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Biotechnological exploitation of marine sponges: innovative technologies for an environmentally-friendly approach (BIOSPONFRI)

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Abstract

Marine sponges are the oldest Metazoan group living on earth. Since their discovery, these organisms have attracted increasing attention from the scientific community. Due to their apparent simplicity, sponges became a fascinating model for scientists of any discipline. From the chemical point of view, these animals are a gold mine of natural products with potential biotechnological applications. On the other hand, the diverse array of microbial hosts found on and within sponges fascinated microbiologists. Nonetheless, cell and evolutionary biologists took these animals to study their cellular and morphological structures.

In the last years, the research community has focused on the extraction and characterization of marine natural compounds. In this thesis, I will discuss about the extraction methods utilized to retrieve possible bioactive molecules from different sponge species and the tests performed to check for their biotechnological potential. During my PhD research our group has isolated a new cytotoxic pyrrole-terpene from the marine sponge *Cacospongia mollior*, and an already known compound from the sponge *Geodia cydonium*. On the other hand, we have defined the molecular responses taking place in the cells when exposed to the bioactive fraction D obtained from *Haliclona (Halichoclona) vansoesti*. Also, sulphated polysaccharides were extracted, purified, and assessed for their potential biotechnological applications from different marine sponge species.

Along with cytotoxicity experiments, I have performed also ecotoxicological tests on marine model organisms. During these we have tested the crude extracts and fraction of three sponges. All of those resulted to be harmful on the sea urchin embryos, whereas eggs seemed to be not affected. On the other hand, diatom extract fractions increased malformations rate compared to the control.

Although the array of the sponges' natural compounds is wide, these are secondary metabolites most of the time, this translates in low extraction rates. In other words, to have a sufficient quantity of the molecules of interest, a very high number of fresh animals is required. This exposes wild stocks to overexploitation.

One way to avoid this is the use of bioinformatics and *in silico* studies to identify and characterize the molecular pathways beyond the synthesis of the compounds of interest. For this reason, during my PhD studies we explored the microbial diversity of four biotechnologically promising sponges sampled in two different sites via Metataxonomic analysis. This confirmed that the growth site influences largely the bacterial composition. In parallel, we carried out a metagenomic study on other biotechnologically promising sponges. This underlined the presence of genes or enzymes involved in the biosynthesis of compound of interest, confirming the biotechnological potential hindered by these species.

A sustainable way to retrieve fresh sponge material is the development of sponge aquaculture that can be carried out in captivity (aquarium tanks), or on site (open waters). Both alternatives have pros and cons, but still, no unique way to culture different sponge species has been defined. Our preliminary results show that a microalgae diet is useful to keep sponge specimens alive for some time but alone it is not sufficient to determine their growth. Another way to avoid overexploitation of wild sponges population is the culture of their cells *in vitro* and eventually, the production of the compound of interest in large quantities in big bioreactors. Unfortunately, the outcome of the experiments carried out during my PhD project underline that there is still much work to do to fill in knowledge gaps present in this field. On the other hand, the same results show that the sponge *Petrosia ficiformis* is a good experimental model for the pursuing of further studies on sponge cell culture.