Journal of the South Carolina Academy of Science
A peer-reviewed electronic publication of the South Carolina Academy of Science

Volume 15
Issue 1 *Governor's Awards Issue*

Article 6

4-1-2017

Differential Response of Coral Symbiotic Dinoflagellates to Bacterial Toxins that Produce Bleaching in Stony Corals

Diego L. Gil-Agudelo
University of South Carolina Beaufort

Yael Ban-Haim
Tel-Aviv University

Eugene Rosenberg
Tel-Aviv University

Garriet W. Smith
University of South Carolina Aiken

Differential Response of Coral Symbiotic Dinoflagellates to Bacterial Toxins that Produce Bleaching in Stony Corals

Abstract

Bleaching of corals and other organisms with symbiotic zooxanthellae is a worldwide phenomenon with increasing importance due to global warming scenarios. Bleaching has been historically related to changes in the environment, especially water temperature increase, that stress corals and provoke the release of zooxanthellae. The discovery of Vibrio shilonii, a bacterium causing bleaching under thermal stress in corals of the Mediterranean Sea has changed our thinking about the cause (or explanation) for bleaching of corals worldwide. During this study, we evaluated the effect of a proline rich toxin, extracted from Vibrio shilonii, on zooxanthellae obtained from: Oculina patagonica from the Mediterranean Sea, two species from the Gulf of Elait (Red Sea), four species from the Caribbean Sea, and five Symbiodinium species extracted from different hosts (corals, jellyfish, zoanthid, and anemones) from different parts of the world. Our results show a differential response of zooxanthellae to the toxin, which implies, that a number of coral species may be affected by this bacterium to different degrees.

Differential Response of Coral Symbiotic Dinoflagellates to Bacterial Toxins that Produce Bleaching in Stony Corals

Diego L. Gil-Agudelo[1*], Yael Ban-Haim[2], Eugene Rosenberg[2], and Garriet W. Smith[3]

[1] *University of South Carolina at Beaufort, Department of Natural Sciences, One University Boulevard, SC 29909, USA*
[2]*Tel-Aviv University, Department of Molecular Microbiology & Biotechnology, Ramat Aviv 69978, Israel.*
[3]*University of South Carolina at Aiken, Department of Biology and Geology, 471 University Parkway, Aiken, SC 29801, USA.*

Bleaching of corals and other organisms with symbiotic zooxanthellae is a worldwide phenomenon with increasing importance due to global warming scenarios. Bleaching has been historically related to changes in the environment, especially water temperature increase, that stress corals and provoke the release of zooxanthellae. The discovery of *Vibrio shilonii*, a bacterium causing bleaching under thermal stress in corals of the Mediterranean Sea has changed our thinking about the cause (or explanation) for bleaching of corals worldwide. During this study, we evaluated the effect of a proline rich toxin, extracted from *Vibrio shilonii*, on zooxanthellae obtained from: *Oculina patagonica* from the Mediterranean Sea, two species from the Gulf of Elait (Red Sea), four species from the Caribbean Sea, and five Symbiodinium species extracted from different hosts (corals, jellyfish, zoanthid, and anemones) from different parts of the world. Our results show a differential response of zooxanthellae to the toxin, which implies, that a number of coral species may be affected by this bacterium to different degrees.

Introduction

Bleaching of reef organisms has been among the most important stressors of coral reefs for the last two decades (Glynn, 1991a and 1993; Brown, 1997, Hoegh-Guldberg, 1999). Bleaching has caused the death of significant amounts of corals in different parts of the world and the consequent impacts on marine ecosystems and human economy have been considerable (Glynn, 1991b; Hoegh-Guldberg, 1999). Although bleaching has been correlated with changes in the environment (increase of water temperature, sedimentation, changes in salinity of the water, and light stress, among others) (see Glynn 1991), evidence of bacterial induced bleaching has been found on at least two occasion (Kushmaro et al., 1996, 1997, and 2001, Ben-Haim et al., 2003).

Bacterial bleaching of *Oculina patagonica* by *Vibrio shilonii* in the Mediterranean Sea has been extensively studied. Kushmaro et al. (1997 and 2001), Ben-Haim et al. (1999), and Banin et al. (2001) described the infectious mechanisms resulting in inhibition of photosynthesis (drop in the charge separation efficiency or Quantum Yield of the photosystem II). In general, after the penetration of *V. shilonii* into *O. patagonica*, the bacteria produce extracellular toxins (including toxin P) that block photosynthesis and lyse the symbionts, thus, resulting in bleaching (Banin et al, 2001). Toxin P is produced in response to thermal stress and shows an effect on the Quantum Yield (QY) in the presence of NH_4Cl. It has been proposed that Toxin P forms channels or pores that allow NH_4^+ to pass through the membrane destroying the pH gradient and consequently inhibiting photosynthesis (Banin et al, 2001; Rosemberg and Ben-Hain, 2002).

The effect of Toxin P has been tested in symbiotic dinoflagellates from a number of coral species from the Mediterranean and the Red Sea (Ben-Haim et al., 1999). Its effects on symbiotic dinoflagellates associated with corals from other regions and with symbiots of other reef organisms have not been evaluated to date. This paper evaluates the effect of toxin P on symbiotic dinoflagellates from the Mediterranean, the Red Sea, and the Caribbean corals. In addition, the effect on cultured zooxanthellae, and the possible implications of massive coral bleaching by *V. shilonii* was investigated.

Materials and Methods

Acquisition of the symbiots

Coral samples (about 5 cm pieces) of *Oculina patagonica*, *Acropora euristema*, *Pocillopora damicornis*, *Millepora alcicornis*, *Montastaea annularis*, *M. faveolata*, and *Porites astreoides* were collected from the Mediterranean, the Red, and Caribbean Seas. These coral pieces were transported to laboratories at the University of Tel-Aviv or to the Puerto Morelos' field station (Universidad Nacional Autónoma de México). Upon arrival, coral tissue was removed from the skeleton using water picks and approximately 100 ml of filtered sea water. The resultant mix of dinoflagellates and coral tissue was centrifuged at 14,000 rpm for 30 min. The pellet was recovered and resuspended in 100 ml of filtrated sea water and centrifuged again for 4 min. at 10,000 rpm. The resultant pellet (zooxanthellae still with coral tissue residues) was resuspended in 1.5 ml of filtered sea water and centrifuged a final time for 21 min. at 1,200 rpm in an Ependorf microcentrifuge.

Strains of the symbiotic dinoflagellates *Symbiodinium pilosum*, *Symbiodinium kawagutii* (extracted from the Pacific coral *Montipora verrucosa*), *Symbiodinium microadriaticum* (from the jelly fish *Cassiopeia xamachana*), *Symbiodinium. pulchlorum* (from the anemone *Aiptasia pulchella*), and *Symbiodinium sp.* (extracted from the anemone *Discosoma sancti thomae*) were also used to test the effect of the toxin. These species were cultured at the Universidad Nacional de Mexico's field station. Zooxanthellae from culture were grown in ASP-8A medium for 7 days at 14/10 hour light and darkness cycles. Cells were concentrated by centrifugation to obtain a final concentration of 106 cells/ml.

Preparation of crude toxin P and trials

For the isolation of the crude toxin, a pure strain of *Vibrio shilonii* was cultured in Marine broth (Difco) or a modification of Artificial Sea Water broth (Smith and Hayasaka, 1982) with glycerol omitted due to its inhibition of the production of the toxin P by *V. shilonii* (Banin et al. 2001). Bacterial strains were incubated at 30ºC for 24 hours to obtain the toxin (Banin et al., 2001). Cultures were centrifuged at 10000 rpm for 10 minutes

and the supernatant was used for the trials, as the source of crude toxin.

To obtain Quantum Yield (QY) measurements, the F_o and F_{max} of the zooxanthellae, were observed using an underwater Pulse-Amplitude-Modulated Fluorometer (PAM) with Mediterranean and Red Sea corals, and with a Plant Efficient Analyzer (PEA) for Caribbean samples. The use of PEA in Caribbean samples also permitted the analysis of induction curves during the treatments. This analysis allows the determination of possible damage of the photosystem II (PS II) due to the action of the toxin, and if this disruption happened in the donor or acceptor side of electrons of the PS II complex. Readings of these parameters were performed two minutes before adding the supernatant, just after adding it, and every two minutes for 10 minutes thereafter.

Controls

Three different control sets were established. In the first one using filtered seawater (64µm) to replace the sample of supernatant from *V. shilonii*. In the second one, NH_4Cl (final concentration of 12.5 mmol) replaced the supernatant of the bacteria. This control was performed in order to determine the effect of ammonia produced by bacteria on the symbiots and the concentration used was similar to the one used by Ben-Haim et al. (1999) and Banin et al. (2001) in their experiments. This concentration is also similar to the one found in the control supernatant of *V. shilonii* after the incubation period of 24 hours (Ben-Haim et al., 1999). For the third control, Marine Broth medium (Difco) replaced the supernatant of the bacteria to test the possible effects on the symbiots. In addition, the effect of Marine broth and Artificial Sea Water broth was evaluated by substituting the water with culture media. The possibility of a combined effect of broth and ammonia on the zooxanthellae, was also evaluated by adding NH_4Cl to the control (to a final concentration of 12.5 mmol).

The effect of the culture media in the QY of cultured zooxanthellae was also evaluated by performing trials with added ASPA-8A medium and NH_4Cl (12.5 mmol final concentration) instead of the crude toxin. Measurements were performed at similar intervals to those used during trials with the supernatant and the toxin.

Results

Water, broth (both Marine and Artificial Sea Water broths) and ASPA-8A controls showed minimal changes in the percentage of the Quantum Yield (%QY) during the 12 minute experiments. Results showed are the average of the %QY of the replicas (n=3) ± the standard deviation. In general, water controls from zooxanthellae recently isolated from corals (from the Mediterranean, Red, and Caribbean seas) varied between an average of 92.45% ± 0.60 (*P. damicornis* 6 min. into the experiments) and 105.10% ± 1.32 (*A. euristema* 12 min. into the experiment) from initial values (Supplemental Tables 1, 2, and 3).

Marine broth and Artificial Sea Water broth behaved similar to water controls. Average values of %QY of these controls oscillated between 92.36% ± 1.60 (*M. faveolata* 10 min. into the experiment) and 103.35 ± 0.68% (*M. alcicornis* 12 min. into the experiment) for recently isolated coral symbiots and between 90.26% ± 2.17 (135 10 min) and 97.47% ± 0.90 (*S. pulchlorum* 2 min. into the experiment) for cultured zooxanthellae. ASPA-8A controls (performed only for symbiots in culture) showed a larger variation in the %QY, oscillating between 101.12% ± 0.31 (*S. microadriaticum* after 2 min.) and 88.99% ± 0.85 (*S. kawagutti* after 8 min.) from the initial values.

Changes in the %QY were more noticeable when 12.5 mmol (final concentration) of NH_4Cl were added to the controls. In the case of *O. patagonica*, a reduction of more than 22% (%QY = 77.65% ± 3.45) were detected only 4 minutes after the beginning of the experiment (2 min. after adding NH_4Cl). *P. damicornis* showed an even larger decrease, with a %QY falling almost 30% (71.03% ± 5.25) after 12 minutes.

In some cases, reduction in the %QY was larger in controls with broth and ammonia than those only with ammonia. This is the case of *M. annularis* and *M. faveolata*, where %QY felt to 65.99% ± 2.56 and 78.02% ± 2.99 when exposed to broth plus 12.5 mmol NH_4Cl, compared to only 88.97% ± 0.95 and 87.56 ± 1.10 with sea water and ammonium chloride respectively.

The symbiots most affected by the action of the crude toxin were extracted from *O. patagonica*. The %QY was reduced by more than 60% (%QY = 39.53 ± 6.86) after only 8 minutes into the experiment (4 minutes after adding the toxin). This was a reduction of almost 40% compared with the NH_4Cl control (Fig. 1). Similarly, *A. eurystema* extracts showed decreases of more than 45% in the %QY after the addition of the crude toxin. %QY in this species was 54.82% ± 1.16 after 12 min. (8 minutes after the addition of the crude toxin). This was a reduction of about 45% of the initial QY and about 27% lower than the NH_4Cl control (Supplemental Table 1, Fig. 2a). *P. damicornis* extracts also showed a reduction in the %QY of about 32% (%QY = 67.22 ± 6.14) after adding the crude toxin. However, this reduction is very similar to that obtained with the ammonium control (%QY = 71.03 ± 5.25) (Supplemental Table 1, Fig 2a).

Extracted zooxanthellae from *M. annularis*, *M. faveloata*, and *M. alcicornis* when treated with the bacterial toxin showed reductions in the %QY significantly lower than the ammonium control. With *M. annularis*, the average value of the NH_4Cl control after 12 minutes was 89.91% ± 1.36, and with the toxin after the same period was 73.20% ± 4.55, a difference of more than 16%. *M. faveolata* also showed a %QY after 12 minutes of 83.42% ± 1.20, more than 5% below the ammonium control (87.56% ± 1.10). However, the Artificial Sea Water broth plus 12.5 mmol NH_4Cl control showed a fall in the %QY to 67.76% ± 4.05 after the same period of time in *M. annularis* and to 79.83% ± 0.60 in *M. faveolata*. *M. alcicornis* symbiots reduced their %QY from 96.90% ± 0.99 with the Artificial Sea Water broth plus 12.5 mmol NH_4Cl control to 91.33% ± 1.40% when exposed to the crude toxin (Supplemental Table 2, Fig 2b). *P. astreoides* extracts did not show changes after being exposed to the toxin. Its %QY remained closed to 100% (98.99% ± 1.41) during the time of incubation. Similarly, the %QY did not change significantly with the controls (Supplemental Table 2, Fig. 2b).

None of the *Symbiodinium* species maintained in cultured and tested during this experiment, showed significant decrease in QY following their exposure to the crude toxin. Slight decreases in the QY were found in the ammonium control trials (79.55% ± 0.64 in *S. pulchlorum*, 86.78 ± 0.88 in *S. pillosum*, 78.81 ± 1.37 in *S. kawagutii*, 86.08 ± 0.97 in *S. microadriaticum*, and 84.99 ± 0.65 in *Symbiodinium sp.*), but with *Symbiodinium sp*. this decrease was not significant compared with controls without ammonium (Supplemental Table 3, Fig. 2c).

Discussion

Figure 1 shows a significant decrease of the QY of zooxanthellae extracted from *O. patagonica* after exposure to the supernatant from *V. shilonii*. This decrease is comparable to the one obtained

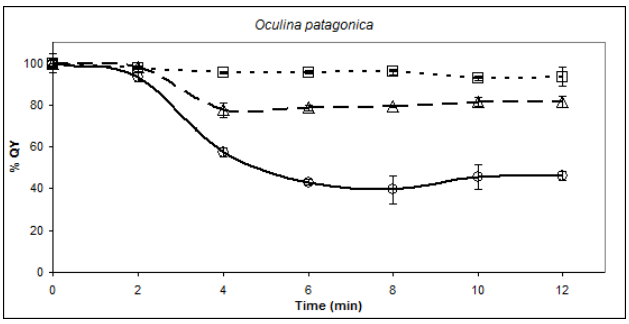

Figure 1 Decrease in the percentage of Quantum Yield (%QY) of zooxanthellae extracted from the Mediterranean coral *Oculina patagonica* after exposure to: crude toxin (—⊕—), NH_4Cl control (-△-), and seawater control (- ▣ - -). Error bars are standard deviations. N=3

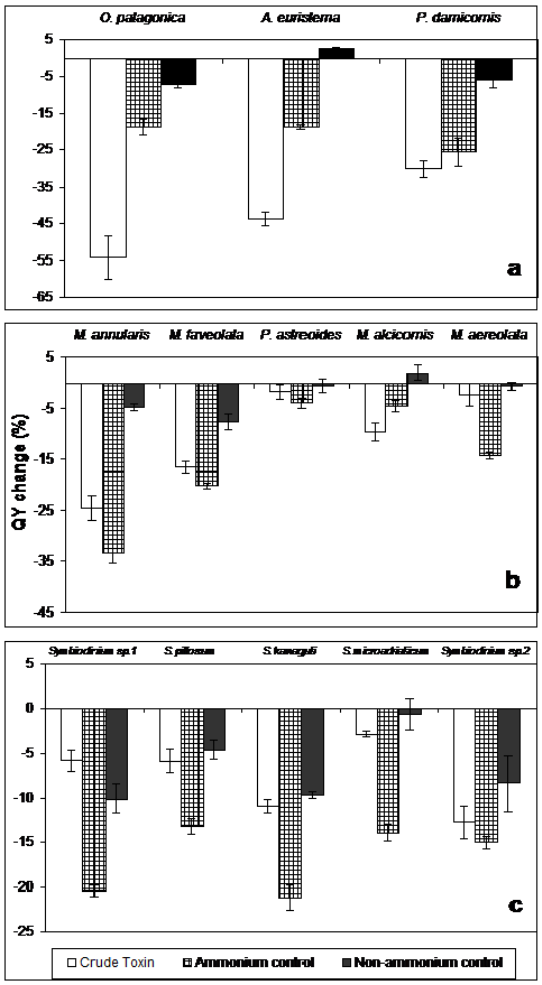

Figure 2. Changes in the Quantum Yield of zooxanthellae from extracted from corals of the Mediterranean and Red Seas (a), Caribbean Sea (b) and cultured zooxanthellae (c).

by Ben-Haim et al. (1999) and by Banin et al. (2001) after exposing the zooxanthellae extracted from this coral to the crude and synthetic toxin respectively. This indicates the presence of a high concentration of toxin P in the supernatant (crude toxin) after the bacteria is incubated under the described conditions. The difference observed between the supernatant and the ammonium chloride control indicates that the drastic reduction of the QY was the product of the toxin and not only the effect of the NH_4Cl.

Results similar to the ones found in *O. patagonica* were obtained in trials using *A. euristema* (Fig. 2a). A significant reduction of the QY followed the addition of ammonium chlorine, as well as the addition of crude toxin. Nevertheless, this reduction was smaller that the one observed in *O. patagonica*. The QY of the zooxanthellae of this species dropped to a QY of approximately 0.35, while the symbiots of *O. patagonica* declined close to or to less than 0.2. Ben-Haim et al. (1999) also observed significant decreases in the percentage of QY in other Acroporid species of the Red Sea (*A. scandens* and *A. humilis*) after exposure to the supernatant.

Fig 2a also shows a decrease of the QY of about 20% after adding NH_4Cl to the symbiots of *P. damicornis*. Using *V. shilonii* supernatant, a reduction of the QY was also evident, but the reduction was noticeably lower than the one observed with symbiots from other species. Furthermore, the reduction was just slightly greater to that observed after adding only NH_4Cl, suggesting that there was no significant difference between either treatments for this species. This indicated a smaller effect of the toxin on these dinoflagelates, suggesting that the toxin had less effect on the symbiots from this species and the change in QY was due to differences in ammonia concentration.

Figure (2b) shows that freshly isolated dinoflagellates from *M. annularis*, *M. faveolata*, *M. aereolata* and *Porites astreoides* did not respond to the toxin over the controls. The hydrozoan *Millepora alcicornis* did show a significant response to the supernatant from *V. shilonii*. This shows a differential response to the toxin among Caribbean species.

Cultured zooxanthellae did not show a significant decrease in the QY with crude toxin treatment over the effect of ammonia alone (Supplemental Table 3, Fig 2c). The effects of ammonia were also less drastic than in freshly isolated species. Lower effects are attributed to changes in algae structures occurred during cultivation, in particular the increase in the thickness of the cell wall which may help in the resistance of the zooxanthellae to ammonia and to the toxin.

The increase in world trade has resulted greater releases of ballast water in all oceans (Carlton and Geller, 1993, Smith et al., 1999). It is known that ballast waters carry numerous species of plants, animals, and bacteria, some of them harmful (Grosholz, 1996). Some are able to establish themselves in new environments. This, in fact, is the case of *O. patagonica*, originally from the Southwest Atlantic that was introduced to the Mediterranean around 1966 (Fine et al, 2001). It is not unreasonable to think that *V. shilonii* has been transported to other areas, including the Caribbean Sea, and that it can be responsible for bacterial bleaching in corals from other latitudes.

Zooxanthellae from different coral species showed a differential response to the toxin P produced by *V. shilonii*. In general, zooxanthellae isolated from Mediterranean and Red Sea corals were more susceptible to the crude toxin that those of Caribbean origin. Differential responses to ammonium was also observed during these experiments. These differential responses of zooxanthellae (both to the toxin and to ammonium) are interesting topics that should be investigated in greater depth, together with the differences in resistance to bleaching by different zooxanthellae clades.

In summary, *V. shilonii* is capable of bleaching zooxanthellae from several coral species, depending on their compliment of clades. Therefore, *V. shilonii* may cause bleaching in coral

species other than *O. patagonica*. It is also possible that a number of other bacteria produce compounds that cause bleaching in various species either in concert or alone, as is the case of *Vibrio coralliilyticus* that affects *P. damicornis* in the Indian Ocean and the Red Sea. The increase in world trade has resulted greater releases of ballast water in all oceans (Carlton and Geller, 1993, Smith et al., 1999). It is known that ballast waters carry numerous species of plants, animals, and bacteria, some of them harmful (Grosholz, 1996). Some are able to establish themselves in new environments. This, in fact, is the case of *O. patagonica*, originally from the Southwest Atlantic that was introduced to the Mediterranean around 1966 (Fine et al, 2001). It is not unreasonable to think that *V. shilonii* has been transported to other areas, including the Caribbean Sea, and that it can be responsible for bacterial bleaching in corals from other latitudes.

Acknowledgements

This work was supported by NSF OCE0326269, NOAA CRES05000038 and the Coral Disease and Coral Bleaching Working Groups of the World Bank Targeted Research Fund through GEF. Additional funds were also provided by the Emerging Diseases Program of the University of Tel-Aviv. Authors also want to acknowledge Dr. Roberto Iglesias-Prieto and Dr. Xavier Hernandez-Pech from the Puerto Morelos' field station of the UNAM for their assistance and support.

Notes and References

**Corresponding author E-mail: dlgil@uscb.edu*

Banin, E., Khare, S.K., Naider, F., and Rosenberg, E. 2001. Proline-rich peptide from the coral pathogen *Vibrio shilonii* that inhibits photosynthesis of zooxanthellae. Appl. Environ. Microbiol. 67(4): 1536-1541

Ben-Haim, Y., Banin, E., Kushmaro, A., Loya, Y., and Rosenberg, E. 1999. Inhibition of photosynthesis and bleaching of zooxanthellae by the coral pathogen *Vibrio shilonii*. Environ. Microbiol. 1(3): 223-229

Ben-Haim, Y., Thompson, F.L., Thompson, C.C., Cnockaert, M.C., Hoste, B., Swings, J., and Rosenberg, E. 2003. *Vibrio coralliilyticus* sp. nov., a temperature-dependent pathogen of the coral *Pocillopora damicornis*. International Journal of Systematic and Evolutionary Microbiology. 53:309-315

Brown, B.C. 1997. Coral bleaching: causes and consequences. Proc. 8th Int. Coral Reef Symp. 1: 65-74

Carlton J.T. and Geller J.B. 1993. Ecological roulette: biological invasions and the global transport of nonindigenous marine organisms. Science 261: 78–82

Fine, M., Zibrowius, H., and Loya, Y. 2001. Oculina patagonica: a non-lessepsian scleractinian coral invading the Mediterranean Sea. Mar. Biol. 138: 1195-1203

Glynn, P.W. 1991a. Coral reef bleaching I n the 1980s and possible connections with global warming. Trends Ecol. Evol. 6: 175-179

Glynn, P.W. 1991b. Elimination of two reef building hydrocorals following the 1982-83 El-niño warming event. Science 253: 69-71

Glynn, P.W. 1993. Coral reef bleaching: Ecological perspectives. Coral Reefs 12 (1): 1-17

Grosholz, E.D. 1996. Contrasting rates of spread for introduced species in terrestrial and marine systems. Ecology 77(6): 1680-1686

Hoegh-Guldberg, O. 1999. Climate change, coral bleaching and the future of the world's coral reefs. Aust. J. Freshwater Mar. Biol. 50:839-866

Iglesias-Prieto R., Matta J.L., Robins W.A. Trench

Kushmaro, A., Loya, Y., Fine, M., and Rosenberg, E. 1996. Bacterial infection and coral bleaching. Nature 380: 396

Kushmaro, A., Rosenberg, E., Fine, M., and Loya, Y. 1997. Bleaching of the coral *Oculina patagonica* by *Vibrio* AK-1. Mar. Ecol. Prog. Ser. 147: 159-165

Kushmaro, A., Banin, E., Stackebrandt, E., and Rosenberg, E. 2001. Vibrio shilonii sp. nov.: the causative agent of bleaching of the coral *Oculina patagonica*. Int. J. Syst. Evolut. Microbiol. 51(4): 1383-1388

Smith, G.W. & Hayasaka, S.S. 1982. Nitrogenase activity associated with Halodule wrightii Roots. Appl. Environ. Microbiol. 143 (6): 1244 - 1248.

Smith, LD; Wonham, MJ; McCann, LD; Ruiz, GM; Hines, AH; Carlton, JT. 1999. Invasion pressure to a ballast-flooded estuary and an assessment of inoculant survival. Biological Invasions [Biol. Invasions], vol. 1, no. 1, pp. 67-87

Supplemental Table 1. Quantum Yield response to different treatments in Mediterranean corals.

Coral Species	Treatment	Time (min)	Quantum Yield		
			Mean	% QY relative to t = 0	S.D.
Oculina patagonica	**Seawater**	0	0.497	100.0	0.010
		2	0.486	97.9	0.004
		4	0.473	95.3	0.002
		6	0.475	95.6	0.004
		8	0.476	95.9	0.007
		10	0.461	92.9	0.005
		12	0.465	93.6	0.022
	NH_4Cl 12.5 mMol	0	0.479	100.0	0.022
		2	0.470	98.3	0.010
		4	0.372	77.6	0.017
		6	0.377	78.8	0.006
		8	0.381	79.5	0.003
		10	0.389	81.3	0.010
		12	0.391	81.8	0.013
	Crude toxin	0	0.479	100.0	0.006
		2	0.448	93.5	0.010
		4	0.275	57.4	0.010
		6	0.206	43.1	0.007
		8	0.189	39.5	0.033
		10	0.219	45.7	0.028
		12	0.221	46.1	0.010
Acropora euristema	**Seawater**	0	0.647	100.0	0.005
		2	0.652	100.8	0.034
		4	0.660	102.0	0.006
		6	0.664	102.6	0.008
		8	0.656	101.4	0.010
		10	0.664	102.6	0.001
		12	0.680	105.1	0.009
	NH_4Cl 12.5 mMol	0	0.659	100.0	0.008
		2	0.611	92.8	0.015
		4	0.544	82.6	0.012
		6	0.546	82.9	0.019
		8	0.551	83.6	0.011
		10	0.535	81.2	0.004
		12	0.540	81.9	0.011
	Crude toxin	0	0.623	100.0	0.015
		2	0.567	91.0	0.024
		4	0.429	68.8	0.007
		6	0.393	63.1	0.011
		8	0.376	60.3	0.033
		10	0.351	56.3	0.012
		12	0.341	54.8	0.007

Supplemental Table 1 (Continued). Quantum Yield response to different treatments in Mediterranean corals.

Coral Species	Treatment	Time (min)	Quantum Yield		
			Mean	% QY relative to t = 0	S.D.
Pocillopora damicornis	**Seawater**	0	0.631	100.0	0.005
		2	0.658	104.3	0.006
		4	0.655	103.8	0.041
		6	0.583	92.4	0.004
		8	0.587	93.0	0.016
		10	0.594	94.2	0.013
		12	0.599	95.0	0.010
	NH_4Cl 12.5 mMol	0	0.640	100.0	0.002
		2	0.607	94.9	0.009
		4	0.490	76.6	0.004
		6	0.478	74.7	0.006
		8	0.487	76.1	0.004
		10	0.477	74.5	0.023
		12	0.454	71.0	0.034
	Crude toxin	0	0.625	100.0	0.003
		2	0.583	93.2	0.006
		4	0.471	75.4	0.012
		6	0.454	72.7	0.013
		8	0.453	72.4	0.017
		10	0.438	70.0	0.015
		12	0.420	67.2	0.038
	Marine broth	0	0.602	100.0	0.001
		2	0.613	101.8	0.001
		4	0.615	102.1	0.006
		6	0.613	101.8	0.009
		8	0.609	101.1	0.013
		10	0.599	99.4	0.014
		12	0.606	100.7	0.003
	Marine broth + 12.5 mMol NH_4Cl	0	0.640	100.0	0.002
		2	0.607	94.9	0.009
		4	0.490	76.6	0.004
		6	0.478	74.7	0.006
		8	0.487	76.1	0.004
		10	0.477	74.5	0.023
		12	0.454	71.0	0.034

Supplemental Table 2. Quantum Yield response to different treatments in Caribbean corals.

Coral Species	Treatment	Time (min)	Quantum Yield		
			Mean	% QY relative to t = 0	S.D.
Montastraea annularis	**Seawater**	0	0.543	100.0	0.009
		2	0.533	98.2	0.005
		4	0.529	97.4	0.002
		6	0.529	97.4	0.006
		8	0.531	97.7	0.003
		10	0.531	97.7	0.006
		12	0.531	97.8	0.003
	NH_4Cl 12.5 mMol	0	0.532	100.0	0.001
		2	0.526	98.9	0.002
		4	0.504	94.7	0.005
		6	0.473	89.0	0.005
		8	0.474	89.0	0.007
		10	0.474	89.1	0.003
		12	0.478	89.9	0.007
	Crude toxin	0	0.552	100.0	0.028
		2	0.494	89.4	0.018
		4	0.432	78.2	0.016
		6	0.418	75.6	0.018
		8	0.424	76.7	0.020
		10	0.417	75.5	0.013
		12	0.404	73.2	0.025
	ASW broth	0	0.533	100.0	0.005
		2	0.520	97.6	0.004
		4	0.518	97.2	0.002
		6	0.509	95.5	0.005
		8	0.510	95.7	0.002
		10	0.507	95.1	0.004
		12	0.501	94.1	0.010
	ASW broth + 12.5 mMol NH_4Cl	0	0.527	100.0	0.001
		2	0.507	96.2	0.009
		4	0.373	70.8	0.013
		6	0.356	67.5	0.010
		8	0.348	66.0	0.014
		10	0.351	66.5	0.010
		12	0.357	67.8	0.021
Montastraea faveolata	**Seawater**	0	0.591	100.0	0.003
		2	0.587	99.3	0.008
		4	0.578	97.8	0.007
		6	0.576	97.4	0.003
		8	0.569	96.3	0.008
		10	0.567	95.9	0.006

Supplemental Table 2 (Continued). Quantum Yield response to different treatments in Caribbean corals.

Coral Species	Treatment	Time (min)	Quantum Yield		
			Mean	% QY relative to t = 0	S.D.
Montastraea faveolata	**NH_4Cl 12.5 mMol**	0	0.606	100.0	0.006
		2	0.582	96.0	0.007
		4	0.561	92.7	0.025
		6	0.538	88.8	0.009
		8	0.536	88.5	0.009
		10	0.530	87.6	0.007
	Crude toxin	0	0.607	100.0	0.007
		2	0.579	95.4	0.008
		4	0.541	89.2	0.006
		6	0.519	85.4	0.005
		8	0.513	84.5	0.002
		10	0.506	83.4	0.007
	ASW broth	0	0.607	100.0	0.005
		2	0.591	97.4	0.006
		4	0.577	95.1	0.006
		6	0.565	93.1	0.006
		8	0.562	92.7	0.001
		10	0.560	92.4	0.010
	ASW broth + 12.5 mMol NH_4Cl	0	0.605	100.0	0.005
		2	0.525	86.8	0.018
		4	0.472	78.0	0.018
		10	0.483	79.8	0.004
Porites astreoides	**Seawater**	0	0.618	100.0	0.006
		2	0.616	99.6	0.007
		4	0.610	98.7	0.007
		6	0.608	98.4	0.004
		8	0.602	97.3	0.004
		10	0.603	97.5	0.003
	NH4Cl 12.5 mMol	0	0.614	100.0	0.004
		2	0.607	98.9	0.005
		4	0.601	97.9	0.003
		6	0.593	96.7	0.003
		8	0.602	98.2	0.010
		10	0.589	96.0	0.006
	Crude toxin	0	0.604	100.0	0.009
		2	0.616	102.0	0.004
		4	0.612	101.3	0.006
		6	0.604	100.1	0.003
		8	0.594	98.4	0.008
		10	0.593	98.3	0.009

Supplemental Table 2 (Continued). Quantum Yield response to different treatments in Caribbean corals.

Coral Species	Treatment	Time (min)	Quantum Yield		
			Mean	% QY relative to t = 0	S.D.
Porites astreoides	**ASW broth**	0	0.613	100.0	0.007
		2	0.614	100.2	0.003
		4	0.612	99.9	0.006
		6	0.614	100.2	0.008
		8	0.612	99.9	0.005
		10	0.610	99.5	0.008

Supplemental Table 3: Quantum Yield response to different treatments in cultured zooxanthellae.

Coral Species	Treatment	Time (min)	Quantum Yield		
			Mean	% QY relative to t = 0	S.D.
Symbiodinium pulchlorum	**Crude toxin**	0	0.643	100.000	0.006
		2	0.628	97.718	0.012
		4	0.613	95.332	0.007
		6	0.611	95.021	0.008
		8	0.609	94.761	0.006
		10	0.605	94.139	0.008
	ASPA	0	0.645	100.000	0.001
		2	0.632	97.934	0.006
		4	0.624	96.746	0.004
		6	0.619	95.971	0.003
		8	0.610	94.576	0.003
		10	0.580	89.928	0.010
	ASPA + 12.5 mMol NH_4Cl	0	0.628	100.000	0.003
		2	0.563	89.644	0.014
		4	0.534	85.077	0.016
		6	0.525	83.643	0.017
		8	0.524	83.537	0.005
		10	0.499	79.554	0.004
	ASW broth	0	0.634	100.000	0.004
		2	0.618	97.478	0.006
		4	0.618	97.478	0.002
		6	0.614	96.795	0.013
		8	0.607	95.691	0.014
		10	0.590	93.011	0.013
Symbiodinium pillosum	**Crude toxin**	0	0.697	100.000	0.001
		2	0.679	97.323	0.006
		4	0.671	96.176	0.005
		6	0.670	96.080	0.005
		8	0.665	95.411	0.004
		10	0.656	94.120	0.009
	ASPA	0	0.704	100.000	0.009
		2	0.685	97.255	0.003
		4	0.678	96.261	0.003
		6	0.674	95.693	0.006
		8	0.674	95.741	0.007
		10	0.672	95.362	0.008
	ASPA + 12.5 mMol NH4Cl	0	0.691	100.000	0.006
		2	0.621	89.870	0.006
		4	0.618	89.387	0.005
		6	0.610	88.326	0.002
		8	0.609	88.085	0.002
		10	0.600	86.782	0.006

Supplemental Table 3 (continued). Quantum Yield response to different treatments in cultured zooxanthellae.

Coral Species	Treatment	Time (min)	Quantum Yield		
			Mean	% QY relative to t = 0	S.D.
Symbiodinium pillosum	**ASW broth**	0	0.701	100.000	0.002
		2	0.684	97.481	0.005
		4	0.678	96.673	0.003
		6	0.673	96.008	0.005
		8	0.667	95.152	0.005
		10	0.666	94.962	0.004
	ASW broth + 12.5mMol NH_4Cl	0	0.706	100.000	0.011
		2	0.661	93.718	0.005
		4	0.656	92.915	0.006
		6	0.649	91.970	0.007
		8	0.643	91.120	0.004
		10	0.641	90.789	0.002
Symbiodinium kawaguti	**Crude toxin**	0	0.617	100.000	0.007
		2	0.579	93.787	0.006
		4	0.562	91.140	0.004
		6	0.557	90.222	0.003
		8	0.557	90.222	0.003
		10	0.550	89.141	0.005
	ASPA	0	0.611	100.000	0.010
		2	0.587	96.020	0.005
		4	0.578	94.547	0.005
		6	0.570	93.293	0.005
		8	0.544	88.986	0.005
		10	0.553	90.403	0.003
	ASPA + 12.5 mMol NH_4Cl	0	0.620	100.000	0.006
		2	0.506	81.711	0.035
		4	0.467	75.417	0.017
		6	0.484	78.053	0.010
		8	0.487	78.644	0.004
		10	0.488	78.806	0.009
	ASW broth	0	0.630	100.000	0.003
		2	0.598	94.974	0.006
		4	0.590	93.704	0.009
		6	0.581	92.222	0.004
		8	0.580	92.063	0.007
		10	0.569	90.265	0.014
	ASW broth + 12.5mMol NH_4Cl	0	0.628	100.000	0.006
		2	0.520	82.803	0.011
		4	0.522	83.068	0.008
		6	0.531	84.501	0.008
		8	0.521	82.962	0.008
		10	0.525	83.599	0.008

Supplemental Table 3 (continued). Quantum Yield response to different treatments in cultured zooxanthellae.

Coral Species	Treatment	Time (min)	Quantum Yield Mean	Quantum Yield % QY relative to t = 0	Quantum Yield S.D.
Symbiodinium microadriaticum	**Crude toxin**	0	0.546	100.000	0.006
		2	0.540	98.962	0.006
		4	0.530	97.129	0.007
		6	0.531	97.251	0.005
		8	0.523	95.907	0.006
		10	0.530	97.129	0.002
	ASPA	0	0.568	100.000	0.011
		2	0.574	101.116	0.002
		4	0.568	100.000	0.006
		6	0.563	99.119	0.006
		8	0.563	99.178	0.008
		10	0.564	99.413	0.010
	ASPA + 12.5 mMol NH_4Cl	0	0.568	100.000	0.011
		2	0.536	94.363	0.009
		4	0.519	91.368	0.007
		6	0.506	89.196	0.010
		8	0.505	88.902	0.012
		10	0.489	86.083	0.006
	ASW broth	0	0.560	100.000	0.006
		2	0.554	98.929	0.008
		4	0.544	97.143	0.006
		6	0.545	97.262	0.014
		8	0.533	95.179	0.012
		10	0.545	97.321	0.007
	ASW broth + 12.5mMol NH_4Cl	0	0.558	100.000	0.009
		2	0.544	97.551	0.010
		4	0.541	96.894	0.015
		6	0.529	94.863	0.013
		8	0.540	96.834	0.013
		10	0.535	95.878	0.009
Symbiodinium sp.	**Crude toxin**	0	0.566	100.000	0.000
		2	0.523	92.344	0.006
		4	0.507	89.517	0.006
		6	0.514	90.813	0.024
		8	0.494	87.338	0.001
		10	0.494	87.279	0.010
	ASPA	0	0.553	100.000	0.012
		2	0.524	94.813	0.017
		4	0.509	92.159	0.006
		6	0.506	91.496	0.008
		8	0.500	90.410	0.013
		10	0.506	91.616	0.017

Supplemental Table 3 (continued). Quantum Yield response to different treatments in cultured zooxanthellae.

Coral Species	Treatment	Time (min)	Quantum Yield Mean	Quantum Yield % QY relative to t = 0	Quantum Yield S.D.
Symbiodinium sp.	**ASPA + 12.5 mMol NH_4Cl**	0	0.553	100.000	0.008
		2	0.505	91.380	0.004
		4	0.487	88.005	0.002
		6	0.476	86.076	0.007
		8	0.480	86.799	0.009
		10	0.470	84.991	0.004
	ASW broth	0	0.542	100.000	0.007
		2	0.502	92.563	0.007
		4	0.491	90.473	0.006
		6	0.476	87.830	0.006
		8	0.480	88.506	0.009
		10	0.470	86.663	0.004
	ASW broth + 12.5mMol NH_4Cl	0	0.567	100.000	0.016
		2	0.534	94.180	0.019
		4	0.524	92.357	0.012
		6	0.510	89.947	0.026
		8	0.516	91.005	0.014
		10	0.497	87.713	0.003