

Review

Marine diazotrophic cyanobacteria: Out of the blue

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Abstract Cyanobacteria are now recognized as the primary nitrogen-fixing (diazotrophic) microorganisms in the oceans as contributing significantly to the biogeochemistry of the global nitrogen cycle. The evolution of a remarkable range of morphologies and metabolic capacities has led to a behavioural versatility that helps them to proliferate and combat nutrient limitations in oceanic ecosystems. Advancement in community gene, genome and metagenome analyses of marine microbial communities will further advance functional interpretations and biotechnological uses. In this review we highlight the diversity, adaptation and function of cyanobacteria in the ocean.

Key words: Cyanobacteria, diazotrophs, diazocytes, heterocystous, *nifH* gene.

Cyanobacteria have radically changed our biosphere during their 3.5 billion years of existence (Schopf 1996). They are among the organisms that contributed to two major evolutionary episodes: the introduction of oxygenic photosynthesis, which led to the oxygenation of the atmosphere, and an endosymbiotic event with a non-pigmented eukaryote, which gave rise to all algae and plants (Miyagishima 2005). Cyanobacteria are now also recognized as the primary diazotrophs (nitrogen-fixing organisms) in the oceans and have thereby revolutionized our view on sources contributing to the global nitrogen cycle. Research on marine cyanobacteria has in recent years generated new insights into their global distribution and diversity, and has stressed their significance as macronutrient scavengers and primary producers (Karl et al. 2002).

Marine planktonic unicellular cyanobacteria

The free-floating, planktonic, marine cyanobacteria dominate quantitatively in oceans and on a global scale, but for unknown reasons, these populations have a more restricted morphological and genetic diversity compared with for instance, their limnic counterparts. Among these, the small ($\sim 1 \mu\text{m}$ cell diameter and a genome of average 2 Mb) unicellular cyanobacterium *Prochlorococcus* is now appreciated to be the most common, and probably the smallest photosynthetic unit on Earth (Rocap et al. 2003). The genus *Prochlorococcus* comprises physiologically distinct eco-types depending on e.g. light and nutrient gradients, and is together with the likewise unicellular and common genus

Synechococcus, of immense importance for carbon sequestration into ocean ecosystems (Bouman et al. 2006; Coleman et al. 2006; Johnson et al. 2006; Rocap et al. 2003). However, while these cyanobacteria are unable to fix nitrogen, some other marine unicellular representatives are now known to be potent nitrogen fixers (Montoya et al. 2004; Zehr et al. 2001). A common ancestry (16S rRNA) was recently proposed for populations of unicellular marine cyanobacteria in the Atlantic and Pacific Oceans, while recent data show that the diazotrophic (*nifH*) representatives within this group apparently diverged later (Falcon et al. 2004).

Filamentous planktonic cyanobacteria: bloom formation and toxin production

Bloom formation (surface accumulations) by cyanobacteria in oceans is a recurrent, poorly understood phenomenon. For example, conspicuous diazotrophic blooms regularly appear in the tropical and subtropical areas of the Atlantic and Pacific Ocean (*Trichodesmium*), along the coast of the southeast Pacific Ocean (*Lyngbya*) and in the Baltic Sea (*Nodularia* and *Aphanizomenon*). In particular, in the Baltic Sea the *Nodularia* and *Aphanizomenon* blooms typically occur in the summer, when water is warm and stratified, but their timing and location have proved difficult to predict. Some bloom-forming cyanobacteria also produce toxins, which are actually more toxic to human and terrestrial mammals than to aquatic biota. Neurotoxins and hepatotoxins produced by some of the bloom-forming cyanobacteria, such as *Nodularia* (Laamanen et al. 2001; Sivonen et al.

1989) and *Lyngbya majuscula* (Li et al. 2001), cause economic losses to the surrounding societies, ranging from disturbance in tourism due to the unpleasant smell, skin irritation and gastrointestinal problems of the swimmers, to death of fish and domestic animals drinking the water. Interestingly, and for an unknown reason, blooms of toxic cyanobacterial species may contain both toxic and non-toxic strains. Since the morphological discrimination between the two types is not possible (Dittmann and Wiegand 2006), PCR-based techniques targeting the toxin biosynthesis genes were therefore developed and became popular for the detection and quantification of toxic strains (Dittmann and Wiegand 2006). Cyanotoxins are secondary metabolites, their biological function and the regulatory mechanisms controlling their biosynthesis remain largely unknown. Toxin production was considered a restricted phenomenon to some cyanobacteria genera until the potential 'slow' neurotoxin (β -methyl-L-amino alanin; BMAA) was discovered to be produced by most cyanobacteria (Cox et al. 2005; see also article by Jonasson et al. in this issue).

Marine benthic cyanobacteria

The bottom-dwelling benthic cyanobacteria occupying coastal marine ecosystems, exhibit a wider morphological and genetic variability, while their total biomass is considerably smaller than that of the planktonic forms. In these oceanic fringes, mixed cyanobacterial morphologies occur as complex biofilms (Bauer et al. 2008; Charpy et al. 2007; Charpy-Roubaud et al. 2001; Díez et al. 2007), as well-organized and laminated mats (Díez et al. 2007; Golubic et al. 1999; Stal 2000), and as ancient stromatolites (Stal 2000; Steppe et al. 2001). Representative cyanobacteria in these habitats are the larger filamentous genera such as *Lyngbya*, and potentially members of the benthic LPP group. Together these groups include several aerobic and anaerobic diazotrophs. The fact that there is a close relationship between geographically widely separated phylotypes of the filamentous small-celled 'LPP-group B' (e.g. *Leptolyngbya*), as shown using 16S rRNA and *nifH* genes (the latter encoding a subunit of the nitrogen-fixing enzyme complex, nitrogenase), may point to a global distribution and ecological importance of the LPP group (Díez et al. 2007). A common origin for the planktonic *Trichodesmium* and the benthic *Blennothrix* (or *Hydrocoleum*) genera was also recently suggested (Abed et al. 2006). The marine cyanobacterial radiation thus consists of lineages of phylogenetically related morpho- and genotypes, although the origin of their evolution and diversification is still unclear.

Nitrogen fixation and cellular differentiation

Although nitrogen is the most abundant gas in the earth biosphere, it is available only for the organisms, such as cyanobacteria, that are capable of nitrogen fixation. Nitrogen fixation is catalyzed by the enzyme nitrogenase which is oxygen sensitive. Cyanobacteria capable of developing the specific nitrogen-fixing cell type known as a heterocyst, are common in soils and limnic environments, but for unknown reasons rare in oceans, and mostly confined to diatom symbioses (such as the heterocystous *Richelia* spp.). The morphology and physiology of heterocysts is adjusted to protect the oxygen-sensitive nitrogenase enzyme and was considered the norm for daytime diazotrophy until a novel cell type, the diazocyte, was discovered in *Trichodesmium* (Figure 1). Like in heterocysts, nitrogenase is contained in the diazocytes, making this cell type functionally equivalent to the heterocyst (El-Shehawey et al. 2003). Heterocysts and diazocytes may owe their differentiation strategies to terrestrial and oceanic adaptations, respectively. A second marine genus developing diazocytes, *Katagnymene*, was recently shown to belong within the *Trichodesmium* radiation (Lundgren et al. 2005). To decipher molecular mechanisms related to diazocyte development and

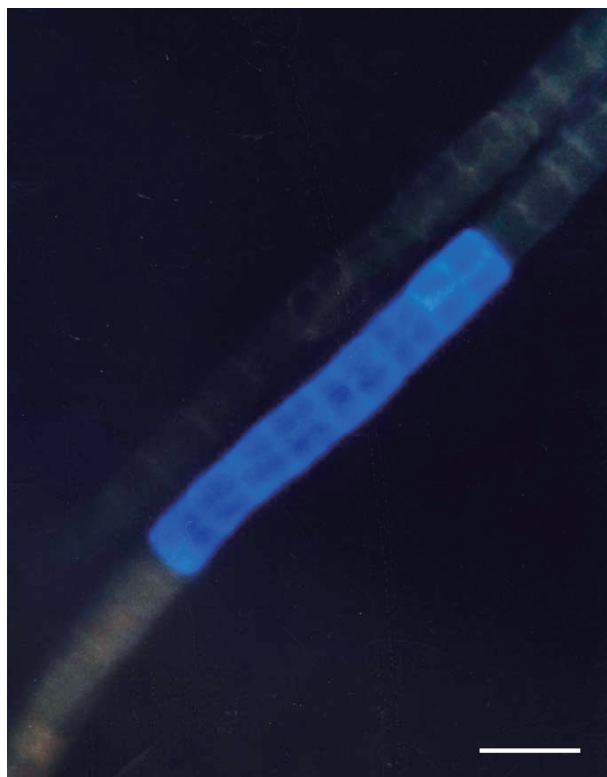


Figure 1. Light micrograph illustrating the occurrence of nitrogenase (blue) in central parts of filaments of a *Trichodesmium* IMS 101 colony. The blue immuno-fluorescence is restricted to 1–4 subsets of cells (10–15%) in each filament. The protocol followed was that of El-Shehawey et al. (2003). Size bar, 10 μ m.

whether limnic species of *Katagnymene* differentiate diazocytes is now of great interest. Why heterocystous cyanobacteria are rare in tropical and subtropical oceans is still a puzzle, although high temperatures have been proposed to play a role (Staal et al. 2003). Information is therefore needed on the occurrence of marine diazotrophic cyanobacteria from the colder oligotrophic Antarctic and Arctic Oceans.

Combating nutrient limitation in the ocean

Because cyanobacteria often live under strong nitrogen limitation in oceans, a substantial proportion of these marine communities consist of nitrogen-fixing cyanobacteria. The planktonic filamentous (non-heterocystous) genus *Trichodesmium* is the main nitrogen-fixer in warmer oceans (Karl et al. 2002). This genus alone, together with some diazotrophic unicellular and symbiotic cyanobacteria, is presently estimated to provide about half of all 'new' nitrogen sequestered into the global biosphere (Karl et al. 2002). Indeed, a recent transatlantic survey suggests that the abundance of *Trichodesmium* colonies is even higher than previously reported, which further underscores their significance as nitrogen scavengers and importance in the global nitrogen cycle (Davis and McGillicuddy 2006). Some other diazotrophic cyanobacteria are more restricted geographically, but may still be tremendously important. These include for instance the planktonic filamentous heterocystous genera *Nodularia* and *Aphanizomenon* spp. found in the temperate Baltic Sea, which, like *Trichodesmium*, form large surface 'blooms'.

Marine cyanobacterial diazotrophs are also well-known endosymbionts of bloom-forming diatoms such as *Hemiaulus* and *Rhizosolenia* found in tropical and subtropical parts of the oceans (Foster and Zehr 2006; Villareal 1991). Cyanobacteria with diazotrophic potential may also occur as symbionts in a coral (*Montastraea cavernosa*) (Lesser et al. 2004) and a dinoflagellate (the dinophysoid *Histioneis*) (Foster et al. 2006b).

Marine cyanobacteria require highly efficient scavenging systems to acquire key elements besides C via their photosynthesis and N via nitrogen fixation. Research in this area has focused on the uptake and transport of phosphate and iron. Evidence shows that marine diazotrophic cyanobacteria are adapted to hydrolyze and scavenge organic phosphate sources, such as phosphonates (*Trichodesmium*; Dyhrman et al. 2006), and phosphomonoesters (*Crocospheera watsonii*; Dyhrman and Haley 2006). The diazotrophic cyanobacteria also exhibit a high demand for iron, which is an integral part of the nitrogenase complex and other cellular processes, including photosynthesis. The various organisms handle iron limitation in different ways. *Trichodesmium* possesses a high-affinity iron scavenging system (Webb et al. 2001),

but no siderophores. The first iron storage protein in marine microorganisms was identified in the genome of *Trichodesmium erythraeum* and belongs to the Dps family (DNA binding protein from starved cells) (Castruita et al. 2006). *Lyngbya majuscula* produces iron-reducing superoxide radicals to increase iron uptake (Rose et al. 2005). It is not known how Baltic Sea cyanobacteria combat iron limitation.

Genome sequencing: filling the gaps

Although cyanobacteria are among the top five sequenced phyla (~thirty cyanobacterial genomes completely sequenced), genome sequences for marine diazotrophic cyanobacteria are largely lacking. The only genome sequence available is that of *Trichodesmium* IMS101. Upcoming sequencing plans, however, include both diazotrophic unicellular cyanobacteria (*Crocospheera watsonii* and *Cyanothece* sp.) and the endosymbiont of diatoms (*Richelia*). The stage may then be set to compare and contrast molecular strategies used by marine diazotrophs (i.e. how to decouple the incompatible N₂ fixation and oxygenic photosynthesis processes). Until today, comparative genomic analyses of cyanobacteria have revealed metabolic pathways, exclusive protein families and frequent lateral gene transfer events (Kechris et al. 2006; Mulikidjanian et al. 2006; Zhaxybayeva et al. 2006). Also, the recent application of metagenomics to oceanic microbes is creating enormous datasets on the genetic diversity and metabolic pathways in marine microbes, only a few unicellular non-diazotrophic cyanobacteria have been encountered (<1 µm cell fractions examined) (Rusch et al. 2007). Genomic reconstruction is also complicated by the complex communities that comprise many genera/species (Tyson et al. 2004). Connecting eDNA metagenomic data to uncultured individual cyanobacterial morphotypes will require visual observations using means such as LM, SEM, or TEM.

Concluding remark

Although marine diazotrophic cyanobacteria are of documented ecological and biogeochemical significance in global N cycling, their cell biology is far from being fully evaluated. Continued research is needed to explore developmental and cellular processes governing nutrient sequestration capacities, toxin production, and their spectacular surface blooms if we are to fully understand their role in oceans. Novel gene and genome sequences will aid in refining timescales for marine cyanobacterial evolution and permit identification of additional diazotrophic actors and diazotrophic strategies and provide rare opportunities to further understand fundamental global events related to marine cyanobacteria. At this

time, a great diversity has been documented among marine diazotrophs, but their quantitative and qualitative significance need further scrutiny.

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