TESI DI DOTTORATO

Titolo: Ciona robusta (formerly Ciona intestinalis type A) as model system for ecotoxicological studies

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ABSTRACT

The invertebrate urochordate *Ciona robusta* (formerly *Ciona intestinalis* type A) is a sessile marine benthic organism distributed worldwide that attaches to the surfaces of both natural and artificial substrates, from shallow water to the deep sea. Based on its phylogenetic position as basal chordate, from more than a century *Ciona* represented an excellent model system for studying developmental biology thanks also to the simplicity of its fast embryogenesis and genome resources already available. In the last decades, in the light of such interesting features, *Ciona* has been used to evaluate the embriotoxicity of legacy pollutants (e.g. heavy metals, pesticides, organic compounds). The rapid embryonic and larval development, resemblance to vertebrates, ease of management, low cost, transparent body and low risk of ethical issues are some of the aspects that make *C. robusta* a valuable embriotoxicological model. Moreover, the genetic, genomic and molecular tools allow a deeper investigation on the molecular mechanisms affected by pollutants and could provide insights on their mode of actions (MoA). Within this thesis, the effects of two classes of contaminants of emerging concerns (CECs) have been tested on the embryogenesis of *C. robusta*. CECs are a group of natural and synthetic chemicals including nanoscale particles and transformation products, which have been increasingly found at low levels in surface waters. These compounds may pose a risk to aquatic life and, thus, it is fundamental to assess their potential effects on marine organisms. In detail, the effects of two dispersants named as A and B, used for cleaning up the petroleum hydrocarbon contamination in case of accidental oil spills at sea, and polystyrene nanoparticles (PS NPs), as proxy for nanoplastics have been investigated on *C. robusta* larval development. The four chapters of the thesis report findings on the effects of the two dispersants and PS NPs bearing different surface charges by using two approaches: 1) embryotoxicity by looking at adverse effects in developing embryos and on sub-lethal biological responses on functional proteins and enzymes; 2) mechanisms of action (MoA) at molecular level using different techniques as Real-Time PCR, RNA sequencing and bioinformatics. We demonstrated how embriotoxicity in *C. robusta* could represent a useful tool to evaluate the impact of dispersants on marine species. The data obtained indicated a different toxicity

between dispersants A and B, confirmed also by phenotype alterations. Moreover, the evaluation of the expression of selected genes involved in stress response (SODa, SODb, MnSOD, GPx, HSP60, HSP70), detoxification (Cyp450, GST, GluR) and cell survival (p38, Cas8) indicates dispersant B as teratogen while dispersant A having less impact on *C. robusta* larvae. Regarding nanoplastic, surface charges seem to play a significant role in the observed embryotoxicity of the amino-modified PS NPs (PS-NH_2) in agreement with their behavior in exposure media. No effects were found for carboxyl-modified PS NPs (PS-COOH) on *C. robusta* embryo development while from mild to severe phenotype alterations were observed upon exposure to PS-NH_2 including behavioral traits (e.g. swimming performances). Among those mostly evident, embryos result unable to hatch and several abnormal phenotypes were found. In addition, induction of oxidative stress linked to an increase of ROS production and the down-regulation of some representative genes involved in stress response (HSP70, HSP60, MnSOD, cytochrome b, p-38 mapk and caspase 8) were observed. The analysis of transcriptome through differential RNA-seq allowed to identify altered pathways affected by PS-NH_2. Several genes resulted dysregulated upon the exposure to PS-NH_2, while the GO analysis, which classified genes in three different subclasses, revealed that the number of genes affected, belonging to different subclasses, have a dose-response relationship with the concentration tested. Going deeply into the bioinformatic analysis, “glutathione synthesis and recycling pathway”, “neurotransmitter clearance pathway”, “passive transport by aquaporins” and “fructose and mannose metabolism”, “starch and sucrose metabolism” and “glycolysis” pathways resulted affected. The alteration of these pathways could be related to the hypoxic microenvironment due to the dense coating of PS-NH_2 around the egg envelopes of *Ciona* embryos. Similar findings in terms of embryotoxicity and phenotype alterations have been observed in another ascidian species, *Phallusia mammillata*, exposed in similar conditions to amino-modified PS NPs (PS-NH_2). Furthermore, the quantitative analyses of *Phallusia* phenotype using the software Toxicosis8, revealed the affection of both central and peripheral nervous system. The use of *C. robusta* embryos as a model to study the effects of dispersants and PS NPs as proxy for nanoplastics proved to be instrumental in shedding light on different aspects of developmental toxicity exerted by those CECs, moreover these results will hopefully provide important information useful for higher and more complex chordates.

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