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ORIGINAL PAPER

Whole-cell optical biosensor for mercury – operational conditions in saline water

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The present study demonstrates the influences of chlorides, fluorides and bromides of potassium and sodium on the growth and Hg^{2+}-induced bioluminescence of bioreporter *Escherichia coli* ARL1. In a Luria–Bertani medium (LB), cell growth was inhibited by concentrations of sodium and potassium fluorides above 0.2 mol L^{-1}. The addition of NaCl increased cell tolerance to the toxic effects of fluorides and bromides. Lag periods of 10 h and more were observed for cultivations in LB without NaCl and with halides (NaCl, KCl, NaBr, KBr, NaF and KF) at concentrations lower than 0.06 mol L^{-1}. In a phosphate buffer (PB), the bioluminescence of *E. coli* ARL1, induced with $HgCl_2$, was increased by the addition of NaCl, KCl, NaBr, KBr, NaF and KF (concentration of 0–0.25 mol L^{-1}). In a saline phosphate buffer (PBS), the maxima of induced bioluminescence declined to 50 %, in the case of NaF (0.12 mol L^{-1}), and to zero for KF. An addition of tryptone to the induction medium increased induced light emission ten-fold. Concentrated artificial sea water (ASW) (70–100 % ASW) inhibited bioluminescence induction. The new detection assay with *E. coli* ARL1 made possible the detection of 0.57 μg L^{-1} of $HgCl_2$ in double-diluted artificial sea water (25 % ASW).

Keywords: mercury detection assay, bioluminescent bioreporter, sea water, *E. coli* ARL1, whole-cell biosensor

Introduction

Bioluminescent bioreporters are analytical tools for the rapid and non-expensive detection of bio-available pollution. Lux strains sensing specific toxic effects afford the advantage of being able to respond to mixtures of contaminants inducing the same effect, hence could be used as a sensor for the sum effect, including the effect of compounds not as yet identified by chemical analysis (Woutersen et al., 2011).

The use of mercury is widespread, particularly in the production of gold, vaccines, antimicrobials, amalgams and electronics. Mercury is a ubiquitous pollutant that, when absorbed, is extremely toxic. Although whole-cell bioluminescent sensors were described before the end of the last century (Selifonova et al., 1993), their real application still lacks a better understanding of their operational conditions. Waters with salt concentrations of > 2 mass % are common both in nature as sea water or salt lakes, as well as in industry wastewaters, recyclable treatment brines and concentrates from membrane purification processes.

Microorganisms require variable amounts of salts for growth and metabolism. In general, the requirement for salts is not an exclusive need for NaCl because many halophiles require low levels of potassium ions, magnesium ions and other cations and anions, in addition to NaCl (Kushner, 1968). Furthermore, for some bacteria such as *Escherichia coli*, the apparent requirement for NaCl is not specific and other salts

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and sugars can be substituted.

Growth of *E. coli* 0157:H7 began to slow down in the presence of 2.5 mass % of NaCl and death occurred in the broth at 8.5 mass % NaCl (Glass et al., 1992). Potassium chloride (1.5 %) decreased the growth of another strain of *E. coli* (Abdulkarim et al., 2009). In contrast to that, *E. coli* ATCC 8739 was adapted to 11 mass % of salt (How et al., 2013) and *E. coli* K-l2 MC 4100 to sea water (Troussellier et al., 1998). Fluoride negatively influenced the growth of *E. coli* (Baker et al., 2012), but the growth of *E. coli* BL21-pGFPuv was increased by low concentrations (0.1–10.0 mmol L^{-1}) of sodium fluoride (Ma et al., 2014).

Similarly, salts' concentrations influenced bioluminescence. In vitro experiments showed that the bioluminescence of the bioluminescent-coupled enzymatic system NADH:FMN–oxidoreductase–luciferase was increased in the presence of potassium bromides and chlorides and decreased in the presence of potassium iodides (Gerasimova & Kudryasheva, 2002). The sensitivity of the mer-lux biosensors to Hg(II) was increased by the introduction of transport functions, *merTPC* in pOS14. The bioluminescence of bioreporters *E. coli* HMS174(pOS14) and *E. coli* HMS174(pRB28) was higher in LB than in a mineral medium (Selifonova et al., 1993) after 40 min of induction. Under aerobic conditions, increasing pH from 5 to 7 led to the lower sensitivity of bioreporter *E. coli* HMS174(pRB28) (Golding et al., 2008).

As a contaminant, mercury is ubiquitous, with the highest concentrations in estuarial waters. In these brackish waters, monitoring of the mercury concentrations and especially mercury bioavailability should prevent the health risk to populations that tend to be dense in estuaries. This study sought to examine free cells *E. coli* (EC100) ARL1 and the *merR::luxCDABE*-based bioreporter (Shi et al., 2014) as a bioluminescent detector of $HgCl_2$, as a form of bioavailable mercury, in brackish and sea waters.

Experimental

General

Escherichia coli (EC100) ARL1 (Dahl et al., 2011) was kindly donated from the collection of microorganisms of CEB University of Tennessee (Knoxville, USA).

All compounds were commercial products. Potassium, sodium and mercury halides, $SrCl_2 \cdot 6H_2O$ p.a. (Lach-Ner, Czech Republic), phosphates, $MgCl_2 \cdot 6H_2O$, $NaHCO_3$, H_3BO_3 (Penta, Czech Republic), $CaCl_2$, (97 %; Pan Reac Appli Chem,Spain), tryptone (Oxoid, UK), yeast extract, kanamycin (kan) and D-glucose (Sigma–Aldrich, USA). Artificial sea water was prepared as detailed by Kester et al., (1967).

Phosphate buffer saline (PBS; pH 7.4) was prepared by dilution from a previously prepared concentrated solution (× 10) of PBS that contained KH_2PO_4 (17 mmol L^{-1}), Na_2HPO_4 (52 mmol L^{-1}), NaCl (1.5 mol L^{-1}). The phosphate buffer (PB) had the same pH and composition as PBS without NaCl. D-Glucose stock solution (2 mol L^{-1} in D_2O) was sterilised by filtration through a syringe filter (pore size 0.22 μm; Millipore, France). Luria–Bertani media (LB) contained tryptone (10 g L^{-1}), yeast extract (5 g L^{-1}), NaCl (10 g L^{-1}), pH 7.2. (Sambrook et al., 1989). The LB + kan medium was prepared by the addition of a stock kanamycin solution (10 g L^{-1}) to the final concentration of 50 mg L^{-1}. The LB + kan – NaCl contained tryptone (10 g L^{-1}), yeast extract (5 g L^{-1}) and kanamycin 50 mg L^{-1}. The tryptone solution (20 g L^{-1}) was sterilised in an autoclave.

Growth of E. coli ARL1 in medium with salts

One colony was inoculated from an LB plate into 50 mL of LB liquid medium with kanamycin and incubated overnight at 37 °C to grow an overnight culture.

Into each well of 96 wells-microplate solutions of salts (0.00 mol L^{-1}, 0.0625 mol L^{-1}, 0.125 mol L^{-1}, 0.25 mol L^{-1}) and LB + kan with a diluted overnight cell suspension-optical density of (OD_{600})-0.04 (measured in 1 cm cuvette with UV-VIS Spectrophotometer HP8452A; Hewlett-Packard, USA) were pipetted. All experiments were performed in triplicate. Cell growth was recorded overnight (at 37 °C every 30 min) using a microplate reader Spectrostar Omega (BMG LABTECH, Germany). Prior to each measurement, the plate was shaken (10 s, 300 min^{-1}).

Induction of bioluminescence

The overnight culture was inoculated into a fresh LB medium with kanamycin and incubated at 37 °C, 200 min^{-1} to OD_{600} approximately 0.6 ($\approx 1 \times 10^8$ colony-forming units per millilitre; measured by UV-VIS spectrophotometer HP8452A). The bacterial cells were centrifuged for 10 min at 2600*g* and a pellet was re-suspended in PBS with D-glucose (40 mmol L^{-1}) to a cell concentration of 2×10^8 colony-forming units per millilitre.

Into each well of 96 wells-microplate, solutions of salts (0.00 mol L^{-1}, 0.063 mol L^{-1}, 0.125 mol L^{-1}, 0.25 mol L^{-1}) were pipetted and solutions of $HgCl_2$ (125 μL) were added to make up concentrations of $HgCl_2$ in wells (0.00 μg L^{-1}, 0.60 μg L^{-1}, 1.25 μg L^{-1}, 2.50 μg L^{-1}, 5.00 μg L^{-1}). After salt additions, the pH was measured and adjusted to pH 7.4 in all experiments. PBS + tryptone solution (final concentration 10 g L^{-1}) was added together with $HgCl_2$. Finally, each well was supplemented with a cell suspension (125 μL) to make a cell concentration of 1×10^8 colony-forming units per millilitre. Bioluminescence was measured overnight using a Spectrostar Omega (BMG Labtech, Germany) luminometer at 37 °C (from

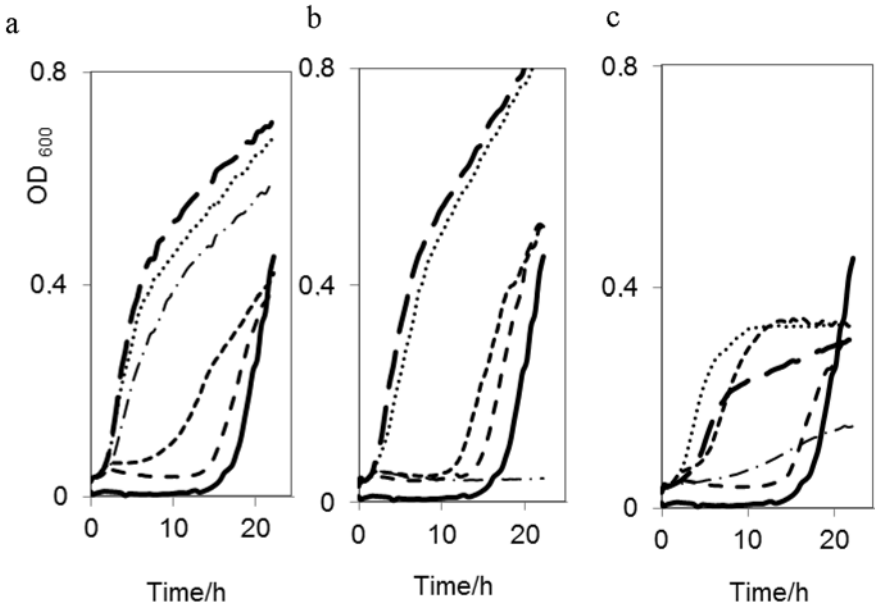

Fig. 1. Growth curves of *E. coli* ARL 1 in LB – NaCl supplemented with NaCl (a), NaBr (b) and NaF (c) at concentrations: – –·– 0.5 mol L^{-1}; – — — 0.25 mol L^{-1}; – · · 0.125 mol L^{-1}; – - - 0.06 mol L^{-1}; – – – 0.03 mol L^{-1}; – —— 0.0 mol L^{-1}.

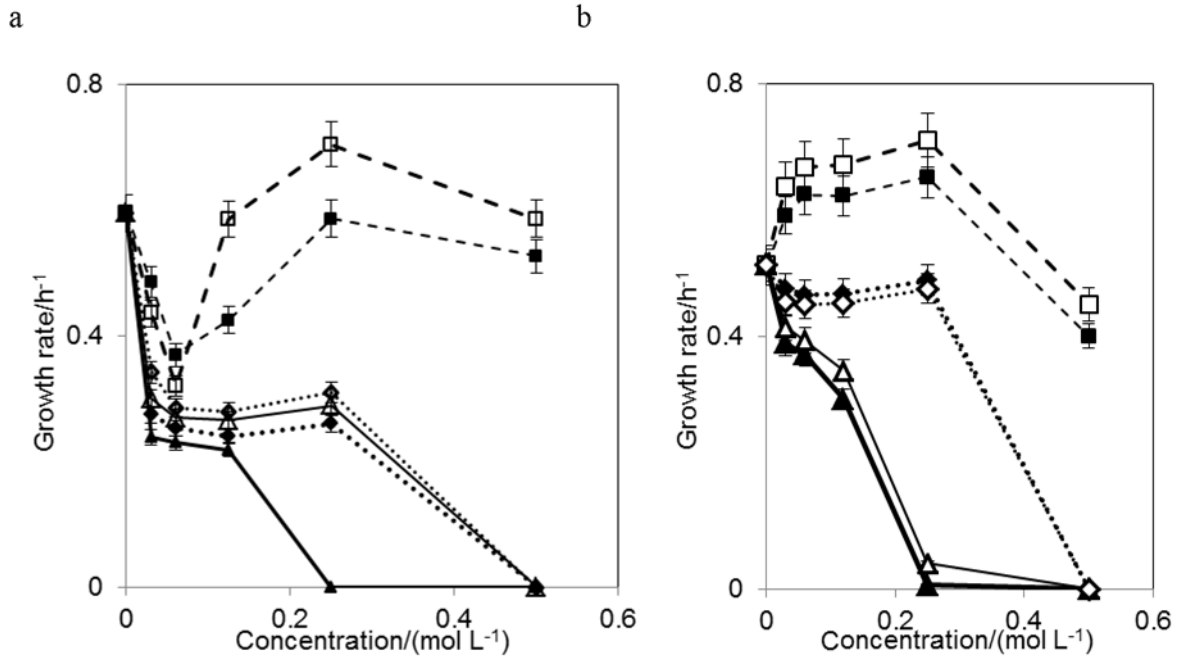

Fig. 2. Influence of halides on maximum growth rate of *E. coli* ARL 1 in LB – NaCl (a); in LB (b): ■ – KCl; □ – NaCl; ▲ – KF; △ – NaF; ◆ – KBr; ◇ – NaBr.

the plate bottom, 4 s every 7 min). All experiments were performed in triplicate. The maxima of relative bioluminescence (RB) were calculated as:

$$\mathrm{RB} = B_{\mathrm{sm}} - B_{0\mathrm{m}} \tag{1}$$

where B_{sm} were the bioluminescence maxima attained in an experiment and $B_{0\mathrm{m}}$ the bioluminescence maxima in wells without an inducer, $HgCl_2$. The sums of bioluminescence emitted after induction ($\sum$bioluminiscence) were calculated as:

$$\sum \text{bioluminesce} = \sum_{t=0}^{t=15\,\mathrm{h}} B_{\mathrm{s}} - \sum_{t=0}^{t=15\,\mathrm{h}} B_0 \tag{2}$$

where B_{s} were the bioluminescence in wells with an inducer and B_0 were the bioluminescence in wells without an inducer.

The limits of detections were determined from the dependences of the maxima of induced bioluminescence on the concentration of $HgCl_2$ in accordance with Hakkila et al. (2004).

The limit of determination for the sensor bacteria (PLOD) was set in accordance with the standard deviation of the luminescence measured with the sample without $HgCl_2$ (Hakkila et al., 2004):

$$\mathrm{PLOD} = 2(B_{0\mathrm{m}} + 3\mathrm{SD}) \quad (3)$$

where SD is the standard deviation.

Results and discussion

Growth of E. coli ARL1 in LB medium with sodium and potassium halides

It is recognised that all cations in low concentrations stimulate bacterial growth and in higher concentrations inhibit it (Winslow et al., 1932). The present study calculated the growth curves of the bacterial bioreporter *E. coli* ARL1 for an aerated LB medium in the presence of varying amounts of added NaCl, NaF, NaBr, KCl, KBr and KF ranging from zero molarity to a molarity of 0.5. The growth curve of *E. coli* ARL1 cultivated in LB medium without salt (LB – NaCl) exhibited a prolonged lag phase and lower optical density after 12 h of incubation (Fig. 1). Lag periods of 10 h and more were observed for cultivations in LB – NaCl with halides concentrations lower than 0.06 mol L^{-1} (Figs. 1 and 4). At these salts contents, the shorter lag periods were accompanied by lower maximum growth rates (Fig. 2a) but the maximal OD_{600} after 12 h of cultivation increased (Fig. 3). A comparison of the growth rates in LB and LB – NaCl (Figs. 2a and 2b) shows that NaCl increased cell tolerance to the toxic effects of fluorides and bromides. Cell growth was inhibited by sodium and potassium fluorides at concentrations above 0.2 mol L^{-1}.

Induction of bioluminescence with mercury chloride in medium with salts

The intensity of the induced bioluminescence is the main factor influencing limits of detection. The content of salts modifies osmolarity, which increases the activity of the MerR promoter (Condee & Summers, 1992) and also modulates the biological availability of mercury (Barkays, 1997). This last might be related to the formation of cell clusters (Hidalgo et al., 2010) or changes in the charges of mercury ions (Golding et al., 2008). The bioluminescence of *E. coli* ARL1 induced with $HgCl_2$ (2.5 μg L^{-1}) increased with an increasing concentration of NaCl up to 250 mmol L^{-1} (Fig. 5). By contrast, Deryabin and Aleshina (2008) observed that non-induced bioluminescence decreased in the presence of NaCl above 150 mmol L^{-1}. The

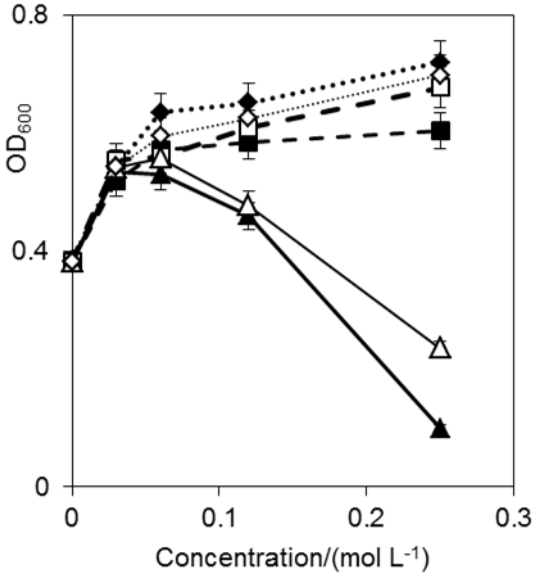

Fig. 3. Maxima of optical densities in stationary growth phase (12 h cultivation) of *E. coli* ARL 1 in LB with halides. Average data of maximal OD_{600} shown from wells without length correction: ■ – KCl; □ – NaCl; ▲ – KF; △ – NaF; ◆ – KBr; ◇ – NaBr.

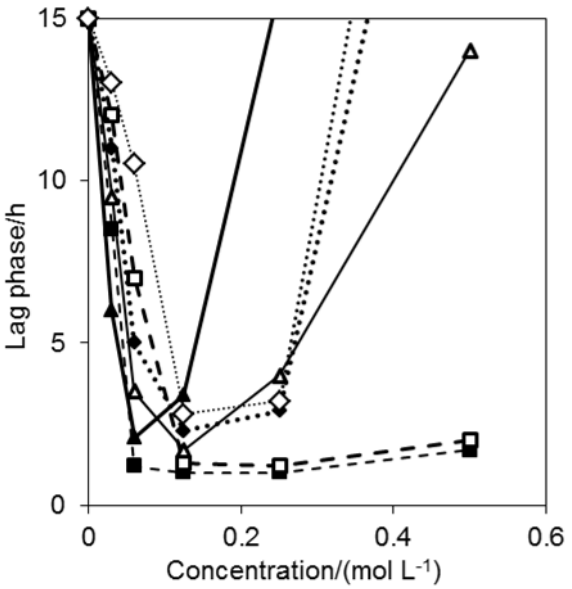

Fig. 4. Lag phases of growth of *E. coli* ARL 1 in LB – NaCl with halides: ■ – KCl; □ – NaCl; ▲ – KF; △ – NaF; ◆ – KBr; ◇ – NaBr.

increase in the induced bioluminescence with an increasing concentration of NaCl at relatively low concentrations of Hg (9 nml L^{-1}) might be explained by the observation of Selifonova and Barkay (1994) that more Hg entered into the cells in an Na-supplemented medium.

In PB, Hg^{2+} -induced bioluminescence was low and it was increased by the addition of all the halides

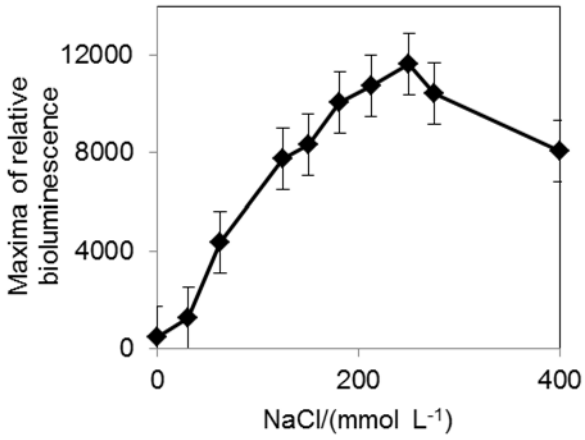

Fig. 5. Influence of NaCl on maxima of bioluminescence induced with $HgCl_2$ (2.5 μg L^{-1}) in PB.

tested (Fig. 6a). In PBS, light emission was suppressed in the presence of fluorides and NaBr. A sodium-coupled mercury transport was proposed as one of mechanisms of mercury uptake. Since the cells in the present study were grown with a high potassium deficiency, the addition of K^+ to the *E. coli* ARL1 assay medium restored the physiological K – Na exchange and the Na gradient and resulted in the stimulation of light induction.

In the presence of fluorides, potassium or sodium, concentrations close to the toxic (0.12 mol L^{-1}) maxima of induced bioluminescence declined to 50 % for NaF and to zero in the case of KF (Figs. 7a and 8a).

However, at concentrations of NaF of 0.06 mol L^{-1}, the cell light emission reduced slowly (Fig. 9). This resulted in higher sums of bioluminescence than in induction without NaF (Fig. 7b).

Induction of bioluminescence in PBS supplemented with tryptone

With aim of increasing bioreporter light emission in the presence of the low mercury concentrations which are common in real samples (Boszke et al., 2002), assays were performed with various compositions of induction media. In addition to mineral media (Golding et al., 2008; Selifonova et al., 1993; Corbisier et al., 1999), phosphate saline buffer with glucose (this study), nutrients added as amino acids (Dahl et al., 2011), casamino acid (Ivask et al., 2007) and LB medium (Selifonova et al., 1993) have been used. In the present study, tryptone was added as a less expensive equivalent of casamino acids. The addition of tryptone (5 g L^{-1}) to the phosphate saline buffer with glucose increased the light emission induced by $HgCl_2$ ten-fold (Fig. 10).

Bioluminescence induction in artificial sea water

In artificial sea water, bioluminescence was inhibited regardless of the high content of $HgCl_2$ (Fig. 11). In diluted sea water, with the content of artificial sea water (ASW) less than 50 %, bioluminescence was markedly induced only with concentrations of $HgCl_2$ above 1 μg L^{-1}.

Tryptone significantly increased the bioluminescence maxima in the medium with diluted ASW

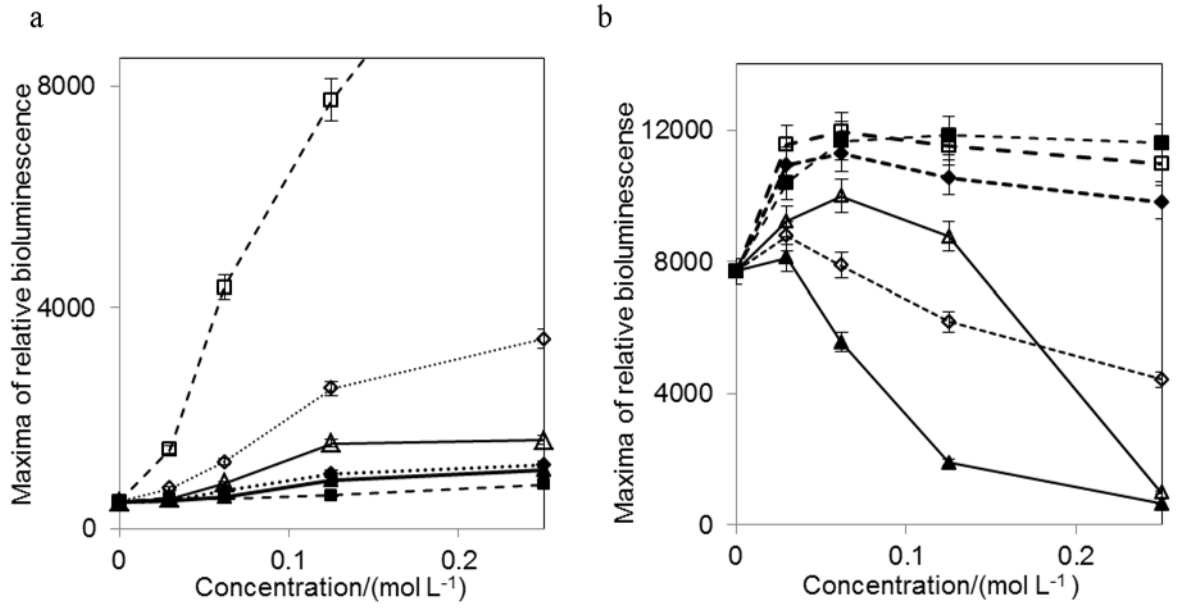

Fig. 6. Influence of halides on induction of bioluminescence with $HgCl_2$ (2.5 μg L^{-1}) in PB (a) and in PBS (b): ■ – KCl; □ – NaCl; ▲ – KF; △ – NaF; ◆ – KBr; ◇ – NaBr.

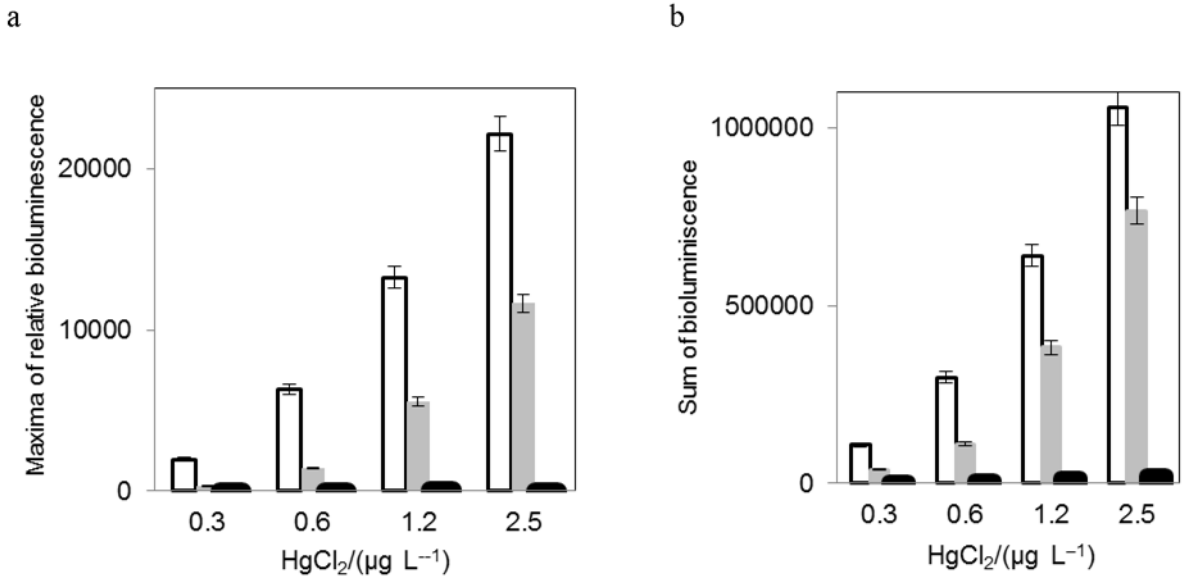

Fig. 7. Bioluminescence induced by $HgCl_2$ in PBS medium + KF: maxima of relative bioluminescence (a); sum of bioluminescence (b) at concentrations of KF: □ – 0 mmol L^{-1}; ■ – 60 mmol L^{-1}; ■ – 120 mmol L^{-1}.

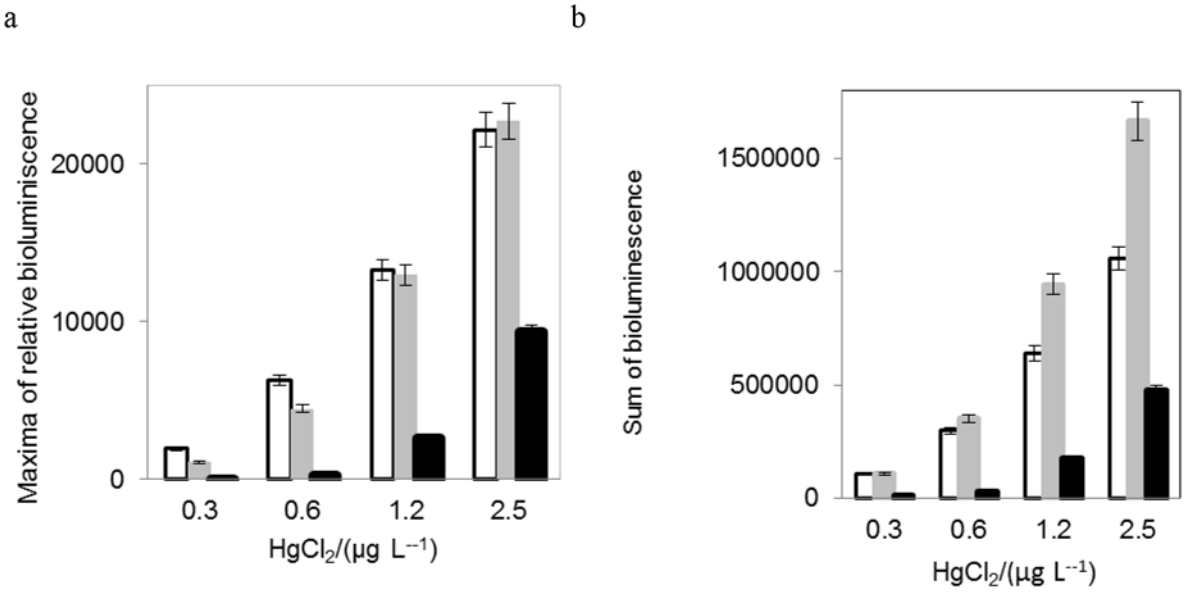

Fig. 8. Bioluminescence induced in PBS medium by NaF: maxima of relative bioluminescence (a); sum of bioluminescence (b) at concentrations of NaF: □ – 0 mmol L^{-1}; ■ – 60 mmol L^{-1}; ■ – 120 mmol L^{-1}.

Table 1. Limits of detection (LOD) and PLOD of mercury chloride found with *E. coli* ARL1 in ASW in PBS and PBS supplemented with tryptone

ASW/%	LOD/(μg L^{-1})		$10^2 \cdot$ PLOD	
	tryptone	PBS	tryptone	PBS
6.25	0.23	1.80	9.25	12.50
12.50	0.24	ND[a]	8.04	ND
25.00	0.57	ND	12.20	ND
50.00	ND	ND	ND	ND

a) LOD was higher than 1.25 μg L^{-1}; ND – not detectable.

(Fig. 12), hence a concentration of $HgCl_2$ of 0.3 μg L^{-1} was detected in ASW diluted to 6 % (Table 1).

Organic nutrients added to an induction medium might have supported the transport of mercury ions. This might be a result of the enhanced production of tranport proteins and facilitation of the passive transport of mercury ions by their coordination with organic compounds.

The limit of detection (0.57 μg L^{-1}) is similar to that achieved by Selifonova et al. (1993), under conditions of triply-diluted estuarine waters with *E. coli* HMS174(pRB28), which implies that the effect of

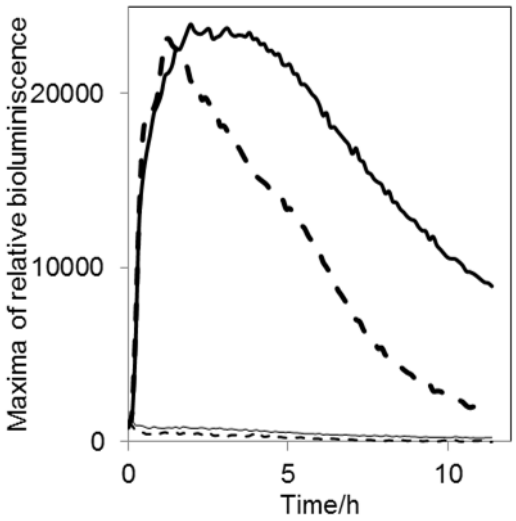

Fig. 9. Comparison of time records of maxima of relative bioluminescence E. coli ARL1 in PBS and with NaF induced by $HgCl_2$ (2.5 μg L^{-1}) in PBS buffer: – - - negative control; – – – PBS; – —— NaF (0.06 mmol L^{-1}).

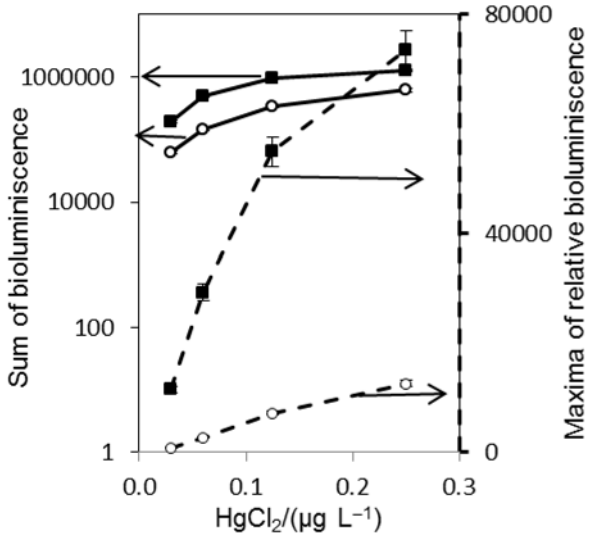

Fig. 10. Induction of bioluminescence in: ■ – PBS; ○ – PBS with tryptone (5 g L^{-1}).

salinity on bioluminescence is slightly influenced by the method of introduction of the reporter genes.

Conclusions

The study demonstrated the influence of chlorides, fluorides and bromides of potassium and sodium on the growth and bioluminescence induction of bioluminescence bioreporter *E. coli* ARL1. In fresh water, modification of the assay with *E. coli* ARL1 improved sensitivity four-fold. Concentrated ASW, 70–100 mass % ASW, inhibited bioluminescence induction. The new bioluminescence detection assay with *E. coli* ARL1 made possible the detection of 0.57 μg L^{-1} of $HgCl_2$ in double-diluted artificial sea water (25 mass % ASW). Such mercury concentrations are below the EPA limits for drinking water (2 μg L^{-1}) (Environmental Protection Agency, 2009) and occur frequently in estuarial and delta waters (Boszke et al., 2002).

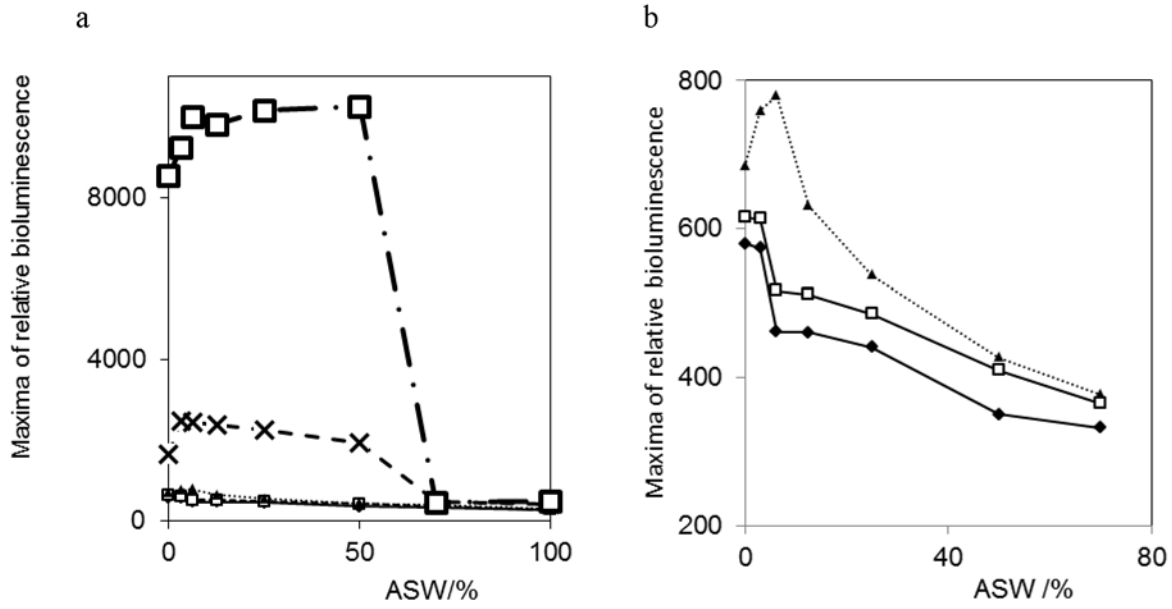

Fig. 11. Bioluminescence *E.coli* ARL1 induced with $HgCl_2$ in ASW + PBS (a) and detail of dependence for concentrations of $HgCl_2$ (b): ◆ – 0.0 μmol L^{-1}; □ – 0.6 μmol L^{-1}; ▲ – 1.2 μmol L^{-1}; × – 2.5 μmol L^{-1}; ☐ – 5.0 μmol L^{-1}.

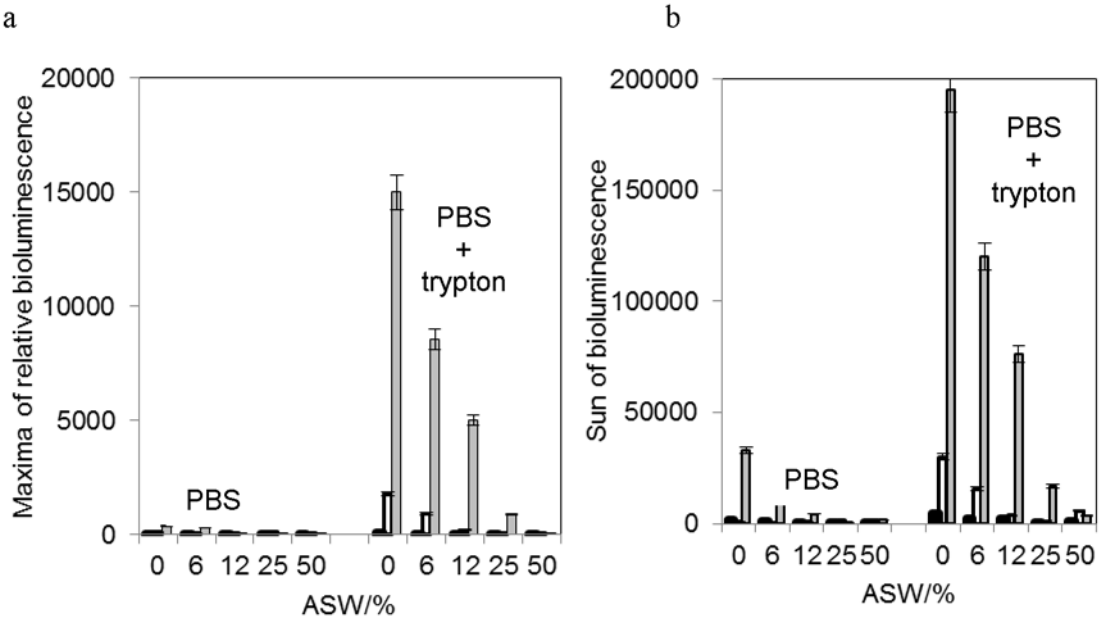

Fig. 12. Bioluminescence *E. coli* ARL1 induced by $HgCl_2$: in ASW + PBS (a) and ASW + PBS + tryptone (b) at different concentrations of $HgCl_2$: ■ – 0.10 μmol L^{-1}; □ – 0.30 μmol L^{-1}; ■ – 0.60 μmol L^{-1}.

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