The role of floridoside in osmoadaptation of coral-associated algal endosymbionts to high-salinity conditions

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The endosymbiosis between Symbiodinium dinoflagellates and stony corals provides the foundation of coral reef ecosystems. The survival of these ecosystems is under threat at a global scale, and better knowledge is needed to conceive strategies for mitigating future reef loss. Environmental disturbance imposing temperature, salinity, and nutrient stress can lead to the loss of the Symbiodinium partner, causing so-called coral bleaching. Some of the most thermotolerant coral-Symbiodinium associations occur in the Persian/Arabian Gulf and the Red Sea, which also represent the most saline coral habitats. We studied whether Symbiodinium alter their metabolite content in response to high-salinity environments. We found that Symbiodinium cells exposed to high salinity produced high levels of the osmolyte 2-O-glycerol-α-D-galactopyranoside (floridoside), both in vitro and in their coral host animals, thereby increasing their capacity and, putatively, the capacity of the holobiont to cope with the effects of osmotic stress in extreme environments. Given that floridoside has been previously shown to also act as an antioxidant, this osmolyte may serve a dual function: first, to serve as a compatible organic osmolyte accumulated by Symbiodinium in response to elevated salinities and, second, to counter reactive oxygen species produced as a consequence of potential salinity and heat stress.

INTRODUCTION

Coral reefs are biodiversity hot spots of immense biological and economical value (1). The relationship between Symbiodinium—an autotrophic endosymbiotic dinoflagellate—and scleractinian corals form the basis of coral reef ecosystems (2). The coral host provides inorganic nutrients and carbon dioxide to the dinoflagellate in exchange for energy in the form of photosynthetically produced carbohydrates (3, 4). This symbiotic relationship is highly sensitive to environmental disturbance. For instance, increases in temperature, salinity, nutrients, and/or high solar irradiance can impair photosynthetic efficiency and enhance the formation of harmful reactive oxygen species (ROS) that must be detoxified by the organism via antioxidants and ROS scavengers (5–8). Otherwise, environmental stress can ultimately lead to coral bleaching, the visual whitening of corals due to loss of their endosymbionts (5, 8). As a consequence, increasing exposure to environmental stress, in particular to rising seawater temperatures, is threatening the existence of coral reefs at a global scale (9, 10).

In comparison to the detrimental effects of elevated seawater temperatures, much less is known about the effects of increased salinities on corals and their endosymbionts. Yet, reef corals from the Red Sea and the Persian/Arabian Gulf (PAG) are commonly found at salinities of up to 41 [Practical Salinity Scale 1978 (PSS-78)] in the Red Sea and up to 50 in the PAG, at summer temperatures exceeding 32° and 35°C in parts of the Red Sea and PAG, respectively (11–14). Both salinity and temperature in these regions are the highest globally to support reef growth (15). Although the osmotic response of Symbiodinium at the molecular level is virtually unknown (16–18), studies on free-living algae suggest that production and accumulation of compatible organic osmolytes (COOs), referred to as osmoadaptation (19), is the most widespread mechanism for adjusting intracellular osmotic pressure in response to elevated salinity; accumulation of inorganic ions and cell volume changes may occur as well. However, because the latter two processes are stressful and can disturb cellular function, they are not considered to represent viable long-term solutions for osmoadaptation (20–23). In contrast, the accumulation of COOs adjusts the osmotic pressure and protects proteins from increased ion concentrations (22).

To test whether Symbiodinium is capable of synthesizing COOs, we subjected Symbiodinium strains from different clades and origins to conditions of high salinity, both in vitro and in their coral hosts (hereafter referred to as in hospite), and screened for the presence of COOs using gas chromatography–mass spectrometry (GC-MS). We hypothesized that Symbiodinium would increase cellular concentrations of COOs in response to elevated salinities and that the response would be similar in vitro and in hospite. Among the COOs employed by Symbiodinium, we identified the carbohydrates floridoside, inositol, and mannitol in vitro and in hospite. These compounds can act as both osmolytes and antioxidants, thereby having the potential capacity to convey osmoadaptation to increased salinities and the ability to counter ROS produced as a consequence of salinity or other forms of stress, including heat stress (6, 8, 24–30).

RESULTS

High levels of floridoside in Symbiodinium exposed to high salinities

We screened Symbiodinium cultures exposed to different salinities (that is, 25 for low salinity, 38 for ambient salinity, and 55 for high salinity) for the presence of carbohydrate COOs. A markedly high abundance of a compound at 31.5-min retention time in the GC-MS trace was detected in all four tested Symbiodinium cultures under high-salinity conditions (that is, Symbiodinium microadriaticum type A1,
Symbiodinium sp. type A1, Symbiodinium minutum type B1, and Symbiodinium psygmophilum type B2) (Fig. 1 and Table 1). This compound was identified as the derivative of floridoside \(2-O\)-glycerol-\(\alpha\)-D-galactopyranoside-(hexa-trimethylsilane) by a search against the National Institute of Standards and Technology Mass Spectrometry (NIST MS) library with a reverse match factor of 971 of 1000 (table S1). Floridoside levels of Symbiodinium strains exposed to high salinity ranged from \(50.3 \pm 4.3\) nmol (\(S.\) minutum) to \(489.5 \pm 31.9\) nmol (\(S.\) psygmophilum) and were consistently represented among the most abundant carbohydrates quantified in this analysis (Table 1). In contrast, floridoside was nondetectable under low-salinity conditions for all Symbiodinium strains (Fig. 2A and Table 1). At an ambient salinity level of 38, floridoside was only detected in \(S.\) psygmophilum at a level of \(51.0 \pm 8.0\) nmol (Fig. 2A and Table 1). This strain also accounted for the highest measured amounts of floridoside under a high-salinity level of 55 (\(489.5 \pm 31.9\) nmol). In comparison, inositol and mannitol were consistently present at low salinities and showed reduced levels at higher salinities for some strains (Table 1).

We identified several other metabolites in the same GC-MS trace and could quantify a total of five additional carbohydrates (that is, glyceraldehyde, glucose, galactose, ribose, and fructose) and four amino acids (that is, glycine, alanine, valine, and proline) that serve as putative osmolytes for each of our samples. Metabolite levels were significantly different

Fig. 1. GC analysis of carbohydrate osmolyte diversity in \(S.\) psygmophilum at three salinity levels. Green, low-salinity level of 25; black, ambient-salinity level of 38; red, high-salinity level of 55. The trace from 26- to 32-min retention time (RT) shows a floridoside peak at 31.5-min RT. The abundances are normalized to an internal standard (ISTD). The chemical reaction depicts synthesis of floridoside (3) as a product of uridine diphosphate (UDP)-galactose (1) and glycerol 3-phosphate (2). Notably, nearly identical traces were obtained for the other three Symbiodinium strains. Gal/gl, galactose/gluose.

Table 1. COO levels at three salinities of four Symbiodinium strains. Carbohydrates (floridoside, inositol, mannitol, glucose, galactose, ribose, and fructose) and amino acids (glycine, alanine, valine, and proline) were quantified by GC-MS. COO levels are provided in nanomoles and normalized to the GC-MS internal standard hydroxy benzylic acid (HBA) and \(1 \times 10^5\) cells ml\(^{-1}\) (that is, concentration (Conc.) is in nanomoles per \(10^5\) cells or in nanomoles per milliliter culture). \(S.\) microadriaticum, type A1; \(S.\) minutum, type B1; \(S.\) psygmophilum, type B2.

| Metabolite          | Salinity | \(S.\) microadriaticum | S. minutum type A1 | S. minutum | S. psygmophilum |
|---------------------|----------|------------------------|-------------------|------------|----------------|
|                     |          | Conc. | SE | Conc. | SE | Conc. | SE | Conc. | SE |
| Floridoside         | 25       | 0.0  | 0.0 | 0.0  | 0.0 | 0.0  | 0.0 | 0.0 | 0.0 |
|                     | 38       | 0.0  | 0.0 | 0.0  | 0.0 | 0.0  | 0.0 | 51.0 | 8.0 |
|                     | 55       | 144.2 | 15.0 | 273.8 | 28.5 | 50.3 | 4.3 | 489.5 | 31.9 |
| Inositol            | 25       | 0.7  | 0.1 | 0.4  | 0.1 | 1.2  | 0.1 | 51.5 | 8.1 |
|                     | 38       | 2.2  | 0.1 | 0.4  | 0.1 | 1.2  | 0.1 | 51.5 | 8.1 |
|                     | 55       | 14.3 | 1.5 | 2.6  | 0.3 | 35.2 | 3.0 | 111.1 | 7.2 |
| Mannitol            | 25       | 3.1  | 0.3 | 3.0  | 0.4 | 3.0  | 0.4 | 0.1  | 0.0 |
|                     | 38       | 3.7  | 0.2 | 2.5  | 0.2 | 7.6  | 0.5 | 1.8  | 0.3 |
|                     | 55       | 2.6  | 0.3 | 2.0  | 0.2 | 4.6  | 0.4 | 1.6  | 0.1 |
| Glycerol            | 25       | 59.5 | 6.2 | 92.9 | 11.7 | 25.7 | 3.4 | 29.1 | 3.6 |
|                     | 38       | 130.2 | 8.3 | 198.8 | 15.2 | 68.2 | 4.3 | 37.4 | 5.9 |
|                     | 55       | 107.0 | 11.1 | 37.2 | 3.9 | 6.9  | 0.6 | 10.9 | 0.7 |
| Glucose             | 25       | 85.3 | 8.9 | 181.0 | 22.7 | 27.3 | 3.6 | 50.8 | 6.4 |
|                     | 38       | 111.9 | 7.2 | 69.9  | 5.4 | 32.7 | 2.0 | 193.9 | 30.5 |
|                     | 55       | 152.8 | 15.9 | 232.8 | 24.2 | 25.0 | 2.1 | 429.4 | 27.9 |

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between different salinities (except proline) and *Symbiodinium* strains, as well as combinations thereof (all $P_{ANOVA} < 0.01$) (Table 2). This indicates that these metabolites are differentially regulated under changing salinities and in different *Symbiodinium* strains. Only the production of floridoside showed a substantial increase at high salinities, whereas the levels of all other metabolites (including mannitol and inositol) showed inconsistent patterns (Table 1).

Because floridoside can be derived from glycerol and glucose/galactose (31), we investigated changes in the abundance of these molecules in detail (Fig. 3). In all *Symbiodinium* strains, a decrease in glycerol coincided with the accumulation of floridoside when comparing low- to high-salinity conditions (Fig. 3 and Table 1). Notably, glucose and galactose were enriched under high-salinity conditions in *S. microadriaticum*, *Symbiodinium* sp. type A1, and *S. psygmophilum*; it was only in *S. minutum* that these sugars remained at the same level or showed a slight decrease between low- and high-salinity conditions (Fig. 3 and Table 1).

High levels of floridoside in *Symbiodinium* from coral holobionts exposed to high salinities

To assess the importance of floridoside in coral holobionts, we exposed the coral *Porites lobata* associated with *Symbiodinium thermophilum* originating from the southern PAG (11) to different salinities and measured floridoside levels. All samples exposed to high salinity displayed a substantial increase of floridoside (~6-fold increase) (Fig. 2B and table S2).

We then compared floridoside levels in *Symbiodinium* from coral holobionts that show different capacities to survive at high salinities. We found that corals that were actively growing and surviving at a salinity of 42 for $>24$ months (that is, *P. lobata* and *H. grandis*) had higher floridoside levels than *Porites lichen*, which is only capable of surviving for a short period of time at this salinity (Fig. 2C and table S2) (11).

We also checked for homologs of the putative enzyme that converts glycerol 3-phosphate to floridoside in the available genomes of the corals *Acropora digitifera* and *Stylophora pistillata* to assess whether coral hosts are, in principle, able to synthesize floridoside. Following the study of Pade *et al.* (31), we used the gene sequence that encodes the enzymatically active floridoside phosphate synthase/phosphatase from the red alga *Galdieria sulphuraria* (*Gasu_26940*) to search for homologs in coral. We found no homologs for the corals *A. digitifera* and *S. pistillata*. Conversely, we found putative full-length homologs of this gene in all available *Symbiodinium* genomes, that is, *S. microadriaticum* (Smic14738, Smic32192, and Smic6078), *S. minutum* (symbB.v1.2.003359, symbB.v1.2.013114, and symbB.v1.2.013196), and *Symbiodinium kawagutii* (Skav203497). Hence, the coral genomes investigated do not harbor the floridoside phosphate synthase/phosphatase enzyme required to produce floridoside.
Fig. 2. Floridoside levels of Symbiodinium in vitro and in hospite at different salinities. Floridoside levels represent measured amounts (in nanomoles) per 10^5 cells ml\(^{-1}\) for cultured Symbiodinium and per dry weight (in milligrams) of isolated Symbiodinium for coral samples. Data obtained for each experiment were normalized to the highest value (set to 100%). (A) Floridoside levels in four cultured Symbiodinium strains in vitro at low (25), ambient (38), and high salinities (55). Smic, S. microadriaticum (type A1); Sym A1, Symbiodinium sp. type A1; Smin, S. minutum (type B1); Spsy, S. psygmophilum (type B2). (B) Floridoside levels for S. thermophilum isolated from P. lobata cultured at salinities of 34 and 42. (C) Floridoside levels of Symbiodinium from corals with a different long-term survival capacity at high salinities after incubation at a salinity of 42. Floridoside levels were determined for Symbiodinium sp. type C96 (P. lichen), S. thermophilum (P. lobata), and Symbiodinium sp. type C40 (Hydnophora grandis). Error bars denote SE. Letters indicate Tukey’s HSD (honestly significant different) post hoc test differences based on pairwise comparisons of analysis of variance (ANOVA) results (groups with different letters are significantly different at \(P < 0.01\) for (A) and \(P < 0.05\) for (C)). Osmolyte levels represent measured amounts (in nanomoles) per 10^5 cells ml\(^{-1}\).

Table 2. Statistical evaluation of metabolite changes of carbohydrates and amino acids at three salinities across four Symbiodinium strains. Symbiodinium strain and salinity level are fixed factors, and strain*salinity serves as the interaction effect. Two-way ANOVA was used. Significance levels at \(P < 0.01\) are in boldface.

| Measured metabolite | Symbiodinium strain | Salinity | Strain*salinity |
|---------------------|---------------------|----------|----------------|
|                     | F       | P        | F       | P        | F       | P        |
| Floridoside         | 84.9329 | <0.0001  | 408.1242| <0.0001  | 61.0533 | <0.0001  |
| Inositol            | 199.4686| <0.0001  | 116.6508| <0.0001  | 37.0999 | <0.0001  |
| Mannitol            | 86.3291 | <0.0001  | 30.0316 | <0.0001  | 14.8469 | <0.0001  |
| Glycerol            | 97.7188 | <0.0001  | 92.3146 | <0.0001  | 22.3281 | <0.0001  |
| Glucose             | 74.5158 | <0.0001  | 66.2231 | <0.0001  | 32.1908 | <0.0001  |
| Galactose           | 77.2113 | <0.0001  | 31.7549 | <0.0001  | 7.7703  | <0.0001  |
| Ribose              | 45.7089 | <0.0001  | 47.2761 | <0.0001  | 33.2876 | <0.0001  |
| Fructose            | 6.0851  | 0.0031   | 23.1370 | <0.0001  | 52.0966 | <0.0001  |
| Glycine             | 55.2650 | <0.0001  | 282.0940| <0.0001  | 56.3923 | <0.0001  |
| Alanine             | 15.1440 | <0.0001  | 123.7890| <0.0001  | 97.8385 | <0.0001  |
| Valine              | 42.2583 | <0.0001  | 71.2012 | <0.0001  | 12.3394 | <0.0001  |
| Proline             | 23.6038 | <0.0001  | 2.8518  | 0.0774   | 57.3734 | <0.0001  |
DISCUSSION

Here, we determined levels of the carbohydrates floridoside, inositol, and mannitol in response to high salinity in *Symbiodinium* in vitro and in hospite to assess the capacity of these COOs to fulfill a function in osmoadaptation to high salinities. Notably, other osmolytes that were not measured in our study, for example, taurines, betaines, and dimethylsulfoniopropionate (32–34), might also contribute to the osmoadaptation of *Symbiodinium*. Consequently, our data should not be considered a complete assessment of all osmolytes in *Symbiodinium*.

However, we identified the osmolyte floridoside consistently and in increased amounts in *Symbiodinium* at high salinities. This shows that cultured *Symbiodinium* cells produce floridoside in response to salinity stress. We also found elevated floridoside levels in *Symbiodinium* of corals capable of long-term survival under high-salinity conditions (*P. lobata* and *H. grandis*). Hence, our work has uncovered a putative key COO that allows *Symbiodinium* to osmoadapt to extreme salinities in vitro and in hospite. The fact that our results show a consistent accumulation of floridoside across a range of *Symbiodinium* strains and experimental conditions provides strong support for the notion that increased floridoside levels constitute part of the osmoadaptive response to high salinities. Our findings also provide insight for our understanding of the role of osmoadaptation in the coral-*Symbiodinium* endosymbiosis, with implications for the coral stress response, as further discussed below.

**Floridoside as a key osmolyte in Symbiodinium**

Synthesis of the osmolyte floridoside has been identified as a conserved pathway in evolutionary distinct organisms, such as red algae, green algae, and Cryptophyceae (24, 31, 35). Floridoside is produced by UDP galactosyltransferases via condensation of glycerol 3-phosphate and UDP-galactose (31). UDP-galactose demands can be supplied via starch mobilization, resulting in increased glucose/galactose pools, as described for the green algae *Dunaliella* sp. under conditions of high salinity (36). A similar mechanism might explain the increased levels of glucose (in cultures of *S. microadriaticum* and *Symbiodinium* sp. type A1) and galactose (in all cultured strains) that we measured in *Symbiodinium* at high salinity (Fig. 3 and Table 1). The consistent

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**Fig. 3. Osmolyte levels of floridoside and intermediates (glycerol, glucose, and galactose) at three salinities across four *Symbiodinium* strains.** Glucose and galactose can be metabolized to glycerol (via the Calvin cycle) and cover UDP-galactose and glycerol 3-phosphate demands for floridoside synthesis. Bar graphs show floridoside, glycerol, glucose, and galactose levels for *S. microadriaticum* (type A1) (A), *Symbiodinium* sp. type A1 (B), *S. minutum* (type B1) (C), and *S. psygmophilum* (type B2) (D) cultures at low salinity (25; light gray), ambient salinity (38; gray), and high salinity (55; black) after 4 hours at 108 μmol photons m⁻² s⁻¹. Clade designation, origin of strain, and respective average salinity are provided following strain abbreviations. Error bars denote SE. Letters indicate Tukey’s HSD post hoc differences based on pairwise comparisons of ANOVA results (groups with different letters are significantly different at *P* < 0.05). Tukey’s post hoc tests were not performed if ANOVAs yielded a nonsignificant *F* ratio, designated as n.s. (not significant). Osmolyte levels represent measured amounts (in nanomoles) per 10⁵ cells ml⁻¹.
increase of glucose and galactose in concert with up-regulation of floridoside suggests that these compounds fulfill a conserved osmotic adjustment function within the genus *Symbiodinium* (Fig. 3 and Table 1). Notably, the different *Symbiodinium* strains exhibited a differential response in regard to absolute floridoside production levels, but these differences did not follow a geographic (considering salinity levels at origin) or phylogenetic pattern (Fig. 3). Besides UDP-galactose, the second component required for floridoside synthesis, glycerol 3-phosphate, is likely supplied from photosynthesis or via the Calvin cycle (37). Glycerol 3-phosphate can be produced from glycerol, which is considered to be one of the main COOs in marine algae (38), although it has been shown to be released under osmotic pressure in *Symbiodinium* (16, 18, 39). Given that our analysis on available *Symbiodinium* genomes confirmed the presence of homologs for the enzyme that produces floridoside, it will be interesting to check for the presence and identity of the enzyme(s) required for floridoside synthesis in ecologically relevant *Symbiodinium* (for example, *S. thermophilum*) and to determine whether gene expression or duplication can be aligned with strain- or species-specific differences (40).

**Osmoadaptation in the coral-*Symbiodinium* endosymbiosis, with implications for the coral stress response**

Our results demonstrate that exposure to high salinities leads to higher endosymbiont floridoside levels in vitro and in hospite. This may point to the fact that elevated floridoside levels increase not only the capacity of *Symbiodinium*, but also that of the holobiont, to cope with the effect of high osmotic pressure in extreme environments. Furthermore, our results suggest that osmolarity changes within the coral tissue are noticed by endosymbiotic *Symbiodinium*; thus, both the coral and *Symbiodinium* respond to salinity changes, presumably by adjusting the inner osmolarity to the higher outside salinity. However, it remains to be determined whether *Symbiodinium* adjust their inner osmolarity the same way in hospite as in vitro (32). Since potentially any metabolite contributes to the osmolarity in hospite, the endosymbiotic environment in coral cells might differ from the in vitro seawater environment (38).

Beyond its function as an osmolyte, floridoside has been shown to act as an antioxidant with ROS-scavenging properties (25, 26). Hence, floridoside has the capability to convey osmoadaptation as well as to counter ROS produced in response to salinity or other forms of stress (6, 8, 24, 26–28). In particular, increased ROS is detrimental to photosystem II in photosynthetic organisms (5, 41–43). Therefore, the production of antioxidants at high salinities is potentially important for *Symbiodinium*, and floridoside represents an osmolyte that fulfills a ROS-scavenging function at the same time (26). Increasing levels of floridoside and oxidative stress in response to increased salinities were shown in the red alga *Gracilaria sordida* (44) and *Gracilaria corticata* (45), respectively. Future work should determine the exact role that floridoside plays in response to conditions of high salinity, as either an antioxidant, a COO, or both, for example, by comparing floridoside and ROS levels at ambient and increased salinities.

The notion that ROS-producing mechanisms for photosynthetic organisms are similar (if not identical) under salinity and heat stress (42) also has interesting implications for our understanding of the response to heat stress in *Symbiodinium* and, by extension, for the coral hosts. Similar to salt stress, heat stress results in malfunction of the photosynthetic machinery of *Symbiodinium* and in the production of ROS that may damage the algal cells and, in the case of the coral-algal endosymbiosis, may trigger bleaching (46). We find that *Symbiodinium* exposed to high salinity in vitro and in hospite accumulate high amounts of floridoside. Hence, elevated floridoside levels in high-salinity environments may increase the ability to tolerate heat stress in *Symbiodinium* and, by extension, their coral hosts through scavenging of increased ROS levels. Consequently, the thermal resilience of coral holobionts may potentially increase under conditions of high salinity because of the accumulation and inherent antioxidative capabilities of floridoside. Experimental data connecting increased floridoside levels to decreased ROS and bleaching levels at increased salinities are in demand to support this potential link.

**MATERIALS AND METHODS**

**Symbiodinium cultures**

*S. microadriaticum* CCMP2467 (type A1; originally isolated from *S. pistillata*, Red Sea, Gulf of Aqaba) (40), *Symbiodinium* sp. type A1 (originally isolated from *Aurelia aurita*, Central Red Sea) (47), *S. minutum* Mf1.05b (type B1; isolated from *Orbicella faveolata*, Florida Keys, United States) (48, 49), and *S. psamnophilum* Mf10.14b.02 (type B2; isolated from *Oculina diffusa*, Bermuda, UK) (50, 51) were cultured in f/2 medium without silicon under a photon flux of 108 μmol m⁻² s⁻¹ at 26°C (52). The f/2 medium was prepared by sterile filtered Red Sea water [with a salinity of 38 and complemented with NaNO₃, NaH₂PO₄, vitamins, and trace metals (53)]. For each strain, we used replicate culture flasks and prepared three salt-adjusted f/2 media [salinities of 25, 38, and 55; following the study of van der Merwe et al. (54)] either by adding appropriate amounts of NaCl or by diluting the media with double-distilled H₂O (ddH₂O). Triplicates of 5 ml of *Symbiodinium* cultures at exponential growth (10⁵ to 10⁶ cells ml⁻¹) were transferred to 35 ml medium without silicon under a photon flux of 108 μmol m⁻² s⁻¹ at 26°C (55). The f/2 medium was additionally cultured under a reduced salinity of ~36.5 for *P. lichen* (41) and *H. grandis* (42). *S. minutum* cultures were confirmed the presence of homologs for the gene(s) required for floridoside synthesis in ecologically relevant *Symbiodinium* (Ochsenkühn et al., Sci. Adv. 2017;3:e1602047 16 August 2017). Notably, the different *Symbiodinium* strains exhibited a conserved osmotic demand to support this potential link.
whereas *P. lobata* with *S. thermophilum* (11, 14) and *H. grandis* with *Symbiodinium* sp. type C40 both show long-term survival.

Survival capacity was determined before the experiment by incubating 10 replicate colonies for >24 months at a salinity of 42. In contrast to other Indo-Pacific species (including *P. lichen* (11)), *P. lobata* and *H. grandis* have not suffered any mortality and have been actively growing during this time (56). In the present experiments, replicate colonies of *P. lichen* and *H. grandis* previously cultured at lower salinities (34 and 36.5) were gradually adjusted to a high salinity of 42 over 2 days before being moved to the high-salinity compartment for 12 days before sampling. Replicate colonies were produced by earlier fragmentation.

Using an airbrush, coral tissue was blasted off the skeleton with ice-cold, sterile-filtered, freshly prepared artificial seawater with the same salinity as the culture-rearing water. Three coral colonies were used per species to extract zooxanthellae. *Symbiodinium* cells were precipitated from the homogenate by centrifugation at 2500 g for 5 min at 4°C. The cells were washed twice (to remove the host tissue fraction and to pre-

Metabolite extraction and recovery

Cell pellets from *Symbiodinium* cultures and *Symbiodinium* extracted from coral tissues were resuspended and washed with 30 ml of sterile seawater on ice, pelleted, and washed for ~30 s, with further 5 ml of ddH2O to remove residual salt. After a further centrifugation step, pellets were resuspended in 5 ml of ddH2O, and cells were disrupted by high-pressure ultrasonication for 4 min at 3-s pulsing and 6-s pause. Cell debris was removed by centrifugation at 20,000 g for 5 min at 4°C. Proteins, DNA, or RNA was removed by ethanol precipitation by adding nine parts of −20°C ethanol to one part of supernatant. The precipitate was pelleted and removed by centrifugation, whereas the supernatant was frozen in liquid nitrogen and lyophilized. Dry samples were dissolved in 240 μl of ddH2O, spiked with 10 μl of internal standard [HBA (1 μg/ml) in ddH2O], transferred into GC vials, and dried under vacuum. For derivatization, 50 μl of MOX reagent (2% methoxamine HCl in pyridine) was added to each sample, and the solution was heated to 75°C for 1 h. Afterward, 100 μl of MSTFA solution [N-(trimethylsilyl)trim trifluoroacetamide, 1% trimethylchlorosilane; Thermo Scientific] was added, and samples were heated for 1 h at 100°C. Each sample vial was centrifuged at 2000g for 10 min, and 100 μl of the supernatant was transferred to glass inserts placed inside GC vials.

**GC-MS analysis, quantification, and analysis**

Derivatized carbohydrates, amino acids, and further intracellular compounds were characterized and quantified by GC-MS. For separation, an HP-5ms column (Agilent Technologies) and a temperature profile starting at 70°C were chosen. Temperature was increased in increments of 6°C min⁻¹ up to 230°C, followed by increments of 60°C min⁻¹ to a maximum of 280°C where it was held for 4 min. Metabolites were quantified by standard curves produced with pure glucose (99.5%; Sigma) and glycero (≥99.5%, ACS Reagent–grade; Sigma), with 60, 30, 10, 1, and 0.1 μg of both compounds. The calibration standards were spiked with 1 μg of HBA, derivatized for GC-MS, and analyzed as described above. All samples were prepared and measured in triplicate. GC-MS data were processed (that is, background subtraction, peak picking, and integration; OpenChrom v. 0.901, Lablicate UG) and MS ionization spectra—identified (NIST MS Software 2.0, Agilent Technologies). Statistical testing was conducted on normalized quantities of metabolites (in nanomoles) using ANOVAs and Tukey’s HSD post hoc tests to assess differences between pairwise comparisons. In the case of cultured *Symbiodinium*, we normalized to 10⁶ cells ml⁻¹. For corals, *Symbiodinium* extracts were normalized over dry weights in milligrams.

**Floridoside homologs in coral and *Symbiodinium* genomes**

We searched for homologs of the putative enzyme that converts glycerol 3-phosphate to floridoside (31) in the available coral and *Symbiodinium* genomes via BLASTp on refeorgenomics.org (57) using an e value cutoff of <10⁻⁵. Briefly, the amino acid sequence for the gene (Gastu_26940) coding for floridoside phosphate synthase/phosphatase from the red alga *G. sulphuraria* was queried against the genomes of *A. digitifera* (58) and *S. pistillata* (59), as well as against the genomes of *S. microadriaticum* (40), *S. minutum* (49), and *S. kawagutii* (60).

**SUPPLEMENTARY MATERIALS**

Supplementary material for this article is available at http://advances.sciencemag.org/cgi/content/full/3/8/e1602047/DC1

*table S1. Metabolites in GC-MS traces and identification information for NIST MS 2.0 Library Search.*

*table S2. Overview of floridoside amounts from Symbiodinium of coral holobionts at different salinities.*

**REFERENCES AND NOTES**

1. M. L. Reaka-Kudla, D. E. Wilson, E. O. Wilson, *Biodiversity II: Understanding and Protecting Our Biological Resources* (Joseph Henry Press, 1996).

2. L. Muscatine, J. W. Porter, Reef corals: Mutualistic symbioses adapted to nutrient-poor environments, *BioScience* **27**, 454–460 (1977).

3. P. G. Falkowski, Z. Dubinsky, L. Muscatine, J. W. Porter, Light and the bioenergetics of a symbiotic coral, *BioScience* **34**, 705–709 (1984).

4. L. Muscatine, J. W. Porter, I. R. Kaplan, Resource partitioning by reef corals as determined from stable isotope composition. *Mar. Biol.* **100**, 185–193 (1989).

5. M. P. Lesser, in *Coral Reefs: An Ecosystem in Transition*, Z. Dubinsky, N. Stambler, Eds. (Springer, 2011), pp. 405–419.

6. J. Wiedenmann, C. D’Angelo, E. G. Smith, A. N. Hunt, F.-E. Legret, A. D. Postle, E. P. Achterberg, Nutrient enrichment can increase the susceptibility of reef corals to bleaching. Nat. Clim. Chang. **3**, 160–164 (2013).

7. N. Rädecker, C. Pogoreutz, C. R. Voolstra, J. Wiedenmann, C. Wild, Nitrogen cycling in corals: The key to understanding holobiont functioning? *Trends Microbiol.* **23**, 490–497 (2015).

8. B. E. Brown, Coral bleaching: Causes and consequences. *Coral Reefs* **16**, 5129–5138 (1997).

9. T. D. Ainsworth, S. F. Heron, J. C. Ortiz, P. J. Mumby, A. Grech, D. Ogawa, C. M. Eakin, W. Leggat, Climate change driven bleaching protection on the Great Barrier Reef. *Science* **352**, 338–342 (2016).

10. C. D’Angelo, B. C. C. Hume, J. Burt, E. G. Smith, E. P. Achterberg, J. Wiedenmann, Local adaptation constrains the distribution potential of heat-tolerant *Symbiodinium* from the Persian/Arabian Gulf. *ISME J.* **9**, 2551–2560 (2015).

11. T. Röthig, M. A. Ochsenkühn, A. Roik, R. van der Merwe, C. R. Voolstra, Long-term salinity tolerance is accompanied by major restructuring of the coral bacterial microbiome. *Mol. Ecol.* **25**, 1308–1323 (2016).
21. A. Sadka, A. Lers, A. Zamir, M. Avron, A critical examination of the role of de novo protein metallocproteinases, floridoside, and D-isofloridoside from marine red alga (2013).

24. B. C. C. Hume, C. R. Voolstra, C. Arif, C. D. Dunaliella undulata provides evidence for a role of nitrogen fixation in coral bleaching.

27. M. S. Burriesci, T. K. Raab, J. R. Pringle, Evidence that glucose is the major transferred across an antagonistic temperature gradient in the Red Sea. Mol. Ecol. 21, 388–405 (2012).

28. B. C. C. Hume, C. R. Voolstra, C. Arif, C. D’Angelo, J. A. Burt, G. Eyal, Y. Loya, J. Wiedenmann, Ancestral genetic diversity associated with the rapid spread of stress-tolerant coral symbionts in response to Holocene climate change. Proc. Natl. Acad. Sci. U.S.A. 113, 4416–4421 (2016).

29. L. S. Coles, Coral species diversity and environmental factors in the Arabian Gulf and the Gulf of Oman: A comparison to the Indo-Pacific region. Atoll Res. Bull. 507, 497–508 (2003).

30. L. P. Suescún-Bolívar, R. Iglesias-Prieto, P. E. Thomé, Induction of glycerol synthesis and release in cultured Symbiodinium. PLOS ONE 7, e47182 (2012).

31. C. Goiran, D. Allemand, I. Galgani, Transient Na⁺ stress in symbiotic dinoflagellates after isolation from coral-host cells and subsequent immersion in seawater. Mar. Biol. 129, 581–589 (1997).

32. L. P. Suescún-Bolívar, G. M. I. Travers, P. E. Thomé, Glycerol outflow in Symbiodinium under osmotic and nitrogen stress. Mar. Biol. 163, 128 (2016).

33. R. H. Reed, Use and abuse of osmo-terminology. Plant Cell Environ. 7, 165–170 (1984).

34. I. Ahmad, J. A. Hellebust, Osmoregulation in the extremely euryhaline marine micro-alga Chlorella autotrophica. Plant Physiol. 74, 1010–1015 (1984).

35. A. Sadka, A. Lers, A. Zamir, M. Avron, A critical examination of the role of de novo protein synthesis in the osmotic adaptation of the halotolerant alga Dunaliella. FEBS Lett. 244, 93–98 (1989).

36. G. O. K. Frost, Salinity tolerance of eukaryotic marine algae. Annu. Rev. Plant Physiol. 41, 21–53 (1990).

37. H. Chen, J. G. Jiang, Osmotic responses of Dunaliella to the changes of salinity. J. Cell. Physiol. 219, 251–258 (2009).

38. K. Hoef-Emden, Osmotolerance in the cryptophytes: Jacks-of-all-trades in the Choomonas clade. Protist 165, 123–143 (2014).

39. M. Kim, Y.-X. Li, P. Dewapriya, B. Ryu, S.-K. Kim, Floridoside suppresses pro-inflammatory responses by blocking MAPK signaling in activated microalgae. BBM Rep. 46, 398–403 (2013).

40. Y.-X. Li, S.-H. Lee, Z.-J. Qian, S.-K. Kim, Inhibitors of oxidation and matrix metalloproteinases, floridoside, and α-floridoside from marine red alga Laurencia foliacea. J. Agric. Food Chem. 58, 578–586 (2010).

41. P. M. Hasegawa, R. A. Bressan, J.-K. Zhu, H. J. Bohnert, Plant cellular and molecular responses to high salinity. Annu. Rev. Plant Physiol. Plant. Mol. Biol. 51, 463–499 (2000).

42. J. Bose, A. Rodrigo-Morenio, S. Shabala, ROS homeostasis in halophytes in the context of salinity stress tolerance. J. Exp. Bot. 65, 1241–1257 (2014).

43. B. Shen, R. G. Jensen, H. J. Bohnert, Mannitol protects against oxidation by hydroxyl radicals. Plant Physiol. 115, 527–532 (1997).

44. C. Pogoreutz, N. Rüdecker, A. Cárdenas, A. Gárdes, C. R. Voolstra, C. Wild, Sugar enrichment provides evidence for a role of nitrogen fixation in coral bleaching. Glob. Chang. Biol. 10.1111/gcb.13695 (2017).

45. N. Pade, N. Linka, W. Ruth, A. P. M. Weber, M. Hagemann, Floridoside and isofloridoside are synthesized by the halophilic 6-phosphate synthase-like enzymes in the red alga Gelidium sulphurans. New Phytol. 205, 1227–1238 (2015).

46. P. H. Yancey, M. Heppenstall, S. Ly, R. M. Andrell, R. D. Gates, V. L. Carter, M. Hagedorn, Betaines and dimethylsulfoniopropionate as major osmoregulators in endosymbiotic dinoflagellates. Physiol. Biochem. Plants. 83, 167–173 (2010).

47. S. K. Pierce, Invertebrate cell volume control mechanisms: A coordinated use of intracellular amino acids and inorganic ions as osmotic solutes. Biol. Bull. 163, 405–419 (1982).

48. L. E. Eimeran, M. P. Hare, Transcriptomic analysis of candidate osmoregulatory genes in the eastern oyster C. virginica. BMC Genomics 15, 503 (2014).

49. A. Eggert, U. Karsten, in Red Algae in the Genomic Age, J. Seckbach, D. J. Chapman, Eds. (Springer, 2010), vol. 13, pp. 443–456.

50. A. J. Liska, A. Shevchenko, U. Pick, A. Katz, Enhanced photosynthesis and redox energy production contribute to salinity tolerance in Dunaliella as revealed by homology-based proteomics. Plant Physiol. 136, 2806–2817 (2004).

51. A. Ben-Amotz, T. Grunwald, Osmoregulation in the halotolerant alga Asteromoseras gracilis. Plant Physiol. 67, 613–616 (1981).

52. A. B. Mayfield, R. D. Gates, Osmoregulation in antherozoid–dinoflagellate symbiosis. Comp. Biochem. Physiol. Part A Mol. Integr. Physiol. 147, 1–10 (2007).

53. M. S. Buriñcki, T. K. Raab, J. R. Pringle, Evidence that glucose is the major transferred metabolite in dinoflagellate–cnidarian symbioses. J. Exp. Biol. 215, 3467–3477 (2012).

54. M. Aranda, Y. Li, Y. J. Li, S. Baumgarten, O. Simakov, M. C. Wilson, J. Piel, A. Ashoor, S. Bouquiaux, Y. B. Bajic, T. Ryu, T. Raya, B. Tauer, G. Bickler, H. Kim, J. Bhak, T. C. LaJeunesse, C. R. Voolstra, Genomes of coral endosymbiont dinoflagellates highlight evolutionary adaptations conducive to a symbiotic lifestyle. Sci. Rep. 6, 39734 (2016).
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