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A high sensitivity iron-dependent bioreporter used to measure iron bioavailability in freshwaters

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Abstract

A *Nostoc* sp. PCC 7120 iron bioreporter containing iron-regulated schizokinen transporter gene *alr0397* promoter fused to the *luxAB* genes was examined to optimize its response to bioavailable iron. Dose–response relationships between luciferase activity and free ferric ion (Fe^{3+}) concentrations pFe ($-\lg [Fe^{3+}]$) were generated by measuring luciferase activities of the bioreporter in trace metal–buffered Fraquil medium with various incubation times. The results were best demonstrated by sigmoidal curves (pFe 18.8–21.7, $Fe^{3+} = 10^{-18.8}$–$10^{-21.7}$ M) with the linear range extending from pFe 19.6–21.5 ($Fe^{3+} = 10^{-19.6}$–$10^{-21.5}$ M) after a 12-h incubation time. Optimal conditions for the use of this bioreporter to sense the iron bioavailability were determined to be: a 12-h exposure time, initial cell density of $OD_{730\,nm} = 0.06$, high nitrate (100 μM), high phosphate (10 μM), moderate Co^{2+} (0.1–22.5 nM), Zn^{2+} (0.16–12 nM), Cu^{2+} (0.04–50 nM), and wide range of Mn^{2+} concentration (0.92–2300 nM). The applicability of using this iron bioreporter to assess iron availability in the natural environment has been tested using water samples from eutrophic Taihu, Donghu, and Chaohu lakes. It is indicated that the bioreporter is a useful tool to assess bioavailable iron in various water quality samples, especially in eutrophic lakes with high bioavailable iron.

Introduction

Iron is an essential nutrient for organisms. As the fourth most abundant element in the crust of the earth, it generally exists in two forms, Fe^{2+} and Fe^{3+}, in aquatic environments. In oxic environments, Fe^{2+} can be quickly oxidized into Fe^{3+} and then transformed into insoluble and inaccessible ferric hydroxide. In addition, iron also exists in the form of colloids and can be complexed by organic ligands. Although various iron chelates, including siderophores and grazing byproducts, and iron-organic compounds have been shown to act as sources of iron to phytoplankton (Hutchins *et al.*, 1999; Poorvin *et al.*, 2004), iron bioavailability is still low in many aquatic environments and constrains phytoplankton growth in areas of the open ocean characterized as 'high-nutrient, low-chlorophyll' regions (Martin *et al.*, 1991; Coale *et al.*, 1996), coastal waters (Hutchins *et al.*, 1998), and some freshwater systems (Twiss *et al.*, 2000).

Although rapid and reliable chemical protocols are available to measure absolute levels of iron in water samples, whole-cell bioreporters provide data on the capacity of the biota to acquire and assimilate iron. Recombinant bioluminescent bacterial strains have been successfully applied in monitoring iron (Durham *et al.*, 2002; Mioni *et al.*, 2003) and the availability of other metal ions (Peca *et al.*, 2008) in environmental samples. The bicistronic *isiAB* operon is in part regulated by the iron-dependent repressor Fur (ferric uptake regulator) in cyanobacteria (Ghassemian & Straus, 1996). The first gene *isiA* codes for a protein that is very similar to CP43, a chlorophyll-binding core protein of photosystem II. Flavodoxin coded by gene *isiB* has been revealed to have the ability to replace ferredoxin as carrier in the electron transfer chain. The expression of gene *isiAB* is greatly up-regulated under iron deficiency, so gene *isiAB* can be recognized as a marker for iron deficiency in aquatic systems (Straus, 1994). Bioreporters constructed from *Synechococcus* sp. PCC 7942 and *Synechococcus* sp. PCC 7002 using *luxAB* as reporter genes fused to *isiAB* promoter can assess iron availability of water samples through measuring luciferase activity (Durham *et al.*,

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2002; Porta *et al.*, 2003; Hassler *et al.*, 2006; Boyanapalli *et al.*, 2007). In addition, a bioreporter in *Pseudomonas putida* was constructed using *fepA–fes* promoter of *Escherichia coli* (an enterobactin biosynthesis gene regulated by the Fur system) fused to a *luxCDABE* cassette and was used to measure the iron bioavailability in Lake Erie (Mioni *et al.*, 2003). However, these bioreporters possess a relatively narrow range of application and might be inappropriate for use in lakes with high bioavailable iron.

Nostoc sp. PCC 7120 is a filamentous nitrogen-fixing cyanobacterium, and its outer membrane contains a highly specific transporter of siderophore–iron complexes for iron acquisition. Alr0397 has been shown to be a TonB-dependent schizokinen (a dihydroxamate-type siderophore) transporter (Nicolaisen *et al.*, 2008), and the transcription of *alr0397* is highly inducible by iron deficiency (Nicolaisen *et al.*, 2008; Dong & Xu, 2009). In this study, we examined a *Nostoc* sp. PCC 7120 bioreporter, named as $P_{alr0397}$-*luxAB*, using the gene *alr0397* promoter fused to the *Vibrio fischeri luxAB* genes, to optimize the response to bioavailable iron. Our bioreporter can be used to assess bioavailable iron in various water quality samples, especially in eutrophic lakes with high total iron.

Materials and methods

Strains and growth conditions

Nostoc sp. PCC 7120 was from the Freshwater Algal Culture Collection at the Institute of Hydrobiology of the Chinese Academy of Sciences. Plasmid pHB4232 (Km^r Sp^r; Sp, spectinomycin; Km, kanamycin) constructed by fusing the promoter $P_{alr0397}$ to *luxAB* genes is from Dong & Xu (2009). The 700-bp fragment of *alr0397* promoter of *Nostoc* sp. PCC 7120 was recovered by PCR amplification with primers $P_{alr0397}$-Fw (5′-gctagcgagcctcactaatggcaatcc-3′, the site of restriction is underlined) and $P_{alr0397}$-Rev (5′-ctcgaggttgcgactggattatggct-3′), cloned in the T-vector pMD18-T (Takara) and confirmed by sequencing to obtain plasmid pHB4207 (Ap^r, ampicillin). The 4.4-kb fragment of *luxAB*-Ω digested with SmaI from pRL58 (Black *et al.*, 1993) was inserted into the XhoI site of plasmid pHB4207, transformed into the competent cells of *E. coli* DH5α, and screened by PCR amplification using primers $P_{alr0397}$-Fwt (5′-gctaaagtacctgcaccagc-3′) and luxAB-rev (5′-gccacaaccttcagacgct-3′) to make sure that the promoterless *luxAB* reporter genes were driven by the promoter $P_{alr0397}$ in the resulting plasmid pHB4227 (Ap^rSp^r). The 5.2-kb fragment of $P_{alr0397}$-*luxAB*-Ω was restricted with SphI and SmaI from plasmid pHB4227, blunted with T4 DNA polymerase, and ligated into shuttle vector pRL278 after its digestion with SpeI to construct plasmid pHB4232 (Km^rSp^r). According to Elhai *et al.* (1997), plasmid pHB4232 was conjugated into *Nostoc* sp. PCC 7120, integrated into the chromosome by homologous single-crossover, and selected for spectinomycin to create iron bioreporter $P_{alr0397}$-*luxAB*.

Trace metal–buffered Fraquil medium (Morel *et al.*, 1975) containing 10 μg mL^{-1} spectinomycin was used in the experiments of various iron concentrations. Fraquil medium containing various levels of total iron ($FeCl_3$) from 10 to 3000 nM was prepared. Iron exists mainly (92.15%) in the form of Fe–EDTA complexes ($[Fe–EDTA]^-$ and $[FeOH–EDTA]^{2-}$) in the medium. The free ferric ion concentration (pFe = −lg [Fe^{3+} free ferric]) and Fe(III)′ (the sum of the major inorganic iron species) were calculated using MINEQL+ software (Schecher & McAvoy, 1992): 10 nM (pFe 21.7, Fe(III)′ = 0.20 pM), 15 nM (pFe 21.5, Fe(III)′ = 0.30 pM), 30 nM (pFe 21.2, Fe (III)′ = 0.60 pM), 50 nM (pFe 21.0, Fe(III)′ = 1.01 pM), 70 nM (pFe 20.8, Fe(III)′ = 1.42 pM), 100 nM (pFe 20.7, Fe(III)′ = 2.04 pM), 300 nM (pFe 20.2, Fe(III)′ = 6.39 pM), 500 nM (pFe 20.0, Fe(III)′ = 11.12 pM), 1000 nM (pFe 19.6, Fe(III)′ = 25.05 pM), 3000 nM (pFe 18.8, Fe(III)′ = 151.45 pM). Early exponential phase cells grown for two generations in Fraquil medium with 100 nM Fe^{3+} were collected by centrifugation at 3900 ***g*** for 5–8 min, washed three times with iron-free Fraquil medium, inoculated into 300 mL polycarbonate flasks containing 100 mL Fraquil medium with various concentrations of Fe^{3+} (initial inoculum density $OD_{730\,nm}$ = 0.06), cultured at 30 °C under continuous illumination of 25 μmol photons m^{-2} s^{-1} with shaking (135 r.p.m.), and sampled to measure luciferase activity, respectively, after 0, 12, 24, and 48 h.

To minimize trace metal contamination, all culture materials were soaked in 10% HCl and rinsed thoroughly with Milli-Q water prior to use, and all solutions were prepared with Milli-Q water.

Optimization of parameters

To optimize the measurement parameters, the luciferase activity of bioreporter $P_{alr0397}$-*luxAB* in Fraquil medium with 10, 100, and 1000 nM Fe^{3+} was measured at different inoculum cell densities ($OD_{730\,nm}$ = 0.02, 0.04, 0.06, 0.08, and 0.1), and different concentrations of nitrogen (1, 10, 100, 300, and 900 μM), phosphorus (0.1, 1, 10, 30, and 90 μM), Co^{2+} (0.1, 0.5, 2.5, 7.5, 22.5, and 250 nM), Mn^{2+} (0.92, 4.6, 23, 69, 207, and 2300 nM), Zn^{2+} (0.16, 0.8, 4, 12, 36, and 400 nM) and Cu^{2+} (0.04, 0.2, 1, 10, 50, and 100 nM), respectively. The bioreporter cells were cultured in Fraquil medium as stated previously and sampled to measure luciferase activity after 12 h.

Measurement of luciferase activity

Luciferase activity was measured according to Elhai & Wolk (1990). One microliter *n*-decanal (Sigma) was added into 1 mL bovine serum albumin (20 mg mL^{-1}) and vortexed to obtain the reaction substrate. One milliliter of bioreporter was supplemented with 100 μL reaction substrate, gently mixed, and measured in a tube luminometer (Junior LB 9509) after dark treatment for 2 min to record relative light units (RLU). Luciferase activity was calculated as relative luminescence units per microgram of chlorophyll *a*. Chlorophyll *a* contents were calculated by the formula of Chl*a*(μg mL^{-1}) = 13.14 × $OD_{665\,nm}$ with 100% methanol extracts (Marker, 1972).

Treatment and the total iron content of water samples

Taihu, Donghu, and Chaohu lakes are all shallow lakes with the average depth of 2.1, 2.2, and 3.0 m, respectively. Water from the surface layer (0.5 m) was sampled using a Ruttner water sampler at Donghu Experimental Station of Lake Ecosystem, Taihu Lake Laboratory Ecosystem Research Station, and Yichen Station of Chaohu Lake of China during September 2010. Samples were immediately filtered through 0.45-μm nitrocellulose membrane (filtration equipments were soaked in 10% HCl, rinsed with Milli-Q water, and sterilized before use) and stored in clean polycarbonate bottles at 4 °C. The bioreporter cells precultured in 100 nM Fe^{3+} Fraquil medium as mentioned previously were collected and inoculated into three filtered water samples, and then the luciferase activity was measured after 12 h. Addition of 1000 nM $FeCl_3$ (decreases the luciferase activity) and alternatively 1000 nM desferrioxamine mesylate (DFB; Sigma), a specific chelator of iron (increases the luciferase activity), respectively, served as negative and positive controls when assessing iron bioavailability of water samples. The dissolved iron of water samples was determined by graphite furnace atomic absorption spectrometry (GFAAS, Perkin-Elmer AA-800) at Test Center of Wuhan University.

Results and discussion

Dose–response curve of bioreporter

Luciferase activities of bioreporter cells increased sigmoidally along with the increase in pFe at incubation time of 12, 24, or 48 h and reached the highest at 12 h (Fig. 1a). A long incubation time could result in depletion of nutrients and biological variations in culture medium, which might influence the response of bioreporters to iron thus constraining the utilization of iron by cells. Thus, the incubation time must be as short as possible while assaying bioavailable iron. A 12-h incubation time was appropriate for the bioreporter in our study. Furthermore, a dose–response curve of the bioreporter at pFe ranging from 18.8 ($Fe^{3+} = 10^{-18.8}$ M) to 21.7 ($Fe^{3+} = 10^{-21.7}$ M) was generated at 12 h, with a linear range extending between pFe 19.6 ($Fe^{3+} = 10^{-19.6}$ M) and pFe 21.5 ($Fe^{3+} = 10^{-21.5}$ M; Fig. 1b). At 12 h of incubation, the sigmoidal curve and linear regression equations of the bioreporter were described as follows:

$$y = 2349794.3 - 2326065.3/\{1 + \exp[(x - 20.49)/0.419]\}, R^2 = 0.9996 \quad (1)$$

$$y = 1110920x - 21559260, R^2 = 0.9757 \quad (2)$$

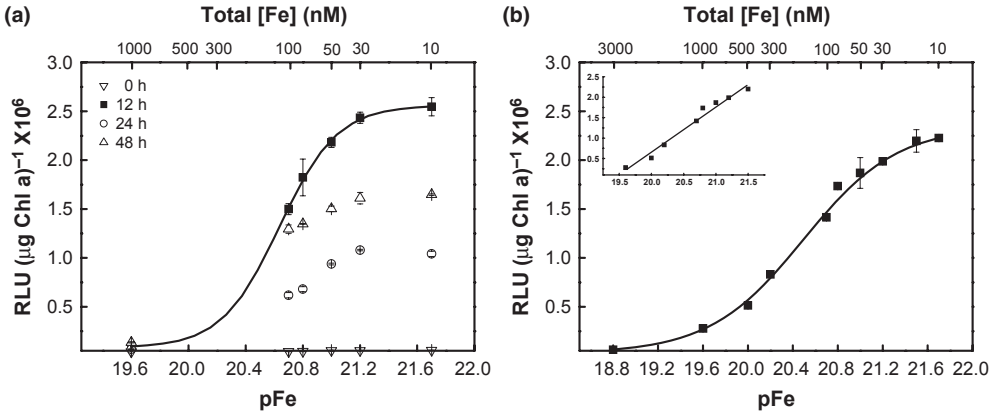

Fig. 1. Dose–response curve of bioreporter $P_{alr0397}$-*luxAB* between pFe 19.6 ($Fe^{3+} = 10^{-19.6}$ M) and pFe 21.7 ($Fe^{3+} = 10^{-21.7}$ M) (a) in Fraquil medium at incubation times of 0, 12, 24, and 48 h or between pFe 18.8 ($Fe^{3+} = 10^{-18.8}$ M) and pFe 21.7 ($Fe^{3+} = 10^{-21.7}$ M) (b) at 12 h of incubation [inset: the linear relationship of luciferase activity and pFe between pFe 19.6 ($Fe^{3+} = 10^{-19.6}$ M) and pFe 21.5 ($Fe^{3+} = 10^{-21.5}$ M)].

The dose–response characterization of pFe and luciferase activity in iron bioreporter *Synechococcus* sp. PCC 7942-KAS101 was described as a typical sigmoidal curve with a linear range between pFe 20.6 ($Fe^{3+} = 10^{-20.6}$ M) and pFe 21.1 ($Fe^{3+} = 10^{-21.1}$ M; Durham *et al.*, 2002; Porta *et al.*, 2003), and the range of its response to Fe^{3+} was narrower compared with $P_{alr0397}$-*luxAB*. However, iron bioreporter *P. putida*, a heterotrophic bioluminescent reporter, boasts a wide pFe range (16.8–19.5; $Fe^{3+} = 10^{-16.8}$–$10^{-19.5}$ M) and is used to analyze iron availability in seawater (Mioni *et al.*, 2005).

Optimal measurement conditions of bioreporter

Environments in lakes, rivers, and other natural freshwaters are complicated, and some of the physical–chemical parameters are not easily measured there. The drift of these parameters might bring out the variation of luciferase activity and, consequently, affect the application of bioreporters in detection of samples from natural aquatic environments. To determine the application range of bioreporter $P_{alr0397}$-*luxAB*, we studied the influence of initial inoculum density and concentrations of nitrogen, phosphorus, Co^{2+}, Mn^{2+}, Zn^{2+}, and Cu^{2+} on luciferase activity of the bioreporter under laboratory conditions in Fraquil medium with 10, 100, and 1000 nM Fe^{3+}. The bioreporter cells were incubated for 12 h under the growth conditions described previously prior to bioluminescence measurement.

Previous research revealed that high biomass of bioreporters (e.g. 10^7 cells mL^{-1}) could be used to increase bioreporter signal intensity (Van Der Meer *et al.*, 2004). However, owing to the high levels of various organic chelants (Powell & Wilson-Finelli, 2003a, b) and slow kinetics of reaction (Hudson & Morel, 1990), the concentration of dissolved iron is very low in some freshwaters (Nriagu *et al.*, 1996; Sterner *et al.*, 2004; Porta *et al.*, 2005). The use of high biomass was likely to result in a bulk depletion of bioavailable iron and affect the practical assessment of iron bioavailability. With the increase in cell inoculum density (from 0.02 $OD_{730\,nm}$ to 0.11 $OD_{730\,nm}$), luciferase activity of bioreporter $P_{alr0397}$-*luxAB* increased firstly and then decreased (Fig. 2). A large consumption of bioavailable iron would promote the biosynthesis of siderophores to complex Fe^{3+} into cells (Ferguson & Deisenhofer, 2002; Wandersman & Delepelaire, 2004), which led to the increase in luciferase activity as cell numbers increased. Under higher Fe^{3+} concentrations (e.g. 100 or 1000 nM), the increase in siderophores because of the increased cell number could accelerate Fe^{3+} transport so as to rapidly enhance iron bioavailability, which might inversely suppress the use of iron to reduce

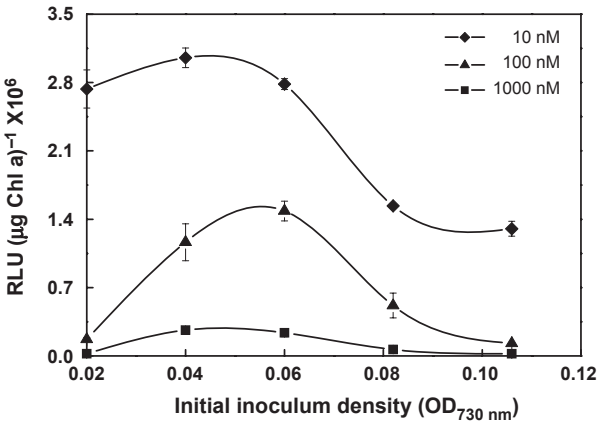

Fig. 2. Influence of initial inoculum density on luciferase activity of bioreporter $P_{alr0397}$-*luxAB* in Fraquil medium with various Fe^{3+} concentrations for 12 h.

luciferase activities of the bioreporter. At lower Fe^{3+} concentrations (e.g. 10 nM), when the cell numbers reached a high level, huge depletion of iron by excessive algal cells might affect the normal function of cells, thus inhibiting luciferase activity. Therefore, an initial inoculum density of $OD_{730\,nm} = 0.06$ was appropriate for the detection of bioavailable iron in water samples by bioreporter $P_{alr0397}$-*luxAB*.

The concentrations of nitrogen (N) and phosphorus (P) are greatly different in freshwaters. For example, the concentrations of total nitrogen (TN) and total phosphorus (TP) are 13.6–42.4 and 0.16–0.28 μM in Lake Erie (Charlton & Milne, 2004; DeBruyn *et al.*, 2004). By contrast, the concentrations of TN and TP in Taihu, Chaohu, and Dianchi lakes of China, respectively, are 116.4–460.7 and 0.74–12.52 μM, 67.9–274.3 and 2.58–13.55 μM, and 214.3–1071.4 and 4.19–45.16 μM (Wang & Chen, 2009; Xu *et al.*, 2010; Li & Xiao, 2011; Wilhelm *et al.*, 2011). The TN and TP of water samples from Taihu, Donghu, and Chaohu lakes, in our study, during September 2010 are shown in Table 1. To observe the impact of N and P concentrations on utilization of iron by bioreporter $P_{alr0397}$-*luxAB*, a series of NO_3^- and PO_4^{3-} concentrations in Fraquil medium with three Fe^{3+} concentrations were set to determine the response of luciferase activities to the concentrations of N and P. In Fraquil medium with 10 or 100 nM Fe^{3+}, luciferase activity of bioreporter $P_{alr0397}$-*luxAB* was enhanced with the increase in NO_3^- concentration and decreased slightly (remaining at a high level) with NO_3^- ranging from 100 to 900 μM (Fig. 3a); similarly, its luciferase activity increased significantly when PO_4^{3-} increased from 0.1 to 1 μM and varied a little with further increase in PO_4^{3-} concentration (Fig. 3b). In Fraquil medium with 1000 nM Fe^{3+}, its luciferase activity increased slightly with the increased N and P

Table 1. Temperature, pH, dissolved O_2 (DO), TN, and TP of water samples from Taihu, Donghu, and Chaohu lakes during September 2010.* Dissolved iron and bioavailable iron measured by bioreporter $P_{alr0397}$-*luxAB* in water samples from the three lakes (mean ± SD, n = 3–4)

Sample station	Temperature (°C)	pH	DO (mg L^{-1})	TN (mg L^{-1})	TP (mg L^{-1})	Dissolved iron (nM)	Luciferase activity [RLU (μg Chla)$^{-1}$]	pFe Equivalent† (linear regression)	Luciferase activity‡ [RLU (μg Chla)$^{-1}$]	
									+DFB	+Fe
Taihu Lake	26.6	6.67	8.27	2.13	0.18	183.1 ± 13.9	228 762 ± 3353	19.61	36 9194	14 182
Donghu Lake	19.8	8.41	7.72	1.40	0.16	147.3 ± 1.3	594 677 ± 7323	19.94	66 5304	78 071
Chaohu Lake	27.3	8.20	8.60	3.20	0.18	131.3 ± 1.2	425 577 ± 17 060	19.79	1 005 992	198 824

*Provided by Professors Dunhai Li and Leyi Ni at the Institute of Hydrobiology, Chinese Academy of Sciences.
†Calculated from linear regression of luminescent response.
‡Positive (addition of 1000 nM DFB) and negative (addition of 1000 nM Fe) control.

concentrations. When the concentrations of N and P were high enough (e.g. 100 μM NO_3^- and 10 μM PO_4^{3-} in this study), further increases in N and P concentrations had little influence on the luciferase activity, showing that iron utilization might not be affected by the uptake of N and P in cells. The variation of N and P concentrations had no effect on luciferase activity of bioreporter in 1000 nM Fe^{3+} concentration condition, which also indicated that iron utilization was not directly related with the uptake of N and P in cells.

Fur acts as a transcriptional repressor of iron-regulated promoters by virtue of its iron-dependent DNA-binding activity to regulate expression of several genes involved in iron homeostasis (Escolar *et al.*, 1999). At high concentrations, Co^{2+} and Mn^{2+} presumably mimic Fe^{2+} (Bagg & Neilands, 1987; Hantke, 1987), and Zn^{2+} can also activate Fur *in vitro* (Bagg & Neilands, 1987). So these metals could possibly interfere with iron detection. In addition, other metals such as Cu^{2+} can compete with iron to chelate dissolved organic siderophores secreted by cells, thus decreasing iron availability (Nicolaisen *et al.*, 2008).

The concentrations of metals in lakes greatly varied and are easily affected by surrounding environments. Take the concentration ranges of Co^{2+}, Zn^{2+}, and Cu^{2+} in Wuhan City (China) for instance; they were 3.7–4.9, 13.1–181.2, and 18.4–83.8 nM in Donghu Lake located in a scenic area, were 7.8–17.6, 1.2–285.1, and 43.1–916.7 nM in Moshui Lake situated in an industrial area, and were 4.9–6.8, 0–0.9, and 58.4–67.7 nM in Houguan Lake in the suburbs (Yu *et al.*, 2007). In an attempt to determine the influence of Co^{2+}, Mn^{2+}, Zn^{2+}, and Cu^{2+} concentrations on iron bioavailability, luciferase activity of bioreporter $P_{alr0397}$-*luxAB* at six concentrations of Co^{2+}, Mn^{2+}, Zn^{2+}, and Cu^{2+} was, respectively, measured in Fraquil medium with three Fe^{3+} concentrations. The increase in Mn^{2+} concentration had no effect on luciferase activity of the bioreporter (Fig. 3d). In Fraquil medium with 10 or 100 nM Fe^{3+}, luciferase activity of the bioreporter decreased significantly with the increase in the metal concentrations when the concentration of Co^{2+} was higher than 22.5 nM (Fig. 3c), Zn^{2+} was higher than 12 nM (Fig. 3e), or Cu^{2+} was higher than 50 nM (Fig. 3f). In Fraquil medium with 1000 nM Fe^{3+}, luciferase activity of the bioreporter was not influenced by the increase in Co^{2+}, Zn^{2+}, and Cu^{2+} concentrations. Therefore, when assessing bioavailable iron by bioreporter $P_{alr0397}$-*luxAB* in natural freshwaters, the concentrations of Co^{2+}, Zn^{2+}, and Cu^{2+} should be taken into account.

Application of bioreporter in water samples

Luciferase activity of bioreporter $P_{alr0397}$-*luxAB* in water samples from Taihu, Donghu, and Chaohu lakes were all

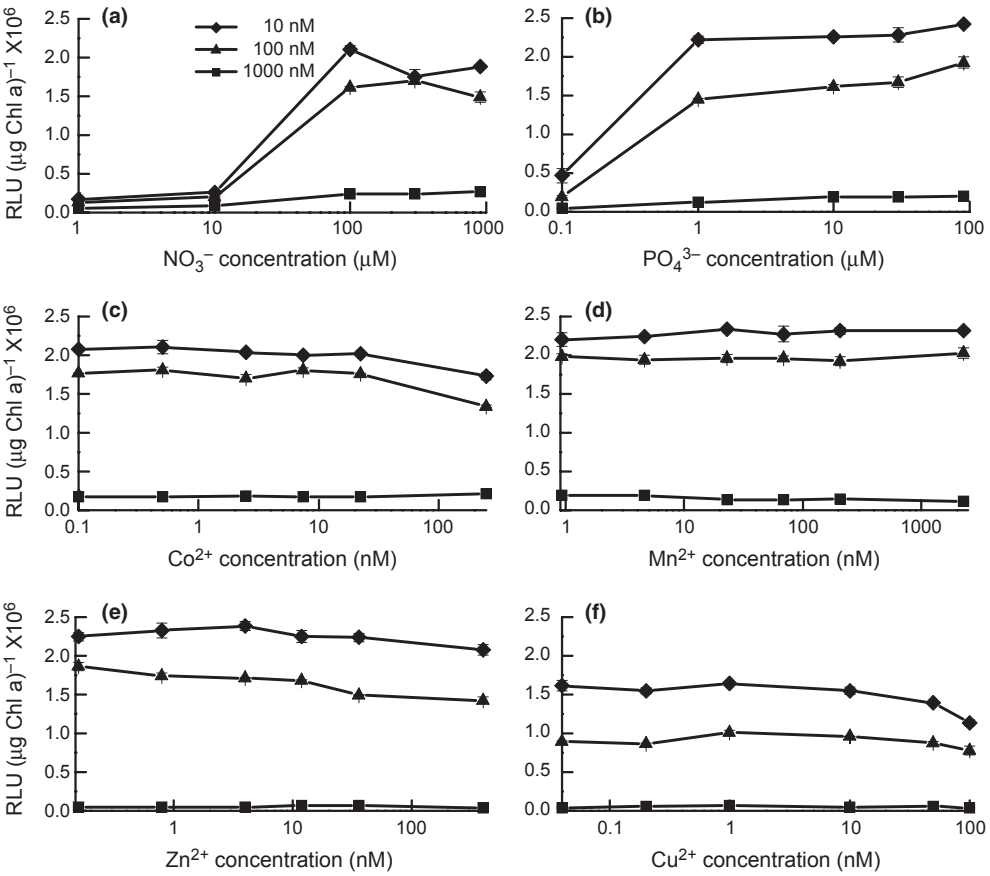

Fig. 3. Influence of NO_3^- (a), PO_4^{3-} (b), Co^{2+} (c), Mn^{2+} (d), Zn^{2+} (e), and Cu^{2+} (f) concentrations on luciferase activity of bioreporter $P_{alr0397}$-*luxAB* in Fraquil medium with various Fe^{3+} concentrations for 12 h.

within the linear range of the dose–response curve. Bioavailable iron concentrations (pFe) of three water samples from Taihu, Donghu, and Chaohu lakes calculated with the linear Eqn. (2) were 19.61 ($Fe^{3+} = 10^{-19.61}$ M), 19.94 ($Fe^{3+} = 10^{-19.94}$ M), and 19.79 ($Fe^{3+} = 10^{-19.79}$ M), respectively, and total dissolved iron in these samples determined by GFAAS was 183.1, 147.3, and 131.3 nM (Table 1). The availability of iron to organisms is dependent on (1) total concentration of the iron; (2) its chemical speciation; and (3) how the physical–chemical properties (such as temperature, pH, and higher-affinity ligands) of a system alter that speciation (Buffle, 1988). In lakes, because of the interaction of iron with dissolved organic matter (DOM), iron binds to the aliphatic and aromatic carboxyl and hydroxyl functional groups of DOM to form dissolved complexes. The chelating properties of DOM and the formation of DOM–Fe and DOM–Fe–P complexes probably make them not directly available to organisms (Maranger & Pullin, 2003). It can be deduced that iron exists mainly in the form of iron chelates in the three lakes. Bioavailable pFe with 20.55 ($Fe^{3+} = 10^{-20.55}$ M) and 20.9 ($Fe^{3+} = 10^{-20.9}$ M) and dissolved iron with 74.6 and 12.1 nM were measured at two stations of Lake Erie by bioreporter KAS101 of *Synechococcus* sp. PCC 7942 (Durham *et al.*, 2002). In addition, because of the different physical–chemical parameters in the aquatic environments, iron availability may not coincide with the increase in the concentration of the dissolved iron (Hassler *et al.*, 2006). High pFe is observed in the water samples from Taihu Lake, which might result from its low pH value. The data of TN and TP in the three lakes indicate that they are all seriously polluted. However, compared with the two other eutrophic lakes, Donghu Lake possesses the lower bioavailable iron, although with a high dissolved iron, indicating a possible explanation of the disappearance of cyanobacterial bloom there.

Different from previous studies, bioreporter $P_{alr0397}$-*luxAB* of *Nostoc* sp. PCC 7120 has wider responsive range of Fe^{3+} (pFe = 18.8–21.7, $Fe^{3+} = 10^{-18.8}$–$10^{-21.7}$ M) and is an ideal quantitative tool to assess bioavailable iron in various water quality samples, especially in eutrophic lakes with high total iron. Optimal conditions for the application of bioreporter $P_{alr0397}$-*luxAB* were determined

to be: cell inoculum density of $OD_{730\,nm}$ = 0.06, 100 μM NO_3^-, and 10 μM PO_4^{3-}; moderate concentrations of Co^{2+} (0.1–22.5 nM), Zn^{2+} (0.16–12 nM), and Cu^{2+} (0.04–50 nM); and wider concentrations of Mn^{2+} (0.92–2300 nM).

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