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 Chan[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.

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 dimethylsulphoniopropionate (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]. *Endozoicomona*s
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

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

82 *dddD* gene in *Endozoicomonas* provides new insights into the evolution of the DMSP cleavage
83 pathway and further confirms the hypothesis that *Endozoicomonas* plays a role in the global sulfur
84 cycle.

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

102

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.

Acknowledgements

This study was supported by funding from Academia Sinica. KT would like to acknowledge the Taiwan International Graduate Program (TIGP) for its fellowship towards his graduate studies. Authors would like to acknowledge support from Dr. Shu-Fen Chiou for GC experiment.

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.

References

1. Veron, JEN. *Coral of the World* Vol. 1-3 (Australian Institute of Marine Science, 2000).
2. Broadent AD, Jones GB, and Jones RJ. DMSP in Corals and Benthic Algae from the Great Barrier Reef. *Estuar. Coast. Shelf Sci.* 2002 **55:** 547-555**.**
3. Raina JB, Tapiolas DM, Foret S, Lutz A, Abrego D, Ceh J *et al.* DMSP biosynthesis by an animal and its role in coral thermal stress response. *Nature* 2013. **502:** 677-680.
4. Broadent AD and Jones GB. DMS and DMSP in mucus ropes, coral mucus, surface films and sediment pore waters from coral reefs in the Great Barrier Reef. *Mar Freshwater Res* 2004 **55:** 849-855.
5. Ayers GP and Gras, JL. Seasonal relationship between cloud condensation nuclei and aerosol methanesulphonate in marine air. *Nature* 1991 **353:** 834-835.
6. Vallina SM and Simo R. Strong relationship between DMSP and the solar radiation dose over the global surface ocean. *Science* 2007 **315:** 506-508.
7. Deschaseaux ESM, Jones GB, Deseo MA, Shepher KM, Kiene RP, Swan HB, et al. Effects of environmental factors on dimethylated sulfur compounds and their potential role in the antioxidant system of the coral holobionts. *Limnol. Oceanogr.* 2014 **59(3):**758-768
8. Raina JB, Dinsdale EA, Willis BL, Bourne DG. Do the organic sulfur compounds DMSP and DMS drive coral microbial associations? *Trends Microbiol.* 2010 **18:** 101-108.

143 9. Reisch CR, Moran MA, Whitman WB. Bacterial catabolism of
144 Dimethylsulfoniopropionate(DMSP). *Front. Microbiol.* 2011 **2:**172.
145 10. Curson ARJ, Todd JB, Sullivan MJ, Johnston AWB. Catabolism of
146 dimethylsulphoniopropionate: microorganisms, enzymes and genes. *Nat Rev Microbiol.*
147 2011 **9**:849-859.
148 11. Raina JB. Tapiolas D, Willis D, Bourne DG. Coral-associated bacteria and their role in the
149 biogeochemical cycling of sulfur. *Appl. Environ. Microbiol.* 2009 **75**:3492.
150 12. Todd JD, Rogers R, Li YG, Wexler M, Bnd PL, Sun L. Structural and regulatory genes required
151 to make the gas dimethylsulfide in bacteria. *Science* 2007 **315:** 666-669.
152 13. Bourne DG, Iida Y, Uthicke S, Smith-keune C. Changes in coral-associated microbial
153 communities during a bleaching event. *ISME J.* 2008 **2:**350-363.
154 14. Ransome E, Rowley SJ, Thomas S, Tait K, Munn CB. Disturbance to conserved bacterial
155 communities in the cold-water gorgonian coral *Eunicella verrucosa. FEMS Microbiol. Ecol.*
156 2014 **90:** 404-416.
157 15. Pike RE, Haltli D, Kerr RG. *Endozoicomonas euniceicola* sp. nov. and *Endozoicomonas*
158 *gorgoniicola* sp. nov., bacterial isolated from the octocorals. *Eunicea fusca* and *Plexaura*
159 sp. *Int. J. Syst. Evol. Microbiol.* 2013 **63:** 4294-4302.
160 16. Tandon K, Chiang PW, Chen WM, Tang SL. Draft Genome Sequence of *Endozoicomonas*
161 *acroporae* Strain Acr-14^{T}, Isolated from *Acropora* Coral. *Genome Announc.* 2018 **6(6):**
162 e01576-17
163 17. Sheu SY, Lin KR, Hsu MY, Sheu DS, Tang SL, Chen WM. *Endozoicomonas acroporae* sp. nov.,
164 isolated from *Acropora* coral. *Int. J. Syst. Microbiol.* 2017 **67:** 3791-3797.

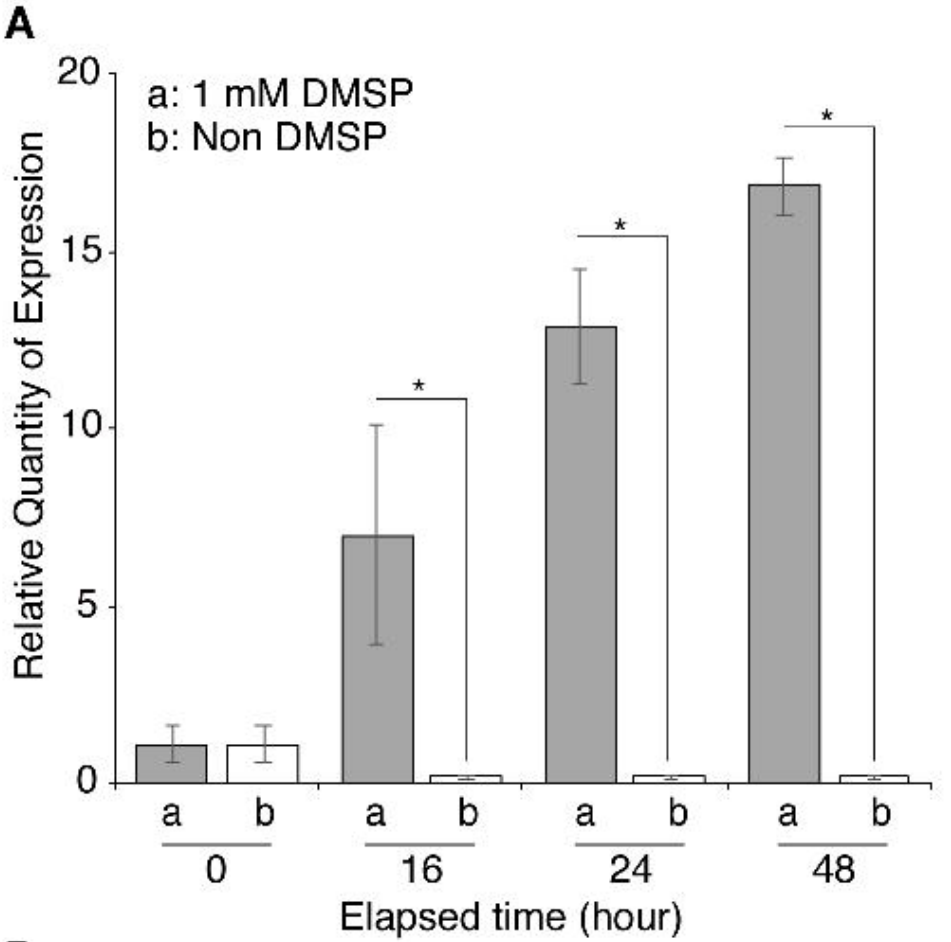
165 18. Neave MJ, Michell CT, Apprill A, Voolstra CR. *Endozoicomonas* genomes reveal functional
166 adaptation and plasticity in bacterial strains symbiotically associated with diverse marine
167 hosts. *Sci. Rep.* 2017 **7:**40579.
168 19. Kwan JC, Donia MS, Han AW, Hirose E, Haygood MG, Schmidt EW, Eric W. Genome
169 streamlining and chemical defense in a coral reef symbiosis. *Proc. Natl. Acad. Sci.* USA
170 2012 **109:** 20655-20660.

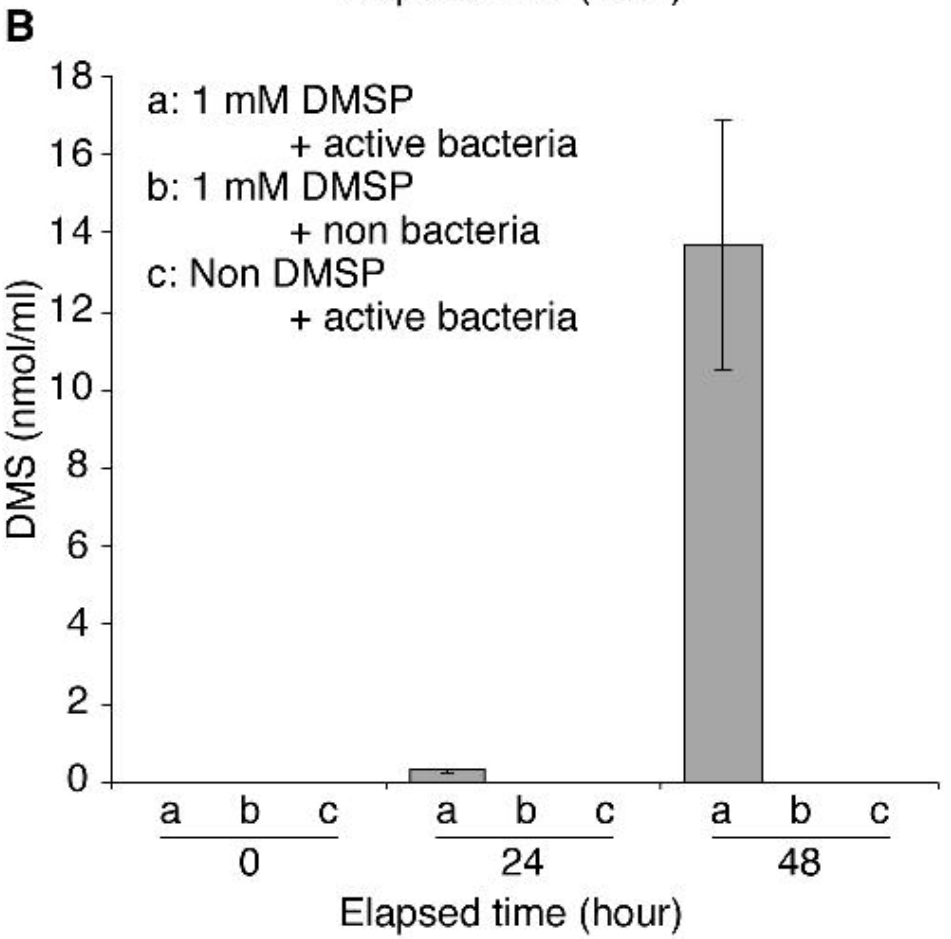
171

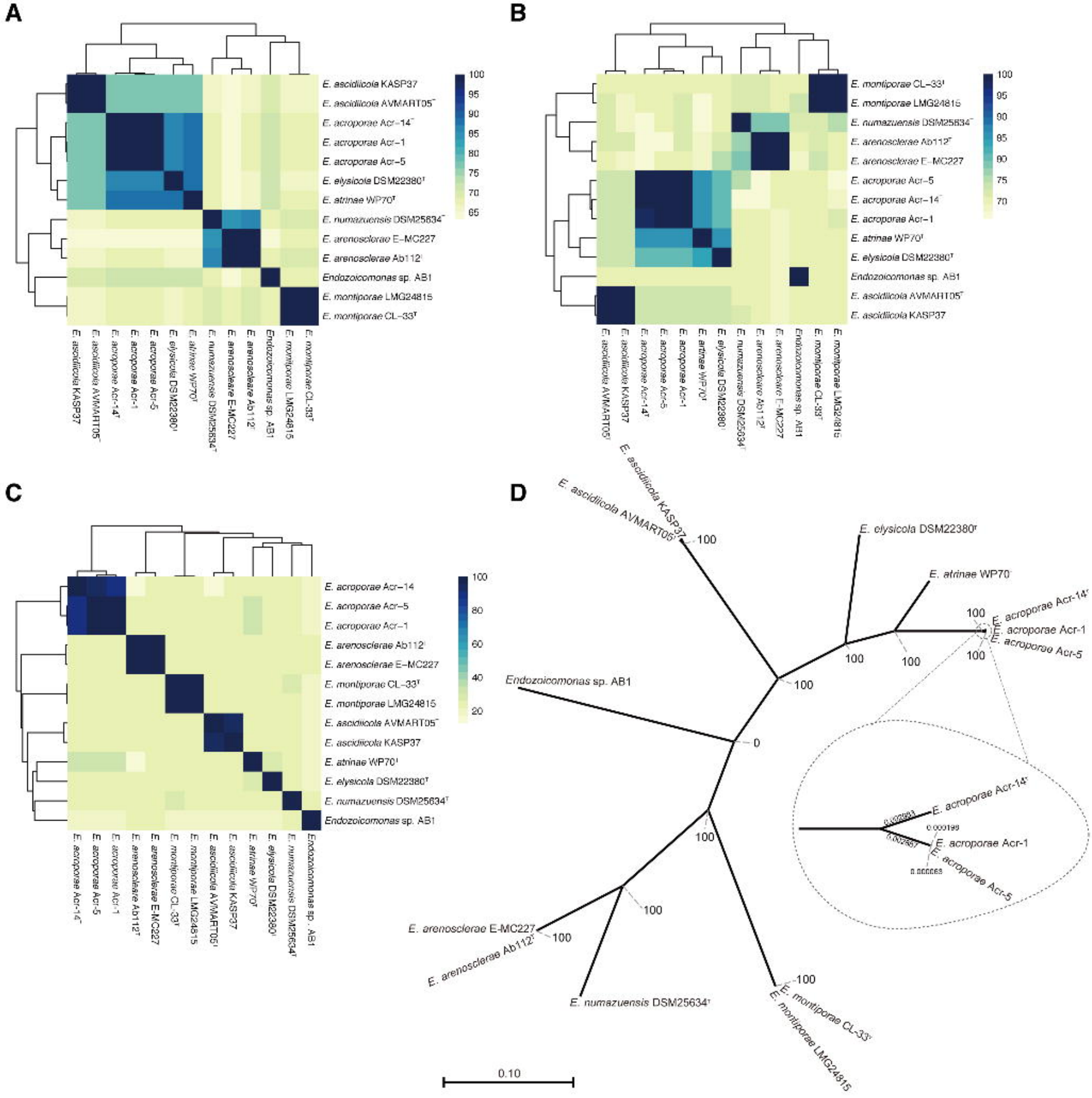
172 ## Figure Legends

173 **Figure 1. A)** RT-qPCR expression analysis; relative *DddD* gene expression increased significantly
174 with time (0, 16, 24, 48 hours) when cultures were grown with 1mM DMSP compared to no DMSP
175 (*t-test p value <0.05*). **B)** Quantification of DMS released, DMS release was only observed in the
176 1mM DMSP+ active bacteria condition, not in conditions b or c.

177 **Figure 2.** Genomic divergence analysis using heat-maps from **A)** AAI, **B)** ANI, and **C)** DDH.
178 Phylogenetic analysis **D)** Core-genome (308 genes)-based unrooted phylogenetic tree with *E.*
179 *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)


A
E. ascidilicola KASP37
E. ascidilicola 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. ascidilicola AVMART05T
E. ascidilicola 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. ascidilicola AVMART05T
E. ascidilicola KASP37
E. atrinae WP70T
E. elysicola DSM22380T
E. numazuensis DSM25634T
Endozoicomonas sp. AB1
100
80
60
40
20
D
E. ascidilicola KASP37
E. ascidilicola AVMART05T
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
0.000199
0.000063
0.10