

1 **Dominant coral bacterium *Endozoicomonas acroporae* metabolizes DMSP**

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21 **Abstract**

22 Dominant coral-associated *Endozoicomonas* bacteria species have been long hypothesized to
23 play a role in the global sulfur cycle by metabolizing Dimethylsulfoniopropionate (DMSP) into
24 Dimethylsulfide (DMS), which releases sulfur into the atmosphere; however, no sequenced
25 genome to date harbors genes for this process. We assembled high-quality (>95 % complete)
26 genomes of two new strains (Acr-1 and Acr-5) of a recently added species *Endozoicomonas*
27 *acroporae* isolated from the coral *Acropora muricata*. We identified and functionally
28 characterized the first DMSP lyase—a *dddD* gene homolog found in all *E. acroporae*, capable of
29 metabolizing DMSP into DMS via the DddD cleavage pathway—using qPCR and GC-MS.
30 Comparative genomic analysis identified that *Endozoicomonas* has high genomic divergence and
31 a high percentage oxidative stress responsive genes and that *E. acroporae* strains have the
32 highest number of Type III secretion system genes (T3SS) in the genus. This study confirms the
33 role of the genus *Endozoicomonas* in the global sulfur cycle.

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40 Introduction

41 The genus *Acropora* contains some of the most abundant reef-building corals in the Indo-Pacific
42 [1], and these corals are also some of the most significant producers of
43 dimethylsulphonioacetate (DMSP) [2,3]. DMSP is present in coral tissue, mucus and
44 symbiotic algae [4]. It is the central molecule in the marine sulfur cycle and precursor to
45 dimethylsulphide (DMS), a climate-active gas [5,6]. DMSP is hypothesized to be part of the coral
46 holobiont antioxidant system [7] and it act as an osmoprotectant against salinity fluctuations
47 [3]. DMSP also acts as a signal molecule that attracts specific bacterial groups, which can form
48 coral holobionts and underpin coral health [8].

49 Coral-associated bacteria use DMSP produced by corals and their symbiotic algae as a
50 reduced sulfur and carbon source [8, 9]; they can also metabolize it into DMS [5,6]. DMSP
51 degradation by marine organisms takes place via two pathways, the cleavage pathway and the
52 demethylation pathway [9, 10]. Raina et al. [11] recently reported that the majority of DMSP-
53 degrading bacteria belong to class *Gammaproteobacteria*, which includes *Alteromonas*-,
54 *Arhodomonas*-, *Idiomarina*-, *Pseudomonas*- and *Spongiobacter*-related organisms. Of these,
55 *Arhodomonas*-, *Pseudomonas*-, and *Roseobacter*-related species harbor a DMSP lyase—i.e. the
56 *dddD* gene, first identified in *Marinomonas* sp. for degrading DMSP [12]. *Endozoicomonas*
57 species, which are predominantly associated with keeping their coral host healthy [13], have
58 been hypothesized to play role in the global sulfur cycle by effectively metabolizing DMSP into
59 DMS [14,15]. However, no previous study has confirmed the genus' role.

60 Material and Methods

61 We *de-novo* assembled high quality (>95 % complete) genomes of two new strains (Acr-1
62 and Acr-5) of a recently added species *Endozoicomonas acroporae* isolated from the coral
63 *Acropora muricata* and identified for the first time a *dddD* gene homolog capable of metabolizing
64 DMSP into DMS via the *DddD* cleavage pathway in all the *E. acroporae* strains. Furthermore, we
65 functionally characterized the expression of the *DddD* gene and quantified the amount of DMS
66 released using qPCR and Gas chromatography-mass spectrometry (GC-MS). Comparative
67 genomic analysis of genus *Endozoicomonas* was performed to ascertain its genomic
68 characteristics and features. We also profiled the abundance of *E. acroporae* species in Penghu,
69 Taiwan and the Red Sea, Saudi Arabia (for details see supplementary data).

70 **Results and Discussion**

71 We assembled high quality genomes (>95 % complete) of the two *E. acroporae* strains and
72 also used the previously assembled type strain *E. acroporae* Acr-14^T [16,17] (Table S1, Fig S1). *E.*
73 *acroporae* species are dominant coral-associated bacteria in the Red Sea, Saudi Arabia (Fig S2A,
74 B) and Penghu, Taiwan (Fig S2C, D). All three strains of *E. acroporae* have a *dddD* gene homolog
75 that encodes a DMSP lyase. qPCR analysis of the *dddD* gene from *E. acroporae* Acr-14^T cultured
76 in 1mM DMSP resulted in 42.77, 56.52, and 91.37 times higher expression than samples cultured
77 without DMSP after 16, 24 and 48hrs, respectively (Fig 1A). The amount of DMS released when
78 the culture (*E. acroporae* Acr-14^T) was incubated in a DMSP-rich environment (Treatment A) was
79 significantly higher (*t-test*, *p-value* <0.05) than controls (Fig 1B). The temporal increase in the
80 concentration of released DMS confirms that *E. acroporae* can metabolize DMSP into DMS. The
81 discovery of the *dddD* gene in *Endozoicomonas* provides new insights into the evolution of the

82 DMSP cleavage pathway and further confirms the hypothesis that *Endozoicomonas* plays a role in
83 the global sulfur cycle.

84 Comparative genomic analysis identified high genomic divergence using Amino-Acid
85 Identity (AAI), Average Nucleotide Identity (ANI) and DNA-DNA Hybridization (DDH) (Fig 2 A, B,
86 and C) in the genus and also a reduced core genome (308 genes) (Fig S3). Genomes of
87 *Endozoicomonas* species are large (5.43 ~ 6.69 Mb) (Table S2) and encode genes for all essential
88 amino-acids [18], giving clues about not predominant genome streamlining as identified in
89 symbiotic bacteria [19] and other symbiotic life stages [20]. Moreover, *E. acroporae* species have
90 the highest numbers of T3SS genes in *Endozoicomonas* (data not shown), suggesting an intricate
91 relationship with their host. Moreover, *E. acroporae* strains have different IS elements than *E.*
92 *montiporae*, hinting that the two coral isolates have different evolution histories (Fig S4).
93 Moreover, diverse phage insertions in *Endozoicomonas* species genomes suggest different
94 infection histories (Table S3). In addition, *E. montiporae* and *E. acroporae* do not share any
95 branches, according to core-genome based phylogenetic analysis; instead, their strains cluster
96 tightly within their clades (Fig 2D). These results indicate that host and *Endozoicomonas* species
97 have a complex nature of co-diversification. All species in this genus have a high percentage of
98 oxidative stress responsive genes, which might be attributed to resistance against low oxygen
99 environment in the ocean as well as highlight the marine adaptation of genus *Endozoicomonas*
100 (Fig S5).

101

102 **Conclusion**

103 This study identified and functionally characterized DMSP lyase—a *dddD* gene homolog—
104 in *E. acroporae* and, in doing so, confirms the role of this coral dominant bacterium in the global
105 sulfur cycle. We also report two high quality genomes for new strains of *E. acroporae* and
106 performed an up-to-date comparative genomic analysis on this genus. We identified a high
107 genomic divergence and high percentage of oxidative stress response genes in all the species of
108 this genus. We compared two coral host-specific *Endozoicomonas* and report a diverse array of IS
109 elements in the genomes, giving clues about genome plasticity.

110 **Data Availability**

111 *E. acroporae* Acr-1 and Acr-5 assembled draft genomes are submitted to GenBank under
112 accession numbers SAUT000000000 and SAUU000000000, respectively.

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116 **Author Contributions**

117 K.T and S.L.T conceived the idea of this study. K.T assembled the genomes, performed
118 bioinformatics analysis and wrote the manuscript. P.W.C cultured the strains and performed qPCR
119 analysis. C.Y.L and Y.F.C performed GC-MS experiments and analysis. S.H.Y and N.H.W helped write
120 the manuscript. P.Y.C, H.Y.C, and M.S.C helped in GC-MS experiments and provided the
121 instruments for conducting the experiment. S.L.T supervised the overall study and modified the
122 manuscript.

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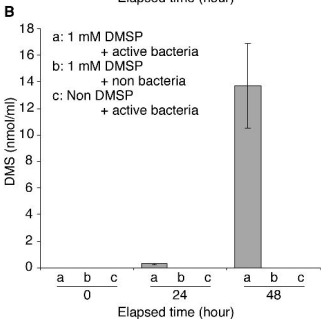
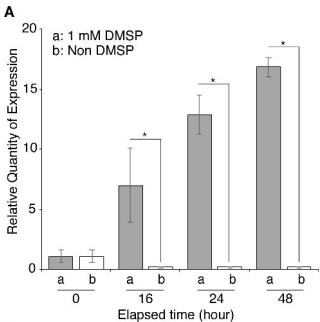
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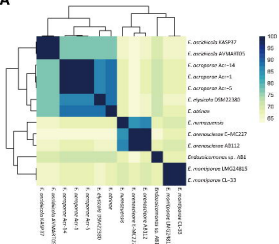
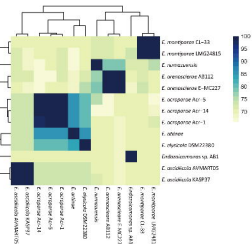
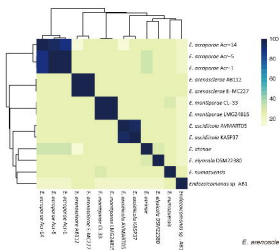
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170 **Figure Legends**

171 **Figure 1. A)** RT-qPCR expression analysis; relative *DddD* gene expression increased significantly
172 with time (0, 16, 24, 48 hours) when cultures were grown with 1mM DMSP compared to no DMSP
173 (*t*-test *p* value <0.05). **B)** Quantification of DMS released, DMS release was only observed in the
174 1mM DMSP+ active bacteria condition, not in conditions b or c.

175 **Figure 2.** Genomic divergence analysis using heat-maps from **A)** AAI, **B)** ANI, and **C)** DDH.
176 Phylogenetic analysis **D)** Core-genome (308 genes)-based unrooted phylogenetic tree with *E.*
177 *acroporae* strains forming a separate clade, as shown in zoomed image.



A**B****C****D**