

Construction and characterization of a cyanobacterial bioreporter capable of assessing nitrate assimilatory capacity in freshwaters

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Abstract

The use of cyanobacterial whole-cell luminescent bioreporters has enhanced our ability to monitor nutrient availability in aquatic ecosystems. We have constructed a *Synechocystis* sp. strain PCC6803 bioluminescent reporter for the assessment of nitrate bioavailability. Specifically, a 380–base pair DNA fragment containing the NtcA/B-dependent nitrate/nitrite-activated *nirA* promoter (regulating expression of genes encoding nitrite reductase) was fused to the bacterial luciferase genes, *luxAB*, and introduced into *Synechocystis* by genetic transformation. Characterization of this strain, designated AND100, yielded dose-dependent increased bioluminescence coincident with increased nitrate added to the growth medium from 1 to 100 μM . Bioluminescence in response to nitrate addition was light dependent up to 50 $\mu\text{mol quanta m}^{-2} \text{ s}^{-1}$. Assessing environmental samples collected from oligotrophic Lake Superior, we demonstrated that the onset of luminescence coincided with the drawdown of nitrate by simultaneously monitoring nitrate depletion from reaction vessels. Nitrate in the Lake Superior samples was consistently underestimated by the bioreporter. Only by following amendment of these samples with phosphate and iron was total nitrate accurately reflected by the cyanobacterial bioreporter. Thus, strain AND100 can be used to elucidate factors that constrain use of nitrate in freshwaters. This is pertinent to a system such as Lake Superior where the concentration of nitrate has increased 6-fold in the last century. Indeed, pilot experiments with the bioreporter suggest that nutrient co-limitation (P and Fe), as well as low light, may reduce the capacity for nitrate assimilation in field samples from Lake Superior.

Cyanobacteria are a dominant component of phytoplankton in marine and freshwater oligotrophic systems (for review, see Stockner 1988 and references therein) where primary production is frequently limited by nutrient availability. Whereas availability of phosphate is traditionally considered as the major factor limiting growth in freshwater ecosystems (Schindler 1977; Hudson et al. 2000), pools of other elements may be depleted, or their availability limited due to speciation effects. With respect to the Laurentian Great Lakes, various studies have documented evidence for low levels of iron (Nriagu et al. 1996; Twiss et al. 2000; Sterner et al. 2004), nitrate (MacGregor et al. 2001), and silicate (Schelske et al. 1986), suggesting that several elements, in addition to phosphate, warrant consideration as possible limiting factors.

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A recently developed approach for the quantification of nutrient availability in freshwater environments is the use of cyanobacterial whole cell luminescent bioreporters (Bachmann 2003; Belkin 2003). Whereas rapid and reliable chemical protocols are available to measure absolute levels of specific nutrients in water samples, bioreporters provide data on the capacity of the biota to acquire and assimilate these nutrients. Recombinant bioluminescent cyanobacterial strains have been successfully applied in monitoring iron (Durham et al. 2002; Porta et al. 2003) and phosphate (Gillor et al. 2002) availability in freshwater. In this study, we constructed a *Synechocystis* sp. strain PCC6803 bioluminescent reporter strain to assess nitrate/nitrite bioavailability in freshwater environments. The construct employs the promoter of the nitrite reductase gene, *nirA*, fused to the bacterial luciferase genes, *luxAB*. The *nirA* promoter is under positive control by two transcription factors, NtcA and NtcB, that together yield elevated transcription when bioavailable nitrate or nitrite is present in the medium (Frias et al. 2000; Aichi et al. 2001). The strain, designated AND100, exhibits NtcA/B-dependent bioluminescence under conditions that favor nitrate/nitrite assimilation, and the intensity of luminescence is a measure of nitrate/nitrite uptake. Combined with additional sensors that respond to ammonium (Gillor et

NtcB-binding motif **NtcA-binding motif** **-10 element**
 5'-ctaaatgcgtaaactgcatatgacctcgcctgagtgtaattacggtacaaatfttaacgaaacgggaaccctatattgatctctac-3'

Fig. 1. Elements of the *Synechocystis* sp. PCC 6803 *nirA* promoter driving *luxAB* expression in bioreporter strain AND100. The 3' end of the sequence corresponds to the 3' end of the PCR amplicon cloned into plasmid pILA.

al. 2003), the capacity to assimilate various nitrogen species can be evaluated in freshwater systems. Because nitrate concentrations in most freshwater systems exceed those of nitrite by more than an order of magnitude (Mortonson and Brooks 1980), the bioreporter can be viewed primarily as a sensor for nitrate bioavailability. As such, this strain will be particularly useful in experiments designed to address factors influencing nitrate use by phytoplankton.

Materials and procedures

Media and growth conditions—For routine laboratory growth of *Synechocystis* sp. PCC6803, BG-11 medium (as described at www.cyanosite.bio.purdue.edu) was employed throughout, except that the concentration of NaNO_3 was reduced to yield a N:P ratio of 10. Experimental manipulation of nitrate concentration was achieved by adding NaNO_3 to nitrate-free BG-11 at concentrations ranging from 1 to 1000 μM . To maintain a constant osmotic strength of the medium, equimolar amounts of NaCl were added as appropriate to replace NaNO_3 . Additionally, the ferric ammonium citrate stock was replaced with equimolar FeCl_3 to avoid interference in those experiments where exogenous ammonium was added. Kanamycin was added to 30 $\mu\text{g mL}^{-1}$ to select for the drug resistant marker in the nitrate bioreporter strain AND100. All cultures were bubbled with air and grown at 22°C in constant light (50 $\mu\text{mol quanta m}^{-2} \text{ s}^{-1}$) provided by cool-white fluorescent lamps. Growth of batch cultures was routinely monitored at daily intervals by measuring light scattering at 750 nm (OD_{750}).

Construction of the PnirA::luxAB fusion strain AND100—The functional components of the *nirA* promoter (*PnirA*) includes a consensus sequence (GTAN_8TAC) for binding of a catabolite activator protein (CAP)-type transcriptional regulator, NtcA, and a motif (ATN_{11}AT), constituting a binding site for a LysR family protein, NtcB (Fig. 1). Transcription of the *nirA* gene is up-regulated by NtcA in ammonium depleted conditions, and NtcB is thought to serve as an enhancer of *nirA* transcription in the presence of nitrate or nitrite (Aichi et al. 1997; Frias et al. 2000; Aichi et al. 2001).

The pILA recombinant plasmid vector described by Kunert et al. (2000) allows the fusion of *KpnI*-adapted promoter fragments upstream from the *Vibrio harveyi luxAB* genes encoding bacterial luciferase. Following plasmid construction and retrieval of plasmid clones from *Escherichia coli* DH5 α , genetic transformation of *Synechocystis* sp. PCC6803 yields the insertion of the promoter::luxAB fusion into the chromosome by homologous recombination. The availability of the complete *Synechocystis* sp. PCC6803 genomic sequence (for review, see

Kaneko and Tabata 1997; Nakamura et al. 1998) allows the rapid cloning of any promoter sequence following PCR amplification. Specifically, a 380-bp fragment including the entire *nirA* promoter was amplified by PCR with the following primers (the *KpnI* site underlined): 5'-TGTAGGTACC-CAAGCTCAGAATGCTGC-3' (forward), and 5'-CAACGTACCAGCCAGATAACAGTAGAGAT-3' (reverse). PCR was performed for 30 cycles of the following temperatures: 94°C, 1 min; 55°C, 2 min; 72°C, 3 min. Following ligation of the *KpnI*-digested PCR products into pILA, transformation of *Synechocystis* sp. PCC6803 yielded strain AND100 following selection on kanamycin BG-11 plates.

Characterization of AND100 as a nitrate bioreporter—Nitrate-dependent luminescence of AND100 was characterized in modified BG-11 media containing NaNO_3 amendments ranging from 1 to 100 μM . Prior to assaying luminescence in media or field samples, cells were prepared by first growing AND100 cultures to late exponential phase ($\text{OD}_{750\text{nm}} \sim 1.0$) in low nitrate BG-11. Cells were harvested by centrifugation at 4000g for 5 min, washed twice in nitrate-free BG-11, and resuspended to a final $\text{OD}_{750\text{nm}}$ of 0.1 in lake water or BG-11 of defined nitrate concentration. Luminescence of AND100 cultures was measured with a Femtomaster FB14 luminometer (ZyLux Corp.) immediately following the addition of 20 μL methanol containing 27 mM *n*-decyl aldehyde, a substrate for bacterial luciferase, to 2 mL of the sample. Whereas direct addition of *n*-decyl aldehyde to a *Synechococcus* sp. PCC7942 bioreporter yielded transient luminescence, suggesting toxicity (Porta et al. 2003), *Synechocystis* sp. PCC6803 exhibited a strong and stable luminescent response under these conditions. Thus, the AND100 bioreporter can be assayed more quickly, avoiding a long incubation in aldehyde vapors as is required for the *Synechococcus* sp. constructs. Luminescence, normalized to $\text{OD}_{750\text{nm}}$ of the sample, was averaged from readings observed from four replicates.

Water collection from Lake Superior—Epilimnetic water (5 m depth) was collected from stations ON-2 (46°58.00'N, 89°21.50'W; 12 September 2002 and 20 May 2004) and HN-210 (47°15.49'N, 88°07.99'W; 30 July 2001), both located in waters offshore from the Keweenaw Peninsula, using a trace metal clean-pumping system (Field and Sherrell 2003; Sterner et al. 2004). Water pumped from the epilimnion was passed through a 0.45- μm capsule filter and collected in acid-cleaned polycarbonate bottles. Samples were either frozen (HN-210 and ON-2 from 2001 and 2002, respectively), or immediately tested with the bioreporter in the shipboard laboratory (ON-2 sample from 2004). All frozen samples were routinely thawed immediately prior to the bioreporter assay.

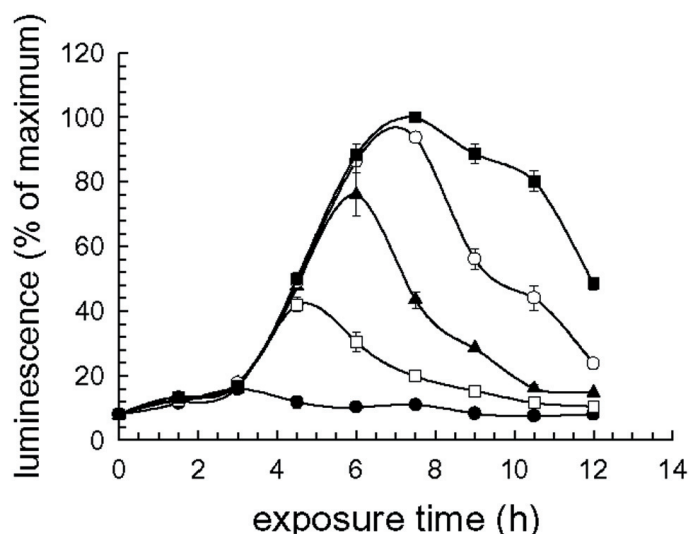


Fig. 2. Bioluminescence of *Synechocystis* sp. reporter strain AND100. Late log phase cells grown in BG11 (modified as described in the text) were transferred at the initial time to BG11 with various concentrations of NaNO_3 (●, 1 μM ; □, 10 μM ; ▲, 25 μM ; ○, 50 μM ; ■, 100 μM). Luminescence was normalized against optical density (750 nm). Data are presented as percent of maximal luminescence obtained in 100 μM NaNO_3 ($2419 \pm 19 \text{ RLU OD}_{750}^{-1}$). Error bars represent standard deviations ($n = 4$).

Monitoring nitrate depletion in bioreporter assays—Assessment of nitrate uptake by the AND100 bioreporter was achieved by measuring nitrate depletion from water sampled at station ON-2 during the course of a bioreporter assay. Nitrate concentration was monitored by using a probe fitted with a biochamber containing denitrifying bacteria defective in nitrous oxide reductase (NO_x^- biosensor; Unisense A/S). Nitrate reduction by bacteria in the biochamber yielded nitrous oxide, which was detected via a Clark-type electrode coupled to a picoammeter (PA2000; Unisense). Electrode polarization was performed according to the manufacturer's instructions. The probe was calibrated to detect micromolar nitrate by constructing a standard curve obtained following incremental spiking of ON-2 water with 1, 2, 5, and 10 μM NaNO_3 . At several time points during a shipboard bioreporter assay, 10 mL aliquots were withdrawn and nitrate concentration measured using the probe.

Assessment

Nitrate-dependent activation of AND100 bioluminescence—Addition of the AND100 bioreporter to BG-11 media containing different concentrations of nitrate showed increased luminescence in response to added nitrate with clear differences resolved between 1 and 50 μM nitrate (Fig. 2). The time course for nitrate-dependent luminescence yielded a maximum after 4 to 7 h incubation, followed by a decline. The transient nature of the luminescent response was likely due to ammonium-dependent NtcA nutritional repression resulting from the intracellular accumulation of ammonium following nitrate reduc-

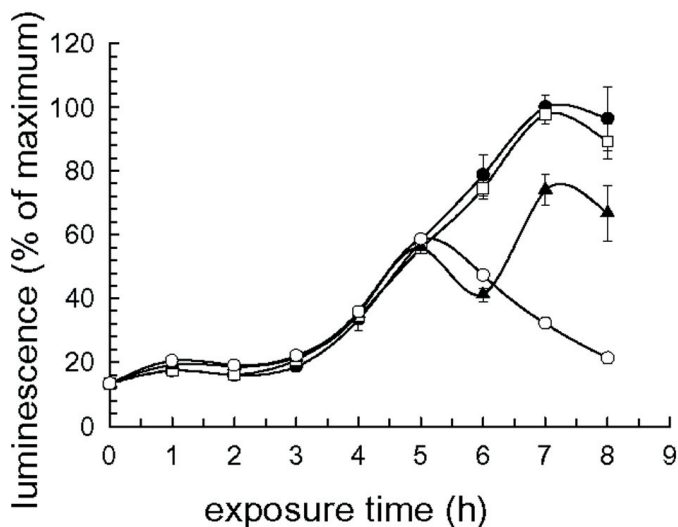


Fig. 3. Effect of ammonium addition on nitrate induced bioluminescence in AND100. Late log phase cells grown in decreased N BG11 were transferred at the initial time to BG-11 containing 100 μM NaNO_3 . At the 5-h time point, various amounts of NH_4Cl were added to the samples (○, final concentration of NH_4Cl [100 μM]; ▲, 10 μM ; □, 1 μM ; ●, no NH_4Cl added). Luminescence was normalized against optical density (750 nm). Data are presented as percent of maximal luminescence obtained when ammonium was not added to the sample ($1390 \pm 49 \text{ RLU} \cdot \text{OD}_{750}^{-1}$). Error bars represent standard deviations ($n = 4$).

tion (Aichi et al. 2001). Indeed, the addition of methionine sulfoximine, an inhibitor of ammonium assimilation, to AND100 incubated in 100 μM nitrate abolished the decline phase seen at 8 h (data not shown). Treatment with methionine sulfoximine resulted in constitutive derepression of nitrate/nitrite dependent *nirA* transcription, as reported previously (Aichi et al. 2001).

A dose-response curve revealed a threshold for nitrate-dependent luminescence in the range of 1 to 10 μM nitrate within 5 h exposure (Fig. 2). Dose-response curves were routinely constructed at the time points varying between 5 and 7 h, depending on the kinetics of induction of luminescence during the course of the assay. Given that spring survey nitrate concentrations in the upper Great Lakes typically fall within the range of 20 to 30 μM (U.S. EPA 2004), the response of our bioreporter is appropriate for assessing nitrate availability in these freshwater systems.

Factors influencing AND100 nitrate-dependent luminescence in BG-11 media—Due to the dual control of *PnirA* by NtcA and NtcB, the output of the AND100 bioreporter is likely affected by the speciation of nitrogen. The NtcA protein yields transcriptional activation under nitrogen deficiency, but nutritional repression coincident with ammonium assimilation (Herrero et al. 2001). Reflecting this, induction of luminescence in 100 μM nitrate was fully repressed by an equimolar addition of ammonium (Fig. 3). Addition of ammonium to a concentration one-tenth that of nitrate yielded transient repression (Fig. 3), reflecting the physiological preference for

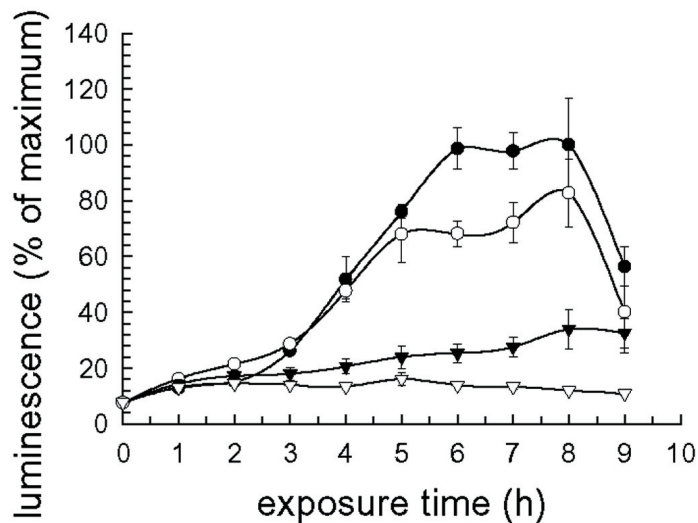


Fig. 4. Effect of light intensity on nitrate induced bioluminescence in AND100. Late log phase cells grown in BG-11 depleted in nitrate were transferred at the initial time to BG-11 containing 100 μM NaNO_3 . ●, 50 $\mu\text{mol quanta m}^{-2} \text{s}^{-1}$; ○, 30 $\mu\text{mol quanta m}^{-2} \text{s}^{-1}$; ▲, 15 $\mu\text{mol quanta m}^{-2} \text{s}^{-1}$; △, 0 $\mu\text{mol quanta m}^{-2} \text{s}^{-1}$. Data are presented as percent of maximal luminescence obtained at 50 $\mu\text{mol quanta m}^{-2} \text{s}^{-1}$ ($2991 \pm 499 \text{ RLU OD}_{750}^{-1}$). Error bars represent standard deviations ($n = 4$).

ammonium over nitrate as a source of nitrogen. Transient repression in 10 μM ammonium was most likely due to uptake of ammonium, yielding decreased luminescence, followed by derepression of luminescence when ammonium became depleted from the medium by assimilation. The observed repression of *PnirA*-dependent gene expression at 10 μM ammonium was consistent with other reports demonstrating repression in cyanobacteria at similar ammonium concentrations (Flores et al. 1980; Dortch 1990).

In addition to photosynthetically derived reducing power needed to reduce nitrate to ammonium during assimilation, more recent studies have shown that NtcA-dependent activation of nitrogen assimilatory genes requires α -ketoglutarate as a coinducer (Tanigawa et al. 2002; Vasquez-Bermudez et al. 2003). Taking this into account, photosynthetic light reactions and carbon fixation together likely influence the activation of transcription during nitrogen limitation. Thus, we tested the induction of luminescence in 100 μM nitrate at several light intensities. Nitrate-dependent transcription occurred only in the light, and increased with increasing light intensity (Fig. 4). Such data support the observation that light and nitrate limitation in cyanobacteria and eukaryotic algae exhibit a synergistic relationship (Rhee and Gotham 1981; Healey 1985). The light-dependent response exhibited by AND100 is potentially useful because of the fact that light can limit phytoplankton growth in lakes such as Lake Superior during periods of both vernal holomixis (Nalewajko and Voltolina 1986) and summer stratification (Nalewajko et al. 1981).

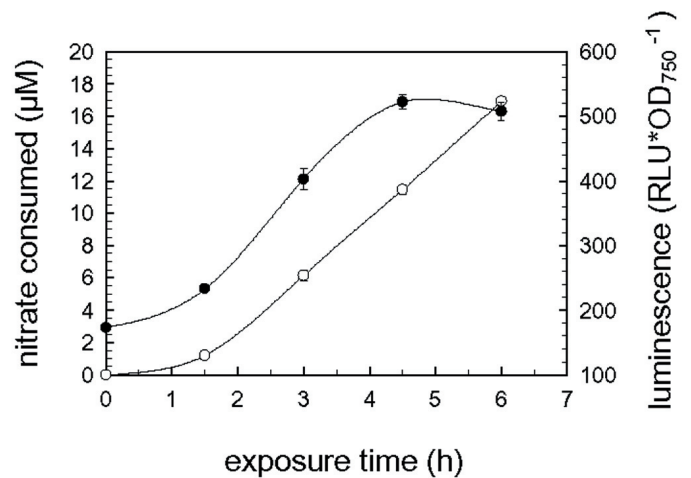


Fig. 5. Coincident induction of luminescence and nitrate uptake. Water samples from pelagic Lake Superior station ON-2 were assayed with the AND100 bioreporter, and nitrate consumption measured together with bioluminescence. ●, luminescence; ○, nitrate consumed.

Induction of bioluminescence during nitrate assimilation—As a result of the dual nitrate/nitrite regulation of the *PnirA* promoter, bioluminescence should be closely coupled temporally to the assimilation of nitrate in the medium. To test whether the onset of luminescence can be correlated with depletion of nitrate from the medium, a nitrate-specific biosensor electrode was employed to monitor nitrate depletion during the course of a bioreporter assay with Lake Superior water collected from pelagic station ON-2. Indeed, nitrate consumption and the induction of luminescence followed the same kinetics (Fig. 5).

Use of the AND100 bioreporter to assess nitrate assimilation in field samples—Lake Superior water collected from pelagic stations ON-2 and HN-210 was tested with the bioreporter to investigate whether the strain could be used to assess nitrate assimilation capacity. A calibration curve yielded a linear response for nitrate concentrations ranging from 10 to 50 μM ($r^2 = 0.985$), and seeding lake water with the bioreporter yielded a luminescent response following 5 to 6 h incubation (Fig. 6). Plotting the bioluminescence onto the calibration curve provided an apparent nitrate concentration of 19 μM at station ON-2 and 25 μM at station HN-210. Since the actual nitrate concentrations at ON-2 and HN-210 were 22.5 μM and 37 μM , respectively, we note that the bioreporter underestimated the true nitrate level, suggesting that nitrate drawdown by the bioreporter was impaired in these samples. By contrast, amendment of water sampled from ON-2 with 2 μM potassium phosphate and 10 nM ferric chloride resulted in an enhanced luminescent response yielding an apparent nitrate concentration of 23 μM (Fig. 6), nearly identical to the chemically derived value.

Further examining the influence of iron and phosphate on AND100 bioluminescence in lake samples, the bioreporter was

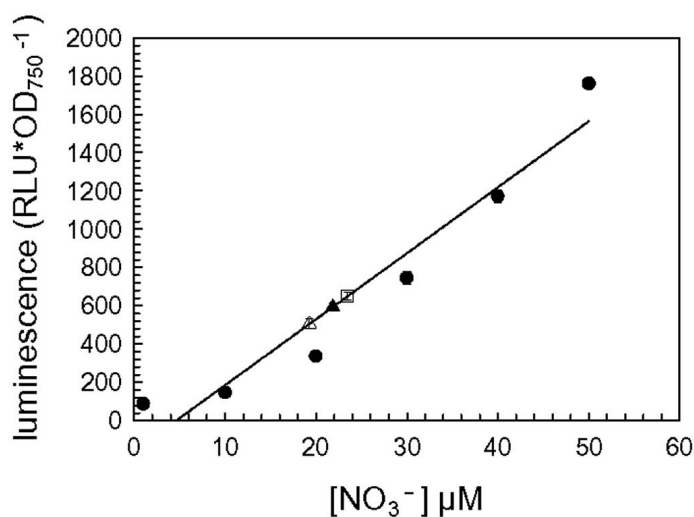


Fig. 6. Extrapolation of nitrate concentration in Lake Superior samples from AND100 luminescence. Bioluminescence of AND100 seeded into water samples from pelagic stations HN-210 and ON-2 are compared to luminescence values in BG-11 media of known nitrate concentration. Δ , unamended ON-2 water; \blacktriangle , ON-2 water amended with 10 nM Fe and 2 μ M phosphate; \square , unamended HN-210 water; \bullet , BG-11 of known nitrate.

used to assay ON-2 water amended by iron and phosphate individually and in combination (Fig. 7). This demonstrated that bioluminescence over 6 h exposure time was enhanced only when iron and phosphate were amended together, suggesting a colimitation of these nutrients as a factor constraining nitrate use.

Discussion

Utility of the bioreporter assay—In this paper, we describe a novel cyanobacterial bioreporter capable of assessing the nitrate assimilatory capacity of freshwater picoplankton. The data reported here indicate that the AND100 bioreporter can be used to yield a signal of suitable sensitivity and reproducibility from which bioavailable nitrate can be quantified. The strain yields nitrate/nitrite-responsive induction of bioluminescence due to the action of the NtcA/B transcriptional activators. The onset of luminescence and nitrate uptake is tightly coupled, thus the intensity of the luminescent signal can be viewed as a measure of nitrate assimilation. Notably, the dynamic range of the AND100 bioreporter is appropriate for measuring nitrate levels that typically occur in the Great Lakes. Whereas we recognize that a bioreporter constructed in *Synechocystis* sp. PCC 6803 may not be fully representative of the diversity of photosynthetic picoplankton of the Great Lakes, the AND100 strain can be viewed as a prototype in which detailed characterization can be carried out prior to expanding the technology to more ecologically relevant phototrophs.

The bioreporter assay can be viewed as an alternative method to measure nitrogen uptake in aquatic systems. Mass

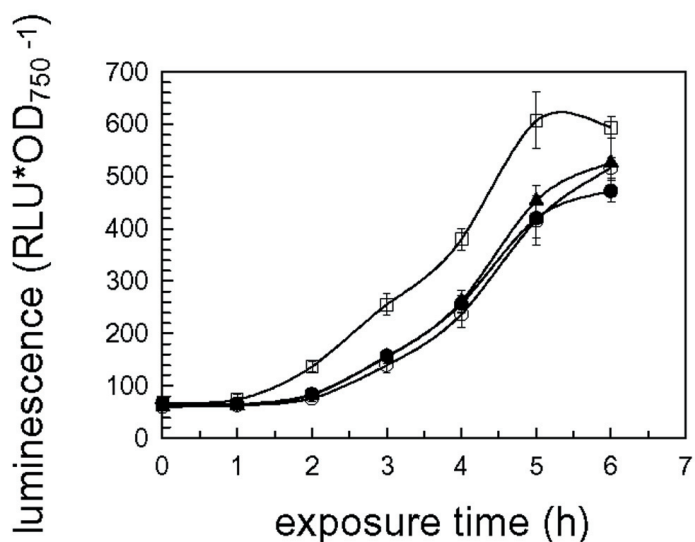


Fig. 7. Time course of AND100 luminescence in water sampled from pelagic station ON-2. Curves represent the following conditions: \bullet , no addition; \blacktriangle , addition of 2 μ M P; \circ , addition of 10 nM Fe; \square , addition of both P and Fe. Error bars represent standard deviations ($n = 4$).

spectrometry techniques that employ the stable isotope ^{15}N can be used to calculate the flux of nitrogenous compounds through aquatic ecosystems (Dugdale and Wilkerson 1986). Such techniques depend upon measuring the uptake and incorporation of ^{15}N by the biota present in the water sample. Since the method is both sensitive and dependent on the natural biological activity resident in the water sample, stable isotope labeling can provide useful measures of nitrogen uptake (Dugdale and Wilkerson 1986). Disadvantages include the expense of ^{15}N substrates and the high cost and availability of instrumentation, contamination of natural abundance ^{15}N , and the time required to analyze the samples (weeks or months). Additionally, separating heterotrophic versus algal uptake with ^{15}N is possible but may be difficult under oligotrophic conditions (J. Finlay, pers. comm. unref.). The AND100 bioreporter, while not an ecologically relevant strain, provides measures of nitrate use that are rapid, inexpensive, reproducible, and are likely indicative of nitrogen assimilation by phototrophs.

Application of the AND100 reporter to Lake Superior—Over the past 100 years, the nitrate concentration in Lake Superior has increased 6-fold. Whereas atmospheric deposition of nitrogen is thought to be the main source of the nitrate that has accumulated in the lake (Bennett 1986), there are likely many factors contributing to such a large change in a major biologically active chemical element. Nitrate may accumulate in Lake Superior as a result of low biotic demand. Alternatively, one or more environmental factors may constrain the ability of phytoplankton to use nitrate in the lake. Owing to the complex seasonal and synergistic relationships between light and nutri-

ent availability in the Great Lakes (Nalewajko et al. 1981; Nalewajko and Voltolina 1986; Millard et al. 1996; Fahnenstiel et al. 2000), the AND100 bioreporter exhibits performance characteristics under varying light regimes and nutrient status that are suitable to assess the influence of these factors on nitrate consumption. The reporter strain thus provides a proxy for the physiological responses of the endogenous cyanobacteria, whose nitrogen assimilatory functions are likely similarly regulated by light levels, nitrogen speciation, and bioavailability of both phosphate and iron. Indeed, the pilot experiments reported here provide evidence that phosphate and iron together constrain nitrate use in Lake Superior because the AND100 strain underestimated chemically derived nitrate levels unless supplemented with both of these nutrients (Fig. 6). These data are supported by a recent study showing that low availability of iron constrains even modest increases in growth response of endemic phytoplankton following amendment of water collected from Lake Superior with phosphate (Sterner et al. 2004). An extensive temporal and spatial survey of Lake Superior combining both traditional bottle amendment and bioreporter assays will be important in sorting out the individual contributions of light and nutrient limitation to the events leading to the long-term nitrifying of Lake Superior.

Comparison to other cyanobacterial N bioreporters—The properties of the AND100 strain differ in many respects from two cyanobacterial nitrogen bioreporters previously described (Mbeunkui et al. 2002; Gillor et al. 2003). These bioreporters are *luxAB* fusions employing the *Synechocystis* sp. PCC6803 *nblA* (Mbeunkui et al. 2002) and *Synechococcus* sp. PCC7942 *glnA* promoters (Gillor et al. 2003), controlling the genes encoding a phycobilisome degradation regulator and glutamine synthetase, respectively. Whereas the dynamic ranges of these strains were similar to AND100, the luminescent response was induced upon nitrogen deficiency, not during nitrogen use as described in this paper. Secondly, the responses of the *Synechococcus* sp. *PglnA* and *Synechocystis* sp. *PnblA* bioreporters were considerably slower, yielding dose-dependent luminescence on the order of 15 to 25 h (Mbeunkui et al. 2002; Gillor et al. 2003). Additionally, the *glnA* strain yielded dose-dependent responses to a wide variety of N species ranging from nitrate, ammonium, urea, and glutamine (Gillor et al. 2003), and *nblA* expression was responsive to nitrate and ammonium (Mbeunkui et al. 2002). Whereas such broader spectrum responses may be very useful properties for the measurement of total nitrogen bioavailability, the nitrate/nitrite specificity of the AND100 bioreporter provides a means for determining the bioavailability of specific nitrogen species, especially when used in concert with the *PglnA* reporter. Indeed, the *PglnA* reporter has been used to document low total nitrogen bioavailability along a west-to-east transect in Lake Erie (Wilhelm et al. 2003). In this context, the AND100 bioreporter likely could provide further insights by focusing on the potential for nitrate use in the Great Lakes. The posi-

tive induction of the AND100 luminescent response will allow one to measure the onset of nitrate use as light levels are manipulated and nutrients are amended to Lake Superior samples. By comparison, the properties of the GSL nitrogen bioreporter, whose bioluminescence is under repression by elevated nitrogen, would be less suitable for such an experiment. Overall, the AND100 strain affords a direct method for determining the role of both chemical and physical factors in regulating nitrate uptake by the endemic phytoplankton.

Concluding remarks: future prospects—Another potential application for the AND100 strain has been suggested based on recent studies examining the use of cyanobacteria in bioremediation efforts aimed at reducing nitrate in drinking water (Hu et al. 2000). Indeed, *Synechococcus* sp. PCC7942 has been proposed as a remedial strain capable of reducing nitrate levels in contaminated reservoirs. Notably, bioassay experiments demonstrated enhanced depletion of nitrate following amendment with both phosphate and a trace metal mixture (Hu et al. 2000). A bioremediation strategy modified by employing strain AND100 would yield a nitrate-dependent real-time bioluminescent signal, providing a means by which the water treatment system could be optimized to maximize nitrate consumption.

Finally, current studies are focusing on the development of more sophisticated bioreporter strains capable of yielding multiple signals. For example, the availability of iron and phosphorus regulated promoters (Durham et al. 2002, 2003; Gillor et al. 2002), along with reporter genes expressing luciferase and green fluorescent protein (GFP) derivatives, will allow the construction of a multichannel sensor strain reporting on bioavailable nitrogen, phosphorus, and iron via spectrally resolvable outputs. Once such strains are characterized, our long-term goal is to improve gene transfer techniques so that this technology can be mobilized into more ecologically relevant marine and freshwater cyanobacterial strains.

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