DEVELOPMENT AND APPLICATION OF A NOVEL TRACER FOR MARINE HETEROCYSTOUS N_2 FIXING ENDOSYMBIOTIC CYANOBACTERIA

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Cyanobacteria are photoautotrophs that play an important role in the global carbon and nitrogen cycle with marine cyanobacteria being the major fixers of dinitrogen (N_2) in modern tropical and subtropical oligotrophic oceans. Cyanobacteria have evolved a range of different strategies in order to combine the incompatible processes of photosynthesis and N_2 fixation (diazotrophy), including the differentiation of specific cells for diazotrophy, known as heterocysts. Free-living heterocystous cyanobacteria are rare in the open ocean; however, heterocystous taxa are abundant as both exo- and endosymbionts in diatoms (Foster et al., 2011; Villareal, 1991), forming symbioses termed diatom-diazotroph associations (DDAs). In all free-living heterocystous cyanobacteria studied to date, the heterocyst cell walls contain heterocyst glycolipids (HGs) comprising a C_6 sugar head group bound to long chain diols, triols, or hydroxyketones. In contrast, DDAs contain novel HGs containing a C_5 sugar rather than a C_6 sugar head group (Bale et al., 2015; Schouten et al., 2013).

In the first part of this study, we developed a liquid chromatography-accurate mass mass spectrometry method using a novel internal standard to analyze the absolute concentration of HG lipids in suspended particulate matter (SPM) from a transect across the tropical north Atlantic including the region affected by the Amazon River plume. The plume supports high concentrations of DDAs such as *Hemiaulus hauckii-Richelia intracellularis and Rhizosolenia clevei-R. intracellularis* (Subramaniam et al., 2008). Two C_5 HGs were detected in the SPM and their concentrations exhibited a significant positive correlation with the number of *Hemiaulus* symbionts, providing the first environmental evidence that C_5 HGs track the abundance and distribution of DDAs. The distribution of C_5 HGs in the surface sediment reflected the transect C_5 HG SPM distribution and provided evidence that HG producers reach the sediment surface, probably enhanced by the silica mineral ballast as well as matrix protection provided by the diatom frustules. The total C_5 HG concentration in surface sediments was more spatially homogenous than the distribution in the SPM which reflects the wide spatial range of the HG-producers through an 'integrated' multi-decadal record of their deposition.

The second part of this study concentrated on the use of HGs in the geological record as a molecular tracer for past diazotrophy. Previously, Bauersachs et al. (2010) found that C_6 HGs were abundant in Pleistocene sapropels from the eastern Mediterranean Sea, which led to the interpretation that heterocystous cyanobacteria were important diazotrophs during sapropel formation. Based on the high numbers of diatoms, in particular rhizosolenoid diatoms and *H. hauckii,* in the sapropels (Kemp et al., 1999), Bauersachs et al. (2010) suggested that the C_6 HGs were likely to be derived from symbiotic cyanobacteria. However, the discovery that modern DDAs produce C_5 HGs, not C_6 HGs, cast uncertainty on this explanation. We analyzed piston-core sediments (64PE406, St 1) from the last interglacial anoxic event in the Eastern Mediterranean (i.e. sapropel S5) to screen for the presence of both C_5 and C_6 HGs. We found that a peak in C_6 HGs occurred soon after initiation of the sapropel deposition (Fig.

1), suggesting that free-living heterocystous cyanobacteria might have been transported and deposited during the discharge of large amounts of freshwater into the Eastern Mediterranean. C_5 HGs were also detected, with their abundance peaking later than the C_6 HGs and in agreement with Ba/Ti ratios (from XRF scanning), used in sapropels as a proxy for organic matter arriving at the seafloor (Fig. 1). The peak in C_5 HGs within the sapropel supports the model of (Kemp et al., 1999), which stated that increased freshwater-induced stratification of surface waters lead to conditions that were ideally suited for those diatoms found in DDAs. Furthermore, significant DDA mass sinking events have been observed in the modern day ocean (Karl et al., 2012). In conclusion, our research has shown that C_5 HGs form unique biomarkers for diazotrophic endosymbiotic cyanobacteria in present and past settings.

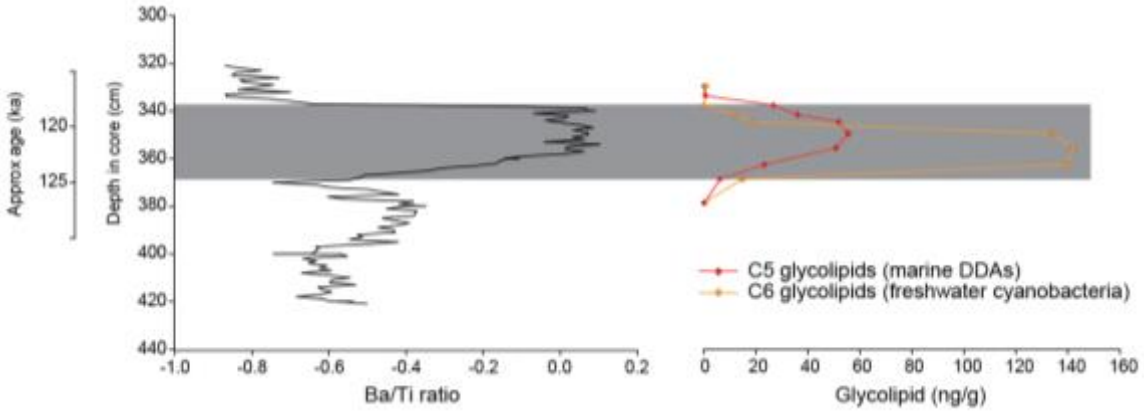

Figure 1. Depth profile if the Eastern Mediterranean piston core (64PE406, St 1) showing the profiles of the Ba/Ti ratio and the summed concentration of C_5 and C_6 HGs (ng/g). The grey box indicates the approximate position of sapropel S5.

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