The Double Edged Role of Nitric Oxide and Hydrogen Peroxide in a Coral Symbiosis

Reporting

Project Information

DENOCS

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Coordinated by
KOBENHAVNS UNIVERSITET
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This project is featured in...

Periodic Reporting for period 1 - DENOCS (The Double Edged Role of Nitric Oxide and Hydrogen Peroxide in a Coral Symbiosis)

Reporting period: 2017-06-01 to 2019-05-31

Summary of the context and overall objectives of the project

Climate changerelated rising sea surface temperatures challenge the integrity of the most diverse marine ecosystem – the coral reefs. Recent years marked the worse global coral bleaching event on record. Corals depend on a symbiotic relationship with microalgae (genus Symbiodinium) that they harbour in their tissue as their primary food source. Coral bleaching leads to a breakdown of this symbiosis and loss of algal cells, eventually leading to coral death if the symbiosis is not re-established.
It is known that oxidative stress and production of reactive chemical species such as NO and H2O2 are involved in the breakdown of coral symbioses. Regulation and mechanisms affecting the dynamics of these reactive species in corals present a significant knowledge gap.This project employed novel microanalytical measuring techniques for NO and H2O2 quantification that enabled detailed studies of a coral stress response in terms of i) the cellular mechanisms , ii) the source of NO iii) direct quantitative measures of H2O2 of intact coral symbioses. Following research questions lead the project:
(1) How can we optimize the assessment of NO and H2O2 in an intact coral symbiosis to study their dynamics and interaction on a quantitative and comparable basis?
(2) Does carbon fixation in Symbiodinium and therefore the coral host's organic carbon supply, decrease under nitrosative stress conditions thus exacerbating a stress response by starving the coral host leading to coral bleaching?
(3) What are the production hotspots of key cellular compounds involved in an oxidative stress response within the coral tissue initiating coral bleaching?

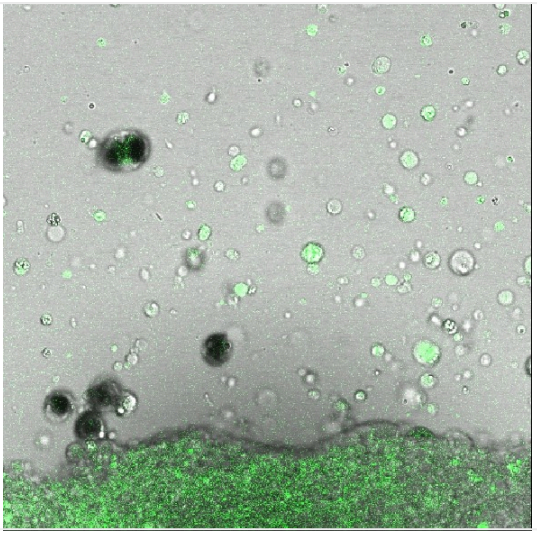
Work performed from the beginning of the project to the end of the period covered by the report and main results achieved so far

In a new advance for coral bleaching studies, NO and H2O2 microsensors were used to describe dynamic radical production. For this, coral explants used as coral models were subjected to environmentally relevant stress scenarios, typically leading to coral bleaching. Treatments included thermal stress, high light and a combination of the two stressors. Coral explants were assessed for their photosynthetic activity (O2 production) and H2O2 production under those conditions. Results showed that H2O2 production was increased when exposed to thermal stress but even more so when incubated in a combination of high light and thermal stress. We could conclude that under thermal stress conditions the cellular radical loads are higher compared to control conditions.
Next, the project aimed to elucidate the stressor-specific production and site of NO production within a coral symbiosis by applying confocal microscopy assays. In order to do this, staining and imaging protocols for coral explants were developed during this project. In the main experiments coral explants were exposed to thermal stress in the dark, a combination of high light and thermal stress as well as saturating light conditions. The treatments were then compared to coral explants from control conditions.Under saturating light alone high NO production levels were observed within the coral symbiosis. To date it was assumed that the coral host is the main NO source within a coral symbiosis. We could show that NO production site within a coral symbiosis is clearly stressor dependent. A new finding is that Symbiodinium was the main source for NO production after 6hrs of incubation at typical coral bleaching conditions (high light and thermal stress), where thermal stress resulted in NO production only under prolonged stress incubation and only in the coral host. These results have implication on our fundamental understanding of the NO related biochemistry going on within a coral symbiosis under environmental stress conditions.
Next, the project focused on the fact that marine organisms are facing progressive hypoxic conditions, once ocean warming is occurring. Hypoxic conditions are forcing certain physiological pathways of the Nitrogen (N) metabolism into producing NO. If this is an operational pathway present in corals has not been described to date. To further assess this, coral explants were incubated under hypoxic conditions in the dark. In addition to this, a treatment with the same conditions and NO synthase inhibitor was applied, to inhibit one of the main NO producing enzymes. High loads of NO production were found to be produced under hypoxic conditions, which was even more exacerbated in coral explants which were incubated with the additional NO synthase inhibitor. In both symbiotic counterparts the NO production was present, indicating that the N metabolism is most likely a relevant NO source within a coral symbioses. Further research is needed to describe the full operation of this N metabolism related NO source in a coral symbiosis.
Reactive nitrogen species can potentially inhibit and modify a phototroph’s photosynthetic machinery. Such impairment of photosynthesis could affect the translocation of photosynthetically fixed carbon in the intact coral symbiosis. Further, it is known that the likelihood of a coral bleaching scenario is dependent on the type of Symbiodinium, which is associated with the coral host. Here we investigated two strains of Symbiodinium, to investigate the impact of NO exposure upon two critical photophysiological pathways, 1) photosynthetic productivity (14C assays) and 2) photosynthetic efficiency (differential chlorophyll a fluorescence). The Symbiodinium cultures were incubated with the addition of NO donors of varying release rate and concentration. We could show that the two strains examined have a very different response towards NO exposure. The results are indicating that the photosystem I functionality is highly impaired in one of the Symbiodinium strains, where the other is able to operate with much lower impairment. Initial results of the detailed photosystem I measures

indicate that the functionality of a back-up valve, regulating enhanced electron flow is most likely operating in the more resilient strain oppose to the other. The implications for a coral symbiosis are, that corals with a Symbiodinium consortia without this photosystem I back-up valve are more likely to be limited in nutrition compared to the other strain and could explain partially at least why some corals are more resilient towards stress conditions compared to others.

Progress beyond the state of the art and expected potential impact (including the socio-economic impact and the wider societal implications of the project so far)

The project further examined the cellular biochemistry of two coral species under bleaching related stress conditions. Corals were incubated under prolonged (3week) thermal stress conditions, which progressively were enhanced to reach a final 5 day incubation at + 5 degrees C. The coral samples were screened for typical cellular biomarkers indicating oxidative stress (i.e. lipid peroxidation, S-nitrotyrosine, general ROS production). In a new advance coral samples were examined for modifications of their amino acid composition using a newly developed mass spectrometry assay. Taken together, the results reveal a differing cellular biochemistry in the two coral species examined, further highlighting the species-specific responses in the redox biology of corals.



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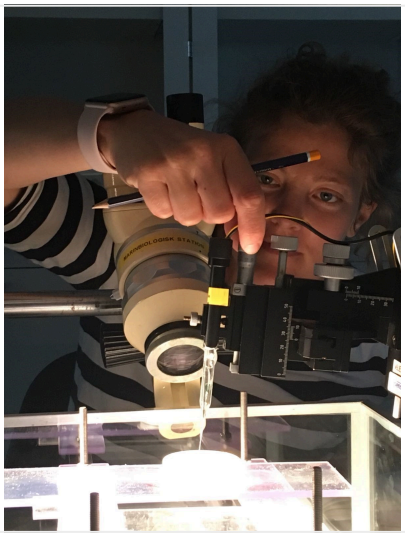

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