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Publication Date
2011-05-09
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Edited by David M. Karl, University of Hawaii, Honolulu, HI, and approved January 26, 2011 (received for review October 29, 2010)

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February 22, 2011
ACKNOWLEDGMENTS:

Assembly and annotations of A. anophagefferens are available from JGI Genome Portal at http://www.jgi.doe.gov/Aureococcus. Genome sequencing, annotation and analysis were conducted by the U.S. Department of Energy Joint Genome Institute is supported by the Office of Science of the U.S. Department of Energy under Contract No. DE-AC02-05CH11231. Efforts were also supported by National Oceanic and Atmospheric Administration Sea Grant Awards NA07OAR4170010 and NA10OAR4170064 to Stony Brook University via New York Sea Grant, National Oceanic and Atmospheric Administration Center for Sponsored Coastal Ocean Research Award NA09NOS4780206 to Woods Hole Oceanographic Institution, National Institutes of Health Grant GM061603 to Harvard University, and National Science Foundation Award IOS-0841918 to University of Tennessee.

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Niche of harmful alga *Aureococcus anophagefferens* revealed through ecogenomics

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Harmful algal blooms (HABs) cause significant economic and ecological damage worldwide. Despite considerable efforts, a comprehensive understanding of the factors that promote these blooms has been lacking, because the biochemical pathways that facilitate their dominance relative to other phytoplankton within specific environments have not been identified. Here, biogeochemical measurements showed that the harmful alga *Aureococcus anophagefferens* outcompeted co-occurring phytoplankton in estuaries and with elevated levels of dissolved organic matter and turbidity and low levels of dissolved inorganic nitrogen. We subsequently sequenced the genome of *A. anophagefferens* and compared its gene complement with those of six competing phytoplankton species identified through metaproteomics. Using an ecogenomic approach, we specifically focused on gene sets that may facilitate dominance within the environmental conditions present during blooms. *A. anophagefferens* possesses a larger genome (56 Mbp) and has more genes involved in light harvesting, organic carbon and nitrogen use, and encoding selenium- and metal-requiring enzymes than competing phytoplankton. Genes for the synthesis of microbial deterrents likely prevent the proliferation of this species, with reduced mortality losses during blooms. Collectively, these findings suggest that anthropogenic activities resulting in elevated levels of turbidity, organic matter, and metals have opened a niche within coastal ecosystems that ideally suits the unique genetic capacity of *A. anophagefferens* and thus, has facilitated the proliferation of this and potentially other HABs.


The authors declare no conflict of interest.

This article is a PNAS Direct Submission.

Fierily available online through the PNAS open access option.

Data deposition: The sequence reported in this paper has been deposited in the GenBank database (accession no. ACJI00000000).

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This article contains supporting information online at www.pnas.org/lookup/suppl/doi:10.1073/pnas.1016106108/-/DCSupplemental.
Results and Discussion
During an investigation of a US estuary, Quantuck Bay, NY, from 2007 to 2009, brown tides occurred annually from May to July, achieving abundances exceeding 10⁶ cells mL⁻¹ or 5 × 10⁶ μm² mL⁻¹ (Fig. 1). A. anophagefferens was observed to bloom after spring diatom blooms and outcompeted small (<2 μm) eukaryotic and prokaryotic phytoplankton (e.g., Ostreococcus and Synechococcus) during summer months (Fig. 1D), a pattern consistent with prior observations (7, 8). Concurrently, dissolved inorganic nitrogen levels were reduced to <1 μM during blooms, whereas dissolved organic nitrogen levels and light extinction were elevated, resulting in a system with decreased light availability and concentrations of dissolved organic nitrogen far exceeding those of dissolved inorganic nitrogen (Fig. 1C). Metaproteomic analyses of planktonic communities were performed to identify phytoplankton that A. anophagefferens may compete with during blooms by quantifying organism-specific peptides among the microbial community. Performing such analyses on the plankton present in this estuary highlights the dominance of A. anophagefferens and coexistence of the six phytoplankton species for which complete genome sequences have been generated (Fig. 1E): two coastal diatom species, Phaeodactylum tricornutum (clone CCMP632) (9) and Thalassiosira pseudonana [clone CCMP 1335 (10) isolated from an embayment that now hosts brown tides (6)], and coastal zone isolates of Ostreococcus (O. lucimarinus and O. tauri) (11) and Synechococcus [clones CC9311 (12) and CC9902] small eukaryotic and prokaryotic phytoplankton, respectively, (Fig. 1 and Table 1). To assess the extent to which the gene set of A. anophagefferens may permit its dominance within the geochemical environment found in this estuary (Fig. 1C), the gene complement of A. anophagefferens was determined by genome sequencing and was compared with those of the six competing phytoplankton species (Fig. 1E and Table 1).

Although phytoplankton genome size generally scales with cell size (15, 16), A. anophagefferens (2 μm) has a larger genome (56 Mbp) and more genes (~11,500) than the six competing phytoplankton species (2.2–32 Mbp and 2,301–11,242 genes) (Table 1 and SI Appendix, Tables S1, S2, S3, and S4). Its small cell size and thus larger surface area to volume ratio allows it to kinetically outcompete larger phytoplankton for low levels of light and nutrients (17), whereas its large gene content and more complex
genetic repertoire may provide a competitive advantage over other small phytoplankton with fewer genes. The A. anophagefferens genome contains the largest number of unique genes relative to the six competing phytoplankton examined here (209 vs. 12–79 unique genes) (Table 1). Many of these unique or enriched genes in A. anophagefferens are associated with light harvesting, organic matter use, and metalloenzymes as well as the synthesis of microbial predation and competition deterrents (SI Appendix; Tables S5, S6, S7, S8, S9, S10, S11, S12, S13, S14, S15, S16, and S17). These enriched and unique gene sets are involved in biochemical pathways related to the environmental conditions prevailing during brown tides (Fig. 1) and thus, are likely to facilitate the dominance of this alga during chronic blooms that plague estuarine waters.

**Light Harvesting.** Phytoplankton rely on light to photosynthetically fix carbon dioxide into organic carbon, but the turbid, low-light environment characteristic of estuaries and intense shading during dense algal blooms (Fig. 1 B and C) can strongly limit photosynthesis. A. anophagefferens is better adapted to low light than the comparative phytoplankton species, which requires at least threefold higher light levels to achieve maximal growth rates (Fig. 2A). Its genome contains the full suite of genes involved in photosynthesis, including 62 genes encoding light-harvesting complex (LHC) proteins (Fig. 2A). This is 1.3–5 times more than other eukaryotic phytoplankton sequenced thus far (Fig. 2A and SI Appendix, Table S7) and a feature that likely enhances adaptation to low and/or dynamic light conditions found in turbid estuaries. LHC proteins bind antenna chlorophyll and carotenoid pigments that augment the light-capturing capacity of the photosynthetic reaction centers (18, 19). Twenty-six A. anophagefferens LHC genes belong to a group that has only six representatives in T. pseudonana and one representative in P. tricornutum (branch PHYMKG in Fig. 3 and SI Appendix, Fig. S1) but are similar to the multicellular brown macroalgae, Ectocarpus siliculosus (20). Similar LHC genes in the microalga Emiliania huxleyi have recently been shown to be up-regulated under low light (21). We hypothesize that these LHC genes encode the major light-harvesting proteins for A. anophagefferens and that the enrichment of these proteins imparts a competitive advantage in acquiring light under the low-irradiance conditions that prevail during blooms (Fig. 1C).

**Organic Matter Use.** In addition to being well-adapted to low light, A. anophagefferens also outcompetes other phytoplankton in estuaries with elevated organic matter concentrations (6) (Fig. 1C), and can survive extended periods with no light (22). Consistent with these observations, the genome of A. anophagefferens contains a large number of genes that may permit the degradation of organic compounds to support heterotrophic metabolism. For example, its genome encodes proteins involved in the transport of oligosaccharides and sugars that are not found in competing phytoplankton, including genes for glycerol, glucose, and d-xylose uptake (SI Appendix, Table S8). The A. anophagefferens genome also encodes more nucleoside sugar transporters and major facilitator family sugar transporters than other comparative phytoplankton species (SI Appendix, Table S8). It is highly enriched in genes associated with the degradation of mono-, di-, oligo-, and polysaccharides as well as sulfonated polysaccharides. A. anophagefferens possesses 47 sulfatase genes, including those targeting sulfonated polysaccharides such as glucosamine-(N-acetyl)-6-sulfatases, whereas the diatoms contain a total of three to four sulfatases and the comparative picoplankton contain none (SI Appendix, Table S9). A. anophagefferens also possesses many more genes involved in carbohydrate degradation than competing phytoplankton (85 vs. 4–29 genes in comparative phytoplankton), including 29 such genes present only in A. anophagefferens (Fig. 4 and SI Appendix, Tables S10 and S11). Collectively, these genes (SI Appendix, Tables S9, S10, S11, and S12) provide this alga with unique metabolic capabilities regarding the degradation of an array of organic carbon compounds, many of which may not be accessible to other phytoplankton. In an ecosystem setting, such a supplement of organic carbon would be critical for population proliferation within the low-light environments present in estuaries, particularly during dense algal blooms (Fig. 1C).

A. anophagefferens, like many HABs, blooms when inorganic nitrogen levels are low but organic nitrogen levels are elevated (Fig. 1C) (1–3), and A. anophagefferens is known to efficiently metabolize organic compounds for nitrogenous nutrition (6, 23). Notably, this niche strategy is reflected within the A. anophagefferens genome, which encodes transporters specific for a diverse set of organic nitrogen compounds including urea, amino acids, purines, nucleotide sugars, nucleosides, peptides, and oligopeptides (SI Appendix, Table S8) (24). Relative to competing phytoplankton, A. anophagefferens is enriched in genes encoding enzymes that degrade organic nitrogen compounds, such as nitriles, asparagine, and urea (Fig. 2B). A. anophagefferens is also the only species among the phytoplankton genomes examined that possesses a membrane-bound dipeptidase, several histidine ammonia lyases, tripeptidyl peptidase, and several other enzymes (SI Appendix, Table S13) that could collectively play a role in metabolizing organic nitrogen compounds that are not bioavailable to other phytoplankton. Furthermore, the A. anophagefferens genome also contains enzymes that degrade amino acids, peptides, proteins, amidase, and nucleotides, often possessing more copies of these genes than competing phytoplankton (SI Appendix, Table S13). This characteristic, along with its unique gene set, may provide A. anophagefferens with a greater capacity to use organic compounds for nitrogenous nutrition compared with its com-

Table 1. Major features of the genomes of A. anophagefferens and six competing algal species: P. tricornutum (9), T. pseudonana (10), O. tauri (11), O. lucimarinus (11), Synechococcus (CC9311) (12), and Synechococcus (CC9902)

<table>
<thead>
<tr>
<th></th>
<th>A. anophagefferens</th>
<th>P. tricornutum</th>
<th>T. pseudonana</th>
<th>O. tauri</th>
<th>O. lucimarinus</th>
<th>Synechococcus CC9311</th>
<th>Synechococcus CC9902</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cell diameter (μm)</td>
<td>2.0</td>
<td>11.0</td>
<td>5.0</td>
<td>1.2</td>
<td>1.3</td>
<td>1.0</td>
<td>1.0</td>
</tr>
<tr>
<td>Cell volume (μm³)</td>
<td>6</td>
<td>61</td>
<td>88</td>
<td>1.8</td>
<td>2.0</td>
<td>1.2</td>
<td>1.2</td>
</tr>
<tr>
<td>Genome size (Mb)</td>
<td>57</td>
<td>27</td>
<td>32</td>
<td>13</td>
<td>13</td>
<td>2.6</td>
<td>2.2</td>
</tr>
<tr>
<td>Predicted gene number</td>
<td>11,501</td>
<td>10,402</td>
<td>11,242</td>
<td>7,892</td>
<td>7,651</td>
<td>2,892</td>
<td>2,301</td>
</tr>
<tr>
<td>Genes with known functions</td>
<td>8,560</td>
<td>6,239</td>
<td>6,797</td>
<td>5,090</td>
<td>5,322</td>
<td>1,607</td>
<td>1,469</td>
</tr>
<tr>
<td>Genes with Pfam domains</td>
<td>6,908</td>
<td>5,398</td>
<td>5,791</td>
<td>4,763</td>
<td>4,214</td>
<td>1,636</td>
<td>1,488</td>
</tr>
<tr>
<td>Genes with unique Pfam domains</td>
<td>209</td>
<td>79</td>
<td>75</td>
<td>23</td>
<td>51</td>
<td>55</td>
<td>12</td>
</tr>
</tbody>
</table>

Genes with known functions were identified using Swiss-Prot, a curated protein sequence database, with an e-value cutoff of <10⁻⁵ (13). Pfam domains are sequences identified from a database of protein families represented by multiple sequence alignments and hidden Markov models (14). The compressed nature of P. tricornutum cells (11 × 2.5 μm) makes its biovolume smaller than T. pseudonana.
petitors, a hypothesis supported by its dominance in systems with elevated ratios of dissolved organic nitrogen to dissolved inorganic nitrogen and the reduction in dissolved organic nitrogen concentrations often observed during the initiation of brown tides (6, 25).

Metalloenzymes. *A. anophagefferens* blooms in shallow, enclosed estuaries (6) where the concentrations of metals and elements like selenium are elevated (26–28), but it never dominates deep estuaries or continental shelf regions (6) that are characterized by lower metal and trace element inventories (26–28). *A. anophagefferens* has a large and absolute requirement for some trace elements, such as selenium (Fig. 2C). In comparison, phytoplankton, such as *Synechococcus*, do not require this element, whereas others, such as *T. pseudonana* and *P. tricornutum*, have lower selenium requirements for maximal growth (Fig. 2C). The *A. anophagefferens* genome is consistent with these observations, being enriched in numerous classes of proteins that require metals and elements like selenium as cofactors (Fig. 2C). It possesses at least 56 genes encoding selenocysteine-containing proteins, two times more than the diatom genomes (Fig. 2C). *A. anophagefferens* selenoprotein family includes nearly all known eukaryotic selenoproteins as well as selenoproteins that were previously described only in bacteria (29) and several selenoprotein families are represented by multiple isoforms (SI Appendix, Table S14). One-half of the selenoproteins are methionine sulfoxide reductases, thioredoxin reductases, glutathione peroxidases, glutaredoxins, and peroxiredoxins (SI Appendix, Table S14). Together, these enzymes help protect cells against oxidative stress in the dynamic and ephemeral conditions present in estuaries through the removal of hydroperoxides and the repair of oxidatively damaged proteins. Moreover, selenocysteine residues are often superior catalytic groups compared with cysteine (30–32), and thus, they allow *A. anophagefferens*...
A. anophagefferens to more efficiently execute multiple metabolic processes and increase its competitiveness relative to other phytoplankton in the anthropogenically modified estuaries where it blooms.

The A. anophagefferens genome is also enriched in genes encoding for molybdenum-, copper-, and nickel-containing enzymes (Fig. 2C). For example, the A. anophagefferens genome includes two times the number of genes encoding molybdenum-containing oxidases found in competing species (6 vs. 1-3 genes) (Fig. 2C and SI Appendix, Tables S15 and S16) and has the largest number of molybdenum-specific transporters (SI Appendix, Table S8). Similarly, A. anophagefferens possesses four times more genes that encode copper-containing proteins than its competitors (27 vs. 1-6 genes) (Fig. 2C), including 5 multicopper oxidases and 20 tyrosinase-like proteins (SI Appendix, Tables S15 and S16). Several of the A. anophagefferens tyrosinase and multicopper oxidase family proteins are heavily glycosylated (more than four glycosylation sites) (SI Appendix, Table S16) and thus, are likely secretory proteins, whereas the few present in the other comparative algal species are not. These copper-containing enzymes degrade lignin, catalyze the oxidation of phenolics, and can have antimicrobial properties (33, 34) and thus, may provide nutrition or confer protection to A. anophagefferens cells. A. anophagefferens is also the only phytoplankton species with a homolog of the CucC copper homeostasis protein, which permits efficient cellular trafficking of this metal (SI Appendix, Table S8). With three nickel-requiring ureases, A. anophagefferens has more nickel-containing enzymes than other comparative phytoplankton (Fig. 2B and C). Consistent with its ecogeographic profile, these ureases allow A. anophagefferens to meet its daily N demand from urea, whereas other phytoplankton do not (35). Perhaps to support the synthesis and use of ureases, A. anophagefferens is the only comparative phytoplankton species with a high-affinity nickel transporter (HoxN) (36). A. anophagefferens is not universally enriched in metalloenzymes, because other phytoplankton contain equal numbers of cobalt-containing enzymes (Fig. 2C). However, the formation of blooms exclusively in shallow estuaries ensures that A. anophagefferens has access to a rich supply of the selenium, copper, and nickel required to synthesize these ecologically important and catalytically superior enzymes (30, 31, 37).

Microbial Defense. Although genes associated with the adaptation to low light, the use of organic matter, and metals permit A. anophagefferens to dominate a specific geochemical niche found within estuaries, genes involved in the production of compounds that inhibit predators and competitors may further promote blooms (2). Although specific toxins have yet to be identified in A. anophagefferens, it is grazed at a low rate during blooms (2, 6), and its genome contains two to seven times more genes involved in the synthesis of secondary metabolites than the comparative phytoplankton genomes (SI Appendix, Fig. S2). A. anophagefferens also possesses a series of genes involved in the synthesis of putative antimicrobial compounds that are largely absent from the competing phytoplankton species (SI Appendix, Table S17). For example, A. anophagefferens has five berberine bridge enzymes involved in the synthesis of toxic isoquinoline alkaloids (38, 39) (SI Appendix, Table S17). A. anophagefferens uniquely possesses a membrane attack complex gene and multiple phenazine biosynthesis genes (SI Appendix, Table S17) that encode enzymes that may provide defense against microbes and/or protistan grazers (40, 41). There are two- to fourfold more ATP-binding cassette (ABC) transporters in A. anophagefferens than in competing species (112 vs. 30-54 ABC transporters) (SI Appendix, Table S8), and it is specifically enriched in ABC multidrug efflux pumps that protect cells from toxic xenobiotics and endogenous metabolites (42, 43). Finally, the A. anophagefferens genome encodes 16-fold more Sel-1 genes (130 vs. 0-8 genes) (Table S6), fourfold more ion channels (82 vs. 1-19 ion channels) (SI Appendix, Table S8), fourfold more protein kinases, and twofold more WD40 domain genes than other phytoplankton (SI Appendix, Table S6). These genes may collectively mediate elaborate cell signaling and sensing by dense bloom populations (44-46), processes that would be important for detecting competitors, predators, other A. anophagefferens cells, and the environment. Together, genes involved in the synthesis of microbial deterrents, export of toxic compounds, and cell signaling may contribute to the proliferation of this species with reduced population losses and thus, assist in promoting these HABs (2).

Conclusions. The global expansion of human populations along coastlines has led to a progressive enrichment in turbidity (47), organic matter, including organic nitrogen (1, 47, 48), and metals (26, 28) in estuaries. Matching the expansion of HAB events around the world in recent decades, A. anophagefferens blooms were an unknown phenomenon before 1985 but have since become chronic, annual events in US and South African estuaries (6), with the potential for further expansion. The unique gene complement of A. anophagefferens encodes a disproportionately greater number of proteins involved in light harvesting and organic matter use as well as metal and selenium-requiring enzymes relative to competing phytoplankton. Collectively, these genes reveal a niche characterized by conditions (low light, high organic matter, and elevated metal levels) that have become increasingly prevalent in anthropogenically modified estuaries, suggesting that human activities have enabled the proliferation of these HABs. In estuaries that host A. anophagefferens blooms, anthropogenic nutrient loading promotes algal growth and as a result, elevated levels of organic matter and turbidity (6), whereas high concentrations of metals have been attributed to maritime paints and some fertilizers (27, 49). Collectively, these findings establish a context within which to prevent and control HABs, specifically by ameliorating anthropogenically altered aspects of marine environments that harmful phytoplankton are genomically predisposed to exploit. Like A. anophagefferens, many HAB-forming dinoflagellates are known to exploit organic
forms of carbon and nitrogen for growth (1–4), grow well under low light (50), and have elevated requirements of copper, molybdenum, and selenium (51, 52). Continued ecogenomic analyses of HABs will reveal the extent to which these events can be attributed to human activities that have transformed coastal ecosystems to suit the generic capacity of these algae.

Materials and Methods

The environmental conditions and plankton community composition within a brown tide-prone estuary (Quantuck Bay, NY) were monitored biweekly from spring to fall of 2007, 2008, and 2009. Nutrient levels were assessed by wet chemical and combustion techniques, whereas the composition of the plankton community was assessed by immunofluorescent assays, flow cytometry, and standard microscopy. Metaproteomes were generated using 2D nano-LC-MS/MS, tandem MS (LC-MS/MS), and spectra were analyzed using SEQUEST and DTAlgo search algorithms. The genome of A. anophagefferens was sequenced using the whole-genome shotgun approach using the Sanger platform assemblers with the JAZZ assembler and annotated using JGI Annotation tools. Complete information regarding all methods used for all analyses reported here is available in SI Appendix.

ACKNOWLEDGMENTS. Assembly and annotations of A. anophagefferens are available from JGI Genome Portal at http://www.jgi.doe.gov/Aureococcus. Genome sequencing, annotation, and analysis were conducted by the US Department of Energy Joint Genome Institute supported by the Office of Science of the US Department of Energy under Contract No. DE-AC02-05CH11231. Efforts were also supported by National Oceanic and Atmospheric Administration Sea Grant Awards NA07OAR4170010 and NOAANOA170064 to Stony Brook University via New York Sea Grant, National Oceanic and Atmospheric Administration Center for Sponsored Coastal Ocean Research Award NA09NOS478206 to Woods Hole Oceanographic Institution, National Institutes of Health Grant GM06163 to Harvard University, and National Science Foundation Award IOS-0841918 to University of Tennessee.