Dominant coral bacterium *Endozoicomonas acroporae* metabolizes Dimethylsulfoniopropionate

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Abstract

Dominant coral-associated *Endozoicomonas* bacteria species are hypothesized to play a role in the global sulfur cycle by metabolizing Dimethylsulfoniopropionate (DMSP) into Dimethylsulfide (DMS); a climate active-gas, which releases sulfur into the atmosphere; however, no sequenced genome to date harbors genes for this process. We assembled high-quality (>95% complete) genomes of two new strains (Acr-1 and Acr-5) of a recently added species *Endozoicomonas acroporae* isolated from the coral *Acropora muricata*. We identified the first DMSP lyase—a *dddD* gene homolog found in all *E. acroporae,* and functionally characterized bacteria being capable of metabolizing DMSP into DMS via the DddD cleavage pathway using RT-qPCR and Gas chromatography (GC). This study confirms the role of *Endozoicomonas* in the global sulfur cycle.

Introduction

The genus *Acropora* contains some of the most abundant reef-building corals in the Indo-Pacific [1], and these corals are also some of the most significant producers of dimethylsulphoniopropionate (DMSP) [2, 3]. DMSP is present in coral tissue, mucus and endosymbiont dinoflagellates (Symbiodiniaceae) [4, 5]. It is the central molecule in the marine sulfur cycle and precursor to dimethylsulphide (DMS), a climate-active gas [6, 7]. DMSP is hypothesized to be part of the coral holobiont antioxidant system [8] and it act as an osmoprotectant against salinity fluctuations [3]. DMSP also acts as a signal molecule that attracts specific bacterial groups, which can form coral holobionts and underpin coral health [9].

Coral-associated bacteria use DMSP produced by corals and their symbiotic algae as a reduced sulfur and carbon source [9, 10]; they can also metabolize it into DMS [6, 7]. DMSP degradation by marine organisms takes place via two pathways, the cleavage pathway and the demethylation pathway [10, 11]. A recent study reported that the majority of DMSP-degrading bacteria belong to class *Gammaproteobacteria*, which includes *Alteromonas-, Arhodomonas-, Idiomarina-, Pseudomonas-* and *Spongiobacter*-related organisms [12]**.** Of these, *Arhodomonas-, Pseudomonas-,* and *Roseobacter*-related species harbor a DMSP lyase—i.e. the *dddD* gene, first identified in *Marinomonas* sp. for degrading DMSP [13]. *Endozoicomona*s species, which are predominantly associated with keeping their coral host healthy [14], have been hypothesized to play role in the global sulfur cycle by effectively metabolizing DMSP into DMS [15, 16]**.** However, no previous study has confirmed the genus' role. Here, we provide the conclusive evidence that one of *Endozoicomona*s species metabolize DMSP into DMS.

Material and Methods

We *de-novo* assembled high quality (>95% complete) genomes of two new strains (Acr-1 and Acr-5) of a recently added species *Endozoicomonas acroporae* isolated from the coral *Acropora muricata* and identified for the first time a *dddD* gene homolog capable of metabolizing DMSP into DMS via the *DddD* cleavage pathway in all the *E. acroporae* strains. Furthermore, we functionally characterized the expression of the *dddD* gene and quantified the amount of DMS released using RT-qPCR and Gas chromatography (GC). Comparative genomic analysis of genus *Endozoicomonas* was performed to ascertain its genomic characteristics and features. We also profiled the abundance of *E. acroporae* species from two previous studies on different species of corals in Penghu, Taiwan [17] and the Red Sea, Saudi Arabia [18] (for details see supplementary data: material and methods).

Results and Discussion

E. acroporae species are dominant coral-associated bacteria in the Red Sea, Saudi Arabia (Fig S2A, B) and Penghu, Taiwan (Fig S2C, D), depicting their wide distribution among different coral species. When comparing *E. acroporae* Acr-1, Acr-5 with previously assembled type strain *E. acroporae* Acr-14^{T} [19, 20] (supplementary results Table S1, Fig S1), all three strains of *E. acroporae* have a *dddD* gene homolog that encodes a DMSP lyase. RT-qPCR analysis of the *dddD* gene from *E. acroporae* Acr-14^{T} cultured in 1 mM DMSP resulted in 42.77, 56.52, and 91.37

79 times higher expression than samples cultured without DMSP after 16, 24 and 48hrs,
80 respectively (Fig 1A). The amount of DMS released when the culture (*E. acroporae* Acr-14^{T}) was
81 incubated in a DMSP-rich environment was significantly higher (t-test, p-value <0.05) than
82 controls (Fig 1B). The temporal increase in the concentration of released DMS confirms that *E.*
83 *acroporae* can metabolize DMSP into DMS. The discovery of the *dddD* gene in *Endozoicomonas*
84 provides new insights into the evolution of the DMSP cleavage pathway and further confirms
85 the hypothesis that *Endozoicomonas* plays a role in the global sulfur cycle.

86 Comparative genomic analysis identified that only *E. acroporae* have the DMSP
87 metabolism gene(s) so far (Table S2). Further, we report high genomic divergence using Amino-
88 Acid Identity (AAI), Average Nucleotide Identity (ANI) and DNA-DNA Hybridization (DDH) (Fig 2A,
89 B, and C) in the genus and also a reduced core genome (308 genes) (supplementary data:
90 results, Fig S5). Genomes of *Endozoicomonas* species are large (5.43 ~ 6.69 Mb) (Table S2) and
91 encode genes for all essential amino-acids [21], giving clues about not predominant genome
92 streamlining as identified in symbiotic bacteria [22] and other symbiotic life stages [21].
93 Moreover, *E. acroporae* species have the highest number of T3SS genes in *Endozoicomonas*
94 (supplementary data: results, Table S3), suggesting an intricate relationship with their host**.**
95 Besides, *E. acroporae* strains have different Insertion Sequence (IS) elements than *E.*
96 *montiporae*, hinting that the two coral-isolates have different evolution histories [23]
97 (supplementary data: results, Fig S3). Furthermore, diverse phage insertions in *Endozoicomonas*
98 species genomes suggest different infection histories (supplementary data: results, Table S4). In
99 addition, *E. montiporae* and *E. acroporae* do not share any branches, according to core-genome
100 based phylogenetic analysis; instead, their strains cluster tightly within their clades (Fig 2D).

These results indicate that host and *Endozoicomonas* species have a complex nature of co-diversification. All species in this genus have a high percentage of oxidative stress responsive genes, which might be attributed to resistance against low oxygen environment in the ocean as well as highlight the genus *Endozoicomonas'* adaptation to marine environments (supplementary data: results, Fig S4).

Conclusion

Endozoicomonas is the most dominant coral-associated bacterial group. Here, we link their function in the global element cycle. In addition, comparative genomic analysis of the genus *Endozoicomonas* gives clues about high genomic divergence and genome plasticity. Although, current understanding of the interaction among coral-microbe-sulfur cycle is still not clear, the results from this study will be beneficial for investigating the global change in the reef ecosystem functioning with the changing environment.

Data Availability

E. acroporae Acr-1 and Acr-5 assembled draft genomes are submitted to GenBank under accession numbers SAUT00000000 and SAUU00000000, respectively.

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Author Contributions

K.T and S.L.T conceived the idea of this study. K.T assembled the genomes, performed bioinformatics analysis and wrote the manuscript. P.W.C cultured the strains and performed RT-qPCR analysis. C.Y.L and Y.F.C performed GC experiments and analysis. S.H.Y and N.W helped write the manuscript. P.Y.C, H.Y.C, and M.S.C helped in GC experiments and provided the instruments for conducting the experiment. W.M.C provided the cultures. S.L.T supervised the overall study and modified the manuscript. All authors read and approved the manuscript.

Conflict of Interest

The authors declare that they have no conflict of interest.

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Figure Legends

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

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

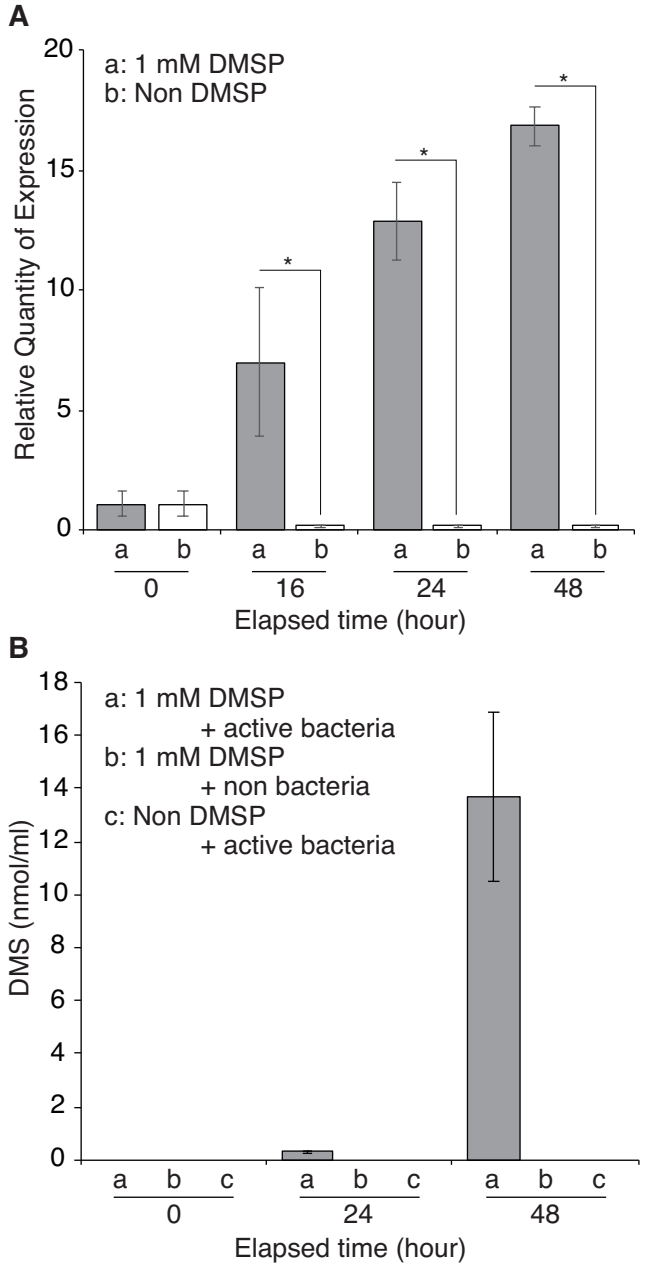
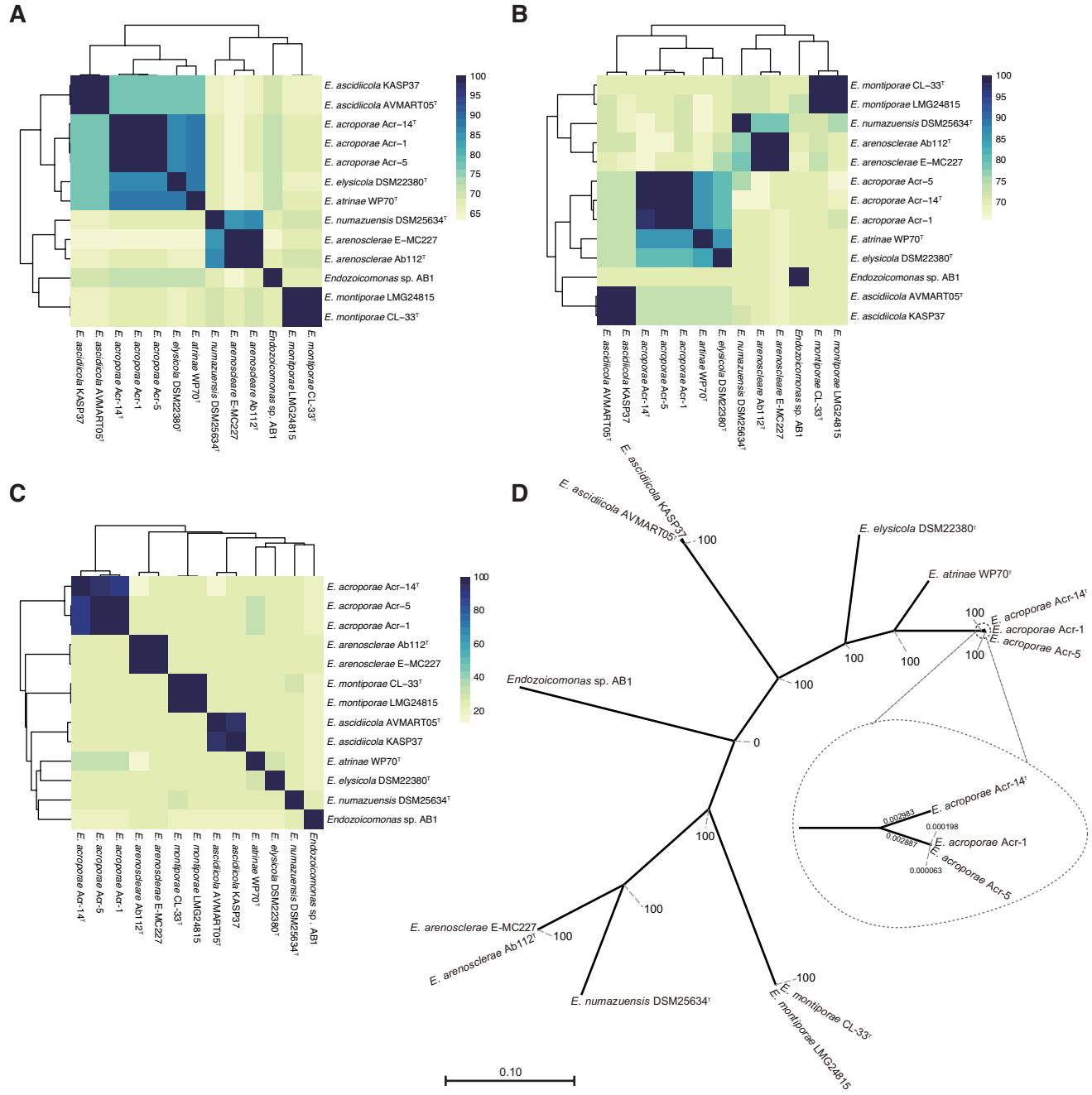

A
a: 1 mM DMSP
b: Non DMSP
Relative Quantity of Expression
20
15
10
5
0
*
*
*
a b a b a b a b
0 16 24 48
Elapsed time (hour)
B
a: 1 mM DMSP
+ active bacteria
b: 1 mM DMSP
+ non bacteria
c: Non DMSP
+ active bacteria
DMS (nmol/ml)
18
16
14
12
10
8
6
4
2
0
a b c a b c a b c
0 24 48
Elapsed time (hour)

Fig. 2


A
E. ascidiicola KASP37
E. ascidiicola AVMART05T
E. acroporae Acr−14T
E. acroporae Acr−1
E. acroporae Acr−5
E. elysicola DSM22380T
E. atrinae WP70T
E. numazuensis DSM25634T
E. arenosclerae E−MC227
E. arenosclerae Ab112T
Endozoicomonas sp. AB1
E. montiporae LMG24815
E. montiporae CL−33T
100
95
90
85
80
75
70
65
B
E. montiporae CL−33T
E. montiporae LMG24815
E. numazuensis DSM25634T
E. arenosclerae Ab112T
E. arenosclerae E−MC227
E. acroporae Acr−5
E. acroporae Acr−14T
E. acroporae Acr−1
E. atrinae WP70T
E. elysicola DSM22380T
Endozoicomonas sp. AB1
E. ascidiicola AVMART05T
E. ascidiicola KASP37
100
95
90
85
80
75
70
C
E. acroporae Acr−14T
E. acroporae Acr−5
E. acroporae Acr−1
E. arenosclerae Ab112T
E. arenosclerae E−MC227
E. montiporae CL−33T
E. montiporae LMG24815
E. ascidiicola AVMART05T
E. ascidiicola KASP37
E. atrinae WP70T
E. elysicola DSM22380T
E. numazuensis DSM25634T
Endozoicomonas sp. AB1
100
80
60
40
20
D
E. ascidiicola AVMART05T
E. ascidiicola KASP37
E. elysicola DSM22380T
E. atrinae WP70T
E. acroporae Acr−14T
E. acroporae Acr−1
E. acroporae Acr−5
Endozoicomonas sp. AB1
E. arenosclerae E−MC227
E. arenosclerae Ab112T
E. numazuensis DSM25634T
E. montiporae CL−33T
E. montiporae LMG24815
100
0
0.002983
0.000198
0.002887
0.000063
0.10