

SHORT COMMUNICATION

Bacterial predation in a marine host-associated microbiome

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In many ecological communities, predation has a key role in regulating community structure or function. Although predation has been extensively explored in animals and microbial eukaryotes, predation by bacteria is less well understood. Here we show that predatory bacteria of the genus *Halobacteriovorax* are prevalent and active predators on the surface of several genera of reef-building corals. Across a library of 198 16S rRNA samples spanning three coral genera, 79% were positive for carriage of *Halobacteriovorax*. Cultured *Halobacteriovorax* from *Porites asteroides* corals tested positive for predation on the putative coral pathogens *Vibrio corallyticus* and *Vibrio harveyi*. Co-occurrence network analysis showed that *Halobacteriovorax*'s interactions with other bacteria are influenced by temperature and inorganic nutrient concentration, and further suggested that this bacterial predator's abundance may be driven by prey availability. Thus, animal microbiomes can harbor active bacterial predators, which may regulate microbiome structure and protect the host by consuming potential pathogens.

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Introduction

Host–microbe relationships are so important for the health of many plants and animals that the term ‘holobiont’ has been coined to describe the sum of a host and its symbionts (Hosokawa *et al.*, 2006). In addition to their interactions with an animal host, host-associated microbes simultaneously compete and cooperate with one another, and alterations to these interactions can affect holobiont function (De Boer *et al.*, 2007). Although top-down control via predation is known to structure many animal communities (Baum and Worm, 2009), the role of predation by bacteria on the structure of microbial communities is less well studied. Both specialist and generalist predatory strategies impact ecological communities by shifting community composition and abundance, but through different mechanisms. Specialists' predation via viral infections drives an evolutionary arms race within their specific prey population and can destroy entire clonal blooms of phytoplankton (Martínez *et al.*, 2007). Conversely, generalist predators have more influence over the

diversity and dynamics of their aggregate prey community.

Bdellovibrionales and like organisms (BALOs) are an order of predatory δ -proteobacteria that prey exclusively on other bacteria, including many known pathogens (Schoeffield and Williams, 1990). Predation by BALOs also releases nutrients (Martínez *et al.*, 2013), affecting biogeochemical cycling and production in nutrient-limited environments, similar to the effects of phage (Fuhrman, 1999; Brussaard *et al.*, 2008). In contrast to viruses, which are the most abundant biological entity in the marine environment and maintain abundances approximately in an order of magnitude greater than their hosts, BALOs do not need to be in high initial concentrations in the environment to drive significant bacterial mortality of their prey (Richards *et al.*, 2012; Williams *et al.*, 2016). One group of *Bdellovibrionales*, the marine *Halobacteriovorax*, are broadly distributed in marine waters across temperature, salinity and pH gradients (Pineiro *et al.*, 2007). *Halobacteriovorax* predation represents a regulatory mechanism that can alter the structure of bacterial communities (Li *et al.*, 2014).

Scleractinian or hard corals are widely studied because of their fundamental role as ecosystem engineers that build tropical reefs. Previous metagenomic and 16S amplicon studies suggest that *Halobacteriovorax* are members of coral microbiomes, as they were detected in *Porites*

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(Wegley *et al.*, 2007) and *Acropora* species (Sweet and Bythell, 2015). However, active predation by coral-associated *Halobacteriovorax* strains and the ecological interactions of this genus with other coral-associated microbes remain unexplored. Here we use a combination of cultivation, predation assays, microscopy, and 16S rRNA gene amplicon 454 pyrosequencing (Supplementary Methods) to assess *Halobacteriovorax*'s role in the microbiomes of three corals (*Siderastrea siderea*, *Agaricia* spp. and *Porites* spp.) in a 3-year data set.

Results and Discussion

We confirmed that *Halobacteriovorax* are members of coral microbiomes by culturing isolates from the three coral genera sampled in the time-series experiment and *Montastraea cavernosa* corals adjacent to the experimental plots (Figures 1a–d; Supplementary Table S1). Using full-length 16S analysis, the phylogenetic relatedness of these isolates to other BALOs was inferred (Figure 1e). Our *Halobacteriovorax* isolates were all identical at the 16S rRNA sequence level (accession #KR493097), and grouped within the previously established *Halobacteriovorax* cluster XIII (Figure 1e; Pineiro *et al.*, 2007).

We then explored whether our strains targeted other members of the coral microbiome for predation by selecting one of our *Porites astreoides* isolates, *Halobacteriovorax* sp. PA1, for the plaque assay technique that determines prey range (Schoeffield and Williams, 1990). *Halobacteriovorax* PA1 successfully attacked and consumed the known coral pathogens *Vibrio coralliilyticus* and *Vibrio harveyi*, as well as a *Vibrio fortis* strain (accession no. AJ440005, CP009468.1 and KT626460, respectively), we isolated from an apparently healthy *P. astreoides* colony. Like free-living isolates, host-associated *Halobacteriovorax* followed a biphasic lifecycle consisting of growth and attack phases (Supplementary Figure S1) visible in scanning electron microscopy (SEMs; Figures 1b–d).

We then examined the prevalence of *Halobacteriovorax* across coral microbiomes using a 3-year time series of 16S rRNA libraries generated from the surface mucus layer of corals (Supplementary Methods) that were exposed to increased nitrogen and phosphorus to simulate nutrient pollution, environmental factors that indirectly contribute to coral disease (Vega Thurber *et al.*, 2014). *Halobacteriovorax* was prevalent in >79% of all samples ($n=198$) and not detected in the other samples. Prevalence of *Halobacteriovorax* in nutrient-enriched corals (81%; $n=115$) was not significantly different ($P=0.28$) from controls (78%; $n=83$); *Halobacteriovorax* relative abundance was also not significantly different ($P=0.14$) between the two data sets. The mean relative abundance of *Halobacteriovorax* across all libraries was low ($0.40\% \pm 0.04$ SEM),

similar to their abundances in estuarine systems (Pineiro *et al.*, 2013) and as characteristic of predators across most ecosystems (Baum and Worm, 2009). The high prevalence of this taxon and its predatory lifestyle suggests it is an important low-abundance member of the core coral microbiome (core members are detected in $\geq 75\%$ and are consistent components of the microbial assemblage). Given their low abundance, false negatives cannot be established with certainty, and the reported prevalence values likely represent a lower bound on *Halobacteriovorax* prevalence.

To study the possible interactions between predatory bacteria and other members of the host microbiome, we constructed co-occurrence networks from our coral microbiome data set using the CoNet plugin (Faust and Raes, 2012; Faust *et al.*, 2012) for Cytoscape (Shannon *et al.*, 2003). Networks were generated from the taxonomic order level and provide the net effect of all BALO predation. CoNet uses a permutation-renormalization and bootstrap (ReBoot) method to mitigate the effects of spurious correlations induced by compositionality while assessing the significance of an association. Networks were constructed separately for two known drivers of shifts in the coral microbiome, temperature and nutrient enrichment. Thus, networks were binned by (a) temperature (2 °C intervals) and (b) nutrient enrichment, as well as by (c) coral host (Supplementary Table S2).

Changing thermal regimes affected bacterial predator–prey interactions (Figure 2, Supplementary Table S3). The largest number of significant taxon co-occurrences (network edges) between BALOs and other bacterial taxa occurred at the highest and lowest extremes of temperature (Figure 2). Networks from the highest-temperature data sets formed two distinct clusters. BALOs are a component of the larger cluster in both the control and nutrient networks, where their edges in the network interact with several taxa, including *Myxococcales*, another predatory bacterial taxa, suggesting that bacterial predators in the network respond to common drivers. Bacterial predator–prey interactions in the networks also fundamentally changed under nutrient enrichment (Figure 2). Only three shared co-occurrence taxa (*Chromatiales*, *NB1-j* and unclassified79) were detected in the networks from the nutrient treatment samples (Supplementary Table S3), and only a single taxa (*Cytophagales*) was shared between the nutrient and control host network.

Siderastrea and *Agaricia* coral species contributed a majority of the BALO interactions in the host networks, whereas *Porites* accounted for only 8% (Supplementary Table S4). Some of the interactions were different among the different coral host. For example, the *Bdellovibrionales* co-occurred with *Vibrionales* on *Agaricia* corals, but not the two other host taxa (Supplementary Table S4). Interestingly, in all the networks generated, BALOs had exclusively

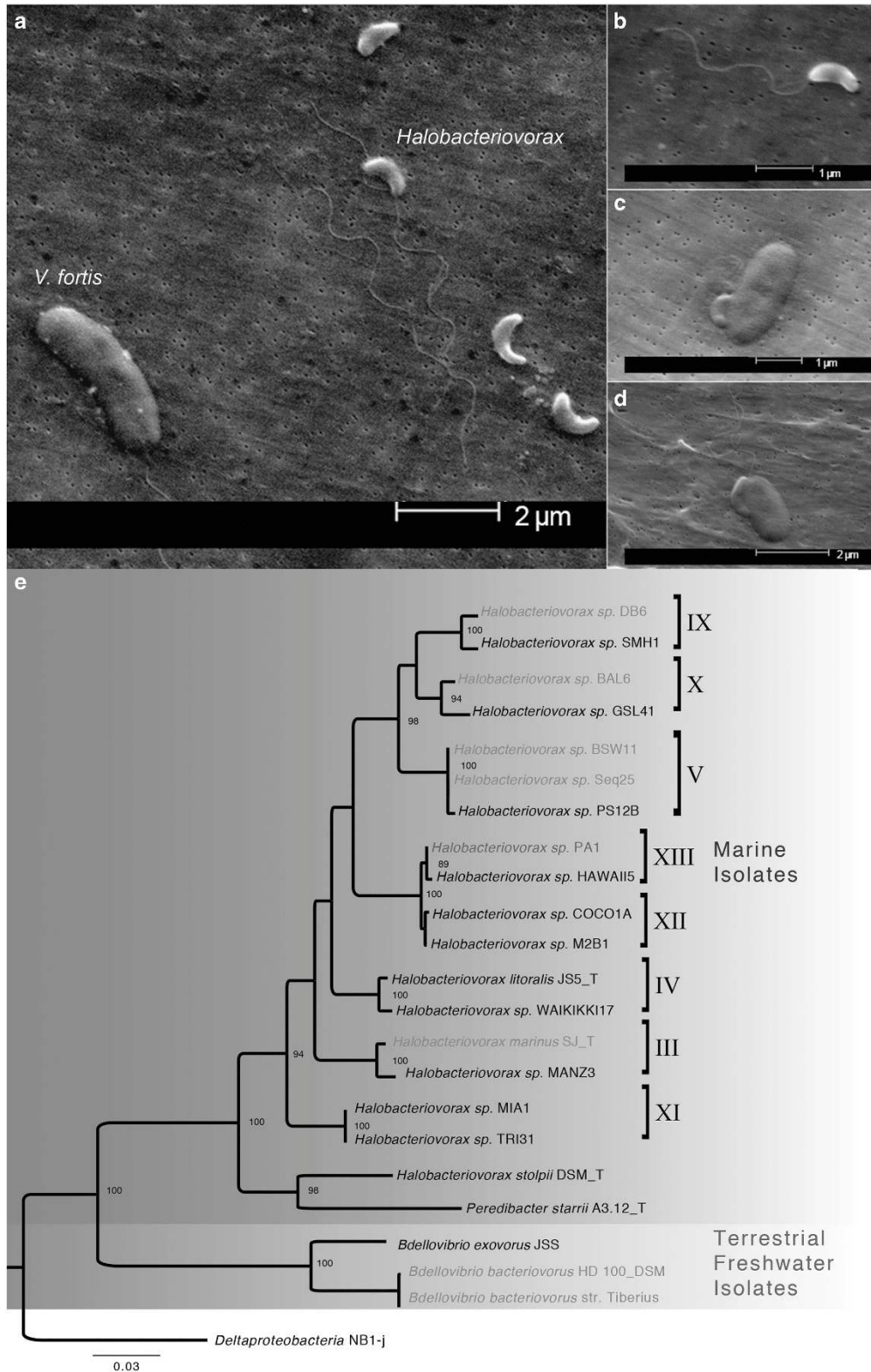


Figure 1 Electron micrographs of our coral-associated strain, *Halobacteriovorax* PA1, including (a) several attack-phase cells and a *Vibrio fortis* prey (the single larger cell), (b) a single attack-phase cell, (c) a cell in attachment phase on the surface of a *Vibrio fortis* prey cell and (d) a *Halobacteriovorax* entering the prey periplasm. (e) Molecular phylogenetic analysis is based on 16S rRNA gene sequences of the order *Bdellovibrionales* by the maximum likelihood method. The coral-isolated strain (PA1) used in this study is blue, and strains with whole-genome sequences are red. The bootstrap consensus tree inferred from 500 replicates and bootstrap values >80% are reported. The analysis involved 23 nucleotide sequences. All positions containing gaps and missing data were eliminated. There were a total of 1216 positions in the final data set. Evolutionary analyses were conducted in MEGA6 [3].

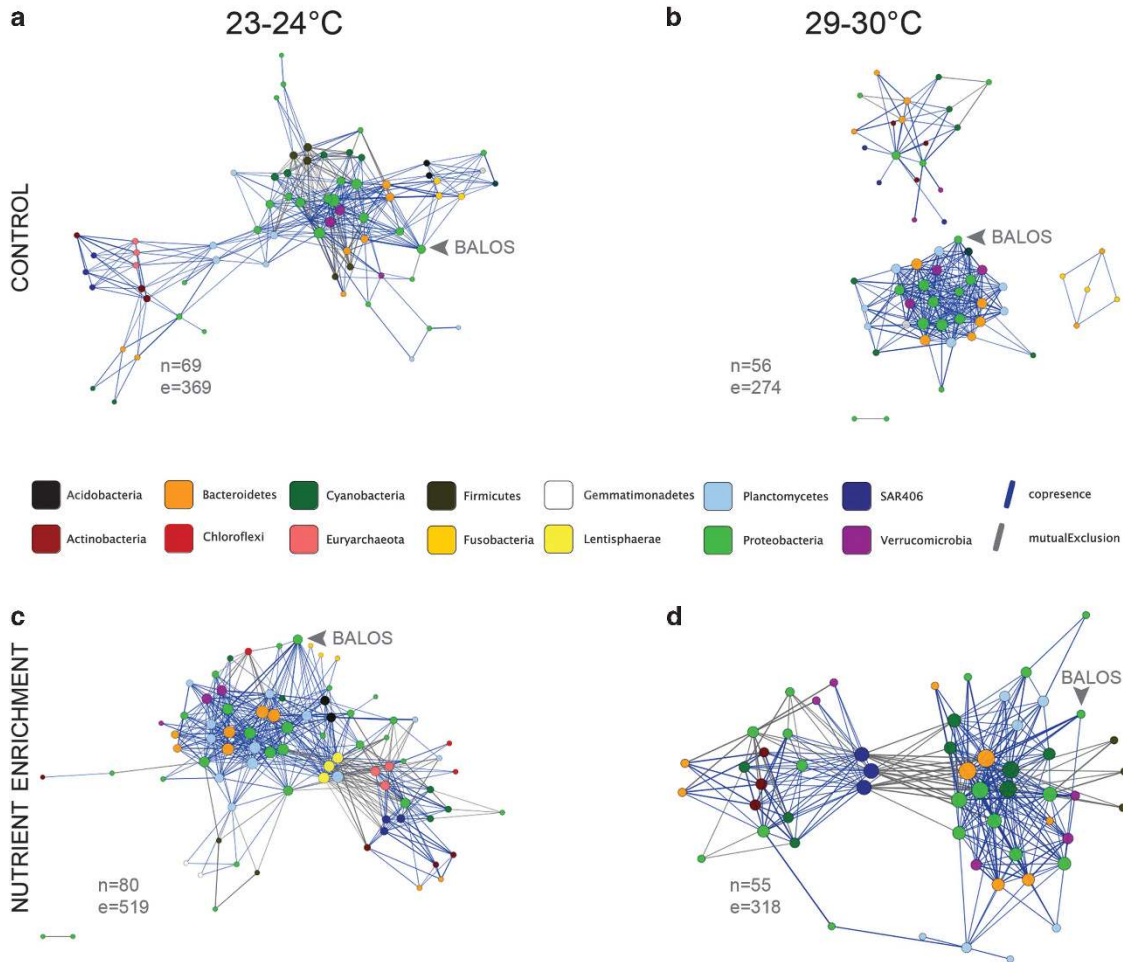


Figure 2 Host-associated microbiome networks focused on members of the *Bdellovibrionales* and like organisms, which include the *Halobacteriovorax*. Networks were constructed for control samples falling within the temperature bin (a) 23–24 °C and (b) 29–30 °C, and nutrient enrichment samples within the temperature bin (c) 23–24 °C and (d) 29–30 °C. Circles in the networks represent bacterial nodes at order taxa level, color-coded by phylum according to the legend in the center. Blue lines connecting node pairs indicate significant co-occurrence patterns, whereas gray lines denote significant mutual exclusion patterns ($P \leq 0.05$) as determined by the ReBoot randomization procedure in the CoNet Cytoscape package (Faust and Raes, 2012; Faust *et al.*, 2012). Network layout was calculated using edge-weighted spring embedded layout in Cytoscape (Shannon *et al.*, 2003).

positive co-occurrence interactions (blue) and never exhibited mutual exclusions (gray; Figure 2). Co-occurrence interactions suggest that (a) *Bdellovibrionales* populations are increasing because of the increase in their bacterial prey populations, (b) conditions are favorable to *Bdellovibrionales* and the co-occurring taxa and/or (c) the predatory bacteria are removing competitors of co-occurring taxa allowing other members to increase. Given the well-known role of prey abundance in other predator–prey systems, response to prey abundance would parsimoniously explain the co-occurrence data. An alternative hypothesis is that low read depth precludes detection of negative interactions by *Halobacteriovorax*. We examined whether negative interactions were observed for other low-abundance microorganisms. Bacterial relative abundance showed little correlation with the number of negative edges detected (Pearson correlation, $r^2 = 0.00159$). The same relationship was held when restricting

regression analysis to taxa equally or less abundant than *Halobacteriovorax* (Pearson correlation, $r^2 = 0.007$).

Active predation by host-associated bacteria opens an intriguing area of research into the structure and function of animal microbiomes. Longitudinal analysis of the coral co-occurrence networks simultaneously delivers both a broad census of the microbiome along with a condensed list of potentially interesting biological interactions that can be targeted in future studies using truly quantitative methods (for example, qPCR of predator–prey cycles). Such truly quantitative approaches would be costly and potentially infeasible without *a priori* knowledge of which members to target.

Our findings establish that regardless of their low abundance, *Halobacteriovorax* predation may significantly impact microbiome dynamics in a context-dependent manner and, as a corollary, global change could alter predator–prey dynamics on host-associated microbiomes.

Conflict of Interest

The authors declare no conflict of interest.

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