EXPLORING THE BACTERIA-DIATOM METAORGANISM USING SINGLE-CELL WHOLE GENOME AMPLIFICATION

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ABSTRACT

Diatoms are responsible for a large fraction of oceanic and freshwater biomass production and are critically important to sequestration of carbon to the deep ocean. As with most surfaces present in aquatic systems, bacteria colonize the exterior of living diatom cells, and interact with the diatom and each other. The health, success and productivity of diatoms may be better understood by considering them as metaorganisms composed of a host cell together with its attached bacterial assemblage. There is ample evidence that this diatom-associated bacterial assemblage is very different from free-living bacteria, but its composition, functional capabilities and impact on diatom health and productivity are poorly understood. In this study, I examined the relationship between diatoms and bacteria at the single-cell level. Samples were collected in a nutrient-limited system (Station ALOHA, 22° 45'N, 158° 00'W) at the deep chlorophyll maximum. Flow cytometry followed by multiple displacement amplification was used to isolate and investigate the bacterial assemblages attached to 40 individual host cells. Thirty-four host cells were diatoms, including 27 *Thalassiosira* spp., 3 *Chaetoceros* spp., and one each of *Pseudo-nitzschia* sp., *Guinardia* sp., *Leptocylindrus* sp., and *Delphineis* sp. The remaining host cells included dinoflagellates, coccolithophorids, and flagellates. The bacteria associated with each host were identified by amplifying, cloning, and sequencing a region of 16S rDNA using primers designed to select against plastid and cyanobacterial sequences. Bacterial sequences were recovered from thirty-two of the forty host cells. For comparison, sequence libraries were also constructed for samples of the free-living and particle-associated bacterial assemblages. Network connectivity and sequence-based statistical analyses were conducted to assess similarities and differences among diatom host cells with regard to their bacterial associates, and among bacterial phylotypes with regard to their typical hosts. The data suggest host-cell specificity in one bacterial genus (*Arthrobacter*), which was found predominantly on *Thalassiosira* spp. cells, but most bacterial phylotypes were not specific to *Thalassiosira* spp. or other diatom hosts, and there was substantial variation in bacterial assemblages even among closely related host cells. Principal coordinate analyses suggest that libraries derived from individual host cells can be placed in distinct groups that are explained by the phylogenetic relatedness of their component bacteria. That is, each group of libraries included a suite of closely related bacteria that were found in most libraries within the group, and were almost exclusively found in that group. Other phylotypes were found in more than one group and did not appear to be diagnostic of any one group. I propose that there is strong evidence for the existence of identifiable assemblages of bacterial phylotypes attached to diatom host cells; further work must be done to validate this hypothesis. As yet, the functional implications are unknown.

INTRODUCTION

Algal-bacterial interactions have been studied for decades (Bell & Mitchell, 1972; Delong, Franks & Alldredge, 1993; Grossart et al., 2005), and the communities of bacteria closely associated with diatoms have been found to be distinct from free-living bacteria (Grossart 1999; Grossart et al., 2007). I argue that diatoms, their attached bacteria, and viruses associated with either host or bacteria may constitute a metaorganism as described by Bosch et al. (2011). Paraphrasing Bosch et al. and others (e.g. Biagi et al., 2011), a metaorganism is a polygenomic, composite organism derived from millennia of co-evolution with microbes. Host-microbiome symbioses are very well known in terrestrial systems (e.g. termites or ruminants and their cellulose-digesting gut microbes). In marine systems, the metaorganism concept has been studied extensively in coral and sponges where the term “holobiont” is used (Olson et al., 2010). Comparatively, very little is known regarding host-microbiome associations in diatoms (Grossart et al., 2010). If diatom-bacterial associations indeed act as a metaorganism, i.e. the properties of the diatom and bacteria acting together are distinct from each organism acting independently, then understanding this interaction may provide insight into the ecological and biogeochemical