Supporting Information

Frommlet et al. 10.1073/pnas.1420991112

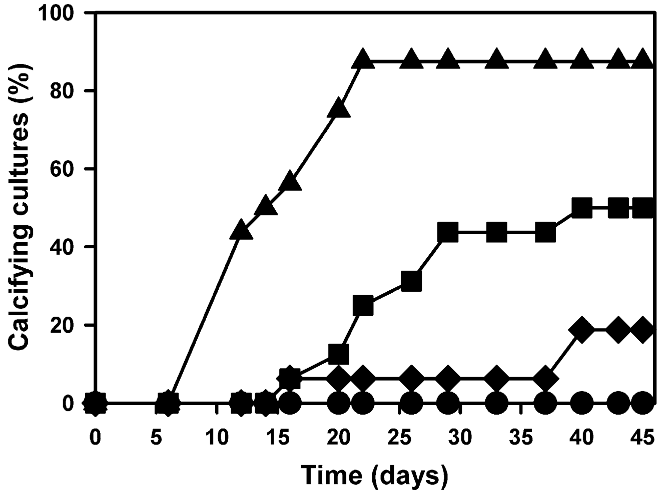


Fig. S1. Relevance of bacteria in symbiolite formation. Percentages of calcifying cultures in the presence of antibiotics (●), following the antibiotics treatment (◆), and following the antibiotics treatment with an inoculum of either *N. phycophila* (■) or *P. atlantica* (▲) (n = 16). Also see Table S4.

Table S1. *Symbiodinium* strains and their capacity to form symbiolites

Culture (NCMA no.)	Species*	Clade or ITS type (ref.)	Host species	Geographic origin	Symbiolite formation
61 (2464)	*S. microadriaticum* Freudenthal 1962	A1 (1)	*Cassiopeia xamachana*	Florida	+
362 (2458)	*S. microadriaticum* Freudenthal 1962	A1 (1)	*Cassiopeia andromeda*	Gulf of Aqaba	+
370 (2467)	*S. microadriaticum* Freudenthal 1962	A1 (1)	*Stylophora pistillata*	Gulf of Aqaba	+
23		A2 (1)	*Bartholomea annulata*	Barbados	+
24		A2 (1)	*Bartholomea annulata*	Barbados	+
89		A2 (1)	*Gorgonia ventalina*	Bermuda	+
97		A2 (1)	*Gorgonia ventalina*	Puerto Rico	+
104		A2 (1)	*Heliopora* sp.	Enewetak	+
130		A2 (1)	*Meandrina meandrites*	Jamaica	+
185 (2461)	*S. pilosum* Trench and Blank 1987	A2 (1)	*Zoanthus sociatus*	Jamaica	+
PHMS TD1e		A3a (2)	*Tridacna* sp.	Philippines	—
292 (2465)		A3 (1)	*Tridacna maxima*	Palau	+
379 (2456)		A4 (1)	*Plexaura homomalla*	Bahamas	—
FLAp1		A (3)	*Aiptasia* sp.	Florida Keys	+
Culture X		A12 (2)	Unknown	Aquarium tank	+
80 (2469)		A13 (1)	*Condylactis gigantea*	Jamaica	+
m. mirabilis		A14 (2)	*Madracis mirabilis*	Florida	+
2 (2460)	*S. minutum* LaJeunesse, Parkinson and Reimer 2012	B1 (1)	*Aiptasia pallida*	Florida	+
12 (2463)		B1 (1)	*Aiptasia tagetes*	Puerto Rico	+
13		B1 (1)	*Aiptasia tagetes*	Bermuda	+
64		B1 (1)	*Cassiopeia xamachana*	Jamaica	+
74		B1 (1)	*Cassiopeia xamachana*	Jamaica	+
Pk702		B1 (3)	*Plexaura kuna*	San Blas	+
Pk704		B1 (3)	*Plexaura kuna*	San Blas	+
Pk706		B1 (3)	*Plexaura kuna*	San Blas	+
146		B1 (1)	*Oculina diffusa*	Bermuda	+
147 (2470)		B1 (1)	*Pseudoterogorgia bipinnata*	Jamaica	+
M. capitata		B1 (1)	*Montipora capitata*	Hawaii	+
351		B1 (1)	*Pocillopora damicornis*	Hawaii	+
141 (3320)	*S. psygmophilum* LaJeunesse, Parkinson and Reimer 2012	B2.1 (1)	*Oculina diffusa*	Bermuda	+
385 (2462)		B3 (2)	*Dichotomia* sp.	Caribbean	+
FLAp2		B (3)	*Aiptasia pallida*	Florida	+
152 (2466)	*S. goreaui* Trench and Blank 1987	C1 (1)	*Discosoma sancti-thomae*	Caribbean	—
203		C2 (1)	*Hippopus hippopus*	Palau	+
HHC1B		C2 (2)	*Hippopus hippopus*	Philippines	+
401		D (4)	Unknown	Unknown	+
Ap31		D (5)	Unknown anemone	Okinawa	+
383 (3420)	*S. voratum* Jeong et al. 2014	E1 (1)	*Anthopleura elegantissima*	California	+
135 (2468)	*S. kawagutii* Trench and Blank 1987	F1 (1)	*Montipora verrucosa*	Hawaii	+
133 (2455)		F2 (1)	*Meandrina meandrites*	Jamaica	+

NCMA, National Center for Marine Algae and Microbiota.
*Species names are provided for cultures that are deposited in a collection and are either identified to species level by the collection or could be clearly matched with the holotype in the respective species description.

1. LaJeunesse TC (2001) Investigating the biodiversity, ecology, and phylogeny of endosymbiotic dinoflagellates in the genus *Symbiodinium* using the ITS region: In search of a 'species' level marker. *J Phycol* 37(5):866–880.
2. LaJeunesse TC, Lambert G, Andersen RA, Coffroth M-A, Galbraith DW (2005) *Symbiodinium* (Pyrrhophyta) genome sizes (DNA content) are smallest among dinoflagellates. *J Phycol* 41(4):880–886.
3. Santos SR, Taylor DJ, Coffroth MA (2001) Genetic comparisons of freshly isolated versus cultured symbiotic dinoflagellates: Implications for extrapolating to the intact symbiosis. *J Phycol* 37(5):900–912.
4. Thornhill DJ, Lord JB (2010) Secondary structure models for the internal transcribed spacer (ITS) region 1 from symbiotic dinoflagellates. *Protist* 161(3):434–451.
5. Santos SR, et al. (2002) Molecular phylogeny of symbiotic dinoflagellates inferred from partial chloroplast large subunit (23S)-rDNA sequences. *Mol Phylogenet Evol* 23(2):97–111.

Table S2. Symbiolite formation in different growth media

			f/2	ASP-8A Tris-buffered	ASP-8A bicarbonate-buffered
Culture	Clade or ITS type	Alkalinity, meq/L	2.11	5.93	1.57
61	A1		+	—	+
362	A1		+	—	+
370	A1		+	—	+
23	A2		+	—	+
89	A2		+	—	+
97	A2		+	—	+
104	A2		—	—	+
130	A2		+	—	—
185	A2		+	—	—
292	A3		+	—	—
FLAp1	A		+	—	—
Culture X	A12		+	—	+
80	A13		+	—	—
2	B1		+	—	+
12	B1		+	—	+
13	B1		+	—	+
64	B1		—	—	—
74	B1		+	—	+
146	B1		+	—	+
147	B1		+	—	—
351	B1		+	—	—
141	B2.1		—	—	—
385	B3		+	—	+
FLAp2	B		+	—	—
203	C2		+	—	—
HHC1B	C2		+	—	—
401	D		+	—	—
Ap31	D		+	—	+
383	E1		+	—	—
135	F1		—	—	—
133	F2		—	—	+

Table S3. Growth of *Symbiodinium* strains with antibiotics and isolated bacterial strains

Culture	Clade or ITS type	Growth with antibiotics*	Bacterial isolates† (sequence similarity)	Accession no.
61	A1	+		
362	A1	+	*Muricauda aquimarina* (99.17%)	KP645212
			Marinobacter salsuginis (99.93%)	KP645213
370	A1	+	*Marinobacter adhaerens* (99.73%)	KP645214
23	A2	+		
24	A2	+		
89	A2	—		
97	A2	+	*Muricauda aquimarina* (99.17%)	KP645205
			Marinobacter salsuginis (99.93%)	KP645206
104	A2	+		
130	A2	+	*Marinobacter salsuginis* (99.93%)	KP645208
185	A2	+		
PHMS TD1e	A3a	+		
292	A3	+		
379	A4	+		
FLAp1	A	+		
Culture X	A12	+		
80	A13	+		
M. mirabilis	A14	+		
2	B1	—		
12	B1	+	*Neptunomonas phycophila* (100%)	KM591221
			Pseudoalteromonas atlantica (99.93%)	KP645203
13	B1	+		
64	B1	+		
74	B1	+	*Alteromonas macleodii* (99.86%)	KP645204
Pk702	B1	+	*Marinobacter salsuginis* (99.93%)	KP645216
Pk704	B1	+		
Pk706	B1	—		
146	B1	+		
147	B1	+		
M. capitata	B1	+		
351	B1	+		
141	B2.1	+		
385	B3	+		
203	C2	+	*Marinobacter salsuginis* (99.93%)	KP645210
			Thalassospira permensis (100%)	KP645211
HHC1B	C2	+		
135	F1	+		
133	F2	+		

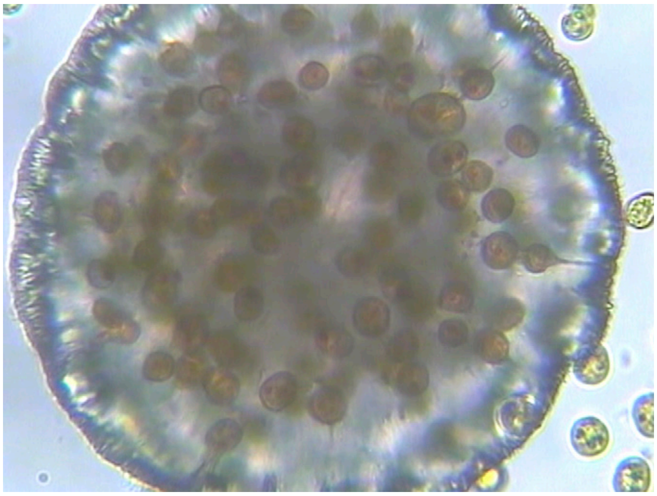
*Growth with antibiotics refers to culture growth, not to symbiolite formation. Growth of three cultures (89, 2, and Pk706) was strongly reduced. None of the cultures grown with antibiotics calcified.

†Bacterial species names are the closest phylogenetic relatives identified on the basis of 16S sequences in the Eztaxon database (www.ezbiocloud.net/eztaxon). *N. phycophila* was first isolated in the course of the present study and has been described as a new species elsewhere (1).

1. Frommlet JC, Guimarães B, Sousa L, Serôdio J, Alves A (2015) *Neptunomonas phycophila* sp. nov. isolated from a culture of *Symbiodinium* sp., a dinoflagellate symbiont of the sea anemone *Aiptasia tagetes*. *Int J Syst Evol Microbiol* 65(Pt 3):915–919 10.1099/ijs.0.000039.

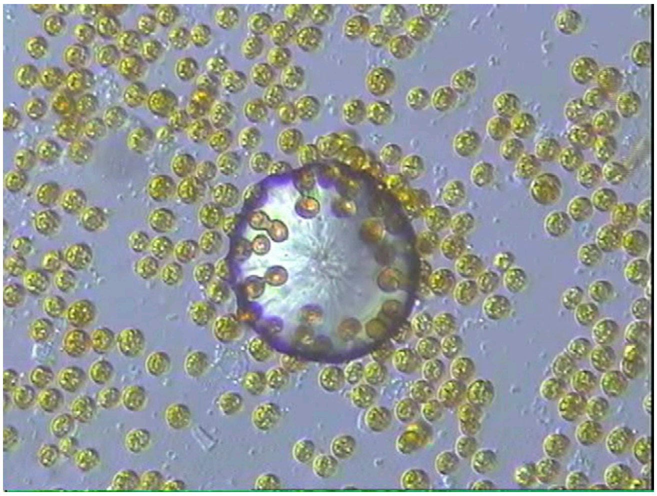
Table S4. Symbiolite formation after antibiotic relaxation and inoculation with bacterial isolates

Culture	Number of days from inoculation to the appearance of symbiolites		
	Previously inoculated with antibiotics	Previously inoculated with antibiotics + *N. phycophila*	Previously inoculated with antibiotics + *P. atlantica*
12	40	—	14
13	—	26	16
23	—	—	20
24	—	—	—
61	—	16	20
64	—	20	—
74	—	—	20
97	—	—	22
104	40	40	12
130	16	29	22
141	—	—	12
146	—	29	12
147	—	—	12
185	—	22	12
203	—	22	12
362	—	—	12



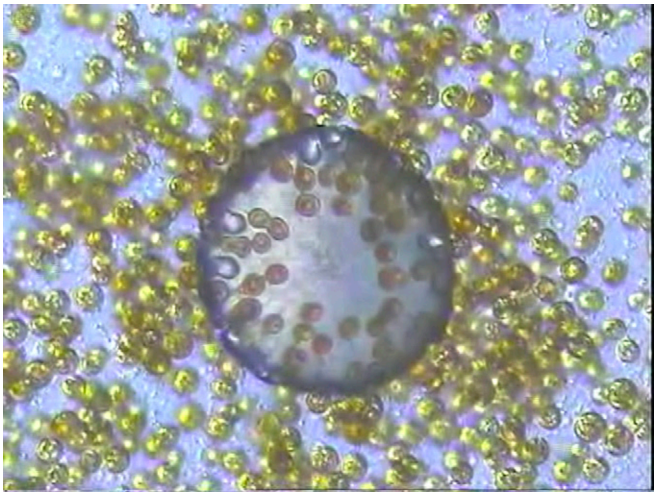
Movie S1. Symbiolite discovery. Real-time movie of the symbiolite discovered in culture 362. The movie plays at 25 frames/s. Scale is shown in Fig. 1*A*.

Movie S1



Movie S2. Symbiolite growth. Time-lapse movie of symbiolite growth in culture 130 over five consecutive light/dark periods (the entire sequence is repeated once). The movie plays at 90,000 times real speed (recorded at one frame/h during the light phases and played at 25 frames/s). Dark periods were removed. Scale is shown in Fig. 3*A*.

Movie S2



Movie S3. *Symbiodinium* cells vacate symbiolite. Time-lapse movie of symbiolite growth and emerging *Symbiodinium* cells in culture 130 over seven consecutive light/dark periods after medium exchange. Arrows highlight two cells that emerge during the sequence. The movie is shown at ~90,000 times real speed (recorded at one frame/h during the light phases and played at 25 frames/s but with two periods of higher frame rate to resolve the process of cell emergence). Dark periods were removed. Scale is shown in Fig. 3 *E*–*G*.

Movie S3