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ORIGINAL ARTICLE

Quorum sensing control of phosphorus acquisition in *Trichodesmium* consortia

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Colonies of the cyanobacterium *Trichodesmium* are abundant in the oligotrophic ocean, and through their ability to fix both CO_2 and N_2, have pivotal roles in the cycling of carbon and nitrogen in these highly nutrient-depleted environments. *Trichodesmium* colonies host complex consortia of epibiotic heterotrophic bacteria, and yet, the regulation of nutrient acquisition by these epibionts is poorly understood. We present evidence that epibiotic bacteria in *Trichodesmium* consortia use quorum sensing (QS) to regulate the activity of alkaline phosphatases (APases), enzymes used by epibionts in the acquisition of phosphate from dissolved-organic phosphorus molecules. A class of QS molecules, acylated homoserine lactones (AHLs), were produced by cultivated epibionts, and adding these AHLs to wild *Trichodesmium* colonies collected at sea led to a consistent doubling of APase activity. By contrast, amendments of (*S*)-4,5-dihydroxy-2,3-pentanedione (DPD)—the precursor to the autoinducer-2 (AI-2) family of universal interspecies signaling molecules—led to the attenuation of APase activity. In addition, colonies collected at sea were found by high performance liquid chromatography/mass spectrometry to contain both AHLs and AI-2. Both types of molecules turned over rapidly, an observation we ascribe to quorum quenching. Our results reveal a complex chemical interplay among epibionts using AHLs and AI-2 to control access to phosphate in dissolved-organic phosphorus.

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Introduction

Cyanobacteria of the genus *Trichodesmium* are key members of the phytoplankton community in the oligotrophic regions of the ocean. Nutrients are very scarce in these environments, yet *Trichodesmium* are highly successful, contributing a significant fraction of CO_2 fixation in their environment (Capone *et al.*, 1997). One of the key adaptations of *Trichodesmium* to its environment is the ability to fix N_2 (Capone *et al.*, 2005), which is highly abundant. However, the scarcity of other nutrients can limit the rates at which these organisms fix both N_2 and CO_2 (Sañudo-Wilhelmy *et al.*, 2001; Mills *et al.*, 2004). Phosphorus limitation of *Trichodesmium* has been predicted by models of ocean biogeochemistry (Krishnamurthy *et al.*, 2007), and demonstrated in field studies employing a variety of incubation-based approaches (Sañudo-Wilhelmy *et al.*, 2001; Mills *et al.*, 2004; Moutin *et al.*, 2005; Krauk *et al.*, 2006; White *et al.*, 2006; Webb *et al.*, 2007). Phosphate concentrations can be very low in the tropical and subtropical oceans, particularly the western North Atlantic (Wu *et al.*, 2000; Cavender-Bares *et al.*, 2001; Lomas *et al.*, 2010). It is clear that *Trichodesmium* meet their phosphorus demand through expression of alkaline phosphatases (APases) (Sohm and Capone, 2006; Hynes *et al.*, 2009; Orchard *et al.*, 2009; Orchard *et al.*, 2010), enzymes used to hydrolyze bioavailable phosphate from comparatively abundant dissolved-organic phosphorus molecules (Karl *et al.*, 2001; Lomas *et al.*, 2010).

In the open ocean, *Trichodesmium* colonies contain a diversity of other types of cells (Paerl *et al.*, 1989; Nausch, 1996; Sheridan *et al.*, 2002; Hewson *et al.*, 2009; Hmelo, 2010), including heterotrophic

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bacterial epibionts that, together with *Trichodesmium*, form a consortium that is highly adapted to the phosphorus-depleted conditions of the open ocean. Cell densities of epibionts in *Trichodesmium* colonies can be three orders of magnitude greater than cell densities in surrounding waters (Sheridan *et al.*, 2002), and APase expression by these epibionts is frequently observed (Dyhrman *et al.*, 2002; Hynes *et al.*, 2009). However, the distribution of APase activity among epibionts and *Trichodesmium* within colonies is highly variable even within the same environment (Dyhrman *et al.*, 2002; Hynes *et al.*, 2009), and the mechanisms driving the variability in this behavior are unknown. Proteobacteria related to those within *Trichodesmium* consortia (Hewson *et al.*, 2009; Hmelo, 2010) are known to employ quorum sensing (QS; a cell-density dependent signaling system), based on acylated homoserine lactones (AHLs; Supplementary Figure 1) to coordinate their behavior (Wagner-Döbler *et al.*, 2005; Case *et al.*, 2008). Furthermore, it was recently shown that AHL-based QS is sustainable at the cell densities typical of *Trichodesmium* colonies (Hmelo and Van Mooy, 2009; Hmelo *et al.*, in press). Given the importance of APases to phytoplankton in the subtropical oceans where phosphate is scarce (Perry, 1972; Sañudo-Wilhelmy *et al.*, 2001; Dyhrman *et al.*, 2002; Hynes *et al.*, 2009; Orchard *et al.*, 2009), and our limited understanding of QS in these same environments, we sought to explore the relationship between APase activity and AHL-based QS within *Trichodesmium* consortia.

Methods

Sampling was conducted in the subtropical North Atlantic Ocean during two cruises. The first cruise was to the Bermuda Atlantic Time-Series station (31.8°N 64.1°W), aboard the R/V *Atlantic Explorer* in September 2008. The second cruise was a transect from Woods Hole, MA, USA, to Barbados aboard the R/V *Oceanus* in November 2010. Sampling in the subtropical North Pacific Ocean was conducted aboard the R/V *Kilo Moana* in July 2010 at the approximate location of the Hawaiian Ocean Time-series station (22.7°N 158°W). Colonies of *Trichodesmium* were collected at sea (roughly from the top 25 m), with a 130-μm mesh plankton net. Before being processed as described below, individual colonies were carefully picked and then gently rinsed twice with 0.2-μm-filtered local seawater, to remove organisms not closely associated with the colonies.

During the first cruise to the North Atlantic, epibionts were isolated by streaking *Trichodesmium* colonies on seawater-based agar plates with tryptone ($1\,g\,l^{-1}$), and without tryptone. Epibiont colonies that grew on the plates were restreaked for purity, and shipped back to the laboratory in Woods Hole. Phylogeny of the epibionts was assessed using 16S rRNA gene sequences. Epibionts were grown overnight in seawater containing tryptone ($1\,g\,l^{-1}$), their DNA was extracted using standard methods, and 16S rRNA genes were amplified using the forward primer 27F (5′-AGAGTTTGATCMTGGCTCAG-3′) and reverse primer 1492R (5′-TACGGYTACCTTGTTA CGACTT-3′) as described (Hmelo, 2010). End sequencing was performed offsite either by MWG-Operon (Huntsville, AL, USA) or Agencourt Biosciences (Beverly, MA, USA). Sequences were analyzed and phylogenetic trees constructed as described (Hmelo, 2010).

Cultured epibionts were subsequently screened for AHL production. Cultures were grown overnight at room temperature in seawater containing tryptone ($1\,g\,l^{-1}$). These seawater cultures were then spotted onto soft agar plates seeded with the AHL biosensor *Agrobacterium tumefaciens* NTL4(pZLR4) as described (Farrand *et al.*, 2002; Hmelo *et al.*, in press). Epibiont cultures yielding a positive response to the biosensor were further screened for the presence of AHLs by high performance liquid chromatography/tandem mass spectrometry (HPLC/MS/MS) as described (Hmelo *et al.*, in press).

Incubations were conducted with *Trichodesmium* colonies during the second cruise in the North Atlantic and during the cruise in the North Pacific. Incubations were initiated by placing *Trichodesmium* colonies in 0.2-μm-filtered local seawater, and then amending these incubations with AHLs or autoinducer-2 (AI-2) to a total initial concentration of $\sim 500\,nmol\,l^{-1}$. The incubations were maintained under approximately 10% surface photosynthetically active radiation at *in situ* temperatures. All incubations were conducted in triplicate. After 24 h, APase activities of *Trichodesmium* colonies were determined; in the North Atlantic, APase assays were conducted with 6,8-difluoro-4-methylumbelliferyl phosphate as described by Orchard *et al.* (2009), whereas in the North Pacific, the assays were conducted with 4-methylumbelliferyl phosphate as described by Hoppe (1993) and adapted by Hmelo *et al.* (in press). Cell-specific APase activities were assessed by microscopy as described (Dyhrman *et al.*, 2002).

In the North Pacific, samples were taken for AHL and AI-2 analysis from both fresh *Trichodesmium* colonies and the aforementioned 24-h incubations. For AHL analysis, samples were spiked with a ^{13}C-labeled C8-HSL (Supplementary Figure 1) as a recovery standard, and immediately extracted three times with ethyl acetate (0.1% formic acid). The extracts were dried under nitrogen, shipped in liquid nitrogen to the laboratory in Woods Hole, and analyzed by HPLC/MS/MS as described (Hmelo *et al.*, in press). As described by Campagna *et al.* (2009), samples for AI-2 analysis were first spiked with ^{13}C-labeled AI-2 as a recovery standard. After this, tagging agent was added to derivatize and stabilize AI-2 (Campagna *et al.*, 2009). Finally, the samples were frozen at −80 °C, shipped on dry ice to

the laboratory at the University of Tennessee, and analyzed by HPLC/MS/MS as described (Campagna *et al.*, 2009). Process blanks were collected regularly (approximately every 15th extraction) using the same 0.2-μm-filtered seawater used to set up the aforementioned incubations. The concentrations of AHLs and AI-2 through time in the incubations were fit with a first-order exponential curve to determine their degradation rate (Hmelo and Van Mooy, 2009), which was then used to calculate the half-life of the molecules.

Results and discussion

Stimulation of APase activity by saturated AHLs

During our first cruise to the oligotrophic western North Atlantic Ocean, we collected *Trichodesmium* colonies and isolated bacterial epibionts from these colonies. Of the 141 isolates we obtained, genera from Gammaproteobacteria (24%) and Alphaproteobacteria (27%) were prevalent (Supplementary Figure 2). In *Vibrio* isolates, we observed the production of 3-oxo functionalized, medium chain-length AHLs (e.g. 3-oxo-octanoyl homoserine lactone (3-oxo-C8-HSL); Supplementary Table 1 and Supplementary Figure 1), similar to what has been observed in *Vibrio* isolated from corals (Tait *et al.*, 2009a). By contrast, the *Erythrobacter* isolates produced saturated (i.e., no 3-oxo group), long chain-length AHLs (e.g., tetradecane homoserine lactone (C14-HSL); Supplementary Table 1 and Supplementary Figure 1). The distribution of AHLs produced by the *Erythrobacter* isolates is similar to AHL distributions reported in other Alphaproteobacteria (Wagner-Döbler *et al.*, 2005), and Alphaproteobacteria were recently found to be prevalent within *Trichodesmium* consortia by using cultivation-independent methods (Hmelo, 2010). Although the few AHL-producing *Erythrobacter* we isolated probably represented only a fraction of the AHL-producing epibionts within *Trichodesmium* colonies, Decho *et al.* (2009) reported that the same saturated, long-chain AHLs were also prevalent in natural cyanobacterial mat communities. These observations led us to surmise that if AHL-based QS was occurring between *Trichodesmium* epibionts, then saturated, long chain-length AHLs would likely be involved.

On the subsequent cruise to the oligotrophic, western North Atlantic Ocean, we conducted incubation experiments designed to test whether AHLs affected rates of APase activity in *Trichodesmium* colonies. We constructed a cocktail of saturated long chain-length AHLs (C10-, C12- and C14-HSL), which was added to incubations containing *Trichodesmium* colonies. At three stations across the western North Atlantic, we consistently found that AHLs elicited a roughly two-fold increase in APase activities (Kruskal–Wallis, $P = 0.0064$; Figure 1). At the station at 28.6°N 65.1°W, *Trichodesmium*

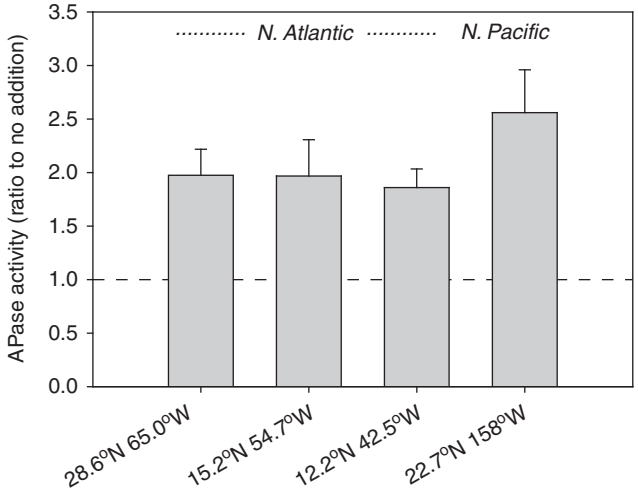

Figure 1 APase activity of *Trichodesmium* colonies in response to amendment with a cocktail of saturated, long-chain AHLs (C10-HSL, C12-HSL and C14-HSL) in the North Atlantic and North Pacific. Error bars indicate the magnitude of the range for triplicate incubations. As the assays were conducted with slightly different methods in the North Atlantic and North Pacific, data are presented as ratios to the no amendment control incubations. The horizontal dashed line represents no response to the amendments.

colonies were also assayed using a cell-specific APase method (Dyhrman *et al.*, 2002), and microscopic inspection clearly showed APase activity by epibionts in the colonies (Figure 2), particularly in the colonies exposed to the AHL cocktail (although this method is qualitative and not amenable to rigorous statistical comparisons (Hynes *et al.*, 2009)). Given this observation, and the fact that available genomes from cyanobacteria, including *Trichodesmium*, lack clear homologs of genes encoding known AHL receptors (Case *et al.*, 2008), we posit that the increase in APase activity (Figure 1) primarily reflected the response of bacterial epibionts and not the response of *Trichodesmium*. Although *Trichodesmium* does contain genes that align weakly at the amino acid level to authentic LuxR homologs, the key residues in the AHL-binding regions (Vannini *et al.*, 2002; Pantankar and González, 2009) are absent, indicating that these *Trichodesmium* genes are unlikely to have AHL-binding functionality (Supplementary Figure 3). It should be noted that protein expression in a terrestrial, epilithic cyanobacterium changed in response to exogenous AHLs (Sharif *et al.*, 2008), although enhanced APase expression was not observed and it is unknown if this particular cyanobacterium possesses a LuxR homolog.

We conducted a similar experiment in the oligotrophic North Pacific. APase activity is still frequently detected in *Trichodesmium* colonies in this region (Hynes *et al.*, 2009), even though the phytoplanktonic community is not thought to be limited by phosphorus (Van Mooy and Devol, 2008; Duhamel *et al.*, 2010) and dissolved phosphate is

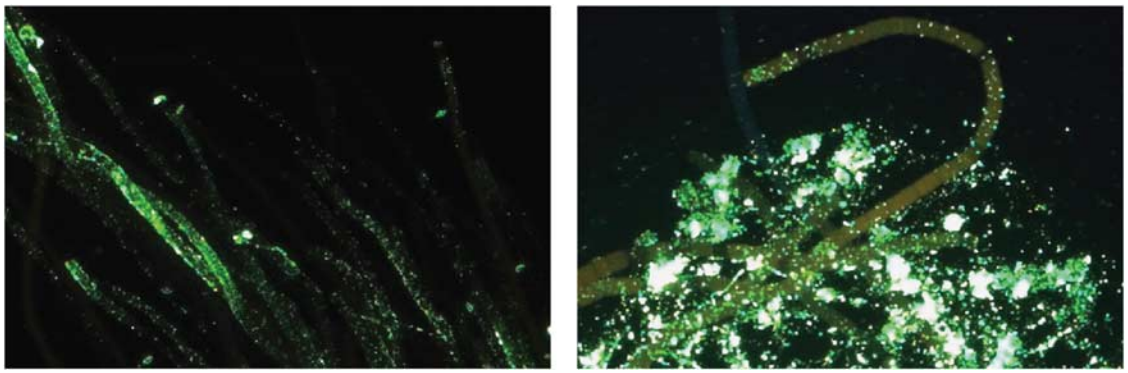

Figure 2 Representative photomicrographs of *Trichodesmium* colonies from the North Atlantic showing the cell-specific response to an enzyme labeled fluorescence assay of APase activity. The bright white and green areas on or near the orange autofluorescent *Trichodesmium* trichomes indicate localized APase activity. Left: endogenous *Trichodesmium* APase activity from a colony that did not receive an AHL amendment. Right: a colony from an incubation amended with the AHL cocktail, which shows the APase activity of epibiotic bacteria.

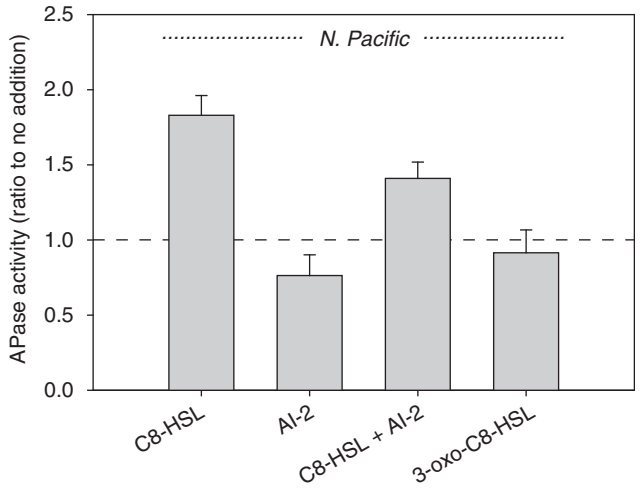

Figure 3 APase activity of *Trichodesmium* colonies in response to various AHL and AI-2 amendments (shown in legend) at a station in the North Pacific Ocean. Error bars indicate the magnitude of the range for triplicate incubations. Data are presented as ratios to the no amendment control incubations. The horizontal dashed line represents no response to the amendments.

more abundant than in the North Atlantic (Wu *et al.*, 2000; Cavender-Bares *et al.*, 2001; Hynes *et al.*, 2009). In the North Pacific, we observed the same approximate doubling in APase activities in response to the long-chain AHL cocktail amendment as we observed in the North Atlantic (Mann–Whitney, $P = 0.0495$; Figure 1). This result suggests that in addition to phosphate (Sohm *et al.*, 2008; Hynes *et al.*, 2009), APase activity within *Trichodesmium* colonies may be influenced by AHL-based QS. Furthermore, our observations of AHL-stimulated APase activity in both oceans suggest that this phenomenon could be globally distributed.

Incubations in the North Pacific were also amended with C8-HSL and 3-oxo-C8-HSL to distinguish possible differences in the effects of saturated and functionalized AHLs on APase activity (Figure 3). The C8-HSL amendment yielded nearly the same response as the saturated, long-chain cocktail, whereas the 3-oxo-C8-HSL had little effect. This result suggests that the saturated AHLs observed in marine cyanobacterial mats (Decho *et al.*, 2009) are recognized by Apase-producing epibionts, whereas the 3-oxo-functionalized AHLs are not. This result also indicates that the increase in APase activity that we observed in incubations amended with saturated AHLs (Figures 1 and 3) is a response to the specific signaling properties of those AHLs and is not a response to the potential nutritional value that would be common to all AHL molecules (e.g. dissolved organic nitrogen).

Attenuation of APase activity by AI-2

We also conducted incubations amended with (*S*)-4,5-dihydroxy-2,3-pentanedione (DPD), the molecule that spontaneously converts to AI-2 QS molecules in seawater (Chen *et al.*, 2002; Miller *et al.*, 2004; Supplementary Figures 1 and 4); AI-2 is thought to be a universal interspecies bacterial QS signal (Chen *et al.*, 2002; Henke and Bassler, 2004; Miller *et al.*, 2004). We observed that amendments of AI-2 appeared to attenuate APase activity (Mann–Whitney; $P = 0.0495$; Figure 3). Furthermore, in incubations amended with both AI-2 and C8-HSL, the AI-2 amendment appeared to counteract the stimulation of APase activity by C8-HSL. Thus, saturated AHLs and AI-2 appeared to have opposing roles in the modulation of APase activity within *Trichodesmium* consortia. We speculate that one subpopulation of bacteria was using AHL-based QS to upregulate APase activity, whereas another presumably broader subpopulation was using AI-2 to do the opposite. An alternative explanation is that a single subpopulation simultaneously employed AHLs to upregulate APase activity when it benefitted themselves (i.e., efficiency sensing; Hense *et al.*, 2007), but used the universal AI-2 signaling molecule to downregulate APase activity in situations

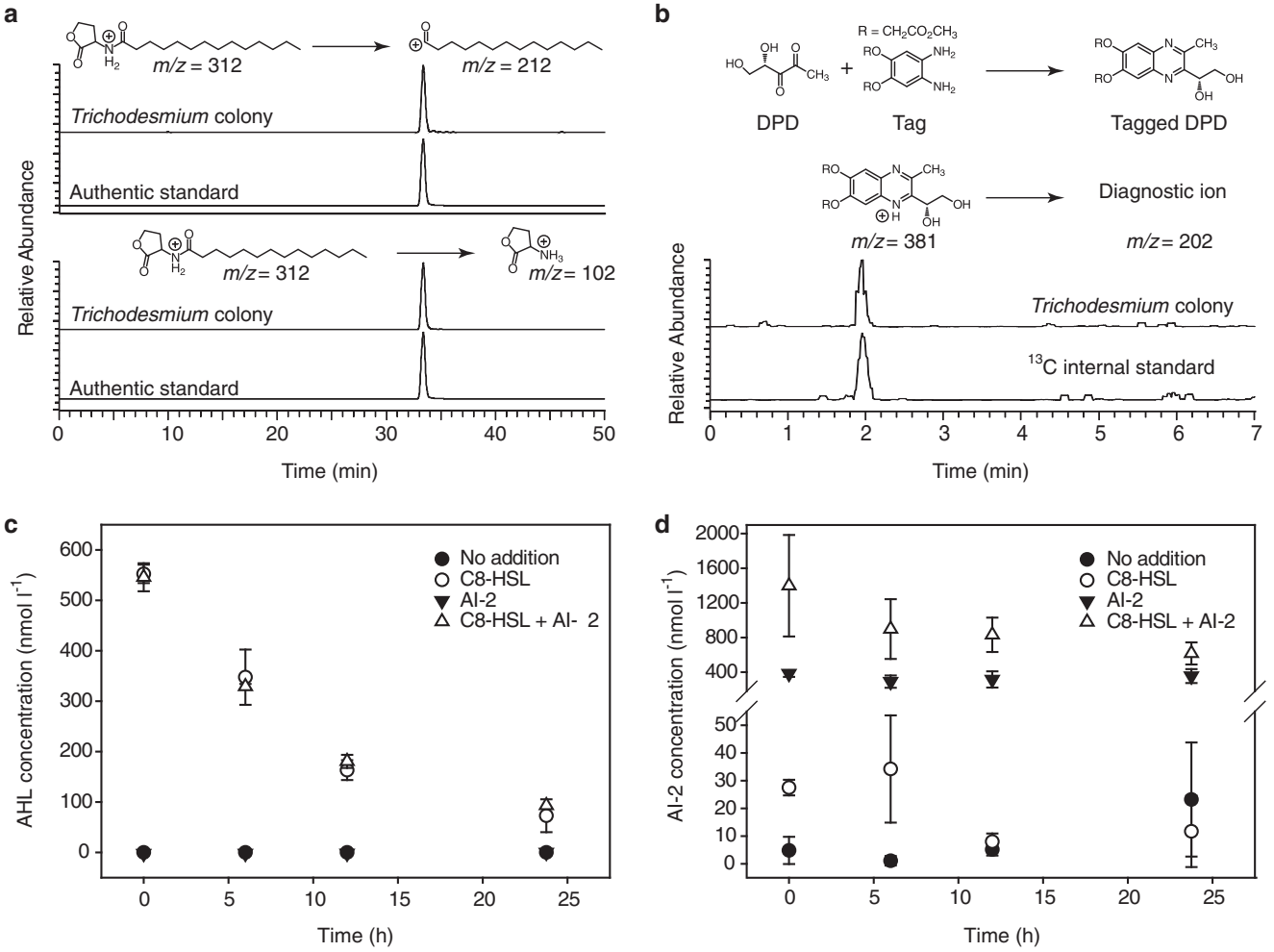

Figure 4 AHL and AI-2 in *Trichodesmium* colonies and amendment experiments. (**a**) HPLC/MS/MS chromatograms showing the presence of C14-HSL in *Trichodesmium* colonies. The top chromatogram shows the selected reaction monitoring (SRM) transition from the parent ion of C14-HSL to the acyl chain ion, whereas the bottom chromatogram shows the transition to the lactone ring ion. (**b**) Chromatogram showing the SRM transition from the parent ion of tagged DPD to a selective fragment ion. Scheme for the tagging of AI-2 is also shown. Tagging is necessary to both stabilize the molecule and make AI-2 amenable to HPLC/MS/MS detection (Campagna *et al.*, 2009). (**c** and **d**) Plots showing the concentrations of C8-HSL and AI-2 through time in incubations containing *Trichodesmium* colonies. Amendments are shown in the legend. Error bars indicate the magnitude of the range for triplicate incubations.

where overall cell abundances were greater and, thus, other competing subpopulations might benefit.

Presence and fate of AHLs and AI-2

Using HPLC/MS/MS, we analyzed *Trichodesmium* colonies collected on the North Pacific cruise and found that both C14-HSL and AI-2 (via DPD derivatization; Campagna *et al.*, 2009) were present (Figure 4; these molecules were absent in all process blanks). In natural seawater, AHLs are short-lived and subject to both abiotic lactonolysis and enzymatic degradation (Decho *et al.*, 2009; Hmelo and Van Mooy, 2009; Tait *et al.*, 2009b). Thus, the presence of AHLs in *Trichodesmium* colonies indicates that QS was active among epibiotic bacteria. As mentioned above, C14-HSL was also observed in cultures of epibionts, lending further support to the idea that saturated, long-chain AHLs may be ubiquitous QS molecules in *Trichodesmium* colonies. Until this study, AI-2 had yet to be observed in a marine environment, and both its half-life and functional role in mixed communities were largely unknown.

We also quantified C8-HSL and AI-2 in the aforementioned incubations by HPLC/MS/MS and found that the turnover rates of these signaling molecules in *Trichodesmium* colonies varied (Figure 4). We observed a rapid decline in C8-HSL concentration with the measured half-life ($t_{1/2} = 7.6 \pm 0.4$ h) being much shorter than expected from abiotic lactonolysis alone ($t_{1/2} = 25.6 \pm 1.9$ h; Hmelo and Van Mooy, 2009); this observation is indicative of active enzymatic degradation (i.e., quorum quenching) of AHLs by members of the *Trichodesmium* consortia (Figure 4). Indeed, the open-ring by-products of lactonolysis

were scarce in the incubations and their accumulation accounted for only about 2.0 ± 0.4% of the C8-HSL that degraded during the incubations (Supplementary Figure 5). This result shows that additional AHL degradation pathways such as enzymatic hydrolysis at acyl-amide bonds (Decho *et al.*, 2011 and references therein, Leadbetter and Greenberg, 2000) were dominant in our incubations. The half-life of C8-HSL was the same whether AI-2 was added to the incubations or not, and thus, quorum quenching of AHLs was unaffected by AI-2.

In contrast to AHLs, AI-2 was relatively stable by itself. However, when incubations were additionally amended with C8-HSL, we observed an initial threefold spike in AI-2 concentrations, followed by a rapid reduction ($t_{1/2} = 18.6 \pm 7.3$ h; Figure 4). The observed initial spike in AI-2 concentrations is consistent with AHL-stimulated induction of AI-2 synthesis, whereas the subsequent decrease could be the result of AHL-stimulated quorum quenching of AI-2, either by extracellular degradation or by uptake via a low-affinity transporter and subsequent intracellular degradation. We know of no previous evidence to support either of these mechanisms, although it is recognized that some bacteria (e.g., *Vibrio harveyi* and *Vibrio fischeri*) utilize both AHLs and AI-2 to synergistically regulate behavior (Henke and Bassler, 2004; Lupp and Ruby, 2004) and that others (e.g., *Pseudomonas aeruginosa* and *Escherichia coli*) are able to degrade AHLs and AI-2 (Xavier and Bassler, 2005; Bokhove *et al.*, 2010). We speculate that the observed quorum quenching of both AHLs and AI-2 is an indication that microbes within the consortia were attempting to employ chemical warfare to disrupt QS regulation of APase activity.

Conclusions

Our results suggest that members of *Trichodesmium* consortia use QS to coordinate the processing and acquisition of phosphorus, a critical nutrient resource in oligotrophic open-ocean environments. Although APase activity in phytoplankton is canonically regulated by the availability of phosphate (Perry, 1972), our data indicate that microscale microbial interactions (e.g., Hewson *et al.*, 2009; Malfatti and Azam, 2009) may also be important to the success of *Trichodesmium* in obtaining phosphate in the oligotrophic ocean. APase activity in *Trichodesmium* colonies may be detectable even when phosphate is relatively abundant (Sohm *et al.*, 2008; Hynes *et al.*, 2009), and our findings indicate that QS could be responsible for this observation. A recent metatranscriptomic study revealed the potential for a high degree of signal processing and information exchange amongst epibionts (Hewson *et al.*, 2009), but at this time, we cannot conclude with any certainty how QS-regulated APase activity impacts the quantity of phosphorus available to *Trichodesmium*, or whether rates of CO_2 or N_2 fixation are ultimately affected by QS. Clearly, in addition to QS, the bioavailability of phosphate and dissolved-organic phosphorus *in situ* will also affect APase activity. It has been suggested that *Trichodesmium* colonies are sources of dissolved inorganic and organic nutrients to surrounding waters (Nausch, 1996; Capone *et al.*, 2005). Therefore, the outcome of the QS-regulated phosphorus cycling within *Trichodesmium* consortia could have profound impacts on the productivity of the broader planktonic community and on the exchange of phosphorus between organic and inorganic reservoirs in the oligotrophic surface ocean.

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Supplementary Information accompanies the paper on The ISME Journal website (http://www.nature.com/ismej)

Supplementary Information

Quorum sensing control of phosphorus acquisition in *Trichodesmium* consortia.

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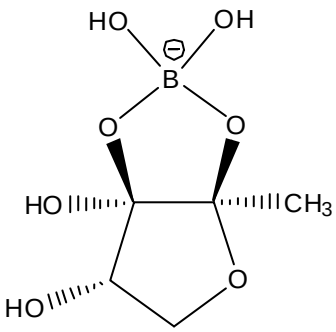
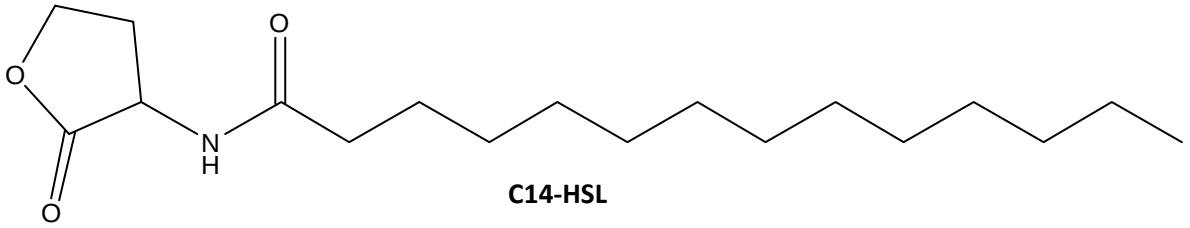
Supplementary Table 1 – List of AHLs detected in cultures of epibionts isolated from *Trichodesmium* colonies. The table indicates whether AHL concentrations were > 3 times higher than background (++), < 3 times above background (+), or not detected (-). Representative structures of the AHLs 3-oxo-C8-HSL, and C14-HSL are given in Supplementary Figure 1.

Accession number	Nearest match in GenBank (percent sequence identity)	3-oxo-C8-HSL	3-oxo-C9-HSL	3-oxo-C10-HSL	3-oxo-C11-HSL	3-oxo-C12-HSL	C12-HSL	C13-HSL	C14-HSL
HM032187	*Vibrio vulnificus* strain MP-4 (100%)	-	++	++	++	++	+	-	-
HM032340	*Vibrio vulnificus* strain MP-4 (100%)	++	++	++	++	++	-	-	-
HM032339	*Vibrio vulnificus* strain MP-4 (100%)	++	++	++	++	++	+	-	-
HM032229	*Vibrio vulnificus* strain MP-4 (100%)	+	++	++	++	++	+	-	-
HM032334	*Vibrio sp.* S1162 (99%)	+	++	++	++	-	-	-	-
HM032194	*Erythrobacter citreus* strain PR52-9 (100%)	-	-	-	-	-	++	++	++
HM032217	*Erythrobacter vulgaris* strain 022 2-10 (100%)	-	-	-	-	-	++	++	++

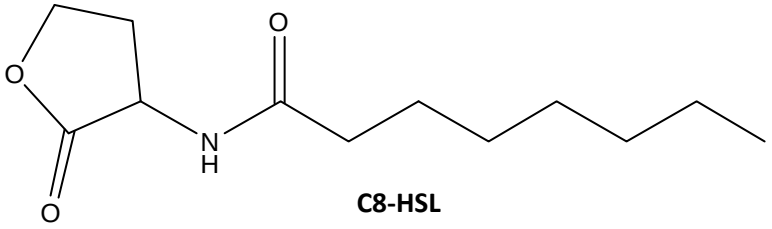
Supplementary Figure 1 – Structures of QS molecules discussed in the main manuscript.



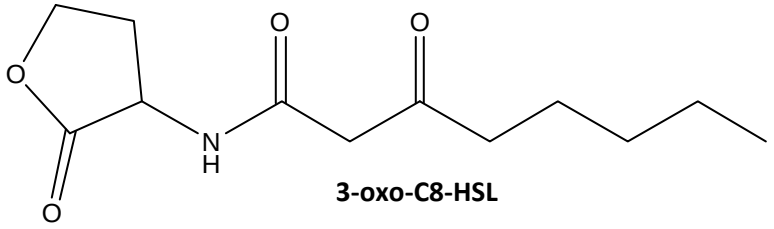
AI-2



C14-HSL

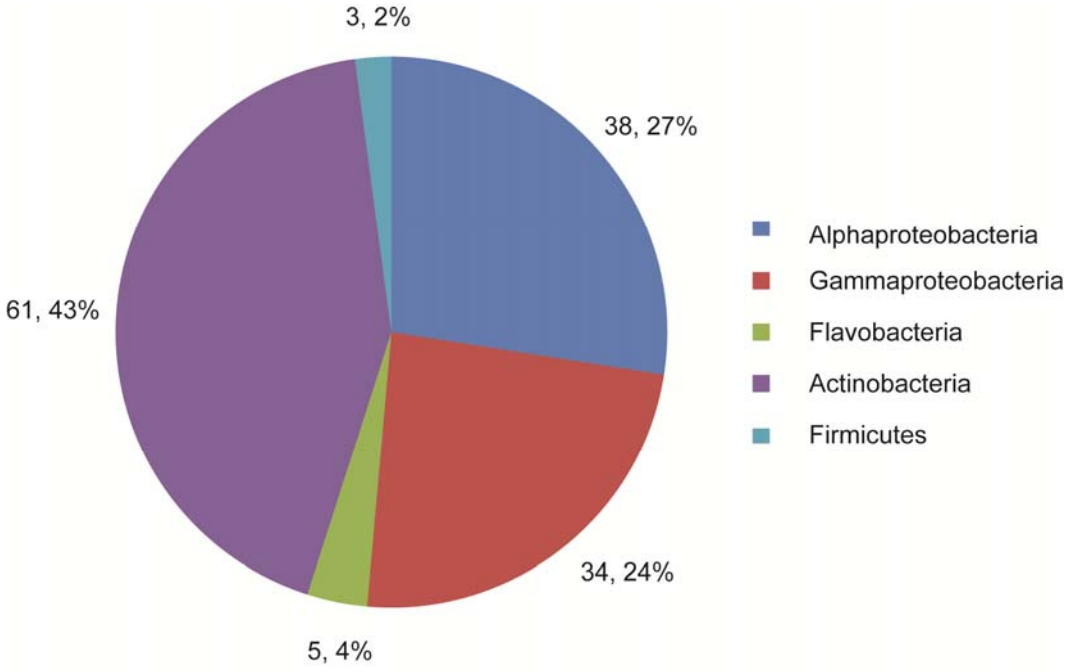


C8-HSL



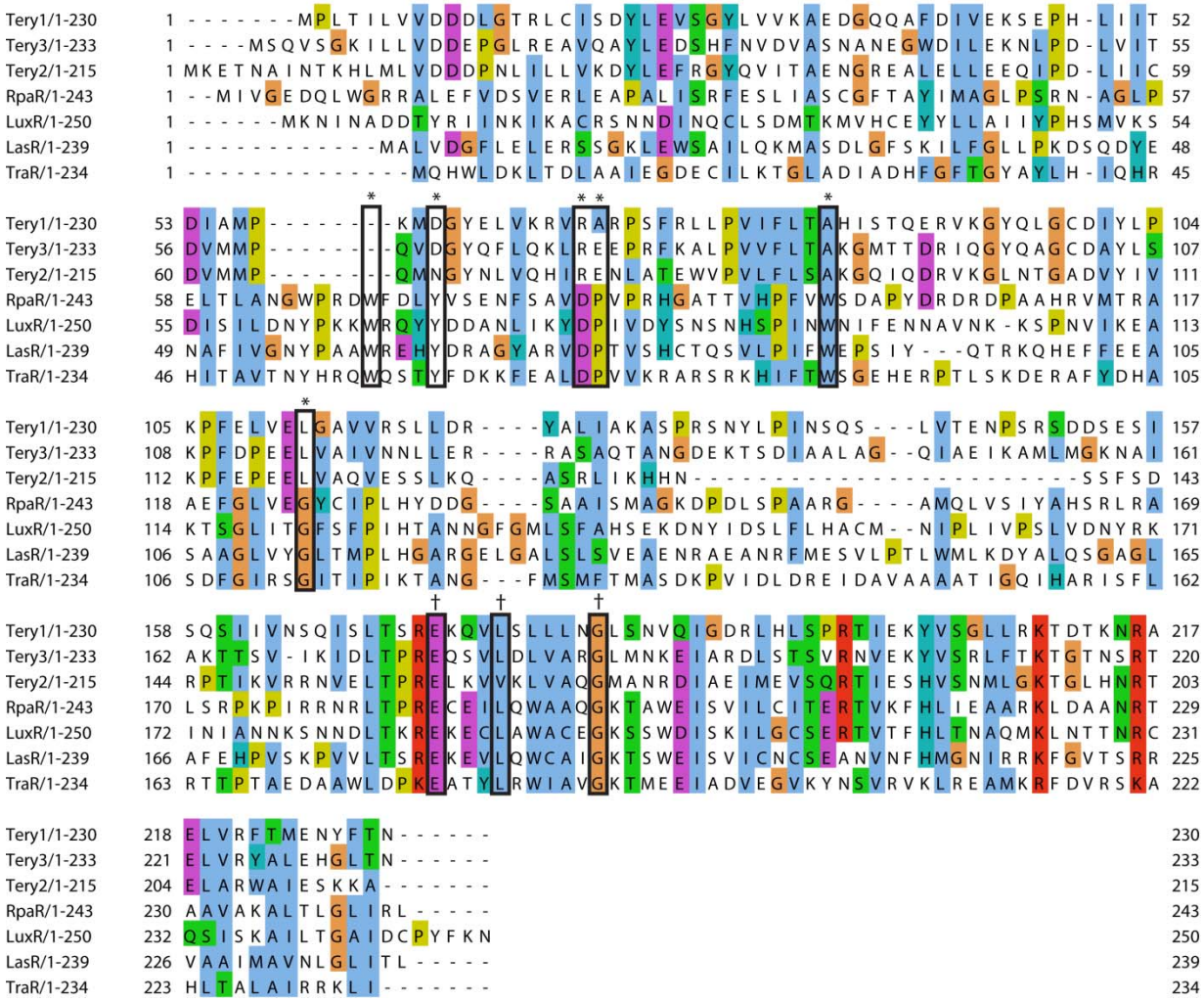
3-oxo-C8-HSL

Supplementary Figure 2a – A total of 141 isolates were obtained from *Trichodesmium* colonies collected in the oligotrophic North Altantic Ocean in the proximity of the Bermuda Atlantic Time-Series (BATS) station (31.8^{o} N 64.1^{o} W).



Supplementary Figure 2b – A neighbor-joining phylogenetic tree based on 16S rRNA genes of the Gammaproteobacteria isolated from *Trichodesmium* colonies in the North Atlantic (accession numbers in parentheses). Red arrows delineate the isolates that were found to produce AHLs. Asterisks indicate 60% or greater bootstrap support from 1.000 sampled tree topologies.

Supplementary Figure 2c - A neighbor-joining phylogenetic tree based on 16S rRNA genes of the Alphaproteobacteria isolated from *Trichodesmium* colonies in the North Atlantic (accession numbers in parentheses). Red arrows delineate the isolates that were found to produce AHLs. Asterisks indicate 60% or greater bootstrap support from 1000 sampled tree topologies.

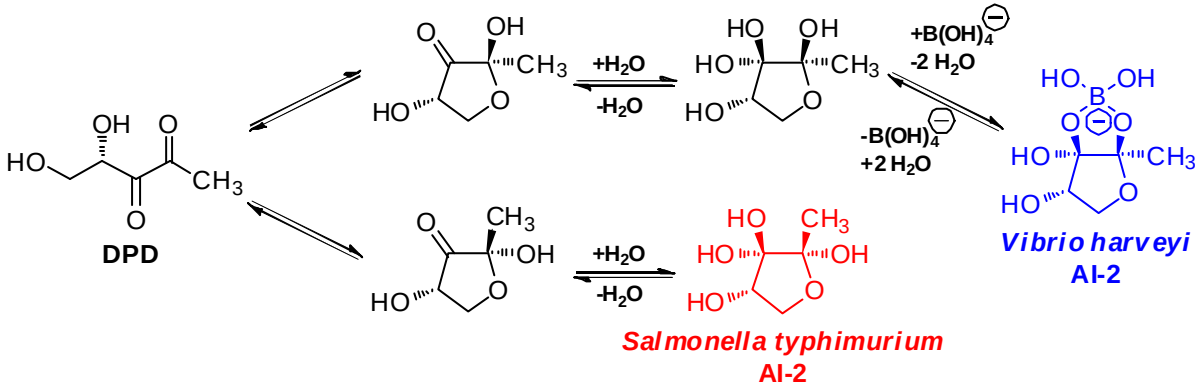


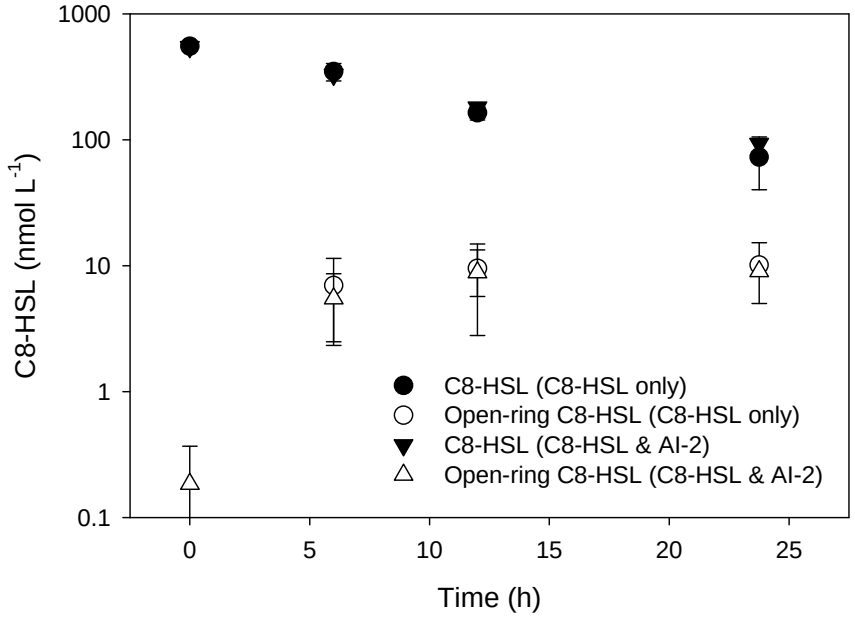
* - Conserved amino acid residues of $TraR_{A.tumefaciens}$ in AHL-binding domain.
† - Conserved amino acid residues of $TraR_{A.tumefaciens}$ in DNA-binding domain.

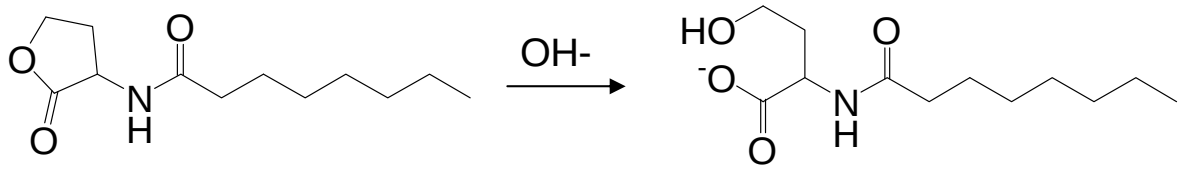
Supplemental Figure 3 – Amino acid alignment of potential LuxR-type response regulators identifed in the genome of *Trichodesmium erythraeum* IMS101 (GenBank CP000393). The LuxR-type response regulators can have an enormous amount of variability even at the amino acid level. Thus, to enable the widest possibility of finding a gene similar to LuxR, a search using COG 2197 "Response regulator containing a CheY-like receiver domain and an HTH DNA-binding domain" was employed. This COG-based approach retrieved five potential LuxR-like genes in the *Trichodesmium erythraeum* genome using the Find Function tool on the DOE Joint Genome Institute, Integrated Microbial Genomes Website (http://img.jgi.doe.gov/cgi-bin/pub/main.cgi). Two of the five open reading frames retrieved were excluded from further analyses for the following reasons: 1) a large potential ORF of 649 amino acids was lacking all AHL and DNA binding domain specific residues; 2) a short ORF of 87 amino acids included only DNA binding residues and was missing the entire AHL binding domain. The remaining

three LuxR-like full-length hypothetical ORFs included in this figure (Tery1, Tery2 and Tery 3) were aligned with Clustal W using default parameters together with RpaR, LuxR, LasR and TraR (all authentic transcriptional regulators involved in quorum sensing from *Rhodopseudomonas palustris*, *Vibrio fischeri* and *Pseudomonas aeruginosa* and *Agrobacterium tumefaciens*, respectively)(Fuqua et al 1996, Schaefer et al 2008). These authentic quorum sensing receptors were included since they respond to a wide range of AHLs(Fuqua et al 1996, Schaefer et al 2008). This diagram is based on that of Vaninni et *al*.(2002) and reviewed by Patankar and González (2009). Critical residues are marked with a black rectangle, and show that conserved AHL-binding residues are lacking in T. *erythraeum,* while only the conserved DNA-binding residues are present.

Supplementary Figure 4 ─ (*S*)-4,5-dihydroxy-2,3-pentanedione (DPD) is the precursor to a series of molecules that collectively give rise to autoinducer-2 (AI-2) quorum sensing activity. To date, two of these molecules have been shown to be biologically relevant (highlighted in blue and red; Chen et al., 2002, Miller et al., 2004). Note that other borated forms are also possible, although they are not shown here. All of these molecules are in equilibrium and are readily interconverted. Due to this, the detection of DPD is sufficient to monitor AI-2 activity.







Supplemental Figure 5 – Concentrations of open-ring C8-HSL in incubations containing *Trichodesmium* colonies. Note that the concentrations of C8-HSL (i.e. closed ring form) are taken from Figure 4 in the main text. Also shown is the reaction of the base-catalyzed lactonolysis that occurs in seawater at pH ≈ 8.2. Quantification of open-ring AHLs was conducted as described by Hmelo et al (submitted) but with an open-ring C8-HSL external standard curve.

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