Microbiomes of *Gambierdiscus lewisii* from the Great Barrier Reef, Queensland, Australia

Shikder Saiful Islam[1,2]*, Andrew Bridle[1], Shauna Murray[3], Christopher Bolch[1]

[1]Institute for Marine and Antarctic Studies, University of Tasmania, Hobart, Tasmania, Australia
[2]Fisheries and Marine Resource Technology Discipline, Khulna University, Khulna, Bangladesh
[3]University of Technology Sydney, School of Life Sciences, Sydney, NSW, Australia
Corresponding author's email: shikdersaiful.islam@utas.edu.au

Abstract

The microbiomes associated with algae can have a substantial influence on host algal cell physiology and health. We examined the relative abundance and core microbiomes of the toxic dinoflagellate *Gambierdiscus lewisii* cultured from inner (Palm Islands) and outer (Bramble Reef) reef locations on the Great Barrier Reef (Queensland, Australia). Cell-associated (attached) and culture medium-associated microbiomes (unattached) were compared. Both attached and unattached microbiomes of inner reef cultures were dominated by Proteobacteria (Gammaproteobacteria and Alphaproteobacteria) whereas outer reef culture microbiomes which dominated by Gammaproteobacteria and Flavobacteriia. Core attached microbiomes of outer reef cultures were more diverse, including *Thalassospira australica*, *Varunaivibrio sulfuroxidans*, *Thalassospira povalilytica*, *Marinobacter salarius* and *Labrenzia aggregata* as additional taxa. In conclusion, this study indicated that microbial community within species may differ due to variations in geographical distribution or ecosystem structure.

Key words: microbiomes, *Gambierdiscus lewisii*, microalgae

Introduction

Microbiomes are microorganisms that share the space and products of host environment (Ho & Bunyavanich, 2018). In the ocean, organic host substrates, physical contact of algae, and their close association often create an opportunity for ecological and chemical interactions (Tarazona-Janampa et al., 2020). The diversity and structure of microbiomes are shown to affect the physiology and health of the host by providing, competing for, or exchanging micronutrients, vitamins, and minerals (Amin et al., 2012; Croft et al., 2005). Micrbiomes also change in reponse to changes in nutrients and organic matter exuded by different species (Park et al., 2017).

A wide range of studies also indicate that microbiomes exhibit species-specificity (Jackrel et al., 2021; Martin et al., 2021), and those biological interactions are a stronger determinant of aquatic microbial community structure than environmental factors (Pushpakumara et al., 2023). These host-microbiome interactions are often obligatory for the host alga and may contribute to bloom formation (Bolch et al., 2011; Bolch et al., 2017; Grossart et al., 2005).

Microbiomes of many planktonic HAB dinoflagellates are relatively well characterised, however those of epiphytic dinoflagellates remain poorly documented. In this study, we use Oxford Nanopore amplicon sequencing of near full-length 16S rRNA genes to compare microbiome diversity among cultures of four cultures of the toxic dinoflgellate *Gambierdiscus lewisii* from tropical waters of the Great Barrier Reef (GBR), Australia.

Materials and methods

Gambierdiscus isolates culture, sample collection and processing

Clonal cultures of *G. lewisii* were isolated in Sept. 2019 from two reef locations on the mid-GBR region; Cultures OIRS33 and OIRS57 from the Palm Islands (75 km NNW of Townsville, Qld), and OIRS413 and OIRS438 from the outer reef atoll Bramble Reef (95 km north of Townsville. All strains were cultured in 75 ml cell culture flasks with 40 ml of 35ppt f/10 medium (+L1 trace metals) at 25°C and 70 µmol photons.$m^{-2}s^{-1}$ (14h:10h L:D). DNA was extracted from mid-log phase cultures. Unattached microbiomes samples were collected by pelleting *Gambierdiscus* cells at low-speed (400*g*, 5 min.) and the supernatant removed to separate tubes (culture medium, CM) microbiomes. The CM was then centrifuged at high-speed (12,000*g*, 15 min.), the supernatant removed carefully, and pellets

retained from DNA extraction. The *Gambierdiscus* cell pellets (attached microbiome) were washed with an equal volume of sterile seawater, centrifuged at 400*g* for 5 min., supernatants removed, centrifuged at 1000*g* for 5, medium removed, and cell pellets extracted as below.

DNA extraction, PCR, library preparation and sequencing

Total nucleic acid was extracted by an ammonium acetate-based protocol modified from Ooi et al. (2020). Briefly, 400 µl of lysis buffer (4 M urea, 0.5% SDS, 10% glycerol, and 0.2 M NaCl) and 5.0 µl of proteinase K were added, the sample heated at 55°C for 30 mins and vortexed for 5 sec. Tubes were incubated on ice for 5 mins, 210 µl 7.5M ammonium acetate added, tubes vortexed for 20 sec and centrifuged at 14 000 *g* for 5 mins at 18°C. Supernatants were transferred to a new 1.5 ml microtube, an equal volume of isopropanol added, mixed by inversion (40×) and centrifuged at 14 000 *g* for 10 mins. Supernatants were decanted, the pellet washed twice with 500 µl of 70% molecular grade ethanol, and resuspended in 50 µl of molecular grade water.

An initial PCR was carried out using primers 27F-1492R in 20µl reaction volumes, using MyTaq HS mix (Bioline, Australia), and primers at 0.5 µM. PCR cycling comprised: denaturation at 95°C for 3 mins, then 25 cycles of denaturation at 95°C for 15 sec, annealing at 50°C for 30 sec, and extension at 72°C for 45 sec, followed by extension at 72°C for 3 mins. A second PCR was performed in 50 µl volumes, consisting of 25 µl LongAmp Hot Start Taq 2X Master Mix, 10 µl of each 16S barcode-tailed primers, 14 µl molecular grade water, and 1 µl first-round PCR product. Thermal cycling comprised denaturation at 95°C for 1 min, then 15 cycles of: denaturation at 95°C for 15 sec, annealing at 55°C for 30 sec and extension at 72°C for 60 sec, and a final extension at 72°C for 3 mins. PCR products were purified using an AMPure XP kit (Beckman-Coulter, Aust.), quantified using a Qubit4 Fluorometer, and used for Oxford Nanopore library preparation (SQK-16S024, Oxford Nanopore Technologies, UK). Sequencing was performed on the MinION R9.4.1 flow cell (Oxford Nanopore, UK).

Bioinformatics

Sequencing data acquisition used MinKNOW core software (v. 4.0.5) and Guppy (v. 4.4.2) for base-calling, demultiplexing and removal of adapter sequences (Wick et al., 2019). Emu was run with a species detection threshold of 0.01% on 16S sequencing data (Curry et al., 2022). Microbiome taxon relative abundance used the marker data profiling module of MicrobiomeAnalyst (Chong et al., 2020). Data were normalized using total sum scaling to address the variability in sampling depth. The core microbiomes in the attached and unattached microbial community were determined using 75% prevalence and 0.01% detection threshold of bacterial taxa.

Results and Discussion

Proteobacteria dominated microbiomes, (attached, 78%-87%; unattached, 66%-82%) of all *G. lewisii* strains except the unattached microbiome of OIRS 413 (Bacteroidetes 53%, Proteobacteria 44%); Fig. 1).

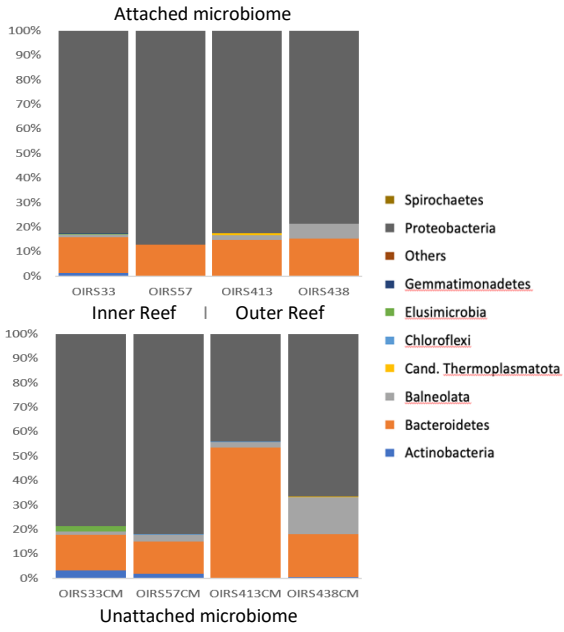

Fig. 1. Phylum relative abundance of attached and unattached microbiomes associated with *G. lewisii*.

At class level, γ- and α-proteobacteria (60% and 25%) dominated the attached microbiome of inner reef and outer reef (47% and 33%) *G. lewisii* cultures (Fig. 2). Unattached microbiomes of inner reef cultures comprised 58% γ-proteobacteria and 6% Flavobacteriia,

compared to 36% and 30% in outer reef *G. lewisii* microbiomes (Fig. 2).

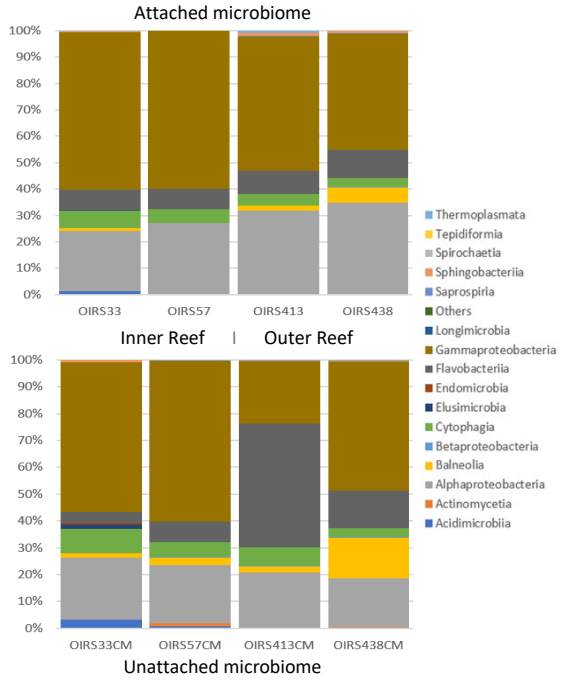

Fig. 2. Class relative abundance of attached and unattached microbiomes of cultured *G. lewisii*.

Both the attached and unattached core microbiomes of *G. lewisii* shared a number taxa (Figs. 3 and 4), indicating that the majority of more abundant bacteria were associated both with *G. lewisii* cell surfaces and the surrounding medium. However, *Gambierdiscus* cultures exude considerable amounts of extracellular polysaccharides that likely contributed to inefficient partitioning of the unattached versus attached components of the microbiome during our extraction process.

Despite the similarities, there were clear differences in composition and rank order of prevalence of core microbiome taxa. Attached microbiomes of inner reef cultures were predominantly a subset of the unattached core microbiome except for *Pontibacterium* and *Labrenzia* and thus appear to be primarily cell or EPS-associated using our conservative prevalence/abundance thresholds for core taxa. In contrast, outer reef cultures retained a much higher attached core microbiome diversity (18 taxa) compared to core unattached (11 taxa); all but three (*Thalassopsira australica, Methylobacter marinus, Spongibacter marinus*) were also part of the core offshore attached microbiome (compare Figs. 3 & 4).

Of particular note is the presence of *Marinobacter* and *Labrenzia* that form part of only the attached core microbiome of *G. lewisii*.

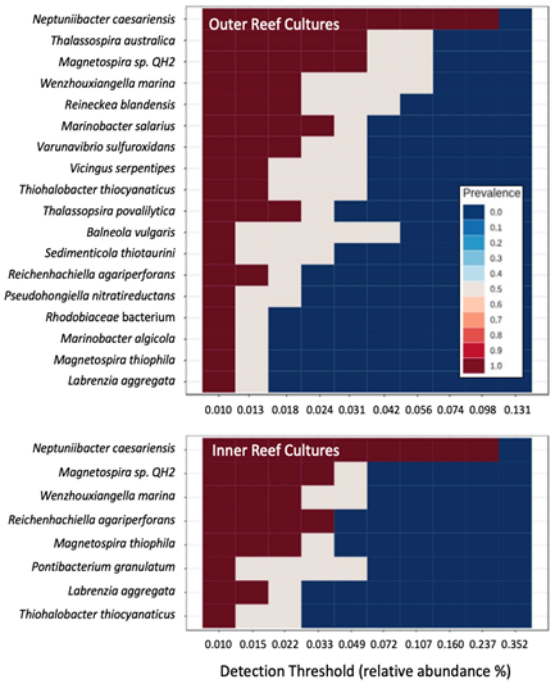

Fig. 3. Core attached microbiomes (≥75% sample prevalence; ≥0.01% rel. abund.) of cultured *G. lewisii*.

Both bacteria are known growth-supporting associates of dinoflagellates (Bolch et al., 2017) and other phytoplankton (Green et al., 2015). *Labrenzia aggregata* has also been shown to increase bleaching resilience of corals (Doering et al., 2023), and may perform a similar oxidative stress management function for *G. lewisii*.

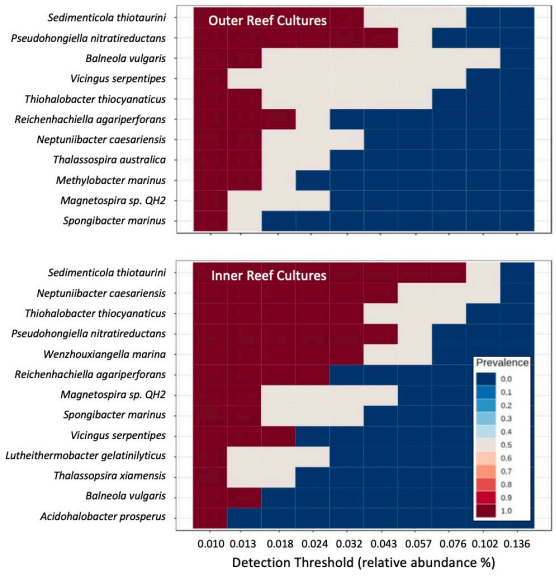

Fig. 4. Core unattached microbiomes (≥75% sample prevalence; ≥0.01% rel. abund.) of cultured *G. lewisii*.

The overall similarity of the community structure of the four *G. lewisii* microbiomes is indicative of a level of species-specificity. However, changes in class dominance, the higher diversity of core microbiomes at Bramble Reef indicate that the environment also partly shapes the microbiome (Pushpakumara et al., 2023). The observed differences in microbiomes between inner and outer reef locations may arise from different physiochemical conditions at inner and outer reef sites. Alternatively, the physiological influence of these differences on *G. lewisii* may in-turn modify selection and recruitment of bacteria to the *G. lewisii* microbiome.

Acknowledgements

This study was funded through the Australian Research Council (ARC) Linkage Project, DP1800001 to SM & CB, and Discovery Project DP210102493 to CB. We thank Sam Murray of the Cawthron Institute (Nelson, New Zealand) for assistance with epiphyte and coral sample collection.

References

Amin, S. A., Parker, M. S., Armbrust, E. V. (2012). Microbiol. Mol. Biol. Rev., 76(3), 667-684.

Bolch, C. J., Subramanian, T. A., Green, D. H. (2011). J Phycol, 47(5), 1009-1022.

Bolch, C. J. S., Bejoy, T. A.,Green, D. H. (2017). Front. Microbiol., 8, 670.

Chong, J., Liu, P., Zhou, G.,Xia, J. (2020). Nat. Protoc., 15(3), 799-821.

Croft, M. T., Lawrence, A. D., Raux-Deery, E., Warren, M. J. *et al.*, (2005). Nature, 438(7064), 90-93.

Curry, K. D., Wang, Q., Nute, M. G., Tyshaieva, A., *et al*., (2022). Nat. Methods, 19(7), 845-853.

Doering, T., Maire, J., van Oppen, M. J.,Blackall, L. L. (2023). Microbiol. Aust., 44(1), 36-40.

Green, D. H., Echavarri-Bravo, V., Brennan, D., Hart, M. C. (2015). Biomed Res. Int., 2015, 194540.

Grossart, H. P., Levold, F., Allgaier, M., Simon, M., *et al*., (2005). Environ. Microbiol., 7(6), 860-873.

Ho, H. E. and Bunyavanich, S. (2018). Curr. Allergy Asthma Rep., 18(4), 1-11.

Jackrel, S. L., Yang, J. W., Schmidt, K. C., Denef, V. J. (2021). ISME J., 15(3), 774-788.

Martin, K., Schmidt, K., Toseland, A., Boulton, C. A., *et al*., (2021). Nat. Commun., 12(1), 5483.

Ooi, M. C., Goulden, E. F., Trotter, A. J., Smith, G. G., *et al*., (2020). Front. Microbiol., 11, 573588.

Park, B. S., Guo, R., Lim, W. A., Ki, J. S. (2017). Mar. Ecol., 38(6), e12474.

Pushpakumara, B. L. D. U., Tandon, K., Willis, A., Verbruggen, H. (2023). Sci. Rep., 13(1), 2743.

Tarazona-Janampa, U. I., Cembella, A. D., Pelayo-Zárate, M. C., Pajares, S., *et al*., (2020). Front. Mar. Sci., 7, 569.

Wick, R. R., Judd, L. M., Holt, K. E. (2019). Genome Biol., 20(1), 129.