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EXPERIMENTAL ARTICLES

Aliivibrio logei KCh1 (Kamchatka Isolate): Biochemical and Bioluminescence Characteristics and Cloning of the *lux* Operon

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Abstract—A new strain of luminescent marine bacteria of the genus *Aliivibrio* (formerly *Vibrio*) was isolated from the intestines of a goby *Cottida* sp. (Sea of Okhotsk basin; the strain was marked as KCh1). The basic conditions of growth on laboratory media were determined for the strain. Strain KCh1 was shown to be a psychrophilic bacterium with an optimal growth temperature of approximately 15°C. The nucleotide sequence of the 16S rRNA gene was determined and shown to be almost identical to the 16S rRNA gene sequence of *Aliivibrio logei* and *A. salmonicida* but considerably different from that of *A. fischeri*. The biochemical characteristics (nitrate reduction, lysine decarboxylation, and D-galactose fermentation) of strain KCh1 matched those of *A. logei* and *A. fischeri* strains. An antibioticogram for strain KCh1 was determined. The *lux* operon of the strain was cloned in *Escherichia coli* cells and partially sequenced, the results show a high homology with the *lux* operon of *A. salmonicida*.

Key words: Aliivibrio logei, bioluminescence, *lux* operon.

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The luminescent marine bacteria *Aliivibrio logei* were initially characterized in 1978 [2], when they were described as a separate species of the genus *Vibrio* (now *Aliivibrio* [1]). Formerly, these bacteria were considered members of a psychrophilic subspecies of *A. fischeri* that grow at 4°C, but not at 30°C, unlike the standard *A. fischeri* strains, which do not grow at 4°C, but do grow at 30°C. Subsequently, *A. logei* were shown to be symbionts of squids, similarly to *A. fischeri*. However, the squids whose light organs (photophores) are inhabited by *A. fischeri* are largely representatives of the genus *Euprymna* found in hot water regions near the Hawaiian Islands and in the western part of the Pacific. By contrast, squids of the genus *Sepiola*, the sole host for the bacterium *A. logei*, dwell in the deeper and colder waters of the Atlantic [3]. As demonstrated in the same study, when compared by a range of biochemical characteristics (lysine decarboxylation, nitrate reduction, and D-galactose fermentation), *A. logei* appears to be similar to *A. fischeri*, while they differ considerably from *A. salmonicida*, a related species pathogenic to Atlantic salmon. However, comparative analysis of the variable 16S rRNA sites revealed their identity in *A. logei* and *A. salmonicida* and considerable differences between these fragments in *A. logei* and *A. fischeri* [3].

Here we report an investigation of a strain of luminescent marine bacteria KCh1, isolated from the intestines of a goby *Cottida* sp. (Sea of Okhotsk basin, Kamchatka).

MATERIALS AND METHODS

Bacterial strains and plasmids. Bacteria *Escherichia coli* TG-1 F$^-$ *thi hsd*Δ5, supE, Δ(*lac-pro*)/F'(*tra*D36 *pro*AB$^+$ *lac*I^{q}Z-M15); *E. coli* MC1061 F' *ara*D139 Δ(*ara leu*)769 *lac*X74 *gal*U *gal*K *hsd*R *mcr*B *rps*L *thi*.

A. fischeri strains MJ-1 and MGU-6 were obtained from the collection of Department of Microbiology, Biological Faculty, Moscow State University. We also used a vector pUC19 and a hybrid plasmid pVFR1—vector pR322 bearing the gene *lux*R and the *A. fischeri lux* operon regulatory region introduced into the vector before the *Photorhabdus luminiscens lux*CDABE cassette, the latter used as reporter genes [4].

Culturing. The luminescent marine bacteria were grown on SWT medium containing the following (%, wt/vol): tryptone, 0.5; yeast extract, 0.25; sea salt, 1.5; and glycerol, 0.3. *E. coli* were grown in LB broth. Solid media were prepared using 1.5% agar. The plasmid-bearing strains were grown on media supplemented with ampicillin (100 μg/ml).

Enzymes and chemical substances. The enzymes used were from Fermentas (Lithuania); *N*-3-oxyhexanoyl L-homoserine lactone (the autoinducer (AI)) and *n*-decanal (the substrate for luciferase) were from Sigma (United States).

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Table 1. The correlation between the growth of bacterial strains KCh1, *A. logei*, *A. fischeri*, and *A. salmonicida* and the cultivation temperature

Strain	Culture temperature		
	4°C	30°C	Optimal temperature, °C
KCh1	+	–	15
A. fischeri MJ-1	–	+	25
A. fischeri MGU-6	–	+	25
*A. logei**	+	–	15
A. salmonicida	+	–	15

"+" and "–" stand for positive and negative, respectively.
*Data for *A. logei* and *A. salmonicida* were taken from [3].

The biochemical tests were done with Kit no. 2 SIB for Intergenus and Interspecies Differentiation of Enterobacteria (13 tests).

Biochemical tests. In order to detect the specific reaction for nitrate reduction, the bacteria were grown in a liquid medium containing 0.5% peptone and 0.2% potassium nitrate. After 24-h incubation at 20°C, 0.5 ml of a 0.8% sulfanilic acid solution in 5 N acetic acid and 9.5 ml of a 0.6% α-naphthylamine solution in 5 N acetic acid (Griess reagent) was added to the suspension. Red coloration indicated nitrite formation.

Construction of antibioticograms. Antibioticograms were constructed using the standard technique, by placing discs on the agar surface inoculated with a young test culture.

DNA isolation. Isolation of the hybrid plasmids and vectors was carried out by the alkaline extraction method. The chromosomal DNA of *A. logei* was extracted from late-exponential-phase cells by treating them with lysozyme and SDS and subsequently with phenol, followed by reprecipitation in ethanol.

Restriction and ligation of DNA fragments, agarose gel electrophoresis, isolation of DNA fragments from the agarose gel by electroelution method, and transformation of calcium cells were performed according to [5].

Cloning of the *lux* operon genes. For the construction of the pSV1 plasmid containing a *lux* operon of *A. logei*, chromosomal DNA was restricted at the *Mbo*I sites (producing an incomplete restrict) and cloned into a vector pUC19 at the site *Bam*HI. For the construction of pSV2, the chromosomal DNA of *A. logei* and the vector pUC19 were restricted at the *Hin*dIII sites (Fig. 1).

The *Hin*dIII and *Bam*HI fragments of KCh1 were separated in agarose gel. A band of 5–10 kb was eluted from the gel and ligated into the vector pUC19. The transformed *E. coli* MC1061 cells were inoculated on an agarized ampicillin-containing (150 μg/ml) medium; the luminescent clones were selected visually (in the case of *n*-decanal, a solution of *n*-decanal was applied to the lid of the petri dish). One clone out of the total of approximately 2000 clones was selected that could produce luminescence in the absence of *n*-decanal. Three clones were selected that luminesced only in the presence of *n*-decanal.

Sequencing of 16S rRNA genes and of the *lux* operon. The DNA sequencing was performed using (di)deoxynucleoside triphosphates, according to Sanger et. al [6].

The following primers were used for the PCR amplification and sequencing of 16S rRNA:

REV16S salm 5'-AGCCGGTTTTGTTTCTGCCCTC-3'

Dir16S salm 5'-CAACCTTGGCAATCTGTGTGAACA-3'

Lux16SD 5'-CGAGCGGCGGACGGGTGAGTAATG-3'

Lux15SR 5'-TGCAGCCCACTCCCATGGTGTGAC-3'

Bioluminescence of the cells containing the hybrid plasmids was measured with a luminometer consisting of an FEU-85 photomultiplier and a V2-15 microvoltmeter. The intensity of bioluminescence (I) was measured in special cuvettes containing 200 μl of the preparation, at room temperature. When necessary, 2–3 μl of the substrate for luciferase (0.001% alcoholic solution of *n*-decanal (Sigma)) was added to the suspension prior to the measurements.

RESULTS

Optimal growth temperature. The data presented in Table 1 show that strain KCh1 was psychrophiles: it could grow at 4°C, but not at 30°C (with the optimal growth temperature of 15°C). By contrast, the bacteria of the species *A. fischeri* grow well at 30°C and do not grow at 4°C.

16S rRNA data. The gene was sequenced that encodes the 16S rRNA of strain KCh1. As shown by the data presented in Table 2, the nucleotide sequence of the 16S rRNA gene from strain KCh1 was significantly different from that of *A. fischeri* (with an average of 65 distinctions; GenBank data for five *A. fischeri* strains were used in the table). The 16S rRNA gene nucleotide sequence of the strain KCh1 was closely related to those of *A. logei* and *A. salmonicida* (similarly, this was shown using the GenBank data for five *A. logei* strains and six strains of *A. salmonicida*).

The biochemical tests. Comparative analysis was performed of three biochemical characteristics of the strain KCh1 and *A. fischeri* strains (the data for the symbiont strains of *A. logei* and *A. salmonicida* were taken from [3]) (Table 3). In previous investigation [3], the strains of *A. logei* and *A. fischeri* isolated as symbionts from the light organs of squids of the genus *Sepiola* exhibited similar parameters of nitrate reduction, lysine decarboxylation, and D-galactose fermen-

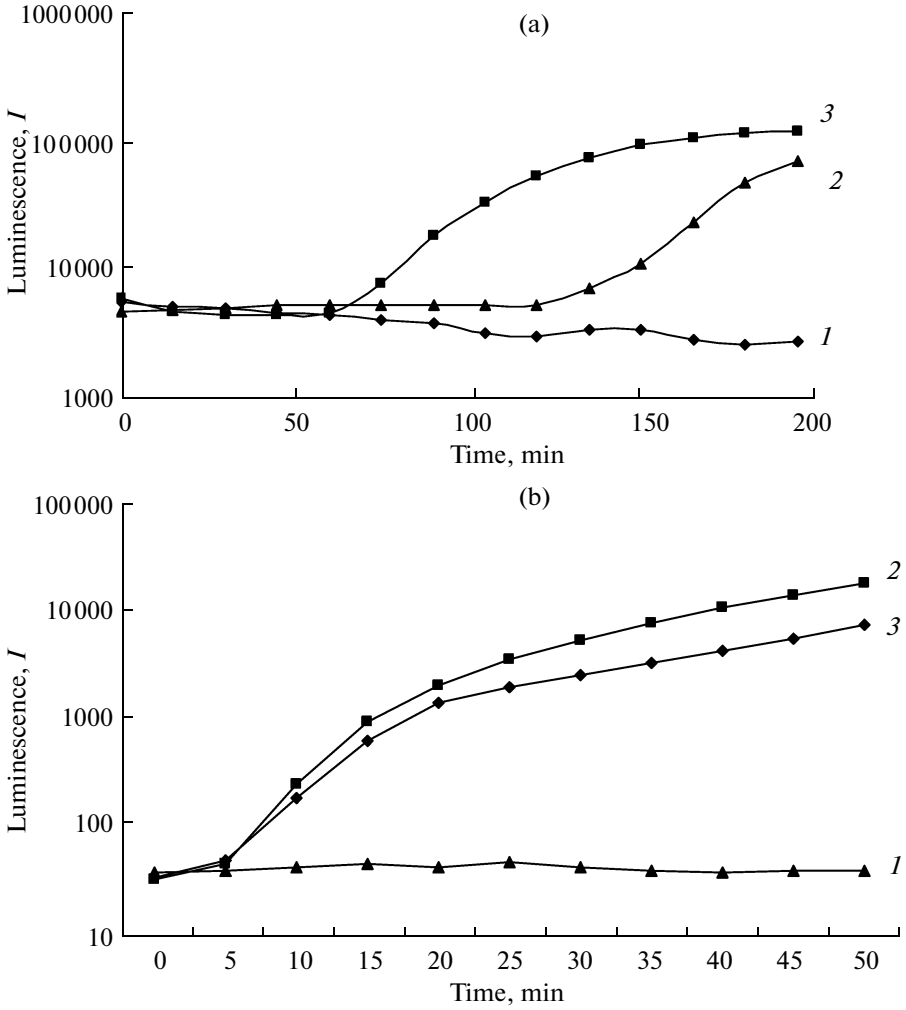

Fig. 1. The role of the quorum sensing system in the bioluminescence induction in the cells of strain KCh1: (a) effect of AI on bioluminescence induction in the cells of strain KCh1. The KCh1 cells were grown in SWTm at 11°C to $OD = 0.1–0.2$; the sample was separated in halves; then either AI (*N*-3-oxyhexanoyl L-homoserine lactone), to the final concentration 10^{-6} M, or the supernatant, obtained by centrifugation of the stationary KCh1 culture, was added to the test sample. The samples were incubated at 11°C without stirring; 200-μl aliquots were taken repeatedly at equal time intervals, and the bioluminescence intensity was measured. Plotted along the ordinate is the relative bioluminescence intensity (*I*). Plotted along the abscissa is time in minutes. intact cells (control) (*1*); cells with the supernatant (*2*); and cells with AI, 10^{-6} M (*3*). (b) Effect of the supernatant (obtained by centrifugation and passing through a 0.22-μm membrane filter of the stationary KCh1 culture) on the bioluminescence induction in *E. coli* MC1016 (pVFR1) cells. *E. coli* MC1016 (pVFR1) cells were grown at 37°C to $OD = 0.3–0.4$. The samples were incubated at 30°C without stirring at 10-min intervals; 200-μl aliquots were taken, and the bioluminescence intensity was measured. Plotted along the ordinate is the relative bioluminescence intensity (*I*). Plotted along the abscissa is time in minutes. Intact cells (control) (*1*); cells with the supernatant (*2*); and cells with AI, 10^{-6} M (*3*).

tation, while differing from *A. salmonicida* in these parameters. As shown in Table 3, the biochemical activity of strain KCh1 matches those of the *A. logei* and *A. fischeri* strains and differs from that of *A. salmonicida*.

Sensitivity of the bacteria *A. logei* KCh1, *A. fischeri* MJ-1, and *A. fischeri* MGU-6 to antibiotics. Sensitivity to antibiotics is usually applied as an accessory parameter in bacterial systematics, and their antibioticograms are used in species taxonomy to assess the phenotypic similarity. Table 4 represents data on the sensitivity of *A. logei* KCh1 to antibiotics and to two synthetic antibacterial drugs, as compared to *A. fischeri* strains MJ-1 and MGU-6. It can be seen that the *A. fischeri* strains and the *A. logei* KCh1 isolate from our collection have generally similar patterns of specific antibiotic resistance, though some differences exist. The strain *A. logei* KCh1 was shown to be resistant to aminoglycoside antibiotics (streptomycin, neomycin, and monomycin) and weakly sensitive to kanamycin and gentamycin, unlike *A. fischeri*, which was more sensitive to many antibiotics of this class. Only the strain *A. logei* KCh1 was sensitive to the β-lactam antibiotic carbenicillin; other tested strains were resis-

Table 2. Comparison of 16S rRNA gene sequences of strain KCh1 and *A. logei*, *A. fischeri*, and *A. salmonicida* strains

Strain	GenBank ID	Number of substitutions
KCh1 (the present work)	FJ858206	–
A. fischeri ES191	DQ026825	67
A. fischeri EHP2	DQ530285	96
A. fischeri MJ-1	EF380230	66
A. fischeri etasm. 1.1	EF667055	64
A. fischeri AT1	EU031644	64
A. logei 15382	AY292932	12
A. logei S3-6	AY771721	7
A. logei NCIMB 2252	AJ437616	1
A. logei ATCC 15832	X74708	16
A. logei SL 101	AY292928	21
A. salmonicida PB1-8rrnA	EU091323	10
A. salmonicida PB1-rrnB	EU091324	12
A. salmonicida ssp. H1 651	X71811	21
A. salmonicida NCMB 2262	X70643	23
A. salmonicida	AY292918	21
A. salmonicida LFI1238	FM178379.1	4

Table 3. Comparison of the biochemical characteristics of the strains KCh1, *A. logei*, *A. fischeri*, and *A. salmonicida*

Strain	Biochemical activity		
	Lysine decarboxylation	Nitrate reduction	D-galactose fermentation
KCh1	+	+	+
A. fischeri MJ-1	+	+	+
A. fischeri MGU-6	+	+	+
*A. logei**	+	+	+
A. salmonicida	–	–	–

"+" and "–" stand for positive and negative, respectively.
*Data for *A. logei* and *A. salmonicida* were taken from [3].

tant to the antibiotics of this group. *A. logei* KCh1 was shown to be sensitive to vancomycin and polymyxin, the antibiotics affecting the cell wall structure, while both *A. fischeri* strains were more resistant to these antibiotics. All the strains tested were sensitive to nalidixic acid (DNA gyrase inhibitor), to rifampicin (RNA polymerase inhibitor), to macrolides (erythromycin, azithromycin, and roxithromycin), to antibiotics of the tetracycline group, and to a chemotherapeutic agent, furadonine.

Characteristics of the KCh1 bioluminescence; quorum sensing regulation. When strain KCh1 were grown in liquid medium, a relatively bright luminescence occurred only at temperatures below 20°C. As a rule, the bacteria were grown at temperatures from 8 to 16°C. Such conditions did not facilitate determination of the quorum sensing regulation system activity. Strain KCh1 bacteria grown at temperatures above 20°C exhibited almost no luminescence. On that account, a method described below was used to detect the quorum sensing system in KCh1 cells.

To detect the presence of the gene coding for LuxR (the *lux* operon transcription activator), AI was added to a medium containing KCh1 cells grown to $OD = 0.2$ at 11°C and the incubation was continued. Fig. 1a illustrates the correlation between the bioluminescence intensity of KCh1 cells and the time of incubation at 20°C with aeration (from the moment of AI addition). As shown in the figure, in the presence of AI, the bacteria began to luminesce after awhile, while the control cells (without AI) remained nonluminous.

To detect AI biosynthesis in KCh1 cells, we used the AI biosensor developed earlier (cells of an *E. coli* strain containing the hybrid plasmid pVFR1 with the genes *lux*R and *lux*CDABE). The cells of this biosensor start to luminesce only in the presence of the relevant AI in the medium [4]. As shown in Fig. 1b, this biosensor commenced to luminesce both after addition of an AI *N*-(3-oxohexanoyl)-L-homoserine lactone (control) and after addition of the supernatant obtained by centrifugation of a stationary-phase KCh1 culture with a high level of bioluminescence.

Thus, it is safe to conclude that the genome of strain KCh1 contains the genes *lux*R and *lux*I, coding for the transcription activator LuxR and the protein LuxI responsible for AI synthesis, respectively. The AI of strain KCh1 appears to be structurally similar to that of *A. fischeri* or probably differ insignificantly from it.

Cloning of the KCh1 *lux* operon genes in *E. coli* cells and sequencing. Cloning of the KCh1 *lux* operon genes was carried out using the vector pUC19. The chromosomal DNA fragments were introduced at the sites *Hin*dIII and *Bam*HI. The *E. coli* MC1016 cells transformed by the ligase mixture were plated onto agarized medium and incubated at 28°C, followed by selection of the luminescent clones. Cloning of the *Bam*HI fragments produced several clones that luminesced only after addition of *n*-decanal, the substrate for luciferase. According to the sequencing results, the DNA fragment in this case did not contain the complete *lux*E gene (this variant is represented in Fig. 2 as pSV1). Cloning of the *Hin*dIII restriction fragments produced luminescent clones that did not require *n*-decanal (this variant is represented in Fig. 2 as pSV2).

Fig. 3 shows the sequence comparison for the regulator region, containing the *lux* box and the right pro-

Table 4. Sensitivity of the luminescent bacteria *A. logei* KCh1, *A. fischeri* MJ-1, and *A. fischeri* MGU-6 to antibiotics

Classes of antibiotics	Antibiotics and therapeutic agents	mg per disc	Mechanism of antibiotic action	Bacterial strains		
				A. logei KCh	*A. fischeri* MJ-1	*A. fischeri* 6 MGU
Tetracyclines	Tetracycline	30	Inhibitors of protein synthesis on the ribosomes (targets are different regions of the 30S ribosomal subunit)	s*	s	s
	Doxycycline	30		s*	s	s
Aminoglycosides	Streptomycin	30		r	s	s
	Kanamycin	30		sr	s	s
	Gentamycin	10		sr	s	s
	Neomycin	30		r	sr	sr
	Monomycin	30		r	r	r
Polypeptide	Polymyxin M	300	Damages the cell membrane structure	s	sr	sr
Polycyclic glycopeptides	Ristomycin	30	Inhibitors of the synthesis of bacterial cell wall components	r	r	r
	Vancomycin	30		s	sr	sr
b-Lactam antibiotics	Benzylpenicillin	10		r	r	r
	Ampicillin	30		r	r	r
	Carbenicillin	100		s	r	r
	Oxacillin	10		r	r	r
Macrolides:	Erythromycin	15	Inhibitors of protein synthesis at translation (targets are 50S ribosomal subunits)	s	s	s
Antibacterial macrolides	Azithromycin (Sumamed)	15		s	s	s
	Roxithromycin (Rulid)	30		s	s	s
Antifungal macrolides (tetraen nystatin)	Nystatin	80	Damages the cell membrane (the eukaryotic membrane–sterol complex)	r	r	r
Aminosugar	Lincomycin	15	Inhibits protein synthesis by blocking elongation of the peptide chain (target is 50S ribosomal subunit)	s	s	s
Ansamycins containing a naphthalene ring	Rifampicin	5	Inhibitor of RNA synthesis (complex with the RNA polymerase)	s	s	s
Derivatives of naphthyridine (synthetic agent)	Nalidixic acid (Nevigramon, Negram)	30	Inhibitor of DNA replication (represses gyrase activity)	s*	s	s
Derivatives of nitrofuran (synthetic agent)	Furadonin (Nitrofurantoin)	300	Monoaminoxydase inhibitor	s*	s	s

Designations: r—resistant; s—sensitive; s*—extremely sensitive; sr—weakly resistant.

moter Pr, of the *lux*-operons of strain KCh1, *A. salmonicida*, and *A. fischeri*. The sequence of strain KCh1 exhibited high homology with that of *A. salmonicida* and a considerable difference from that of *A. fischeri*. It is worth mentioning that, in the sequences of KCh1 and *A. salmonicida*, the initial codon ATG (the starting codon of the LuxI protein) was followed by stop-codons TGA and TAG that blocked the translation of the polypeptide LuxI in the cells of these strains.

DISCUSSION

Analysis of the characteristics of the new psychrophilic luminous strain KCh1 showed that it most likely belongs to the species *A. logei*. Notably, it is the first time that bacteria of this species have been isolated in the Sea of Okhotsk basin. *A. logei* have been previously isolated in cold or deep waters of the Arctic, of ice shelf waters (Ross Sea, Antarctic), of the Atlantic, etc. In

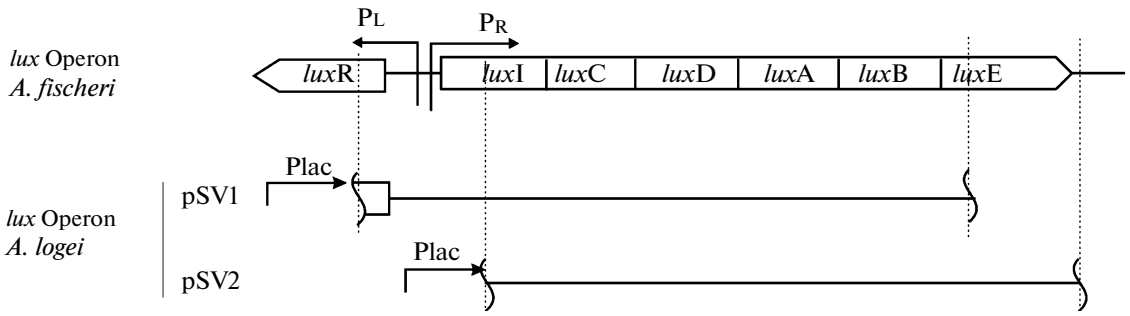

Fig. 2. The structure of *A. fischeri lux* operon and the algorithm for the cloning of KCh1 *lux* genes. The genes *lux*A and *lux*B encode α- and β-luciferase subunits; the genes *lux*C, *lux*D, and *lux*E encode reductase subunits; and *lux*R and *lux*I are regulatory genes (*lux*R encodes the protein LuxR, the *lux* operon transcription activator; *lux*I encodes the protein LuxI, the synthetase that effects AI synthesis). The cloned fragments of the KCh1 *lux*-operon: pSV1—*Bam*HI-fragment, pSV2—*Hin*dIII-fragment.

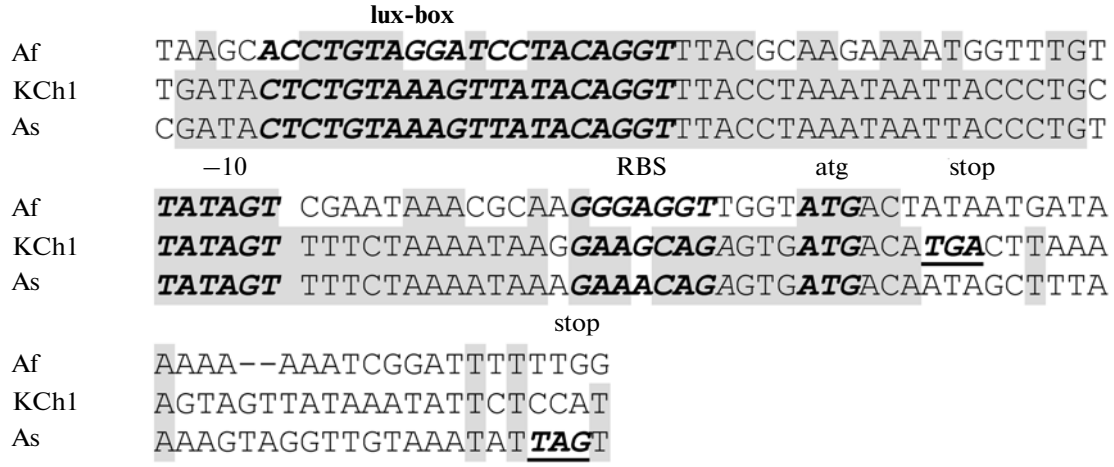

Fig. 3. Nucleotide sequences of the *lux* operon regulator regions of strain KCh1, *A. fischeri*, and *A. salmonicida* (the *lux* box, 10, RBS, ATG-codon, and the stop-codons are highlighted in italics; the stop-codons are also underlined).

addition, they have been isolated as symbionts of squids of the genus *Sepiola* in the Atlantic [2, 3].

Phenotypically, *A. logei* is very similar to *A. fischeri*; however, in contrast to *A. fischeri*, they are evidently psychrophiles, since they are able to grow at 4°C and unable to grow at 30°C, which initially suggested that they be classified as a separate species [2, 7].

Moreover, the close relation between the species *A. logei* and *A. fischeri* is confirmed by the fact that bacteria of these species are often found as joint symbionts in the photophores of squids of the genus *Sepiola* [3].

The group of psychrophilic marine bacteria growing at 4°C also includes *A. salmonicida* [8]. The bacteria of this species attract special attention of researchers, since they are pathogenic, for commercial Atlantic salmon in particular [9, 10]. They had been for a long time considered nonluminous bacteria and were consequently thought to have no genes coding for luciferase. However, the presence of luciferase genes was demonstrated in [11], where a bacterial suspension was reported to produce bright luminescence after addition of a substrate for luciferase, an aliphatic aldehyde. Later, the same group of researchers cloned the *lux* operon of *A. salmonicida* in *E. coli* cells and identified its structure and nucleotide sequence [12].

In [13], a multigene analysis of different strains of the genus *Aliivibrio* was reported, showing that the species *A. salmonicida* and *A. logei* exhibited some distinctions, despite being very closely related. The sequencing of the 16S rRNA gene and the *lux*-operon of strain KCh1 reported in the present work suggests

that this strain cannot be related to one of the above species. However, the biochemical and the bioluminescence characteristics of strain KCh1 match those of *A. logei*, but not of *A. salmonicida*. On this account, we suggest classifying the strain KCh1 with the species *A. logei*.

A comparison of the strains representing the three species (*A. fischeri, A. salmonicida*, and *A. logei*) revealed a high degree of sequence homology between *A. salmonicida* and *A. logei*; however, *A. fischeri* and *A. logei* were more closely related according to biochemical (nitrate reduction, lysine decarboxylation, and D-galactose fermentation) and bioluminescence tests. It is safe to assume that bacteria of the species *A. logei*, being more closely related to *A. salmonicida*, have acquired similar phenotypic characteristics to those of *A. fischeri*, with which they occupy the same econiche.

To conclude, the collection of luminous microorganisms of the Institute for Biophysics, Siberian Branch, Russian Academy of Sciences (Krasnoyarsk), does not contain bacteria of the species *A. logei*. All the strains with an optimal growth temperature of 15°C represented in this collection and retrieved from the Sea of Okhotsk (Kamchatka peninsula) have been classified as a psychrophilic subspecies of *A. fischeri* [14, 15]. However, in the light of our data on identification of the strain KCh1 as *A. logei*, it appears safer to relate these strains as well to the psychrophilic species *A. logei*.

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REFERENCES

1. Urbanczyk, H., Ast, J.C., Higgins, M.J., Carson, J., and Dunlap, P.V., Reclassification of *Vibrio fischeri, Vibrio logei, Vibrio salmonicida* and *Vibrio wodanis* as *Aliivibrio fischeri* gen. nov., comb. nov., *Aliivibrio logei* comb. nov., *Aliivibrio salmonicida* comb. nov. and *Aliivibrio wodanis* comb. nov., *Int. J. Syst. Evol. Microbiol.*, 2007, vol. 57, pp. 2823–2829.

2. Bang, S.S., Baumann, P., and Nealson, K.H., Phenotypic Characterization of *Photobacterium logei* (sp. nov.), a Species Related to *P. fischeri, Curr. Microbiol.*, 1978, vol. 1, pp. 285–288.

3. Fidopiastis, P.M., von Boletzky, S., and Ruby, E.G., A New Niche for *Vibrio logei*, the Predominant Light Organ Symbiont of Squids in the Genus *Sepiola, J. Bacteriol.*, 1998, vol. 180, pp. 59–64.

4. Manukhov, I.V., Kotova, V.Yu., and Zavil'gel'skii, G.B., Involvement of Host Factors in the Regulation of the *Vibrio fischeri lux* Operon in *Escherichia coli* cells, *Mikrobiologiya*, 2006, vol. 75, no. 4, pp. 525–531 [*Microbiology* (Engl. Transl), vol. 75, no. 4, pp. 452–458].

5. Maniatis, T., Fritsh, F., and Sambrook, J., *Molecular Cloning*: *A Laboratory Manual*, New York: Cold Spring Harbor Lab. Press, 1989.

6. Sanger, F., Nicklen, S., and Coulson, A.R., DNA Sequencing with Chain-Terminating Inhibitors, *Proc. Natl. Acad. Sci. USA*, 1977, vol. 74, pp. 5463–5468.

7. Harwood, C.S., Bang, S.S., Baumann, P., and Nealson, K.H., *Photobacterium logei* sp. nov., nom. rev., *Beneckea nereida* sp. nov., nom. rev. and *Beneckea gasogenes* sp. nov., nom. rev., *Int. J. Syst. Bacteriol.*, 1980, vol. 30, p. 655.

8. Egidius, E., Wiik, R., Andersen, K., Hoff, K.A., and Hjeltnes, E., *Vibrio salmonicida* sp. nov., a New Fish Pathogen, *Int. J. Syst. Bacteriol.*, 1986, vol. 36, pp. 518–520.

9. Enger, O., Husevag, B., and Goksoyr, J., Presence of the Fish Pathogen *Vibrio salmonicida* in Fish Farm Sediments, *Appl. Environ. Microbiol.*, 1989, vol. 55, pp. 2815–2818.

10. Enger, O., Husevag, B., and Goksoyr, J., Seasonal Variation in Presence of *Vibrio salmonicida* and Total Bacterial Counts in Norwegian Fish-Farm Water, *Can. J. Microbiol.*, 1991, vol. 37, pp. 618–623.

11. Fidopiastis, P.M., Sorum, H., and Ruby, E.G., Cryptic Luminescence in the Cold-Water Fish Pathogen *Vibrio salmonicida, Arch. Microbiol.*, 1999, vol. 171, pp. 205–209.

12. Nelson, E.J., Tunsjo, H.S., Fidopiastis, P.M., Sorum, H., and Ruby, E.G., A Novel *lux*-Operon in the Cryptically Bioluminescent Fish Pathogen *Vibrio salmonicida* Is Associated with Virulence, *Appl. Environ. Microbiol.*, 2007, vol. 73, pp. 1825–1833.

13. Ast, J.C., Urbanczyk, H., and Dunlap, P.V., Multi-Gene Analysis Reveals Previously Unrecognized Phylogenetic Diversity in *Aliivibrio, Syst. Appl. Microbiol.*, 2009, vol. 32, pp. 379–386.

14. Primakova, G.A., Vorob'eva, T.I., Medvedeva, S.E., and Fish, A.M., Morphology and Ultrastructure of Psychrophilic Luminous Bacteria, *Mikrobiologiya*, 1981, vol. 50, pp. 487–493.

15. *Katalog kul'tur svetyashchikhsya bakterii* (Catalogue of Luminous Bacterial Cultures) Rodicheva, E.K., Ed., Novosibirsk: Nauka, 1997.