TITLE: Correspondence of coral holobiont metabolome with symbiotic bacteria, archaea and *Symbiodinium* communities

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RUNNING TITLE: The coral metabolome links to symbiont communities

SUMMARY:

Microbial symbiotic partners, such as those associated with scleractinian corals, mediate biochemical transformations that influence host performance and survival. While evidence suggests microbial community composition partly accounts for differences in coral physiology, how these symbionts affect metabolic pathways remains underexplored. We aimed to assess functional implications of variation among coral-associated microbial partners *in hospite*. To this end, we characterized and compared metabolomic profiles and microbial community composition from nine reef-building coral species. These data demonstrate metabolite profiles and microbial communities are species-specific and are correlated to one another. Using *Porites spp.* as a case study, we present evidence that the relative abundance of different sub-clades of *Symbiodinium* and bacterial/archaeal families are linked to positive and negative metabolomic signatures. Our data suggests that while some microbial partners benefit the union, others are more opportunistic with potential detriment to the host. Consequently, coral partner choice likely influences cellular metabolic activities and, therefore, holobiont nutrition.

INTRODUCTION

Microbial symbiotic partners mediate biochemical transformations that contribute to host performance, altering individual survival and ecosystem function (McFall-Ngai *et al.*, 2013). Reef-building corals partner with a diverse assemblage of bacterial, archaeal and eukaryotic organisms (i.e., microbiome) that comprise the coral holobiont and cycle organic molecules necessary for life in oligotrophic waters (Rohwer *et al.*, 2002; Gates and Ainsworth 2011). Coral associated micro-algae from the dinoflagellate genus *Symbiodinium* help to sustain large organic biomasses on reefs through carbon production (Muscatine and Porter 1977), while coral-associated bacterial and archaeal assemblages participate in sulfur (Raina *et al.*, 2009) and nitrogen cycling (Rädecker *et al.*, 2015). The breakdown of these symbiotic relationships (e.g., bleaching and disease), leads to mortality and drastic changes in reef structure (Hughes *et al.*, 2003). Because not all symbiotic partners contribute equally to holobiont performance (Stat *et al.*, 2008), investigating how different microbes influence coral physiology is essential to understanding the resilience and resistance of reef ecosystems in a changing ocean environment. To this end, we sought to link microbial community composition to holobiont biochemical profiles using deep sequencing of marker genes and a holistic metabolomic approach.

RESULTS AND DISCUSSION

We sampled the microbial community and metabolite composition from nine coral species within four genera from a single fringing reef in Mo‘orea, French Polynesia (Figure S1; supporting information provides full experimental methodology). We characterized both *Symbiodinium* and bacterial/archaeal communities from each sample by sequencing the internal transcribed spacer 2 (ITS2) and the V3-V4 region of the 16s ribosomal RNA gene, respectively.

Using custom bioinformatics pipelines (FileS1 and FileS4), our analysis identified 80 *Symbiodinium* operational taxonomic units grouped to 97% similarity (OTUs; assigned to clades A, C, D and G) and 618 bacterial/archaeal families through database classification (Figure S2; Table S1); the majority of these families belong within the gamma-, alpha- and delta-proteobacteria but with representatives spread across 130 classes (Figure S2B). In parallel, we assessed coral metabolite profiles from crude extracts using proton-nuclear magnetic resonance spectroscopy (^{1}H-NMR) metabolomic techniques (Sogin et *al.*, 2014). Our spectral binning approach identified 197 peaks from aligned NMR profiles, which were used in subsequent downstream analyses. We unambiguously identified 8 compounds through database and literature matching, and assigned the remaining signals to either carbohydrate, lipid, or aromatic metabolite classes based on spectral identity (Figure S3; Bross-Walch, *et al*, 2005).

Our sequencing and metabolomic profiling data allowed us to test for linkages between microbial community assemblages and metabolite composition. This novel *in hospite* approach provides direction for future efforts seeking to identify specific roles of uncultured complex mixtures of microbial communities in reef corals. Multivariate analysis of *Symbiodinium* and coral metabolite profiles grouped samples into coral species- and genera-specific clusters (Figure 1A and 1C) while bacteria/archaea communities only sometimes separated by genera (Figure 1B). These results confirm previous studies showing that closely related host taxa share similar symbiotic communities and biochemical profiles (Putnam *et al.*, 2012; Rohwer *et al.*, 2002; Sogin *et al.*, 2014). Using a procrustes rotation to statistically compare the multidimensional structure of each dataset, our study demonstrates congruency among coral *Symbiodinium* and bacterial communities with metabolomic profiles (Figure 1D, Figure 1E). Furthermore, these results are robust to our choice of microbial phylogenetic binning. ANOSIM tests of

bacterial/archaeal community structure differences among coral species (R=0.75, p=0.001) and Procrustes correspondence tests of multivariate congruency with metabolite composition (p=0.001) were consistent when bacterial/archaeal OTUs were constructed using a 97% sequence similarity criterion; we use family-level classifications herein as a conservative approach.

Corals host a diverse assemblage of symbiotic partners; therefore finding correlative relationships between metabolites and microbial members across multiple host species is not surprising. Considering different corals exhibit broadly different physiologies (e.g., Loya *et al.*, 2001), it is essential to isolate the influence of the host to identify the effect of microbial communities on holobiont metabolomic signatures. To this end, we applied a hierarchical clustering approach to the metabolite dataset and identified samples originating from *Porites lobata* and *P. rus* colonies as having similar metabolite profiles (Figure 1C, Figure 1H) but different symbiont communities (Figure 1F, Figure 1G). Repeated Procrustes rotation analyses using only the *P. lobata* and *P. rus* samples still detected congruency between microbial communities and metabolite composition (Figure 1H, Figure 1I). This suggests different microbial partners may play similar roles in holobiont nutrition and provide functional redundancy in the system, such that metabolic processes are retained (e.g., Yin et al., 2000). While our approach advances the field by providing a framework to investigate the roles of the symbiotic partners in a complex milieu, further metagenomic approaches will provide a detailed comparison between the functions of different microbes (Dinsdale et al., 2008). Our findings lend weight to growing information on the metabolic role of mixed microbial communities, with various *Symbiodinium* types characterized by different chemical profiles (Klueter *et al.*, 2015) and shifts in associated bacterial/archaeal communities altering the metabolic potential of the holobiont (Wegley *et al.*, 2007).

Using this reduced dataset containing only *Porites sp*. with similar metabolite profiles, we also calculated spearman rank correlation coefficients between the abundance of individual NMR peaks and independently the relative abundances of both *Symbiodinium* and bacterial/archaeal symbionts. We observed significant correlations between coral-associated symbionts and metabolites (multiple comparisons corrected $p < 0.05$), further supporting the hypothesis that microbial communities alter holobiont metabolomic profiles (Figure 2). Hierarchical clustering on correlation patterns group microbial partners based on their association patterns with metabolite peaks, highlighting taxa that are positively and negatively associated with small compounds. While additional efforts are required to determine if these associations are benefiting or harming the holobiont, we can interpret these relationships in context of the current physiological understanding of coral microbial communities. Therefore, using these data, we explore the hypothesis that some symbionts positively contribute to, while others may act in an opportunistic or parasitic manner, with respect to holobiont nutrition (Lesser *et al.*, 2013).

Clade C *Symbiodinium* fixes and translocates more organic carbon than other clades (Stat *et al.*, 2008); our observation that OTU75 assigned to sub-clade C15 correlates positively with carbohydrates but negatively with lipids suggests this sub-type produces metabolically active carbohydrates not used in lipid metabolism (Figure 2A). Our analysis delineates variation in metabolite correlational patterns between closely related 97% *Symbiodinium* clusters (i.e., C15-OTU75 and C15-OTU89), indicating even strain level variants are metabolically distinct. Closely related *Symbiodinium* types differentially influence host-bleaching susceptibility (Sampayo *et al.*, 2008), variation in gene expression profiles (Parkinson *et al.*, 2016) and

holobiont metabolite composition. These findings may help to reconcile performance contrasts in corals, for example, variation in bleaching among individuals (Loya *et al*, 2001).

From our correlation analyses, we also identify clusters of bacterial/archaeal families that are either positively or negatively associated with NMR-metabolite peaks (Figure 2B). We observe positive correlations between several unclassified cyanobacteria families and metabolite signals originating from nitrogen containing branched-chain amino acids and acetate, supporting the hypothesis that cyanobacteria produce nitrogen compounds to support coral nutrition (Lesser *et al.*, 2004; Lesser *et al.,* 2007). We also observe positive associations with *Vibrioaceae*, a bacteria family commonly associated with elevated temperatures and coral stress, and a broad array of metabolites classes providing additional evidence that *vibrio sp.* strongly influences holobiont metabolism (Thurber *et al.* 2009). Additionally, we observe 6 gamma-proteobacteria families are negatively associated with branched-chain amino acids and acetate, including *Oceanospirillaceae* and *Colwelliaceae,* families associated with coral disease (Thompson *et al.*, 2006) and opportunistic colonization following disturbance (Glasl *et al.*, 2016). These data may suggest bacteria families are producing or consuming metabolites, thereby altering *Porites* holobiont metabolism. Considering shifts in coral reef bacteria/archaea communities can alter ecosystem metabolic potential (Haas *et al.*, 2016), our approach can support assessment efforts of reef health by determining metabolic activities of key coral-associated bacterial groups. However, more targeted studies localizing the origin of the compounds to either the host or symbionts are needed to determine causal effects.

Given the global decline in reef ecosystems, it is paramount to identify symbiotic partners that enhance coral resistance and resilience (van Oppen *et al.*, 2015). Our work provides a framework to describe the metabolic impact of mixed symbiotic communities in eukaryotic hosts

and is complementary to metagenomic efforts. The integrative analysis of metabolite and microbial community data identifies symbiotic partners that alter metabolic pathways of corals. These findings emphasize the need to directly investigate the role of the diversity of microbial partners through targeted studies.

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CONFLICT OF INTEREST

The authors declare no conflict of interest

SUPPLEMENTARY INFORMATION

Sequencing data is available under the NCBI accession number PRJNA355371 and PRJNA325688. The metabolomics data is available under the MetaboLights accession number MTBLS342.

Supplemental Methods

TableS1. Sequencing statistics

FileS1. ITS2 bioinformatic pipeline

File S2. ITS2 *Symbiodinium* database

File S3. ITS2 id-to-taxonomy file for *Symbiodinium* database

FileS4. 16S bioinformatics pipeline

FileS5. Quality analysis R script

FileS6. Statistical analysis R script

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FIGURE LEGENDS

Figure 1. Multivariate analysis indicates corals have distinct, but related microbial communities and metabolite composition. Non-metric multidimensional scaling (NMDS) and analysis of similarity (ANOSIM) separate coral species based on (A) *Symbiodinium* (filled circles), (B) bacterial/archaeal communities (filled triangles) and (C) metabolite profiles (open triangles, ANOSIM *p-value* and R annotated on each figure panel). (C) Hierarchical clustering results grouping samples with similar metabolite profiles are plotted as the overlay on the NMDS ordination. NMDS ordinations and ANOSIM results for only *Porites rus* and *P. lobata* samples show statistical separation between species for both (F) *Symbiodinium* and (G) bacterial/archaeal communities, but not (H) metabolite composition. Procrustes rotations comparing metabolite profiles to both *Symbiodinium* (D, I) and bacterial/archaeal communities (E, J) for all species and only the subset of *P. lobata* and *P. rus*, indicate the datasets are congruent ($p < 0.05$).

Figure 2. Significant Spearman rank correlation coefficients (FDR adjusted $p < 0.05$) between metabolite abundances and (A) *Symbiodinium* OTU subtypes or (B) bacterial/archaeal families. Metabolite annotations are based on specific peak matches to known compounds, or are categorized into metabolite classes (i.e., aliphatics, branch chain amino acids, carbohydrates, and lipids) based on peak location and patterning, the latter of which can result in multiple bins of the same general metabolite id. Only OTU/family-metabolite correlations with a Spearman rank value above 0.4 and below -0.4 are presented to facilitate data interpretation.
BCAA = branch chain amino acids.

Figure S1. Coral species collected along a fringing reef in Mo'orea, French Polynesia, including (A, B) Two un-identified species of *Acropora*, (C) *Montipora aequituberculata,* (D) *Montipora*

sp., (E) *Pocillopora meandrina/verrucosa,* (F) *Pocillopora acuta*, (G) *Porites lobata*, (H) *Porites rus* and (I) *Porites irregularis*. (J) Moorea, French Polynesia, the X marks the sampling location.

Figure S2. Relative abundance of coral-associated (A) *Symbiodinium* and (B) bacterial/archaeal communities. Only taxa representing at least 3% of the microbial community structure are presented to facilitate data interpretation.

Figure S3. Aligned and binned representative ^{1}H-NMR metabolomic profiles from 9 coral species. BCAA = Branch chain amino acids

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suggest bacteria families are producing or consuming metabolites, thereby altering *Porites* holobiont metabolism. Considering shifts in coral reef bacteria/archaea communities can alter ecosystem metabolic potential (Haas *et al.*, 2016), our approach can support assessment efforts of reef health by determining metabolic activities of key coral-associated bacterial groups. However, more targeted studies localizing the origin of the compounds to either the host or symbionts are needed to determine causal effects.

Given the global decline in reef ecosystems, it is paramount to identify symbiotic partners that enhance coral resistance and resilience (van Oppen *et al.*, 2015). Our work provides a framework to describe the metabolic impact of mixed symbiotic communities in eukaryotic hosts and is complementary to metagenomic efforts. The integrative analysis of metabolite and microbial community data identifies symbiotic partners that alter metabolic pathways of corals. These findings emphasize the need to directly investigate the role of the diversity of microbial partners through targeted studies.

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CONFLICT OF INTEREST

The authors declare no conflict of interest

SUPPLEMENTARY INFORMATION

Sequencing data is available under the NCBI accession number PRJNA355371 and PRJNA325688. The metabolomics data is available under the MetaboLights accession number MTBLS342.

Supplemental Methods

TableS1. Sequencing statistics

FileS1. ITS2 bioinformatic pipeline

File S2. ITS2 *Symbiodinium* database

File S3. ITS2 id-to-taxonomy file for *Symbiodinium* database

FileS4. 16S bioinformatics pipeline

FileS5. Quality analysis R script

FileS6. Statistical analysis R script

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FIGURE LEGENDS

Figure 1. Multivariate analysis indicates corals have distinct, but related microbial communities and metabolite composition. Non-metric multidimensional scaling (NMDS) and analysis of similarity (ANOSIM) separate coral species based on (A) *Symbiodinium* (filled circles), (B) bacterial/archaeal communities (filled triangles) and (C) metabolite profiles (open triangles, ANOSIM *p-value* and R annotated on each figure panel). (C) Hierarchical clustering results grouping samples with similar metabolite profiles are plotted as the overlay on the NMDS ordination. NMDS ordinations and ANOSIM results for only *Porites rus* and *P. lobata* samples show statistical separation between species for both (F) *Symbiodinium* and (G) bacterial/archaeal communities, but not (H) metabolite composition. Procrustes rotations comparing metabolite

profiles to both *Symbiodinium* (D, I) and bacterial/archaeal communities (E, J) for all species and only the subset of *P. lobata* and *P. rus*, indicate the datasets are congruent ($p < 0.05$).

Figure 2. Significant Spearman rank correlation coefficients (FDR adjusted $p < 0.05$) between metabolite abundances and (A) *Symbiodinium* OTU subtypes or (B) bacterial/archaeal families. Metabolite annotations are based on specific peak matches to known compounds, or are categorized into metabolite classes (i.e., aliphatics, branch chain amino acids, carbohydrates, and lipids) based on peak location and patterning, the latter of which can result in multiple bins of the same general metabolite id. ~~Metabolite annotations are based on peak matches to databases or are placed categorized into metabolite categories classes (i.e., aliphatics, branch chain amino acids, carbohydrates, and lipids) based on peak location and patterns.~~ Only OTU/family-metabolite correlations with a Spearman rank value above 0.4 and below -0.4 are presented to facilitate data interpretation.
BCAA = branch chain amino acids.

Figure S1. Coral species collected along a fringing reef in Mo'orea, French Polynesia, including (A, B) Two un-identified species of *Acropora*, (C) *Montipora aequituberculata,* (D) *Montipora sp.*, (E) *Pocillopora meandrina/verrucosa,* (F) *Pocillopora acuta*, (G) *Porites lobata*, (H) *Porites rus* and (I) *Porites irregularis*. (J) Moorea, French Polynesia, the X marks the sampling location.

Figure S2. Relative abundance of coral-associated (A) *Symbiodinium* and (B) bacterial/archaeal communities. Only taxa representing at least 3% of the microbial community structure are presented to facilitate data interpretation.

Figure S3. Aligned and binned representative ^{1}H-NMR metabolomic profiles from 9 coral species. BCAA = Branch chain amino acids