Light Induced Microphytobenthic Biofilm Oxylipins

I. Objectives and research hypotheses

This PhD project aims to understand the impact of oxidative stress on the lipid metabolism of microphytobenthic biofilms, with a focus on identifying the specific reactive oxygen species (ROS) responsible for these effects on diatoms. The project brings together the complementary expertise of three laboratories: BOREA (microbial ecology), LPP (cold plasma physics), and LBI2M (bioinformatics and evolution of metabolic pathways) and will be carried out at the scale of microbial communities. The study will provide a better understanding of how oxidative stress impacts metabolism and decipher the functional role of the produced metabolites. The results of the study will form a basis for future applied research, given the current lack of basic knowledge on the subject. The project aligns with the objectives of the Ocean Institute, which promotes a transverse approach to the oceanic and coastal specificities of global changes and their impacts on marine biomes.

II. State of the art

Intertidal environments, where the shore is exposed to air at low tide and submerged at high tide, experience significant changes in light conditions. The photosynthetic photon flux density, or the amount of light in the 400-700 nm waveband reaching the sediment and the organisms living there, can vary greatly, from just a few to about 2000 photon.m-2.s-1. This sudden increase in light intensity can cause overproduction of reactive oxygen species (ROS) in benthic microalgae leading to oxidative stress, if an imbalance arises between (i) the production of ROS and (ii) the ability of cells to readily detoxify the reactive intermediates or repair the resulting damage.
For instance, the hydroxyl radical (HO•) can be produced along with peroxyle (HOO•) and is involved in the radical-mediated peroxidation of polyunsaturated fatty acids (PUFA). Lipid peroxidation then promotes loss of physiological cell membrane functions, inactivation of enzymes and other biochemical mechanisms that will have both desirable and undesirable effects (Butel-Ponce et al. 2016).
In diatom dominated intertidal biofilms, oxidation of PUFAs by enzymatic or non enzymatic pathways can cause a variety of cellular responses, ranging from induction of antioxidant enzymes, to apoptotic death (Orefice, et al. 2015). To counter oxidative stress, benthic diatoms have developed photoprotective mechanisms. This includes dissipating excess light energy into heat or migrating to a depth where light intensity is optimal (Cartaxana et al. 2011, Prins et al. 2020). This migration is triggered by light-induced stress and potentially by ROS production, but the exact pathway and signaling process involved is not yet understood.

III. Methodology

The methodology for the study involves combining knowledge from chemical ecology, plasma physics, and bioinformatics. With the help of Cédric Hubas (BOREA, MNHN) and Thierry Dufour (LPP, SU), the PhD student will induce oxidative stress in marine migratory biofilms using two approaches: (1) by modulating the quantity and quality of light provided to the biofilm, and (2) by exposing the biofilms to cold atmospheric plasmas generated as oxidizing atmospheres with low ionization rate and high chemical selectivity. The biofilms will be exposed to cold plasma in a dielectric barrier device (DBD), which will be supplied with a carrier gas such as helium or argon, with/without a reactive gas such as nitrogen or oxygen to produce specific reactive oxygen species (ROS) or radicals among O3, NO•, OH•, or O• (Dufour et al. 2021). ROS in the plasma phase will be measured by optical emission spectroscopy and mass spectrometry while long lifespan reactive species in the aqueous phase (H_2O_2, NO_2^-, CO_3^{2-}, $H_3PO_4^{2-}$, …) will be measured using ion selective probes and routinely-used photospectrometry protocols ($TiOSO_4$, Griess reagent) (Judé et al. 2018).

The PhD student will explore the functional links between the metabolome of intertidal biofilms and specific oxidative stress conditions using high-resolution LC/MS and LC/MS-MS, and GC/MS methods at the BOREA laboratory. This will allow for the analysis of the overall effect of oxidative stress on biofilm metabolism. Lipophilic pigment and fatty acids composition, as well as oxylipins resulting from non-enzymatic lipid peroxidation will be analysed as well. The PhD student will also explore the impact of oxidative stress at the scale of microbial communities using metabarcode approaches.

Finally, the PhD student will investigate the evolution of metabolic pathways (mainly oxylipins and sterols) using phylometabolomics. This approach, directed by Gabriel Markov (LBI2M, CNRS), will allow for the inference of complete metabolic networks/pathways from heterogeneous and fragmented data (Belcour et al, 2020). The PhD student will also examine the effect of synthetic oxylipins on the biofilm metabolome and migratory behavior of benthic diatoms.

IV. Novelty & Adequacy of the project to the Ocean Institute

To unrevealed how oxidative stress impacts the lipid metabolism of biofilms and more specifically identify which ROS are responsible for such effects on diatoms, this interdisciplinary and unprecedented project brings together the complementary expertise of 3 laboratories: BOREA (microbial ecology), LPP (cold plasma physics) and LBI2M (bioinformatics, evolution of metabolic pathways). It will be carried out at the scale of microbial communities and will provide a better understanding of how reactive oxygen stress impacts metabolism and decipher the functional role of the produced metabolites. Given the current lack of basic knowledge on this subject, the results will form a basis on which future applied studies can be conducted.

The fundamental nature and interdisciplinarian nature of the PhD project, which aims to gain a better understanding of the physiological, behavioral and evolutionary responses of biofilm microorganisms to environmental variations, makes it perfectly suited to Axis 1 (The Sea in the History of the Earth, Life and Societies). In addition, the Ocean Institute aims to promote a transverse approach dedicated to the oceanic and coastal specificities of global changes and their impacts on marine biomes (Axis 2). Our project is therefore in line with these objectives because oxidative stress is a basic response of all organisms to environmental stresses, especially those related to global change.

V. References

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