Defining the Core Microbiome of the Symbiotic Dinoflagellate, *Symbiodinium.*

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Abstract

Dinoflagellates of the genus *Symbiodinium* underpin the survival and ecological success of corals. The use of cultured strains has been particularly important to disentangle the complex life history of *Symbiodinium* and their contribution to coral host physiology. However, these cultures typically harbour abundant bacterial communities which likely play important, but currently unknown, roles in *Symbiodinium* biology. We characterised the bacterial communities living in association with a wide phylogenetic diversity of *Symbiodinium* cultures (18 types spanning 5 clades) to define the core *Symbiodinium* microbiome. Similar to other systems, bacteria were nearly two orders of magnitude more numerically abundant than *Symbiodinium* cells and we identified three operational taxonomic units (OTUs) which were present in all cultures. These represented the α-proteobacterium *Labrenzia* and the γ-proteobacteria *Marinobacter* and *Chromatiaceae*. Based on the abundance and functional potential of bacteria harboured in these cultures, their contribution to *Symbiodinium* physiology can no longer be ignored.

Introduction

Abundant bacterial communities occur in association with reef-building corals, where they play critical roles in recycling nutrients and protection against pathogens (Bourne et al. 2016). Recent research has focussed on identifying the stable and consistent members within coral-associated microbial communities in order to better characterise their functional importance (Astudillo-Garcia et al. 2017), with “core coral microbiomes” recently characterised (Ainsworth et al. 2015). However, despite evidence that microalgae typically also live in close association with bacterial partners (Amin et al. 2015; Seymour et al. 2017), little is known about the bacterial consortia associated with the key symbiotic partner of corals, *Symbiodinium*.

Dinoflagellates of the genus *Symbiodinium* are globally important primary producers across coastal ecosystems, and also form a key symbiotic partnership with reef-building corals (Suggett et al. 2017). The genetic and functional diversity of this genus is extremely high (Thornhill et al. 2017; Suggett et al. 2015), and the phylogenetic identity of the dominant *Symbiodinium* symbiont directly influences the environmental stress tolerance and recovery of their coral hosts (Suggett et al. 2017). Consequently, *Symbiodinium* biology and its impact on the ecological success of reef building corals over space and time, has been a central research focus for decades (Warner & Suggett 2016). These dinoflagellates can be cultivated *ex-hospite* in monocultures, which remains a primary platform to understand their biology and the complex role they play in coral functioning (Warner & Suggett 2016). However, these cultures inherently contain abundant bacterial communities co-isolated with *Symbiodinium* (Ritchie 2011; Shoguchi et al. 2013; Frommlet et al. 2015a). Bacteria are involved in sophisticated interactions with microalgae and their complex metabolic exchanges typically influence the health and physiological performance of both partners (Seymour et al. 2017). To date, the potential roles bacteria may play in *Symbiodinium* physiology have been almost entirely overlooked. We characterised the bacterial communities associated with 18 *Symbiodinium* types (spanning 5 clades; Supporting Information Table S1), to define the *Symbiodinium* core microbiome and thus develop a framework for considering the putative functional roles of bacteria in moderating *Symbiodinium* biology.

Results & Discussion

Bacterial cells were on average 65 times more numerically abundant than *Symbiodinium* in culture ($1.04\times10^7 \pm 1.37\times10^6$ versus $1.59\times10^5 \pm 2.38\times10^4$ cells/mL respectively; Fig. 1A), which is consistent with bacterial densities commonly reported from other microalgae

cultures (Amin et al. 2015). These bacterial communities were also diverse (Supporting Information Table S2; Shannon diversity index: 2.72–5.61) and, perhaps notably, their composition and structure differed substantially from that typically observed in other microalgae cultures and coral-associated communities (Neave et al. 2017; Ainsworth et al. 2015). For example, the extremely common coral endosymbiont *Endozoicomonas* (Neave et al. 2017) was not detected in any *Symbiodinium* strains.

Three different OTUs were present in all *Symbiodinium* cultures and were identified as core members of the *Symbiodinium* microbiome (Fig. 2; Supporting Information Table S3). These OTUs belonged to bacterial genera from the α- and γ-proteobacteria (Fig. 2), and included *Labrenzia*, *Marinobacter* and an unclassified purple sulfur bacterium from the *Chromatiaceae* family. The most abundant core member, *Labrenzia*, represented up to 38.4% of the *Symbiodinium*-associated communities (Fig. 1B), has been previously identified in corals and other microalgae cultures (Supporting Information Table S7) and is notable for its ability to produce dimethylsulfoniopropionate (DMSP) (Curson et al. 2017). High concentrations of this compound, which likely plays a role in stress tolerance (Sunda et al. 2002), are present in *Symbiodinium* cultures (Steinke et al. 2011) and have until now been fully attributed to the microalgae. However, the large proportion of the DMSP-producing *Labrenzia* in *Symbiodinium* culture illustrates how bacteria might in fact be partially responsible for some traits solely ascribed to *Symbiodinium*. Another core member, *Marinobacter*, has been demonstrated to produce specific siderophores which can provide enhanced levels of bioavailable iron to phytoplankton (Amin et al. 2009) and could therefore positively impact *Symbiodinium* growth (Ritchie 2011), this bacteria has also been previously identified in *Symbiodinium* cultures (Frommlet et al. 2015b). *Marinobacter*, together with the last core member, an unclassified *Chromatiaceae*, have also both been previously reported in corals and other microalgae cultures (Supporting Information Table S7).

The small numbers of OTUs shared across the 18 *Symbiodinium* strains investigated here is perhaps unsurprising given the large phylogenetic and functional diversity of these dinoflagellates (Fig. 1B). Therefore, we also defined the core microbiome for each *Symbiodinium* clade (Fig. 2). The number of OTUs in these clade-specific cores ranged from 4 in clade A to 35 in clade C (which is potentially a consequence of the lower number of clade C representatives used here). Four bacterial genera were present and abundant in more than one *Symbiodinium* clade. These included the Flavobacteria *Muricauda* and the γ-proteobacteria, *Haliea, Oceanococcus* and *Algiphilus*. Notably, the latter preferentially uses polycyclic aromatic hydrocarbons that accumulate on phytoplankton cell surfaces as primary carbon sources (Gutierrez et al. 2012). Furthermore, Flavobacteria such as *Muricauda* are known for their association with phytoplankton: they can degrade both high and low molecular weight compounds exuded from these cells and secrete surface proteins that might facilitate their attachment (Buchan et al. 2014).

Notably, clade F harboured bacterial communities that were significantly different from all other clades (ANOSIM; significance level = 0.1%) and were also the most uniform (similarity: 63.50%) (Supporting Information Table S4-S5). Furthermore, some unique core members from this clade have potentially been conserved after years of growth in culture (Supporting Information Table S1): *Gilvibacter* represented, on average, 51.4 ± 3.65% of the F1 and F1' *Symbiodinium* type communities, while only having a mean abundance of 0.02±0.003% across all other types. These two F1 strains were isolated from Hawaii and Heron Island (Australia) respectively (Supporting Information Table S1). This bacterium has been isolated from the sediment of tropical marine environments, where F1 *Symbiodinium* is often found in association with Foraminifera (Pochon et al. 2001) and we propose that, given its conserved occurrence across two strains isolated 7000 km apart, it is possible that F1

Symbiodinium might have coevolved in tight association with this nitrate-reducing bacterium (Khan et al. 2007).

Less than 5% of the hundreds of studies on *Symbiodinium* cultures have worked with axenic cultures (Supporting Information Table S6). Our results clearly show that bacteria are abundant and diverse within most *Symbiodinium* cultures and that some bacteria are conserved across the large *Symbiodinium* phylogenetic diversity, and may be potentially responsible for traits that are presently solely attributed to these dinoflagellates. Consequently, the role played by bacteria in *Symbiodinium* physiology should no longer be overlooked and future work should precisely target some of the core members identified here and clearly resolve the metabolic interactions that likely occur between core bacterial taxa and this globally important dinoflagellate.

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Conflict of Interest

The authors declare no conflict of interest.

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Supporting Information

Appendix S1. Descriptive information regarding the methods used within this study.

Table S1. Summary of *Symbiodinium* ITS2 type, strain identification number, location of original isolation, taxa of the cnidarian host species and the time spent in culture. Types are organised alphabetically (determined *via* ITS2 as per Lajeunesse et al. 2012 and Lee et al. 2015); species names are given where known (as per Suggett et al. 2015). **'** indicates same type but different isolation location.

Table S2. Shannon diversity index (H') and number of observed species (O_sp.) calculated using the QIIME alpha_diversity.py command for bacterial communities present 18 ITS2 types (in triplicate). Analysis done on rarefied data (to 14000 reads). Colour scale for H': lowest = white, highest = purple; O_sp.: lowest = white, highest = green.

Table S3. Operational taxonomic units (OTUs) defined as core members of the bacterial communities of *Symbiodinium* cultures and their corresponding GenBank accession numbers.

Table S4. ANOSIM results on the bacterial communities associated with five clades (A, B, C, D & F) of *Symbiodinium* in cultures. Significant values in bold.

Table S5. SIMPER analysis of the bacterial communities associated with five clades (A, B, C, D & F) of *Symbiodinium* in cultures. Showing the main drivers of the cladal similarity and dissimilarity.

Table S6. Web of Science literature search to identify studies on *Symbiodinium* using cultures. Search parameters: Search term "*Symbiodinium* culture" and "axenic *Symbiodinium* culture. These two lists were cross checked to ensure they did overlap and axenic studies were manually checked to ensure viability. All studies that used axenic cultures of

Symbiodinium are highlighted (12 studies out of 268 use axenic cultures). Search date: 27/06/2017.

Table S7. Summary of studies reporting Labrenzia, Marinobacter and Chromatiaceae sequenced or isolated from corals (pale blue) and microalgae (pale green). Literature search completed on the 17th of September 2017 using Google Scholar.

Figure S1. Two-dimensional non-metric MDS plot of bacterial communities associated with 18 *Symbiodinium* types generated in PRIMER6. Bray Curtis similarity was used and a square root transformation was used. Clades are designated by colour, clade A: red, clade B: orange, clade C: yellow, clade D: green, clade F: blue. Clustered according to 60 (grey line) and 80% (dotted black line) similarity. 2D stress: 0.18.

Figure S2. Mean F_v/F_m (± SE, n=3) of *Symbiodinium* cultures maintained in exponential growth. Clades are designated by bar colour according to cladal identity (Red: A, Orange: B, Yellow: C, Green: D, Blue: F), ITS2 type is shown on the x-axis

Figure S3. Rarefaction curves generated in QIIME using alpha_rarefaction.py. Data was then rarefied to 14000 sequences per sample.

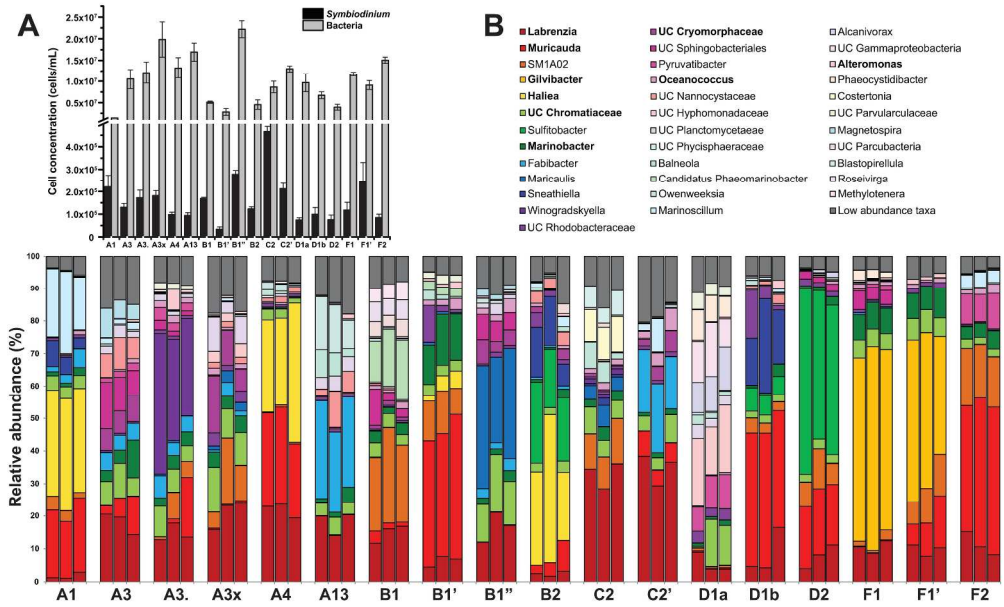

Figure 1. (A) Symbiodinium and bacterial cell concentrations (cells/mL) across 18 cultures of Symbiodinium (spanning 5 clades) maintained in exponential growth in IMK medium (Supporting Information Table S1). Physiological performance of cultures was monitored using Fast Repetition Rate fluorometry and densities were determined by flow cytometry (Appendix S1). (B) Bacterial community composition (relative abundance %) of each Symbiodinium type at the genus level, based on extracted bacterial genomic DNA and 16S rRNA gene amplicon sequencing (Illumina MiSeq). Sequences were processed using a custom pipeline (see Appendix S1 for detail and references) and taxonomy was assigned using SILVA(v128). The low abundance category contains the sum of all genera that made up <5% of the community in at least one replicate. Bacteria listed in bold are members of Symbiodinium clade cores. UC: Unclassified.

263x197mm (300 x 300 DPI)

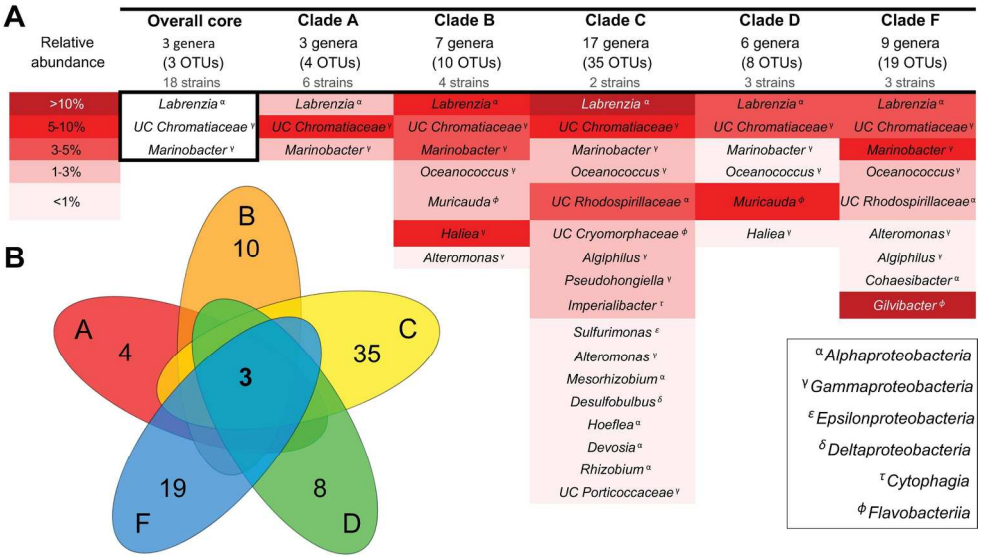

Figure 2. (A) The Symbiodinium core microbiome and the individual clade core microbiomes. To be considered core, a single OTU (clustered at 97% using vsearch, see Appendix S1) must be present in 100% of samples and have a minimum abundance of 0.0001%, each OTU was then identified to the highest taxonomic resolution possible. (B) Diagram showing the number of OTUs in each clade core and the overall core, exclusively. UC: Unclassified.

190x152mm (300 x 300 DPI)