LitR directly upregulates autoinducer synthesis and luminescence in *Aliivibrio logei*

Sergey Bazhenov¹,², Olga Melkina³,⁴, Vadim Fomin¹, Ekaterina Scheglova¹, Pavel Krasnik¹, Svetlana Khrulnova¹,⁵, Gennadii Zavilgelsky³ and Ilya Manukhov¹,²,³

¹Laboratory for Molecular Genetics, Moscow Institute of Physics and Technology, Dolgoprudny, Russia
²Higher School of Economics, Moscow, Russia
³State Research Institute of Genetics and Selection of Industrial Microorganisms of the National Research Center “Kurchatov Institute”, Moscow, Russia
⁴State Research Institute of Genetics and Selection of Industrial Microorganisms of the National Research Centre “Kurchatov Institute”, Kurchatov Genomic Center, Moscow, Russia
⁵National Research Center for Hematology, Moscow, Russia

ABSTRACT

LitR is a master-regulator of transcription in the *ainS/R* and *luxS/PQ* quorum sensing (QS) systems of bacteria from *Vibrio* and *Aliivibrio* genera. Here, we for the first time directly investigated the influence of LitR on gene expression in the *luxI/R* QS system of psychrophilic bacteria *Aliivibrio logei*. Investigated promoters were fused with *Photobacterium phosphoreum luxCDABE* reporter genes cassette in a heterological system of *Escherichia coli* cells,litR A. *logei* was introduced into the cells under control of P<sub>lac</sub> promoter. LitR has been shown to upregulate genes of autoinducer synthase (*luxI*), luciferase and reductase (*luxCDABE*), and this effect doesn’t depend on presence of *luxR* gene. To a much lesser degree, LitR induces *luxR1* but not the *luxR2* — the main *luxI/R* regulator. Enhanced litR expression leads to an increase in a LuxI-autoinducer synthesis and a subsequent LuxR-mediated activation of the *luxI/R* QS system. Effect of LitR on *luxI* transcription depends on *lux*-box sequence in *luxI* promoter even in absence of *luxR* (*lux*-box is binding site of LuxR). The last finding indicates a direct interaction of LitR with the promoter in the *lux*-box region. Investigation of the effect of LitR A. *logei* on *luxI/R* QS systems of mesophilic *Aliivibrio fischeri* and psychrophilic *Aliivibrio salmonicida* showed direct *luxR*-independent upregulation of *luxI* and *luxCDABE* genes. To a lesser degree, it induces *luxR A. fischeri* and *luxR1 A. salmonicida*. Therefore, we assume that the main role of LitR in cross-interaction of these three QS systems is stimulating the expression of *luxI*.

INTRODUCTION

In *lux* regulons of some *Aliivibrio* bacteria, such as *Aliivibrio fischeri*, the regulatory gene *luxR* and structural genes *luxICDABE* are divergently transcribed from a pair of promoters situated between them; the main regulatory operator is *lux*-box (the site of LuxR...
binding) (Devine, Countryman & Baldwin, 1988; Devine et al., 1989; Meighen, 1991; Stevens, Dolan & Greenberg, 1994). Structures of lux regulons of mesophilic and psychrophilic bacteria of the Aliivibrio genus significantly differ: lux regulons of psychrophilic ones, Aliivibrio logei and Aliivibrio salmonicida, are divided into two groups of genes: luxR1-luxCDABEG and luxR2-luxI (Fig. 1) (Fidopiastis, Sørum & Ruby, 1999; Khrulnova et al., 2010; Manukhov et al., 2011).

luxR and luxI genes constitute a quorum sensing (QS) system. luxI-luxR system provides regulation of the expression of lux regulon genes (cell luminescence control) in dependence on cell culture density (Nealson & Hastings, 1979; Fuqua, Winans & Greenberg, 1994; Zavilgelsky & Manukhov, 2001). There are several proteins, which can modulate this QS system’s work. H-NS and CRP regulate the transcription of luxR genes (Dunlap & Greenberg, 1988; Ulitzur et al., 1997; Melkina, Goryanin & Zavilgelsky, 2017; Melkina et al., 2019). The content of the native protein LuxR is post translationally regulated by GroEL/ES chaperons and the Lon protease (Dolan & Greenberg, 1992b; Zavil’gel’skii & Manukhov, 1994; Manukhov, Kotova & Zavil’gel’skii, 2006; Manukhov et al., 2010), in psychrophilic bacteria it is so for the LuxR2 protein, but not the LuxR1 (Khrulnova et al., 2016; Konopleva et al., 2016).

LitR in A. fischeri, being the master regulator of the ainS/R and luxS/PQ QS systems, can positively regulate the luxI/R system by activation of a luxR transcription (Fidopiastis et al., 2002; Miyashiro & Ruby, 2012). The deletion of the litR gene in A. salmonicida leads to an autoinducer (AI, 3-oxo-C6-homoserine lactone) concentration decrease detected at late growth phase (Hansen et al., 2015). It is known that Vibrio harveyi LuxR, which is a close homologue to LitR from A. fischeri, A. logei and A. salmonicida, does not require binding to signaling molecules (Fidopiastis et al., 2002; Rutherford et al., 2011).

In dependence on its own concentration in the cell V. harveyi LuxR binds to the DNA in promoter regions and activates transcription by a direct interaction with RNA-polymerase (Rutherford et al., 2011; Van Kessel et al., 2013). The concentration of LitR is an object of LuxO-mediated regulation by the luxS/PQ and ainS/R QS systems and it increases when these QS pathways are activated (Miyashiro et al., 2010; Rutherford et al., 2011; Van Kessel et al., 2013).
Thus, the effect of LitR enhanced expression on the lux promoters after activation of ainS/R and luxS/PQ QS systems was investigated in mesophilic bacteria A. fischeri, but not the psychrophilic ones (A. logei, A. salmonicida), which have other lux regulon structure. Previously it was supposed that A. salmonicida LitR could activate the luxR1, luxR2 and luxI genes promoters (Khider et al., 2019), but it was not tested experimentally. The aim of this study was to determine whether there is a direct effect of litR on the expression of lux regulon of psychrophilic bacteria A. logei and A. salmonicida, and, if there is such an effect, to determine the mechanism of the effect of litR on proteins or promoters of lux regulon. The effect of A. logei LitR on the expression of regulatory luxR1 and luxR2 and structural luxCDABEG and luxI genes from A. logei and A. salmonicida was investigated in a heterologous model on Escherichia coli cells. Promoters of the A. fischeri lux genes were used as a control.

MATERIALS AND METHODS

Bacterial strains and plasmids
All experiments were conducted with the E. coli MG1655 F−, rph−1 strain (Guyer et al., 1981), which were obtained from VKPM collection (Russia). Plasmids used in this study are listed in Table 1 and illustrated in Fig. S1. Primers and a detailed description of plasmids constructed in this study are presented in a Supplemental File (see Plasmid constructions). Genes and promoters of Aliivibrio bacteria were from A. fischeri MGU-6, A. logei KCh1 (Khrulnova et al., 2010) and A. salmonicida NCIMB 2262T (provided by Jesus L. Romalde (Egidius, Wiik & Andersen, 1986)).

Culture media and growth conditions
Bacteria were grown in Luria-Bertani (LB) broth. The liquid LB medium was composed of 1% tryptone, 0.5% yeast extract and 0.5% NaCl, for the preparation of solid medium, agar was added to a final concentration of 1.5% m/v. The medium was supplemented with appropriate antibiotics: 100 μg/ml ampicillin, 10 μg/ml tetracycline, or their composition. Overnight cultures were grown at 37 °C with continuous agitation and then were used to inoculate liquid LB. The resulting cultures were grown at 22–25 °C with continuous agitation (200 rpm). The optical density (OD) of cell suspensions was measured with a KFK-3 photometer (Zagorsk Optical-Mechanical Plant, Russia). An induction of the litR gene expression (plasmid p15Tc-litR) was made at the early exponential growth phase (OD about 0.1) by addition of IPTG (isopropyl β-D-1-thiogalactopyranoside) to a final concentration of one mM.

DNA isolation, restriction, ligation and transformation
Plasmid DNA was isolated by alkaline lysis. Cell transformation with hybrid plasmids followed standard protocols. Endonuclease restriction, DNA fragment ligation, agarose gel electrophoresis and isolation of DNA fragments from agarose gel were performed according to Green & Sambrook (2012). Restriction and ligation reactions were carried out...
using enzymes from Promega (USA): EcoRI, BamHI and KpnI restriction enzymes and T4 DNA ligase were used for constructing new plasmids (see Supplemental File for details).

### Chemical substances

Isopropyl β-D-1-thiogalactopyranoside (IPTG) was from Anatrace (USA).

### Measurement of bioluminescence

Bioluminescence intensities were measured in volume of 200 μl in plastic microtubes placed in front of a photomultiplier photocathode at room temperature using Biotox-7MB (BioPhisTech, Russia) or in 96-well plates using SynergyHT (Biotek Instruments, Winooski, VT, USA). Luminescence values were expressed in relative light units (RLU).

### Determination of LitR-dependent promoter regulation

The effect of LitR on the expression of genes from lux regulons of the *Aliivibrio* genus bacteria was investigated for three species: *A. fischeri*, *A. logei* and *A. salmonicida*. For this purpose we used the heterologous system of *E. coli* cells transformed with different biosensor plasmids from a set (Table 1, Fig. S1) that carry luxCDABE genes of *P. luminescens* transcriptionally fused with promoters of interest. To introduce the litR gene from *A. logei* into this system, the p15Tc-litR plasmid was constructed. It comprises a p15 origin, the *litR A. logei* gene under a lac promoter, and a *lacI* gene, which lowers the base transcription from P* lac* and allows regulating litR with IPTG.

| Plasmid            | Relevant characteristics                                                                 | Source or References       |
|--------------------|------------------------------------------------------------------------------------------|---------------------------|
| p15Tc-lac         | Vector for cloning of regulatory gene under control of P* lux*; p15 origin, lacI, Tc*     | Bazhenov et al. (2021)    |
| p15Tc-litR        | *litR* gene of *A. logei* under control of P* lux*; p15 origin, lacI, Tc*                | This study                |
| pDEW201           | Promoter probe vector with promoterless operon *Photorhabdus luminescens luxCDABE* genes and the replication origin of pBR322; Ap* | Van Dyk & Rosson (1998)   |
| pDEW201-derivative biosensor plasmids with luxCDABE-reporter for promoters investigation: |                                            |                           |
| pALR1             | lux-reporter for A. logei P* luxR1 + luxR1                                              | Melkina et al. (2019)     |
| pDewP2rev         | lux-reporter for A. logei P* luxR2                                                       | This study                |
| pIVA              | lux-reporter for A. logei P* luxCDABEG + luxR1                                           | Khrul'nova et al. (2011)  |
| pSV16             | lux-reporter for A. logei P* lux + luxR2                                                 | Khrul'nova, Manukhov & Zavil'gel'skii (2011) |
| pAS1              | lux-reporter for A. salmonicida P* luxCDABEG + luxR1                                     | This study                |
| pAS2              | lux-reporter for A. salmonicida P* lux + luxR2                                            | This study                |
| pR2               | lux-reporter for A. logei P* lux without luxR2                                            | Bazhenov et al. (2021)    |
| pAFR              | lux-reporter for A. fischeri P* lux + luxR                                                | Melkina et al. (2019)     |
| pVFR1             | lux-reporter for A. fischeri P* luxCDABEG + luxR                                        | Manukhov, Kotova & Zavil'gel'skii (2006) |
| pD-lb1            | lux-reporter for A. logei P* luxCDABEG                                                    | This study                |
| pD-lb2            | lux-reporter for A. logei P* luxCDABEG with changed lux-box (matches lux-box of luxI promoter) | This study                |

**Note:**

Ap*, ampicillin resistant; Tc*, tetracycline resistant.

**Table 1** List of plasmids, which were used in this study.
Luminescence induction factor calculation

The culture of *E. coli* cells carrying the *A. logei* litR gene under the IPTG-inducible promoter on the p15Tc-litR plasmid and the *P. luminescens* luxCDABE genes under control of the promoter of interest on a biosensor plasmid was grown to OD~0.1 in liquid LB medium, then divided into two equal parts: “control” and “induced”. The “induced” portion was supplemented with one mM IPTG. Further, the cultures were grown under equal conditions with periodic measurements of optical density and luminescence. The induction factor is the induced/control ratio of cell cultures’ luminescence. All figures show representative kinetic curves of biological independent triplicates.

Statistics

Error bars at the graphs are the SD of three independent experiment replications.

Computer analysis

Amino acid sequences alignment was performed using the Vector NTI software (Thermo Fisher Scientific, Waltham, MA, USA). The intrinsic disorder of proteins was predicted by their aa sequences with “Predictor of Naturally Disordered Regions” (pondr.com), one can find description of VL-XT, VSL2, VL3 and Charge-Hydropathy algorithms in Obradovic et al. (2003).

RESULTS

Effect of LitR *A. logei* on promoters of regulatory genes *luxR*, *luxR1* and *luxR2*

Firstly, the effect of LitR on the expression of *luxR* *A. fischeri*, *luxR1* *A. logei* and *luxR2* *A. logei* was investigated. Figure 2 shows the coefficient of increase in luminescence of biosensors after the activation of the litR gene expression.

The data presented in Fig. 2 shows that LitR from *A. logei* is capable of a significant activation of the luxR *A. fischeri* gene transcription. This result is consistent with previous work (Fidopiastis et al., 2002) and means that the LitR homologues from *A. fischeri* and *A. logei* are interchangeable and have a common function. The amino acid sequence of LitR is highly conservative among the *Aliivibrio* species. In particular, LitR proteins from psychrophilic bacteria have almost the same sequences, comparing of them with mesophilic LitR *A. fischeri* gives that DNA-binding regions three compared proteins are identical and whole “helix-turn-helix” domain differs only by several aa substitutions (Fig. S2). Interesting that psychrophilic LitR variants are more ordered than mesophilic one according to analysis with VL-XT, VSL2, VL3 and Charge-Hydropathy instruments at pondr.com (Fig. S3). The increase in LitR content in the cell activates the expression of luxR1 *A. logei*, although to a lesser extent than that of *A. fischeri luxR*, and does not affect the expression of the luxR2 *A. logei* gene. The obtained result was in conflict with the data of Norwegian colleagues on a decrease in the production of an autoinducer in *A. salmonicida* litR mutant cells (Hansen et al., 2015); therefore, it was decided to test the effect of litR on the transcription of the “rightward” promoters of lux regulon.
Effect of LitR \textit{A. logei} on promoters of structural genes \textit{luxICDABEG}

Results of investigation of LitR \textit{A. logei} effect on expression of autoinducer synthase (\textit{luxI}), luciferase and reductase (\textit{luxCDABE}) genes are given in \textit{Fig. 3}. Promoters of different \textit{Aliivibrio} species were tested with LitR \textit{A. logei} in the heterologous system of \textit{E. coli} cells.

In response to the addition of one mM IPTG at OD~0.1 there was an increase in luminescence of \textit{E. coli} MG1655 cells carrying combinations of the plasmid p15Tc-litR (\textit{litR} under \textit{P}_{\text{luxI}} \textit{A. logei} lux-reporter, \textit{P}_{\text{luxCDABEG}} \textit{A. logei} lux-reporter, \textit{P}_{\text{luxCDABEG}} \textit{A. salmonicida} lux-reporter, and \textit{P}_{\text{luxCDABEG}} \textit{A. salmonicida} lux-reporter (\textit{Table 1}, \textit{Fig. S1}).

As can be seen from the graphs, LitR activates the transcription from the promoters of the \textit{luxI} and \textit{luxCDABEG} genes of both psychrophilic luminescent bacteria species \textit{A. logei} and \textit{A. salmonicida}. It should be noted that in both cases the activation of the \textit{luxI} gene promoter in response to an increased expression of the \textit{litR} gene was somewhat stronger than the activation of the \textit{luxCDABEG} gene promoter. It can be seen that the amplitude of activation of \textit{A. salmonicida} promoters is almost twice lower than that of \textit{A. logei} promoters. This can be explained by the influence of other regulatory elements. These promoters are still the subject of research, but it is already known that their regulation is influenced by many factors, such as Lon, GroEL/ES, H-NS, CRP. We assume that the
observed twofold difference may be due to differences in the sequences of promoters and surrounding regions, although the lux-box elements for these species are identical.

The data from Fig. 3C indicates that the induction of litR A. logei in the heterologous system of E. coli leads to a notable (more than one order of magnitude) activation of expression from the A. fischeri P_luxICDABEG promoter. This effect has not been previously described and may indicate that litR is able to induce LuxI/R QS system through stimulation of both luxI and luxR gene expressions in A. fischeri cells. Moreover, the effect on luxI expression was higher in magnitude. The data obtained contradicts the results

Figure 3 The induction of promoters P_luxI and P_luxICDABEG of A. logei (A), A. salmonicida (B) and A. fischeri (C) by enhancement of the litR A. logei expression in E. coli cells. Curves represent the increase in luminescence of E. coli MG1655 cells carrying the luxCDABE genes of P. luminescens under control of (A) P_luxI A. logei (pSV16), P_luxICDABEG A. logei (pIVA), (B) P_luxI A. salmonicida (pAS2), P_luxICDABEG A. salmonicida (pAS1), or (C) P_luxICDABEG A. fischeri (pVFR1) promoters in combination with the litR A. logei gene under control of P_lac (p15Tc-litR) in response to the addition of one mM IPTG at OD=0.1. The ”−litR” curve corresponds to a negative control-analogous cell lines, which lacks the litR gene (p15Tc-lac vector used).
published by (Fidopiastis et al., 2002), which investigated the effect of litR on the expression of lacZ of the gene inserted into luxC ORF. Perhaps it could be connected with the differences in the sensitivity of the reporter genes used. However, LitR proteins from mesophilic and psychrophilic bacteria have some differences and it needs more detailed investigation.

**LuxR-independent activation of promoters by LitR depends on the lux-box sequence**

To make sure that the observed activation of “rightward” promoters determined by LitR occurs independently of the presence of regulatory genes from the luxI/R QS system in the cell, measurements of LitR-dependent activation of A. logei luxI gene transcription were carried out in the presence of the luxR2 regulatory gene and without it. Plasmids pSV16 and pR2 were used (Table 1, Fig. S1), in which the luxCDABE P. luminescens genes are under control of the A. logei P_{luxI} promoter with and without the luxR2 gene, respectively. The litR gene was introduced as before on a separate plasmid p15Tc-litR. Measurement results are shown in Fig. 4A, the increase in luminescence was calculated as the ratio of luminescence of IPTG-induced cells to the luminescence of the same cells without IPTG. As a negative control, cells carrying the p15Tc-lac vector without the litR gene were used.

We assumed that LitR could bind DNA in the promoter upstream region, the same site LuxR binds to (lux-box). To assess the effect of the lux-box sequence on the transcription activation of A. logei P_{luxCDABEG} by the LitR protein, we compared the LitR-dependent activation of two variants of the A. logei P_{luxCDABEG} promoter, which differ only in the lux-box sequences (Fig. 4B). The native P_{luxCDABEG} promoter was truncated exactly upstream of the lux-box and introduced into pDEW201 upstream of P. luminescens luxCDABE gene cassette (line lb1 on Fig. 4B, the pD-lb1 plasmid was used); to obtain another promoter variant, the lux-box was changed to match P_{luxI} (lb2 lines on Fig. 4B, the pD-lb2 plasmid was used). The difference between the obtained chimeric promoters is limited to only five bp (Fig. S4), in both cases, the cells don’t contain any of the luxR, luxR1 or luxR2 genes. All genetic elements, with the exception of lux-boxes, were identical in the compared cultures. The litR gene, as in previous experiments, was introduced into the cell on the p15Tc-litR plasmid, where it was under control of P_{lac}.

As can be seen from the data presented in Fig. 4A, LitR activates P_{luxI} and P_{luxCDABEG} A. logei promoters regardless of the presence of LuxR2 in the cells: luminescence induction in response to litR expression stimulation is almost equal for cells with luxR2 gene and without it (p15Tc-litR in combination with pSV16 and pR2, correspondently). The observed induction of P_{luxI} and P_{luxCDABEG} A. logei and consequently luminescence of cells is completely determined by the litR gene: in its absence (vector p15Tc-lac in combination with pSV16, pR2, pDIb1, or pD-lb2 biosensor plasmids), the luminescence of cells does not change in response to the addition of IPTG. The data in Fig. 4B shows that the change in the lux-box sequence affects the activation of the promoter by LitR. This indicates that the LitR binding site in the promoter region of A. logei luxCDABEG genes coincides or at least intersects with the LuxR binding site.
DISCUSSION

The luxR-luxI regulon could be used for development of expression systems with target protein expression regulation in dependence on bacterial population density (Swennen & Nocadello, 2011; Nocadello & Swennen, 2012). luxI-luxR regulation is modulated by a large number of intracellular factors that ensure its sensitivity to external conditions (Manukhov, Kotova & Zavil’gelskii, 2006; Konopleva et al., 2016), LitR becomes on a par with them, giving a connection with other QS systems (Fidopiastis et al., 2002).

Our experiments showed that promoters of luxI and luxCDABEG genes of all three investigated species of the Aliivibrio genus are activated by LitR from A. logei. Apparently, the interaction between LuxR and LitR proteins does not occur, since Fig. 4 shows the absence of the effect of the luxR2 gene on litR-mediated activation of the luxI gene promoter. That is, the interaction between litR and the lux operon is realized by the direct influence of LitR on promoters. In the psychrophilic species A. logei and A. salmonicida, the induction of promoters from which the luxI gene is transcribed is notably higher than induction of the other promoters of their lux regulons. As LuxI is an autoinducer synthase, its expression induction could stimulate whole QS system. The expression of luxCDABEG genes increases in the presence of LitR too, but the amplitude of this induction is too low to make Aliivibrio cell luminescence visible. Thus, we assume that the main effect of LitR on lux regulons of psychrophilic bacteria of the Aliivibrio genus is the independent of LuxR stimulation of LuxI synthesis (Figs. 3 and 4A). When ains/R and luxS/PQ QS systems triggered before the luxI/R one due to stress conditions, stimulation of LuxI synthesis by LitR brings forth the increase in AI production and gives an initial impulse to activate luxI/R system. Higher concentrations of AI stabilize LuxR2 from A. logei/A. salmonicida, and the luxI/R QS system is triggered (Khrulnova et al.,...
This LitR-dependent luxI induction could appear necessary in stress conditions, when the chaperonin GroEL/ES lacks or the protease Lon content is increased and consequently the QS is not activated even at a high culture density due to the lack of functional LuxR2 (Dolan & Greenberg, 1992a; Zavil’gel’skii & Manukhov, 1994; Khrulnova et al., 2010, 2016).

The LitR binding site is not defined well and is still an object of discussion (Van Kessel et al., 2013; Chaparian et al., 2016). Here we showed that the sequence of lux-boxes has significance for the transcription activation of luxICDABEG by LitR regardless of the presence of luxR1 or luxR2 genes in the cell (Fig. 4). It is an obvious sign that LitR binds to the lux-box or directly next to it.

**CONCLUSION**

The results of this study demonstrated that LitR of psychrophilic bacteria A. logei is able to directly stimulate (independently of LuxR presence) transcription from promoters of luxI.
and luxCDABEG genes and to a lesser degree from promoter of luxRI gene. Thus, 3-OH-C10 and AI-2 in the medium induce expression of litR and could enhance luxI expression and 3-O-C6 synthesis (Fig. 5). But for the effect of 3-OH-C10 and AI-2 on luxI/R system to be significant, the conditions are needed preventing this system from normal function. Our finding could be a clue to understanding the cross-interaction of the luxS/PQ and ains/R QS systems with the luxI/R one in psychrophilic bacteria of A. logei and A. salmonicida species. Such effect was not previously described for the luxI/R QS system of well-known mesophilic bacteria A. fischeri. Main effect of this cross-interaction is LitR-dependent stimulation of autoinducer synthesis through PluxI induction and this pathway could be activated in conditions, when luxI/R QS system was not activated by its own AI until high culture density.

ADDITIONAL INFORMATION AND DECLARATIONS

Funding
The work of Sergey Bazhenov, including biosensor constructing and investigation, was funded by RFBR, project number 19-34-90020. The work of Svetlana Khrulnova, including strategy for constructing of biosensor plasmids and assessment of effectiveness of constructed biosensors for further research and applications, was supported by RFBR, project number 20-34-70132. The work of Ilya Manukhov, project management and conceptualization, was supported by the Ministry of Science and Higher Education of the Russian Federation (agreement # 075-00337-20-03, project FSMG-2020-0003). The work of Olga Melkina, including the help in the constructing of biosensor plasmids, was supported by the Ministry of Science and Higher Education of the Russian Federation (075-15-2019-1658). The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

Grant Disclosures
The following grant information was disclosed by the authors:
RFBR: 19-34-90020 and 20-34-70132.
Ministry of Science and Higher Education of the Russian Federation: agreements # 075-00337-20-03 (project FSMG-2020-0003) and #075-15-2019-1658.

Competing Interests
The authors declare that they have no competing interests.

Author Contributions
- Sergey Bazhenov conceived and designed the experiments, performed the experiments, analyzed the data, prepared figures and/or tables, authored or reviewed drafts of the paper, and approved the final draft.
- Olga Melkina conceived and designed the experiments, authored or reviewed drafts of the paper, and approved the final draft.
- Vadim Fomin performed the experiments, authored or reviewed drafts of the paper, and approved the final draft.
Ekaterina Scheglova performed the experiments, authored or reviewed drafts of the paper, and approved the final draft.

Pavel Krasnik performed the experiments, authored or reviewed drafts of the paper, and approved the final draft.

Svetlana Khrulnova conceived and designed the experiments, authored or reviewed drafts of the paper, and approved the final draft.

Gennadii Zavilgelsky conceived and designed the experiments, authored or reviewed drafts of the paper, and approved the final draft.

Ilya Manukhov conceived and designed the experiments, analyzed the data, prepared figures and/or tables, authored or reviewed drafts of the paper, and approved the final draft.

Data Availability
The following information was supplied regarding data availability:

The raw measurements of luminescence are available in the Supplementary File.

Supplemental Information
Supplemental information for this article can be found online at http://dx.doi.org/10.7717/peerj.12030#supplemental-information.

REFERENCES
Bazhenov S, Novoyatlova U, Scheglova E, Fomin V, Khrulnova S, Melkina O, Chistyakov V, Manukhov I. 2021. Influence of the luxR regulatory gene dosage and expression level on the sensitivity of the whole-cell biosensor to acyl-homoserine lactone. Biosensors 11(6):166 DOI 10.3390/bios11060166.

Bjelland AM, Sørum H, Tegegne DA, Winther-Larsen HC, Willassen NP, Hansen H. 2012. LitR of Vibrio salmonicida is a salinity-sensitive quorum-sensing regulator of phenotypes involved in host interactions and virulence. Infection and Immunity 80:1681–1689 DOI 10.1128/IAI.06038-11.

Chaparian RR, Olney SG, Hustmyer CM, Rowe-Magnus DA, Van Kessel JC. 2016. Integration host factor and LuxR synergistically bind DNA to coactivate quorum-sensing genes in Vibrio harveyi. Molecular Microbiology 101(5):823–840 DOI 10.1111/mmi.13425.

Devine JH, Countryman C, Baldwin TO. 1988. Nucleotide sequence of the luxR and luxI genes and structure of the primary regulatory region of the lux regulon of Vibrio fischeri ATCC 7744. Biochemistry 27(2):837–842 DOI 10.1021/bi00402a052.

Devine JH, Shadel GS, Baldwint TO, Heckel R, Johnston TC, Lin J-W. 1989. Identification of the operator of the lux regulon from the Vibrio fischeri strain ATCC7744 (bioluminescence/ regulation/autoinduction/repression/activator). Proceedings of the National Academy of Sciences of the USA 86:5688–5692.

Dolan KM, Greenberg EP. 1992a. Evidence that GroEL, not σ32, is involved in transcriptional regulation of the Vibrio fischeri luminescence genes in Escherichia coli. Journal of Bacteriology 174(15):5132–5135 DOI 10.1128/jb.174.15.5132-5135.1992.

Dolan KM, Greenberg EP. 1992b. Evidence that GroEL, not sigma 32, is involved in transcriptional regulation of the Vibrio fischeri luminescence genes in Escherichia coli. Journal of Bacteriology 174(15):5132–5135 DOI 10.1128/jb.174.15.5132-5135.1992.
Dunlap PV, Greenberg EP. 1988. Control of *Vibrio fischeri* lux gene transcription by a cyclic AMP receptor protein-LuxR protein regulatory circuit. *Journal of Bacteriology* **170**(9):4040–4046 DOI 10.1128/jb.170.9.4040-4046.1988.

Egidius E, Wiik R, Andersen K. 1986. *Vibrio salmonicida* sp. nov., a new fish pathogen. *International Journal of Systematic Bacteriology* **36**(4):518–520 DOI 10.1099/00207713-36-4.518.

Fidopiastis PM, Miyamoto CM, Jobling MG, Meighen EA, Ruby EG. 2002. LitR, a new transcriptional activator in *Vibrio fischeri*, regulates luminescence and symbiotic light organ colonization. *Molecular Microbiology* **45**(1):131–143 DOI 10.1046/j.1365-2958.2002.02996.x.

Fidopiastis PM, Sørum H, Ruby EG. 1999. Cryptic luminescence in the cold-water fish pathogen *Vibrio salmonicida*. *Archives of Microbiology* **171**(3):205–209 DOI 10.1007/s002030050700.

Fuqua WC, Winans SC, Greenberg EP. 1994. Quorum sensing in bacteria: the LuxR–LuxI family of cell density-responsive transcriptional regulators. *Journal of Bacteriology* **176**(2):269–275 DOI 10.1128/jb.176.2.269-275.1994.

Green MR, Sambrook J. 2012. *Molecular cloning: a laboratory manual*. New York: Cold Spring Harbor Laboratory Press.

Guyer MS, Reed RR, Steitz JA, Low KB. 1981. Identification of a sex-factor-affinity site in *E. coli* as gamma delta. *Cold Spring Harbor Symposia on Quantitative Biology* **45**:135–140 DOI 10.1101/sqb.1981.045.01.022.

Hansen H, Purohit AA, Leiros HKS, Johansen JA, Kellermann SJ, Bjelland AM, Willassen NP. 2015. The autoinducer synthases LuxI and AinS are responsible for temperature-dependent AHL production in the fish pathogen *Aliivibrio salmonicida*. *BMC Microbiology* **15**(1):1–13 DOI 10.1186/s12866-015-0402-z.

Khider M, Hansen H, Hjerde E, Johansen JA, Willassen NP. 2019. Exploring the transcriptome of luxI and dains mutants and the impact of n-3-oxo-hexanoyl-l- and n-3-hydroxydecanoyl-l-homoserine lactones on biofilm formation in *Aliivibrio salmonicida*. *PeerJ* **7**:e6845 DOI 10.7717/peerj.6845.

Khrul’nova SA, Manukhov IV, Zaval’gel’skii GB. 2011. “Quorum sensing” regulation of lux gene expression and the structure of lux operon in marine bacteria *Aliivibrio logei*. *Russian Journal of Genetics* **47**:1596–1603.

Khrulnova SA, Baranova A, Bazhenov SV, Goryanin II, Konopleva MN, Maryshev IV, Salykhovala AI, Vasilyeva AV, Manukhov IV, Zavilgelsky GB. 2016. Lux-operon of the marine psychrophilic bacterium *Aliivibrio logei*: a comparative analysis of the LuxR1/LuxR2 regulatory activity in *Escherichia coli* cells. *Microbiology* **162**(4):717–724 DOI 10.1099/mic.0.000253.

Khrulnova SA, Manukhov IV, Zarubina AP, Zavilgelsky GB. 2010. *Aliivibrio logei* KCh1 (Kamchatka Isolate): biochemical and bioluminescence characteristics and cloning of the lux operon. *Microbiology* **79**(3):349–355 DOI 10.1134/S0026261710030112.

Konopleva MN, Khrulnova SA, Baranova A, Ekimov LV, Bazhenov SV, Goryanin II, Manukhov IV. 2016. A combination of luxR1 and luxR2 genes activates Pr-promoters of psychrophilic *Aliivibrio logei* lux-operon independently of chaperonin GroEL/ES and protease Lon at high concentrations of autoinducer. *Biochemical and Biophysical Research Communications* **473**(3):1158–1162 DOI 10.1016/j.bbrc.2016.04.032.

Manukhov IV, Khrul’nova SA, Baranova A, Zavilgelsky GB. 2011. Comparative analysis of the lux operons in *Aliivibrio logei* KCh1 (a Kamchatka Isolate) and *Aliivibrio salmonicida*. *Journal of Bacteriology* **193**(15):3998–4001 DOI 10.1128/JB.05320-11.

Manukhov IV, Kotova VI, Zavil’gel’skii GB. 2006. Host factors in the regulation of the *Vibrio fischeri* lux operon in *Escherichia coli* cells. *Mikrobiologia* **75**:525–531.
Manukhov IV, Melkina OE, Goryanin II, Baranova AV, Zavilgelsky GB. 2010. The N-terminal domain of Aliivibrio fischeri LuxR is a target of the GroEL chaperonin. Journal of Bacteriology 192(20):5549–5551 DOI 10.1128/JB.00754-10.

Meighen EA. 1991. Molecular biology of bacterial bioluminescence. Microbiological Reviews 55(1):123–142 DOI 10.1128/mmbr.55.1.123-142.1991.

Melkina OE, Goryanin II, Bazhenov SV, Manukhov IV, Zavilgelsky GB. 2019. Comparative analysis of Aliivibrio logei luxR1 and luxR2 genes regulation in Escherichia coli cells. Archives of Microbiology 201(10):1415–1425 DOI 10.1007/s00203-019-01691-3.

Melkina OE, Goryanin II, Zavilgelsky GB. 2017. Histone-like protein H-NS as a negative regulator of quorum sensing systems in gram-negative bacteria. Genetika 53:165–172.

Miyashiro T, Ruby EG. 2012. Shedding light on bioluminescence regulation in Vibrio fischeri. Molecular Microbiology 84(5):795–806 DOI 10.1111/j.1365-2958.2012.08065.x.

Miyashiro T, Wollenberg MS, Cao X, Oehlert D, Ruby EG. 2010. A single qrr gene is necessary and sufficient for LuxO-mediated regulation in Vibrio fischeri. Molecular Microbiology 77(6):1556–1567 DOI 10.1111/j.1365-2958.2010.07309.x.

Nealson KH, Hastings JW. 1979. Bacterial bioluminescence: its control and ecological significance. Microbiological Reviews 43(4):496–518 DOI 10.1128/mmbr.43.4.496-518.1979.

Nocadello S, Swennen EF. 2012. The new pLAI (lux regulon based auto-inducible) expression system for recombinant protein production in Escherichia coli. Microbial Cell Factories 11(1) DOI 10.1186/1475-2859-11-3.

Obradovic Z, Peng K, Vucetic S, Radiwojac P, Brown CJ, Dunker AK. 2003. Predicting intrinsic disorder from amino acid sequence. Proteins: Structure, Function and Genetics 53(S6):566–572 DOI 10.1002/prot.10532.

Rutherford ST, Van Kessel JC, Shao Y, Bassler BL. 2011. AphA and LuxR/HapR reciprocally control quorum sensing in vibrios. Genes and Development 25(4):397–408 DOI 10.1101/gad.2015011.

Stevens AM, Dolan KM, Greenberg EP. 1994. Synergistic binding of the Vibrio fischeri LuxR transcriptional activator domain and RNA polymerase to the lux promoter region. Proceedings of the National Academy of Sciences USA 91(26):12619–12623 DOI 10.1073/pnas.91.26.12619.

Swennen E, Nocadello S. 2011. Expression of recombinant proteins. US patent WO2010136897A3. Available at https://patents.google.com/patent/WO2010136897A3/en (accessed 6 September 2021).

Ulitzur S, Matin A, Fraley C, Meighen E. 1997. H-NS protein represses transcription of the lux systems of Vibrio fischeri and other luminous bacteria cloned into Escherichia coli. Current Microbiology 35(6):336–342 DOI 10.1007/s002849900265.

Van Dyk TK, Rosson RA. 1998. Photorhabdus luminescens luxCDABE promoter probe vectors. Methods in Molecular Biology 102:85–95 DOI 10.1385/0-89603-520-4:85.

Van Kessel JC, Ulrich LE, Zhulin IB, Bassler BL. 2013. Analysis of activator and repressor functions reveals the requirements for transcriptional control by LuxR, the master regulator of quorum sensing in Vibrio harveyi. mBio 4(4):e00378-13 DOI 10.1128/mBio.00378-13.

Zavil’gel’skii GB, Manukhov IV. 1994. Lon-protease participates in the regulation of transcription of the Lux-operator of Vibrio fischeri. Genetika 30:337–341.

Zavilgelsky GB, Manukhov IV. 2001. Quorum sensing, or how bacteria talk to each other. Molecular Biology 35(2):224–232 DOI 10.1023/A:1010439501530.