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Redefining the sponge-symbiont acquisition paradigm: Sponge microbes exhibit chemotaxis towards host-derived compounds

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Originality-Significance Statement: Marine sponges often harbor highly host-specific microbial communities which are stable across broad geographic and temporal scales. These sponge symbionts are generally extremely rare in the surrounding environment and it was previously assumed that sponges primarily maintain these highly-stable partnerships via strict vertical transmission. Our research infers a new mechanism for how sponges can acquire bacterial symbionts from the surrounding environment, revealing an active role of symbionts in finding their host using chemotaxis.
Summary

Marine sponges host stable and species-specific microbial symbionts that are thought to be acquired and maintained by the host through a combination of vertical transmission and filtration from the surrounding seawater. To assess whether the microbial symbionts also actively contribute to the establishment of these symbioses, we performed in situ experiments on Orpheus Island, Great Barrier Reef, to quantify the chemotactic responses of natural populations of seawater microorganisms towards cellular extracts of the reef sponge *Rhopaloeides odorabile*. Flow cytometry analysis revealed significant levels of microbial chemotaxis towards *R. odorabile* extracts and 16S rRNA gene amplicon pyrosequencing showed enrichment of ‘sponge-specific’ microbial phylotypes, including a cluster within the Gemmatimonadetes and another within the Actinobacteria. These findings infer a new mechanism for how sponges can acquire bacterial symbionts from the surrounding environment and reveal an active role of the symbionts in finding their host.
Introduction

Sponges contain microbial symbionts from all three domains of life – Bacteria, Archaea and Eukarya – which can comprise up to 35% of a sponge’s biomass and are essential for host fitness and survival (reviewed in (Webster and Taylor, 2012)). Molecular surveys have revealed that many sponge-associated microbes occur exclusively within sponges (Hentschel et al., 2002; Taylor et al., 2007b) or are exceptionally rare in the surrounding coral reef environment (Taylor et al., 2013; Thomas et al., 2016). These microbes often form monophyletic ‘sponge-specific’ 16S rRNA gene sequence clusters (Hentschel et al., 2002; Taylor et al., 2007a; Simister et al., 2012; Taylor et al., 2013). The occurrence of these ‘sponge-specific’ bacteria in phylogenetically distant sponges from geographically isolated locations, coupled with the rarity of these bacteria in the surrounding environment, has led to interest in the evolutionary mechanisms that have maintained these complex and diverse symbioses (Webster and Thomas, 2016).

Previous research has indicated that sponges likely acquire their symbionts via the dual mechanisms of microbial filtration from the surrounding seawater (Reiswig, 1971; Webster et al., 2010) and vertical transmission from parent to offspring (Schmitt et al., 2007; Sharp et al., 2007; Webster et al., 2010). However, it has remained unclear whether these microbial associations are solely controlled by the sponge or if the microbes are also capable of actively seeking out their hosts on coral reefs. For instance, it has recently been shown that coral-associated microbes display high levels of chemotaxis to chemicals released from the coral holobiont (Garren et al., 2014; Tout et al., 2015). Here we propose that chemotaxis may also be involved in the formation of ‘sponge-specific’ microbial interactions and we assessed this using the model Great Barrier Reef sponge Rhopaloeides odorabile, which hosts a highly diverse and stable microbial community that is critical in regulating host health (Webster and Hill, 2001; Webster et al., 2001b; Webster et al., 2001a; Webster et al., 2008; Webster et al., 2011; Fan et al., 2012). We assessed whether natural populations of coral reef bacteria exhibit chemotaxis towards cellular extracts of R. odorabile and examined whether
previously identified sponge symbionts, which are rare or undetectable in seawater, are among these chemotactic microbes.

This study was conducted in July 2013 at Orpheus Island (18°35.5959’S, 146°28.9559’E) on the Great Barrier Reef, Australia. To assess microbial chemotaxis towards extracts of the sponge *R. odorabile*, we employed a microfluidic-based *in situ* chemotaxis assay (ISCA) (Tout et al., 2015) (Supplementary Information), whereby the strength of microbial chemotaxis was assessed using cytometric cell counts and the composition of the chemotactic microbes was determined using 16S rRNA gene amplicon sequencing.

To prepare sponge cellular extracts, three replicate *R. odorabile* samples were used. A 1 cm³ section of tissue was excised from each sponge and homogenised in 10 ml of 0.2 µm-filtered seawater using a mortar and pestle. The tissue homogenates were carefully filtered through 0.2 µm syringe filters (Millipore) to remove microbial cells and subsequently filtered through Amicon Ultra 0.5 3 Kda filters (Millipore) to remove any remaining DNA. To ensure all microbial DNA had been removed from the sponge extracts, PCR reactions were performed as described in Fig. 2 and no positive amplificates were obtained. The filtered seawater (FSW) controls were prepared by filtering 50 ml of reef water through a 0.2 µm syringe filter (Millipore). The Marine Broth (MB) positive controls were prepared by mixing a 1% marine broth (Difco) solution and filtering through a 0.2 µm syringe filter (Millipore).

Across all ISCAs the chemotactic response of the natural microbial community to the cellular extracts of *R. odorabile* was significantly greater than to the FSW and even the MB positive controls ($t_c = 5.7 \pm 1.3$, $P<0.05$; Fig. 1, Table S1). While the community composition (OTUs defined at 97% sequence similarity) of chemotactic bacteria responding to the *R. odorabile* extracts was not significantly different from the FSW controls (Fig. 2a, $P>0.05$; Table S2), we identified an elevated presence of “sponge-specific” sequences within the *R. odorabile* extract samples. A total of 56 sequence matches to previously defined ‘sponge-specific’ sequence clusters were observed (Table S3), with 96% of
these occurring within the *R. odorabile* cellular extracts (Fig. 2b). Of these, 66% were affiliated to the *Gemmatimonadetes* cluster SC67 and 30% to the *Actinobacteria* cluster SC22 (as defined by Simister et al., 2012). The *R. odorabile* microbiome has previously been described using full length clone sequencing and 454 sequencing of the 16S rRNA gene and was found to be dominated by *Proteobacteria, Acidobacteria, Choloroflexi, Actinobacteria* and *Gemmatimonadetes* (Webster et al. 2001; Webster et al., 2010). While the chemotactic microorganisms that fell into SSSC were not identical matches to published *R. odorabile* sequences, the assignment to *Actinobacteria* and *Gemmatimonadetes* SSSC lineages is nonetheless consistent with the previously described microbial community of *R. odorabile*. The most abundant bacterial families responding to cellular extracts of *R. odorabile* were the *Sphingomonadaceae, Rhodobacteraceae* and *Piscirickettsiaceae*, which represented 12%, 9% and 8% of the total community, respectively (Fig. 2a, Table S4). The primary drivers of the differences between the communities responding to *R. odorabile* extracts and to the FSW control were members of the *Piscirickettsiaceae, Sphingomonadaceae*, and *Vibrionaceae* (each contributed 2% to the total dissimilarity), which were all more abundant in the *R. odorabile* cellular extracts than in the FSW controls (Fig. 2a, Table S5).

The important role of chemotaxis in structuring host-bacterial associations within the marine environment is becoming increasingly apparent (Banin et al., 2001; Rosenberg et al., 2007; Meron et al., 2009; Garren et al., 2014; Tout et al., 2014; Tout et al., 2015). The results presented here are the first observations of chemotaxis by sponge symbionts towards sponge-derived compounds. Importantly, previously defined ‘sponge-specific’ bacteria were not detected by amplicon sequencing of the background coral reef seawater, which is consistent with previous reports that these microorganisms are exceptionally rare outside of sponge hosts (Taylor et al., 2013). However, environmentally rare ‘sponge-specific’ bacteria were present in the *R. odorabile* extract treatment, indicating that they exhibited chemotaxis towards sponge-derived chemicals, highlighting a new mechanism for the establishment of sponge-bacteria associations.
The concept of sponges hosting specific and stable symbiont populations that provide benefit to the host was originally proposed by Vacelet and Donadey (Vacelet and Donadey, 1977) and subsequently validated by extensive molecular research (reviewed in Hentschel et al., 2012; Webster and Taylor, 2012; Webster and Thomas, 2016). For example, members of the Piscirickettsiaceae and Sphingomonadaceae, which were the most differentially represented taxa responding to the sponge extract and the FSW control in the present study, are known to form intimate associations with marine sponges (Thomas et al., 2010) and have been linked by functional metagenomics to the production of enzymes involved in the vitamin B12 synthesis pathway (Thomas et al., 2010). The theory of sponge symbiont specificity was expanded by the discovery that sponges host microbes falling within monophyletic ‘sponge-specific’ 16S rRNA gene sequence clusters (Hentschel et al., 2002; Taylor et al., 2007a; Simister et al., 2012; Taylor et al., 2013). These ‘sponge-specific’ sequence clusters are defined as groups of 16S rRNA gene sequences that share greater similarity to each other than to sequences from non-sponge sources, are derived from at least two or more sponge species (or the same species from at least two different locations) and are supported by at least three independent phylogenetic tree-building algorithms (Hentschel et al., 2002). The observed chemotaxis of microbes within the Gemmatimonadetes and Actinobacteria ‘sponge-specific’ 16S rRNA gene sequence clusters therefore provides evidence that some sponge symbionts may actively find their sponge host on coral reefs using chemotaxis. These microbial phyla are known to associate with a diverse range of sponges (Taylor et al., 2007b) and are highly active within their respective hosts (Kamke et al., 2010), yet have been shown to be rare in the surrounding seawater on the Great Barrier Reef (Webster et al., 2010; Bourne et al., 2013).

This study illustrates that chemotaxis may underpin the establishment of some sponge-bacterial associations. Future studies that further refine the specific chemical cues derived from different sponge hosts are likely to reveal further patterns of sponge symbiont attraction. Our observations indicate that the behaviour of individual microbes can underpin the establishment of an important
animal-microbe symbiosis and adds to the growing evidence that chemotaxis is an ecologically important phenotype in the marine environment.

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Figure Legends

Figure 1 Accumulation of bacteria in response to chemoattractants. Chemoattractants included i) 3 Kda filtered *R. odorabile* cellular extracts, ii) 0.2 μm-filtered seawater controls (FSW), and iii) 0.2 μm-filtered 1% Marine Broth nutrient controls (MB). Three replicate ISCA were deployed onto the Orpheus Island reef flat at 8 m water depth in a region devoid of sponges. For each ISCA deployment, 7 replicates of each chemoattractant were randomly distributed across the ISCA with 4 subsequently used for flow cytometry and 3 used for DNA extraction. A background seawater sample (1L) was also collected from the same location prior to ISCA deployments for 16S rRNA gene sequencing. Over the course of the 4 h deployment, the chemoattractant diffuses into the external environment, creating a chemical gradient in the surrounding seawater (Berg, 1993) that triggers the
migration of chemotactic bacteria into the wells. The accumulation of bacteria was assessed by flow cytometry as described in (Tout et al., 2015) and expressed in terms of a Chemotactic Index, \( I_c \) (mean ± standard deviation), with \( I_c \) calculated as the number of cells responding to the chemoattractant relative to the number of cells responding to the FSW control. Responses above \( I_c = 1 \) represent statistically significant positive chemotaxis. Vertical bars represent mean ± SD (n = 4).

**Figure 2 a)** Taxonomic composition of bacteria responding to the ISCA deployments. To identify the composition of the chemotactic microbes, samples from 3 replicate wells per ISCA were pooled and DNA was extracted from the sponge extract and FSW ISCA samples using the Ultra Clean microbial DNA isolation kit (Mo Bio, Carlsbad, California). DNA was additionally extracted from the bulk 1L seawater sample to screen for the presence of sponge-specific microbial OTUs in the surrounding reef seawater. Extracted DNA was amplified using the 16S primers 803F (5'-ATTAGATACCCTGGTAGTC-3') and 1392R (5'-'ACGGGCGGTGTGTRC-3') under the following cycling conditions: 95°C for 3 min; 25 cycles of 95°C for 30 s, 55°C for 45 s and 72°C for 90 s; followed by a final extension at 72°C for 10 min (Engelbrektson et al., 2010). Amplicons were sequenced using the 454 GS-FLX pyrosequencing platform (Roche) at the Australian Centre for Ecogenomics (University of Queensland, Australia). 16S rRNA gene sequences were analysed using the QIIME pipeline (Kuczynski et al., 2002; Caporaso et al., 2010) and data was submitted to the sequence read archive at NCBI under the accession SUB1639379. Bar charts summarise the bacterial taxonomy at the family level (data are averages of \( n=3 \) ISCAs/treatment). The microbial community identified in the FSW control is representative of organisms that swam into this treatment as a consequence of random motility, rather than chemotaxis due to the lack of any chemical gradient. Thus, this sample provides an overview of the motile, but not necessarily chemotactic proportion of the community. **b)** Composition of chemotactic ‘sponge-specific’ sequence clusters (SC) in the cellular extract of *R. odorabile* and FSW control. A representative sequence (average sequence length of 505 bp) of each
identified OTU was taxonomically assigned (using a BLAST search) with a curated SILVA 16S rRNA database containing 173 previously identified bacterial sponge-specific clusters (SC) and 32 sponge/coral-specific clusters (SCC) (Simister et al., 2012). For each BLAST search, the 10 best hits were aligned in order to determine sequence similarities. A sequence was assigned to an SC/SCC when it was more similar to the sequences comprising that cluster than to other sequences outside the cluster and the similarity to this sequence was at least 75% (Taylor et al., 2013).

References


