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Nutrient stress arrests tentacle growth in the coral model Aiptasia

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Abstract
The symbiosis between cnidarians and dinoflagellate algae of the family Symbiodiniaceae builds the foundation of coral reef ecosystems. The sea anemone Aiptasia is an emerging model organism promising to advance our functional understanding of this symbiotic association. Here, we report the observation of a novel phenotype of symbiotic Aiptasia likely induced by severe nutrient starvation. Under these conditions, developing Aiptasia no longer grow any tentacles. At the same time, fully developed Aiptasia do not lose their tentacles, yet produce asexual offspring lacking tentacles. This phenotype, termed 'Wurst' Aiptasia, can be easily induced and reverted by nutrient starvation and addition, respectively. Thereby, this observation may offer a new experimental framework to study mechanisms underlying phenotypic plasticity as well as nutrient cycling within the Cnidaria – Symbiodiniaceae symbiosis.

Keywords *Exaiptasia pallida* · Model organism · Nutrient starvation · Stoichiometry · Stress phenotype

1 Introduction

Coral reefs are hot spots of biodiversity surrounded by oligotrophic oceans (Reaka-Kudla 1997). The key to understanding the vast diversity and productivity of coral reefs despite these nutrient-limited conditions lies in the ecosystem engineers of these reefs, scleractinian corals (Muscatine and Porter 1977). A mutualistic nutrient-exchange symbiosis with microalgae of the family Symbiodiniaceae (LaJeunesse et al. 2018) allows corals to access autotrophic and heterotrophic energy sources (Rädecker et al. 2015). Indeed, this relationship allows for a highly efficient utilization and recycling of nutrients within the symbiosis, sustaining growth and productivity of corals and forming the foundation of entire ecosystems, i.e. coral reefs (Muscatine and Porter 1977). Yet, despite their ecological success over evolutionary time frames, corals are in rapid decline. Local and global anthropogenic disturbances are undermining the integrity of the coral - algal symbiosis and lead to the degradation of the entire reef ecosystem (Hughes et al. 2017). Slowing or even reverting this development requires in-depth knowledge of the biology of corals and their symbionts (van Oppen et al. 2015). However, given the diversity of corals and their associated microbial symbionts, understanding the intricacies and the functioning of the coral-alga symbiosis has proven challenging.

The sea anemone Aiptasia (sensu *Exaiptasia pallida*) promises to be a powerful new model organism for the study of the coral - alga symbiosis (Rädecker et al. 2018; Voolstra 2013). Just like coral, Aiptasia form an endosymbiotic relationship with Symbiodiniaceae. However, Aiptasia offer distinct advantages for the study of the cnidarian - Symbiodiniaceae symbiosis: (1.) it can be easily propagated under controlled laboratory conditions; (2.) it can be maintained in a symbiont-free stage and is easily re-infected with a diversity of symbionts; (3.) a diversity of genetic and molecular tools are available for the in-depth study of the symbiosis (Voolstra 2013; Baumgarten et al. 2015). As such, understanding the biology and the functioning of this emerging model organism is critical for the progress of coral reef research.

Here, we report the observation of an environmentally-induced altered phenotype of Aiptasia, which may offer

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interesting new insight into cnidarian biology and symbiosis. We have termed this phenotype 'Wurst' (German for sausage) in reference to the altered and distinct morphology of Aiptasia anemones.

2 Material & Methods

2.1 Aquaria maintenance

We unintendedly induced the 'Wurst' phenotype while developing a low-maintenance, long-term culture system for Aiptasia. For this, we set up a 10 L mesocosm containing freshly collected live rock and autoclaved seawater from the Red Sea (Fig. S1). The aquarium was stocked with Aiptasia of the clonal line RS1 (collected from the Red Sea in 2015 and maintained in culture for >2 years (for details see Cziesielski et al. 2018)). Animals were propagated over a three-month period via weekly feeding with *Artemia* nauplii and water exchange with autoclaved seawater (rearing at 30 μmol quanta m^{-2} s^{-1}, 12 h:12 h light/dark cycle, 25 °C, salinity 30–35). Once the Aiptasia had sufficiently proliferated (>300 animals), all feeding and water exchange ceased. Maintenance was minimized to removing filamentous algal growth in the aquarium and regularly topping up the aquarium with reverse osmosis water to adjust salinity. The aquarium was maintained under these conditions for six months until the distinct 'Wurst' phenotype was initially observed in developing Aiptasia.

2.2 Characterization of Aiptasia phenotypes

To provide an empirical measure of phenotypic changes in Aiptasia, we measured the ratio of column length to oral disk diameter for ten fully relaxed polyps of the wildtype as well as the 'Wurst' phenotype of Aiptasia. Symbiont densities as well as total protein content of the host tissue were assessed for five individual animals of each phenotype. Animals were homogenized in 500 μL sterile saline water for 30 s using a Micro DisTec Homogenizer 125 (Kinematica, Switzerland). Algal symbiont cells were separated from host tissue homogenate by centrifugation (3000 g, 5 min). Pelleted cells were resuspended in 200 μL sterile saline water and concentrations were assessed using a hemocytometer. 150 μL of 15-fold diluted host tissue homogenate was used for protein quantification using the Micro BCA Protein Assay kit (Thermo Scientific, USA) according to manufacturer instructions.

2.3 Characterization of seawater conditions

We characterized nutrient conditions in the long-term aquaria culture in comparison to freshly collected autoclaved Red Sea seawater to identify potential causes of the altered Aiptasia phenotype. For inorganic nutrient analysis (ammonium (NH_4^+), nitrate (NO_2^-), nitrate (NO_3^-), phosphate (PO_4^{3-}), silicate (SiO_2)), three 50 mL replicate samples were filtered (0.45 μm), collected in 50 mL centrifuge tubes, and analyzed using the AA3 Continuous Segmented Flow Analyzer (SEAL Analytical, UK). For quantification of dissolved organic carbon (DOC) concentrations, three 20 mL samples were filtered

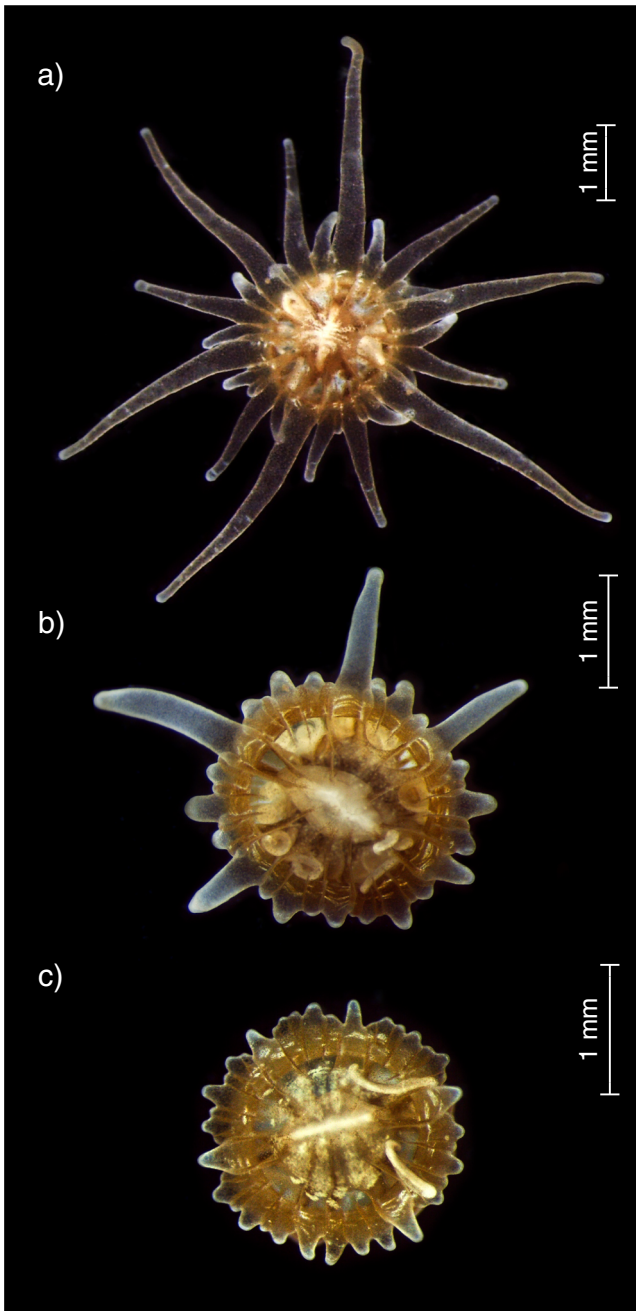

Fig. 1 Phenotype alteration in the coral model anemone Aiptasia induced by nutrient starvation. Long-term aquaria culture in the absence of nutrient input from feeding or water exchange arrests tentacle growth in polyps. While fully grown Aiptasia do not lose their elongated tentacles (**a**), their asexually produced offspring show partially (**b**) or completely (**c**) inhibited tentacle growth. We have termed this new phenotype 'Wurst' (German for sausage) Aiptasia in reference to their elongated, tentacle-free appearance

(0.22 μm), collected in acid-washed 30 ml HDPE bottles, acidified with concentrated phosphoric acid (pH < 2), and analyzed using the TOC-L TOC Analyzer (Shimadzu, Japan).

2.4 Experimental manipulation of aquaria conditions

To confirm that the observed phenotypic changes were the result of altered nutrient availability, we performed a series of consecutive manipulations of culture conditions in the aquarium. We tested three modifications of the culture conditions outlined above: (1.) weekly feeding and water exchange, (2.) weekly feeding and no water exchange, (3.) no feeding and weekly water exchange. These experimental manipulations were performed consecutively using the same aquarium, animals, and procedures. For each of the modifications, animals were first exposed to the altered culturing conditions until all animals had reverted to the wildtype phenotype. Following the recovery of the wildtype, conditions were reverted to the above-mentioned culture conditions with no feeding or water exchange for at least three months until the 'Wurst' phenotype was observed again.

3 Results & Discussion

After Aiptasia were maintained in the absence of feeding or water exchange, the appearance of the new 'Wurst' phenotype was noticed. Curiously, all asexually produced offspring no longer developed the wildtype Aiptasia phenotype. Instead, the new Aiptasia phenotype exhibited partial (some tentacles <1 mm) or complete inhibition of tentacle growth (all tentacles <1 mm; Fig. 1). The loss of tentacles was accompanied by an elongated body shape. Accordingly, 'Wurst' Aiptasia had a significantly higher mean column length to oral disk diameter ratio of 5.98 ± 0.78 compared to 1.87 ± 0.20 in wildtype Aiptasia (mean ± SE; $t_{(18)} = 4.78$, $p < 0.001$). The 'Wurst' phenotype was detectable within days of polyp development and remained stable over time (at least three months). At the same time, already fully developed Aiptasia showed no immediate signs of tentacle-loss or reduction, suggesting that the "Wurst" phenotype was indeed the result of arrested tentacle growth during development rather than an actual loss of tentacles. Of note, 'Wurst' Aiptasia remained fully symbiotic with symbiont densities comparable to those of regular Aiptasia ($t_{(8)} = 0.15$, $p = 0.887$; Supplementary Table S1). However, 'Wurst' Aiptasia showed a significant reduction in host protein content per dry weight by more than 60% compared to regular Aiptasia ($t_{(8)} = 2.98$, $p = 0.017$; Supplementary Table S1). Apart from this observation, 'Wurst' Aiptasia showed no obvious symptoms of impaired health or fitness.

Notably, as the initial tank was not intended for experimental comparisons, the observation of this new phenotype is restricted to one mesocosm for now. However, upon further investigation we found a similar report in an aquarist forum describing the observation of the 'Wurst' phenotype under identical conditions, i.e. long-term culture without feeding and water exchange (http://reefcentral.com/forums/showthread.php?t=2559009, accessed: May 26th, 2018). Hence, we are confident that the observed phenotype is indeed caused by the described long-term culturing conditions.

We hypothesized that the 'Wurst' phenotype may be caused by a stress response of the animal to either the over-accumulation or the depletion of critical nutrients during the long-term culture. Indeed, nutrient analysis of seawater from the rearing aquarium revealed a substantial deviation of nutrient concentrations when compared to regular autoclaved Red Sea seawater used for the initial setup of the tank as well as for water exchanges (Table 1). Notably, DOC, NO_3^-, and PO_4^{3-} concentrations were about 6-, 8-, and 4-fold higher than in regular seawater, respectively. In contrast, NH_4^+, NO_2^-, and SiO_2 were depleted up to 80% in the aquarium rearing water compared to regular seawater. The observed accumulation of some nutrients and depletion of other nutrients are likely not only restricted to the macronutrients reported here, but are probably also reflected in patterns of availability of micronutrients or other compounds (e.g., toxins) in the system as well. While the nutrient data do not allow the identification of the specific nutrient(s) causing the 'Wurst' phenotype, they strongly support the notion that this phenotype represents a stress response to the depletion or over-accumulation of nutrients in the aquarium. On that note, it is important to mention that the Aiptasia host (in contrast to its endosymbiotic

Table 1 Overview of nutrient availability in regular Red Sea and 'Wurst' tank seawater

	DOC μmol L^{-1}	NH_4^+ μmol L^{-1}	NO_2^- μmol L^{-1}	NO_3^- μmol L^{-1}	PO_4^{3-} μmol L^{-1}	SiO_2 μmol L^{-1}
Regular seawater	206.00	0.17	0.25	0.40	0.02	0.50
	± 12.72	± 0.00	± 0.11	± 0.09	± 0.00	± 0.09
'Wurst' seawater	1298.84	0.03	0.10	3.29	0.08	0.12
	± 13.88	± 0.01	± 0.04	± 1.08	± 0.00	± 0.01

DOC = dissolved organic carbon; NH_4^+ = ammonium; NO_2^- = nitrate; NO_3^- = nitrate; PO_4^{3-} = phosphate ; SiO_2 = silica. Note that the 'Wurst' aquarium water showed enriched levels of DOC, NO_3^-, and PO_4^{3-}, whilst being depleted of NH_4^+, $NO2^-$, and SiO_2 compared to regular seawater.

Symbiodiniaceae) lack the cellular machinery for the assimilation of NO_3^- (Lehnert et al. 2014; Rädecker et al. 2015). The strong depletion of NH_4^+ levels in the aquarium coupled with the lack of heterotrophic nitrogen sources, therefore, likely causes strong nitrogen limitation of the host. In this context, the reduced protein content in the tissue of 'Wurst' Aiptasia may point to a disruption of protein biosynthesis as a consequence of nitrogen starvation (Oakley et al. 2017). The observation that the phenotype was only present in developing Aiptasia but not in fully grown Aiptasia further supports the notion of a state of nutrient starvation. Grown animals may be able to fulfill most of their nitrogen demand by efficient recycling of nutrients. In contrast, developing animals rely on environmental nutrient supply in order to support their growth. As such, low environmental nutrient availability is more likely to affect growing rather than fully developed animals.

To confirm that the observed phenotype was the results of nutrient stress, we tested the reversibility of the 'Wurst' phenotype. Indeed, feeding and/or water exchange caused rapid tentacle growth in all 'Wurst' Aiptasia (< 7 days, > 50 animals) resulting in the recovery to the regular phenotype. Notably, the 'Wurst' Aiptasia phenotype re-emerged approximately three months after any further feeding and water exchanges were suspended again.

It is tempting to interpret the lack of tentacles as an acclimation by the host to the absence of food. Yet, our observations show that either feeding or water exchange can revert this phenotype. Hence, it appears that the 'Wurst' Aiptasia is the result of severe nutrient limitation caused by the absence of both heterotrophic as well as inorganic nutrient sources. Given that 'Wurst' Aiptasia remain symbiotic, it is unlikely that carbon limitation alone is the cause of the distinct phenotypic change. Instead, other limiting macro- (e.g., nitrogen) or micronutrients (e.g., copper) may be driving this phenotype.

At this point, it is not possible to disentangle the cellular mechanism underlying the 'Wurst' phenotype. Yet, this observation may open new doors to understanding the functioning of the cnidarian - alga symbiosis. The 'Wurst' phenotype is illustrating the ecological advantages of the cnidarian – Symbiodiniaceae symbiosis as well as its limitations. On the one hand, the translocation of photosynthates by their algal symbionts likely enables 'Wurst' Aiptasia to survive even in the absence of heterotrophic food sources. At the same time, the photosynthates provided are 'junk food': they are high in carbon yet low in nitrogen content (Wiedenmann and D'Angelo 2018). As such, the cnidarian host relies on external nutrient sources to supplement its diet and avoid nutrient starvation.

Given its distinct characteristics and its controlled inducibility and reversibility, the 'Wurst' phenotype provides a promising experimental framework for the study of nutrient cycling in Aiptasia. 'Wurst' Aiptasia may help shed light on the mechanisms underlying phenotypic plasticity in Aiptasia as well as environmental controls of nutrient exchange in the Cnidaria – Symbiodiniaceae symbiosis. Future work integrating genetic and physiological tools will thus not only help to shed light on the cause(s) of the 'Wurst' phenotype, but may offer new insights into the biology and functioning of symbiotic cnidarians, in particular reef-building corals, in general.

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