Distribution and Function of Bacterial Aggregates Within Tissues of the Coral *Acropora loripes*

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BACTERIALAGGREGATES WHY STUDY THEM? **AIM:** Characterization of CAMAs associated with the coral Acropora loripes Due to the close association within the coral to provide novel insights into their Bacteria from the genus *Endozoicomonas* are commonly found as aggregates in a wide host, Endozoicomonas may be developed distribution and functional potential. into a coral probiotic to mitigate coral range of coral species termed coralassociated microbial aggregates (CAMAs). bleaching **METHODS** Genomic data may guide the selection of These structures remain poorly understood Twelve coral genotypes were collected from two probiotic candidates by identifying potential in terms of spatial distribution and function separate reef sites in the central Great Barrier despite extensive efforts studying the coral interactions within the coral holobiont. microbiome Each coral were imaged using a combination of H&E staining and hybridization chain reaction fluorescence in situ hybridization (HCR-FISH) with Endozoicomonas-specific probes CAMA (Endozoicomonas) Endozoicomonas bacteria were isolated and whole-genome sequenced to identify potential interactions within the coral holobiont. Mucus Davies Reef Tentacles Backnumbers Reef NT QLD $\mathbb{W}A$ Mesentery SA NSW Gastric cavity **Epidermis** Gastroderm De novo hybrid assembly of genomes of isolated Endozoicomonas strains using a combination of Illumina and Nanopore Acropora loripes colony long-read sequencing FUNCTIONAL H&E Stain Phylogeny of Endozoicomonas spp. POTENTIAL Host nuclei ---E. numazuensis DSM25634 Spermadine/ arenosclerae ab112 putrescine ---E. arenosclerae EMC227 --E. sp 4G Pho ABC ---- E. marisrubri 6c E. Aloripes C066 E. Aloripes C020 - E. Aloripes D040 E. Aloripes B032 - - E. Aloripes B122 **ROS-Scaveging** Urea ABC E. Aloripes D068 --E. ALB060 H&E images showing location and potential intracellular CAMA GTP → Riboflavin (Vit B2) **---**E. sp AB1-5 Glutamate → Glutathione r - - - - E. ascidiicola KASP37 Aspertate → Nicotinate (Vit B3) ---E. ascidiicola AVMART05 HCR-FISH Pyruvate → Pantothenate (Vit B5) Pacuta CAMA F1 6 A F2 **Biomass** Erythrose → Pyridoxine (Vit B6) - Pacuta CAMA F1 6 A F1 Pimeloyl-ACP → Biotin (Vit B7) Endozoicomonas W5 Kt GTP → Biotin (Vit B7) Endozoicomonas HY Ok ----E. montiporae LMG24815 Host ----E. elysicola DSM22380 ----E. sp SCSIOW0465 ABC ----E. atrinae WP70 ----E. acroporae acr14 ----E. acroporae acr5 **Ankyrin repeat** Sea squirt ---E. acroporae Acr1 ---E. Aloripes E010 amino acids Sea slug ----E. Aloripes B115 Bivalve ----E. Aloripes C013 All Bacteria DAPI (DNA) E. Aloripes B091 Involved in CAMA DAPI (DNA) ■ Transferred to host/ Host colonization? microalgae?

KEGG-based pathway reconstruction of representative

Endozoicomonas strain E. Aloripes ALB60

RESULTS

on 120 gene markers

CAMAs located in tissues lining the gastric cavity

Phylogenetic tree of isolated Endozoicomonas strains based

- Host nuclei found inside CAMAs
- Endozoicomonas genomes show metabolic potential for mitigating oxidative stress and the production vitamins
- Genomic signatures including type VI secretion system and ankyrin repeats could allow host association.

CONCLUSIONS

- First evidence and characterization of CAMAs in the coral *Acropora loripes*.
- CAMAs likely intracellular and enclosed by a membrane.
- 11 isolated *Endozoicomonas* strains comprise two new species.
- Potential for beneficial interactions with host/microalgae





HCR-FISH images showing location of CAMAs and potential

membrane of unknown origin

