Supplementary Materials: Growth and Toxin Production of *Gambierdiscus* spp. Can be Regulated by Quorum-Sensing Bacteria

Bo Wang, Mimi Yao, Jin Zhou, Shangjin Tan, Hui Jin, Feng Zhang, Yim Ling Mak, Jiajun Wu, Leo Lai Chan and Zhonghua Cai

1. Experimental section

Calculation of algal growth rate

During the co-culture experiment, the growth rate (divisions day^{-1}) was calculated using data (n = 5) of algal concentration from the exponential portion of the growth curve by least squares regression [1] as follows:

$$\text{Growth rate} = \frac{n \times \sum^n (t_i \times lnC_i) - [\sum^n (t_i) \times \sum^n (lnC_i)]}{ln2 \times \{n \times \sum^n (t_i^2) - [\sum^n (t_i)]^2\}}$$

where t_i = co-culture day, C_i = algal concentration and n = sample number. The average growth rate was calculated in triplicates.

2. Figures and Tables

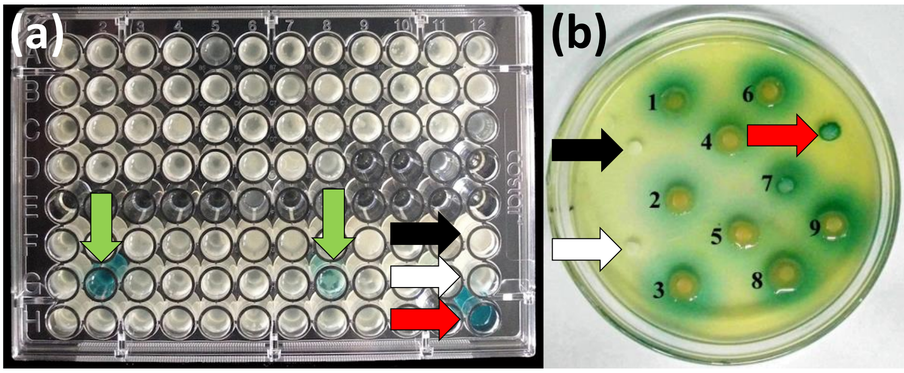

Figure S1. (a) The fast liquid AHL producers screening method in a 96-well microtiter plate and **(b)** the traditional solid agar plate screening results, with indicator strain A136. Black arrow: negative control (*E. coli* DH 5α); white arrow: blank control (water); red arrow: positive control (*P. aeruginosa* PAO1); green arrow: potential AHL-producing bacteria; 1 to 9: the screened AHL-producing strains.

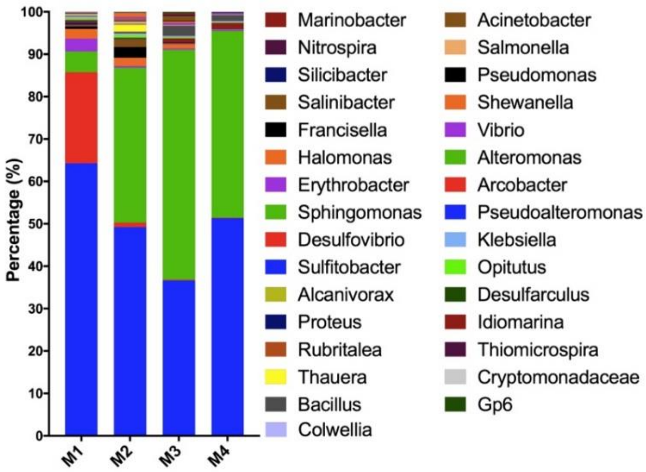

Figure S2. Taxonomic distribution at the genus level of the four sampling sites.

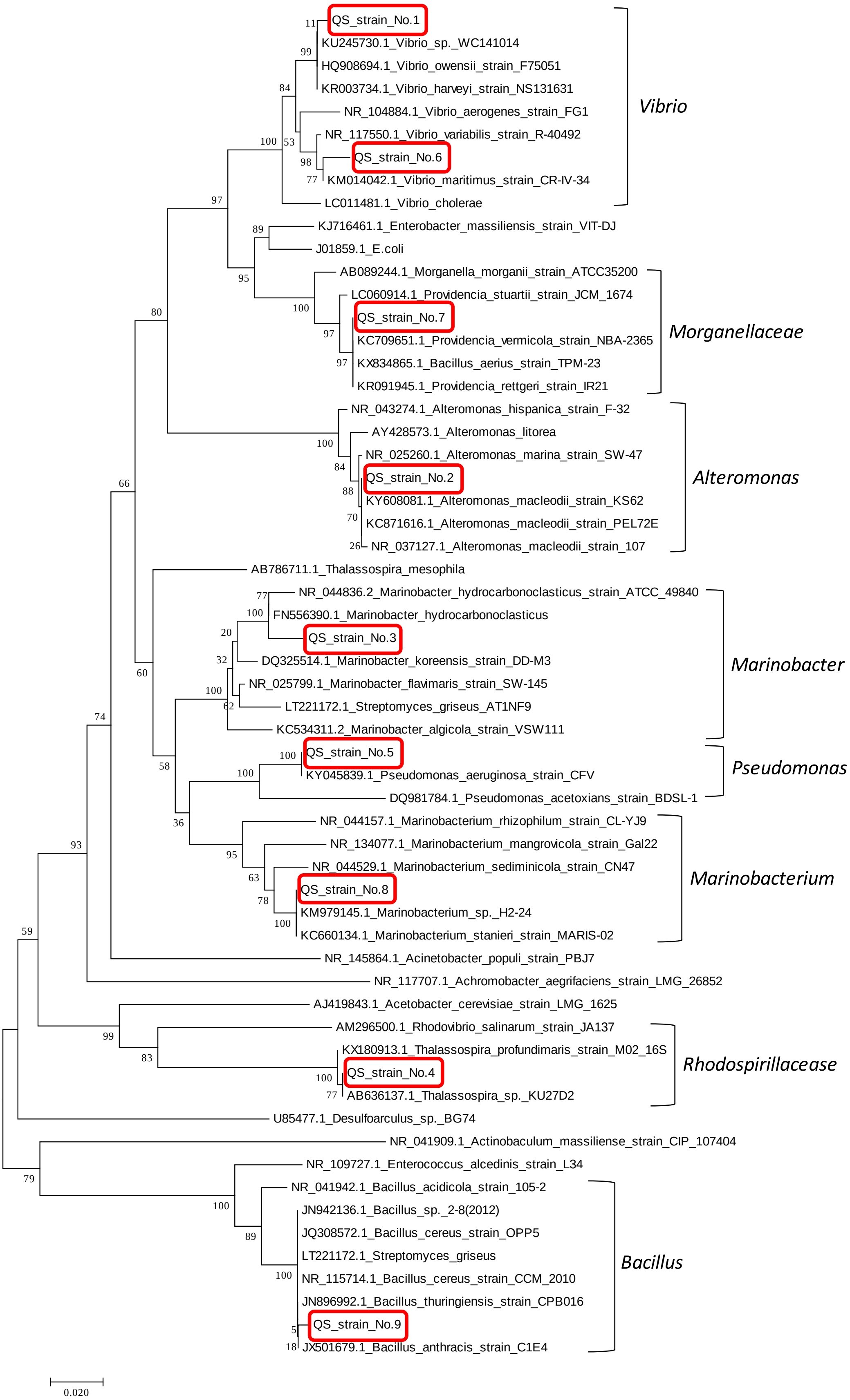

Figure S3. Phylogenetic tree generated using the 16s rRNA gene of the screened AHL-producing bacterial species/phylotypes. Statistical method: Neighbor-joining. Number of bootstrap replications: 500. Model: Maximum Composite Likelihood.

Table S1. Distribution of the corresponding genus of the screened QS bacteria.

Strains	Genus	Distribution of the genus (%)			
		M1	M2	M3	M4
Vibrio sp.	*Vibrio*	3.02	0.36	0.34	0.32
A. macleodii	*Alteromonas*	4.89	36.52	54.2	44.1
M. hydrocarbonoclasticus	*Marinobacter*	0	0	0.25	0.09
Thalassospira sp.	*Thalassospira*	-*	-	-	-
P. aeruginosa	*Pseudomonas*	0.54	2.55	0.03	0.11
V. maritimus	*Vibrio*	3.02	0.36	0.34	0.32
P. vermicola	*Providencia*	-	-	-	-
M. stanieri	*Marinobacterium*	0	0	0.25	0.09
B. anthracis	*Bacillus*	0.2	0.44	2.36	1.31

* Could not be detected at genus level.

Table S2. *Gambierdiscus* strains from Marakei, Republic of Kiribati.

Sample	Species name	Toxicity (pg P-CTX-1 eq cell^{-1})
1022M2C12	*Gambierdiscus* sp. type 5	0.004
1112M1M03	*Gambierdiscus* sp. type 6	0.02
1021M1DC4	*Gambierdiscus* sp. type 6	0

Table S3. The gene fragment for the phylogenetic analyses.

>QS strain No.1
ATGGCGGCAGCTACCATGCAGTCGAGCGGAACGAGTTATCTGAACCTTCGGGGGACGATAACGGC GTCGAGCGGCGGACGGGTGAGTAATGCCTAGGAAATTGCCCTGATGTGGGGGATAACCATTGGAA ACGATGGCTAATACCGCATAATGCCTACGGGCCAAAGAGGGGGACCTTCGGGCCTCTCGCGTCAGG ATATGCCTAGGTGGGATTAGCTAGTTGGTGAGGTAATGGCTCACCAATGCGACGATCCCAAGCTGG TCTGAGAGGATGATCAGCCACACTGGAACTGAGACACGGTCCAGACTCCTACGGGAGGCAGCAGT GGGGAATATTGCACAATGGGCGCAAGCCTGATGCAGCCATGCCGCGTGTGTGAAGAAGGCCTTCG GGTTGTAAAGCACTTTCAGTCGTGAGGAAGGTGGTGTAGTTAATAGCTGTATTATTTGACGTTAGCG ACAGAAGAAGCACCGGCTAACTCCGTGCCAGCAGCCGCGGTAATACGGAGGGTGCGAGCGTTGAT CGGAATTACTGGGCGTAAAGCGCATGCAGGTGGTTTGTTAAGTCAGATGTGAAAGCCCGGGGCTCA ACCTCGGAATAGCATTTGAAACTGGCAGACTAGAGTACTGTAGAGGGGGGTAGAATTTCAGGTGTA GCGGTGAAATGCGTAGAGATCTGAAGGAATACCGGTGGCGAAGGCGGCCCCCTGGACAGATACTG ACACTCAGATGCGAAAGCGTGGGGAGCAAACAGGATTAGATACCCTGGTAGTCCACGCCGTAAAC GATGTCTACTCGGAGGTTGTGGCCTTGAGGCGTGGCTTTCGGAGCTAACGCGTTAAGTAGACCGCCT GGGGAGTACGGTCGCAAGATTAAAACTCAAATGAATTGACGGGGGCCCGCACAAGCGGTGGAGCA TGTGGTTTAATTCGATGCAACGCGAAGAACCTTACCTACTCTTGACATCCAGAGAACTTTCCAGAGA TGGATTGGTGCCTTCGGGAACTCTGAGAGAGGTGCTGCATGGCTGTCGTCAGCTCGTGTTGTGAAAT GTTGGGTTAAGTCCCGCAACGAGCGCAACCCTTATCCTTGTCTGCCAGCGAGTAATGTCGGGAACTC CAGGGAGACTGCCGGTGATAAACCGGAGGAAGGTGGGGACGACGTCAAGTCATCATGGCCCTTAC GAGTAGGGCTACACACGTGCTACAATGGCGCATACAGAGGGCAGCAAGCTAGCGATAGTGAGCGA ATCCCAAAAAGTGCGTCGTAGTCCGGATTGGAGTCTGCAACTCGACTCCATGAAGTCGGAATCGCT AGTAATCGTGGATCAGAATGCCACGGTGAATACGTTCCCGGGCCTTGTACACACCGCCCGTCACAC CATGGGAGTGGGCTGCAAAAGAAGTAGGTAGTTTAACCTTCGGGAGGACGCTACCACTTAGTCTGC
>QS strain No.2
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>QS strain No.3

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>QS strain No.4

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Table S4. Growth rate (divisions day^{-1}) of 1022M2C12 in co-culture experiment.

Sample	Low concentration (5×10^3 cells/mL)	Medium concentration (5×10^4 cells/mL)	High concentration (5×10^5 cells/mL)
Bacteria No.1	0.347 ± 0.012	0.358 ± 0.008	0.326 ± 0.002
Bacteria No.2	0.335 ± 0.010	0.329 ± 0.018	0.332 ± 0.029
Bacteria No.3	0.351 ± 0.003	0.349 ± 0.004	0.056 ± 0.006
Bacteria No.4	0.326 ± 0.009	0.362 ± 0.009	0.349 ± 0.007
Bacteria No.5	0.338 ± 0.013	0.337 ± 0.004	0.350 ± 0.007
Bacteria No.6	0.356 ± 0.017	0.359 ± 0.004	0.394 ± 0.011
Bacteria No.7	0.367 ± 0.019	0.368 ± 0.010	0.381 ± 0.042
Bacteria No.8	0.389 ± 0.013	0.371 ± 0.004	0.394 ± 0.033
Bacteria No.9	0.335 ± 0.009	0.334 ± 0.010	0.284 ± 0.004
Control	0.369 ± 0.012	-	-

References

1. Yamaguchi, M.; Honjo, T. Effects of temperature, salinity and irradiance on the growth of the noxious red tide flagellate *Gymnodinium nagasakiense* (Dinophyceae). *Bull. Jpn. Soc. Sci. Fish. (Japan)* **1989**, *55*, 2029–2036.