Dominant coral bacterium *Endozoicomonas acroporae* metabolizes DMSP

Kshitij Tandon[1,2,3], Pei-Wen Chiang[1], Chih-Ying Lu[1], Shan-Hua Yang[4,5], Ya-Fan Chen[1], Naohisa Wada[1], Ping-Yun Chen[6], Hsiao-Yu Chang[6], Ming-Shean Chou[6], Wen-Ming Chen[7], Sen-Lin Tang[1,2] #

[1] Biodiversity Research Center, Academia Sinica, Taipei 115, Taiwan

[2] Bioinformatics Program, Institute of Information Science, Taiwan International Graduate Program, Academia Sinica, Taipei 115, Taiwan

[3] Institute of Bioinformatics and Structural Biology, National Tsing Hua University, Hsinchu 300, Taiwan

[4] Department of Life Science, Tunghai University, Taichung 40704, Taiwan

[5] Center for Ecology and Environment, Tunghai University, Taichung 40704, Taiwan

[6] Institute of Environmental Engineering, National Sun Yat-Sen University, Kaohsiung 80424 Taiwan, ROC

[7] Laboratory of Microbiology, Department of Seafood Science, National Kaohsiung Marine University, No. 142, Hai-Chuan Rd, Nan-Tzu, Kaohsiung City 811, Taiwan, ROC.

Corresponding author

Corresponding author Email: sltang@gate.sinica.edu.tw

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), 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 and functionally characterized the first DMSP lyase—a *dddD* gene homolog found in all *E. acroporae,* capable of metabolizing DMSP into DMS via the DddD cleavage pathway—using RT-qPCR and GC. Comparative genomic analysis identified that *Endozoicomonas* has high genomic divergence and a high proportion of oxidative stress responsive genes and that *E. acroporae* strains have the highest number of Type III secretion system genes (T3SS) in the genus. 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 symbiotic algae [4]. It is the central molecule in the marine sulfur cycle and precursor to dimethylsulphide (DMS), a climate-active gas [5,6]. DMSP is hypothesized to be part of the coral holobiont antioxidant system [7] 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 [8].

Coral-associated bacteria use DMSP produced by corals and their symbiotic algae as a reduced sulfur and carbon source [8, 9]; they can also metabolize it into DMS [5,6]. DMSP degradation by marine organisms takes place via two pathways, the cleavage pathway and the demethylation pathway [9, 10]. Raina et al. [11] recently reported that the majority of DMSP-degrading bacteria belong to class *Gammaproteobacteria*, which includes Alteromonas-*,* *Arhodomonas-, Idiomarina-, Pseudomonas*- and *Spongiobacter*-related organisms**.** 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 [12]. *Endozoicomona*s species, which are predominantly associated with keeping their coral host healthy [13], have been hypothesized to play role in the global sulfur cycle by effectively metabolizing DMSP into DMS [14,15]**.** However, no previous study has confirmed the genus' role.

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 in Penghu, Taiwan and the Red Sea, Saudi Arabia (for details see supplementary data).

Results and Discussion

We assembled high quality genomes (>95% complete) of the two *E. acroporae* strains and also used the previously assembled type strain *E. acroporae* Acr-14^{T} [16,17] (Table S1, Fig S1). *E. acroporae* species are dominant coral-associated bacteria in the Red Sea, Saudi Arabia (Fig S2A, B) and Penghu, Taiwan (Fig S2C, D). 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 1mM DMSP resulted in 42.77, 56.52, and 91.37 times higher expression than samples cultured without DMSP after 16, 24 and 48hrs, respectively (Fig 1A). The amount of DMS released when the culture (*E. acroporae* Acr-14^{T}) was incubated in a DMSP-rich environment was significantly higher (*t-test, p-value <0.05*) than controls (Fig 1B). The temporal increase in the concentration of released DMS confirms that *E. acroporae* can metabolize DMSP into DMS. The discovery of the *dddD* gene in *Endozoicomonas* provides new insights into the evolution of the DMSP cleavage pathway and further confirms the hypothesis that *Endozoicomonas* plays a role in the global sulfur cycle.

Comparative genomic analysis identified high genomic divergence using Amino-Acid Identity (AAI), Average Nucleotide Identity (ANI) and DNA-DNA Hybridization (DDH) (Fig 2 A, B, and C) in the genus and also a reduced core genome (308 genes) (Fig S3). Genomes of *Endozoicomonas* species are large (5.43 ~ 6.69 Mb) (Table S2) and encode genes for all essential amino-acids [18], giving clues about not predominant genome streamlining as identified in symbiotic bacteria [19] and other symbiotic life stages [20]. Moreover, *E. acroporae* species have the highest numbers of T3SS genes in *Endozoicomonas* (Table S3), suggesting an intricate relationship with their host**.** Moreover, *E. acroporae* strains have different IS elements than *E. montiporae*, hinting that the two coral isolates have different evolution histories (Fig S4). Moreover, diverse phage insertions in *Endozoicomonas* species genomes suggest different infection histories (Table S4). In addition, *E. montiporae* and *E. acroporae* do not share any branches, according to core-genome 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 (Fig S5).

Conclusion

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

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.H.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.

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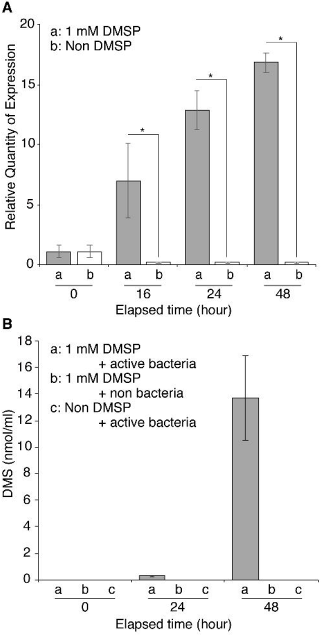
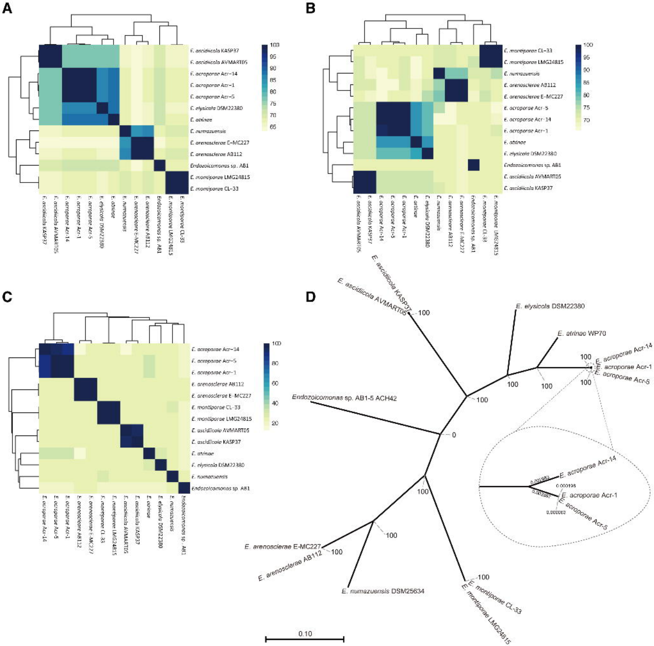
Figure Legends

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
0
5
10
15
20
*
*
*
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)
0
2
4
6
8
10
12
14
16
18
a
b
c
a
b
c
a
b
c
0
24
48
Elapsed time (hour)


A
B
C
D
E. ascidiicola KASP37
E. ascidiicola AVMART05
E. acroporae Acr-14
E. acroporae Acr-1
E. acroporae Acr-5
E. elysicola DSM22380
E. atrinae
E. numazuensis
E. arenosclerae E-MC227
E. arenosclerae AB112
Endozoicomonas sp. AB1
E. montiporae LMG24815
E. montiporae CL-33
100
95
90
85
80
75
70
65
60
40
20
E. atrinae WP70
Endozoicomonas sp. AB1-5 ACH42
E. numazuensis DSM25634
0.002983
0.000198
0.002467
0.000063
100
0
0.10