Giant barrel sponges in diverse habitats: a story about the metabolome

Bayona Maldonado, L.M.

Summary

Marine sponges are important members of reef ecosystems, as they play ecological roles that are essential for the health of the reef. From a chemical perspective, sponges have been widely studied, showing great chemical diversity with biological activity, although this chemical diversity can often not be linked to for instance variation in environmental conditions experienced by sponges. Metabolomics, with its holistic overview of the metabolites present in samples, has provided new tools to gain insight into the function of metabolites in the intricate relationship between sponges and their environment. Among sponges, giant barrel sponges have stood out for their predominance in many reefs, their longevity and the fact that they are widely spread across oceans. In some reefs, giant barrel sponges can cover up to 13% of the available benthic substrate, surpassing those of corals. They can live for more than 2000 years and can be found from New Caledonia to the Caribbean with a presence throughout the Red Sea, the east African coast and the Indo-Pacific region. It has also been shown that what was originally classified as three species (*Xestospongia bergquistia* (confined to the northern Great Barrier Reef), *Xestospongia muta* and *Xestospongia testudinaria*) is actually a species complex that contains at least nine cryptic species. The presence of cryptic species is prevalent in sponge taxa and is one of the main difficulties in the classification of these animals. All these features make giant barrels sponges a good model organism to understand the influence that environmental, biological and genetic factors might have on the metabolic production of marine sponges.

As part of the recognition of metabolomics as an approach that enables the comprehensive study of the metabolism of marine sponges and marine organisms in general, a literature review of the methods and applications of metabolomics in this field was done and included in the thesis as Chapter 2. The first step in a metabolomics study is the extraction method, which is crucial for obtaining an extract that accurately represents the metabolites present in the sample. The influence of extraction conditions (pressure, temperature, solvent polarity, and number of cycles) on the chemical diversity of *Xestospongia* spp. extracts was studied using a pressurized extraction system. It was found that temperature, solvent polarity and number of extraction cycles influenced the chemical diversity of the extracts and that under the set experimental conditions, the extraction with 100% ethanol at lower temperatures provided an extract that best represented the chemical diversity of the sponge (Chapter 3).

Continuing with the exploration of the metabolome of giant barrel sponges, differences in the metabolome of the sponge related to variations in environmental conditions were

investigated using LC-MS and NMR based metabolomics (chapters 4, 5, and 6). Firstly, considering the worldwide distribution of these sponges, the metabolic composition of sponges collected in four locations around the world (Martinique, Curaçao, Taiwan and Tanzania) was compared. Results showed that samples were clearly distinguished according to their location. However, no correlation was found between the antibacterial activity against *Staphylococcus aureus* and *Escherichia coli* exhibited by some samples and their location. This finding indicates that the production of active compounds could be affected by environmental conditions occurring at a smaller scale or that other aspects, such as genetic factors, play an important role in this area. Additionally, using the sample set collected in Curaçao, the effect of sea depth (7-43 m) on the metabolome was evaluated, showing that environmental changes along a depth gradient only had a significant effect on one of the genetic groups (putative species) of *Xestospongia* spp. studied. Lastly, the effect of sea surface temperature (SST) and pH was evaluated on a sample set collected in the Spermonde archipelago, SW Sulawesi (Indonesia). Again, in this case, only one of the genetic groups studied in this region displayed a change in the metabolome related to SST. Moreover, the pH of the seawater did not affect any of the genetic groups, suggesting that these sponges exhibit some resilience when faced with ocean acidification scenarios. In all the cases, when environmental changes altered the metabolome of any of the sponges, the modification was mostly related to lipid type compounds including brominated, hydroxylated and/or polyacetylenic fatty acids that exist either in their free form or as an acyl chain in glycerophospholipids.

Another condition that was considered to be potentially reflected in the metabolome of the sponges is the stage of development throughout their lifespan. Sponges lack age-related markers and *X. muta* was the first sponge for which it was possible to establish the age of the specimen. Using the age of giant barrel sponges as a factor and LC-MS based metabolomics together with molecular networking, it was possible to establish that age indeed has an effect on the metabolome of the sponges (Chapter 5). Moreover, the discriminant signals related to older sponges coincided with phosphatidylcholine glycerophospholipids containing two acyl groups. These types of lipids are well known to be membrane lipids, suggesting a higher rate of cellular division in older sponges. However, additional ecological roles of these compounds cannot be disregarded.

The presence of cryptic species in giant barrel sponges raises the question of whether these putative species could also be distinguished based on their metabolome. In this case samples collected in Indonesia were classified into one of the three genetic groups reported to be present in this region. Using LC-MS and NMR-based metabolomics together with molecular

networking it was possible to observe differences in the chemical production between the genetic groups (Chapter 6). Interestingly, the compounds found to be responsible for the separation were *lyso*-phospholipids which have also been reported to vary in sponges as a response to changes in environmental conditions. This indicates that these genetically closely related sponges might be reacting differently to similar environmental conditions.

In conclusion, this thesis showed how environmental, genetic and biological factors individually or as a result of their interaction can influence the metabolome of giant barrel sponges. This is only the first step towards a better understanding of the role that chemical compounds play in interactions between sponges and the surrounding environment. The advances in this field will rely, among others, on the development of technologies that would allow real time measurement of the metabolome and the study of the interaction between the metabolome and other omics, like the microbiome.