-1.74	8.25E-04
VSAL_I4010s	VSsrna11 VSsrna11 undefined product 65235:65406 forward	-1.77	6.11E-04
VSAL_p320_XX	Ingen CDS, Fjernet fra ny annotering	-1.79	6.11E-04
VSAL_I1011	probable phage regulatory protein	-1.79	1.21E-03
VSAL_p320_14	hypothetical protein	-1.79	6.11E-04
VSAL_II0868	putative lipoprotein	-1.80	6.14E-04
VSAL_I3179s	VSsrna154 small RNA 6S / SsrS RNA	-1.82	4.98E-04
VSAL_I1786	putative iron-regulated protein	-1.85	7.60E-04
VSAL_p320_06	membrane protein	-1.89	9.06E-04
VSAL_p840_11	traU conjugative transfer protein TraU	-1.90	3.09E-03
VSAL_I3165s	VSsrna114 small RNA Qrr	-1.91	8.69E-04
VSAL_I4009s	VSsrna10 VSsrna10 undefined product 54553:54707 forward	-1.93	4.44E-04
VSAL_I0278	wzz O-antigen length determinant protein	-1.94	1.73E-02
VSAL_p320_23	putative exported protein	-1.94	5.58E-04
VSAL_p320_07	hypothetical protein	-1.96	7.53E-04
VSAL_I1045	tail fiber protein	-1.96	6.74E-04
VSAL_p320_24	putative transposase	-1.98	4.02E-04
VSAL_p320_05	hypothetical protein	-1.99	6.14E-04
VSAL_I1038	hypothetical protein, putative phage gene	-2.05	5.14E-04
VSAL_p320_16	hypothetical protein	-2.10	7.05E-04
VSAL_p320_04	hypothetical protein	-2.10	4.98E-04
VSAL_p54_03	putative replication initiation protein	-2.11	9.75E-04
VSAL_p320_02	putative exported protein	-2.14	7.60E-04
VSAL_I3216s	VSsrna193 CsrA-regulating small RNA CsrB3	-2.16	9.06E-04
VSAL_I4002s	VSsrna3 VSsrna3 undefined product 20957:21238 forward	-2.18	3.78E-04
VSAL_I1864	putative outer membrane protein	-2.18	6.14E-04

VSAL_I2480	intA integrase, phage family	-2.21	3.71E-04
VSAL_p320_21	hypothetical protein	-2.24	4.98E-04
VSAL_I0963	hypothetical protein	-2.26	3.73E-02
VSAL_p320_09	hypothetical protein	-2.29	4.49E-04
VSAL_I1034	hypothetical protein, putative phage gene	-2.47	7.05E-04
VSAL_I3166t	tRNA-Ser tRNA-Ser undefined product 2016247:2016331 forward	-2.48	1.07E-03
VSAL_II2045s	VSAsrna46 VSAsrna46 undefined product 1006466:1006637 forward	-2.49	6.11E-04
VSAL_I1036	probable rRNA transcription initiatior protein, putative phage gene	-2.53	6.87E-04
VSAL_I0714	suhB inositol-1-monophosphatase	-2.55	3.66E-02
VSAL_p320_15	hypothetical protein	-2.56	3.71E-04
VSAL_I1033	hypothetical protein, putative phage gene (pseudogene)	-2.73	3.71E-04
VSAL_I1754	hmuT heme transporter protein HuvB, putative periplasmic binding protein	-2.79	3.78E-04
VSAL_I3071r	16S rRNA 16S rRNA undefined product 88493:90029 forward	-2.83	1.08E-03
VSAL_I1035	probable tail tube protein	-2.92	5.14E-04
VSAL_p320_19	putative antirestriction protein	-3.02	1.97E-04
VSAL_p320_20	putative membrane protein	-3.22	2.22E-04
VSAL_I3102s	VSsrna22 small RNA RyhB	-3.23	3.08E-04
VSAL_p43_02	\acetyltransferase\""	-3.25	1.97E-04
VSAL_p320_31	\putative phage intergrase\""	-3.32	3.71E-04
VSAL_I1040	hypothetical protein, putative phage gene	-3.33	3.13E-04
VSAL_I3072r	23S rRNA 23S rRNA undefined product 90756:93646 forward	-3.37	1.04E-03
VSAL_I3144t	tRNA tRNA transfer RNA-Leu 842679:842760 reverse	-3.46	8.25E-04
VSAL_I1751	tonB1 TonB protein (pseudogene)	-3.56	3.71E-04
VSAL_p54_01	acyltransferase	-3.58	4.98E-04
VSAL_I0137	TonB-dependent iron-siderophore receptor precursor	-3.58	3.08E-04
VSAL_I3157t	tRNA-Ser tRNA-Ser undefined product 1120410:1120497 reverse	-3.64	3.13E-04
VSAL_I1039	probable exported protein, putative phage gene	-3.72	2.49E-04
VSAL_I3073r	5S rRNA 5S rRNA undefined product 93740:93859 forward	-3.82	7.53E-04
VSAL_I0134	L-2,4-diaminobutyrate decarboxylase	-3.84	2.49E-04
VSAL_p43_01	replication initiation protein	-3.92	1.24E-04
VSAL_p54_02	putative mobilization protein	-3.93	3.71E-04
VSAL_I0135	alcA siderophore biosynthetis protein	-4.04	3.71E-04
VSAL_I3104s	VSsrna24 small RNA sRNA2	-4.10	2.97E-04
VSAL_I1027	gpO phage capsid scaffolding protein	-4.10	3.71E-04
VSAL_I4155s	VSsrna156 VSsrna156 undefined product 2696120:2696308 forward	-4.20	6.14E-04
VSAL_I0136	iucC siderophore biosynthesis protein	-4.42	1.97E-04
VSAL_I4069s	VSsrna70 VSsrna70 undefined product 1049106:1049299 forward	-4.72	4.98E-04
VSAL_I1029	gpM phage terminase, endonuclease subunit	-5.02	1.24E-04

VSAL_I0985	mrdA penicillin-binding protein 2	-5.06	2.49E-04
VSAL_II2002s	VSAsrna3 VSAsrna3 undefined product 31834:32043 reverse	-5.12	1.46E-04
VSAL_p320_13	putative peptidase, S24-like	-5.16	2.49E-04
VSAL_I1028	gpN major capsid protein	-5.17	1.46E-04
VSAL_I3178s	VSsrna149 tmRNA	-5.19	1.24E-04
VSAL_I3103s	VSsrna23 small RNA Spot 42	-5.60	1.24E-03
VSAL_I0768	hypothetical protein, putative phage gene	-5.62	1.24E-04
VSAL_II0612	HTH-type transcriptional regulator, LysR family (pseudogene)	-5.71	1.24E-04