PhD Programme in
"Models and Methods for Material and Environmental Sciences" (XXXVIII cycle)

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Thesis Abstract

**Tardigrade resistance mechanisms:
evolution and adaptations to extreme environments in relation to climate change**

Background Many taxa, from bacteria and yeasts to multicellular organisms, have evolved mechanisms that allow complete desiccation, while maintaining reversible suspension of macromolecular/metabolic activities and viability after rehydration. This life suspension is known as anhydrobiosis. Tardigrades (known also as water bears) are among the most resilient organisms: they are biostable in a dry state for decades and are able to survive extreme temperatures, pressures, radiations, and xenobiotics [1]. Therefore, they are an excellent model to study the molecular basis of anhydrobiosis. Recently, anhydrobiotic capability of tardigrades has been studied to explore alternative bio-storage options based on water subtraction techniques to induce reversible block of macromolecular interactions, allowing cells storage at non-cryogenic temperatures [2]. In particular, the comparison of -omics data obtained from both dry and hydrated tardigrades led to the identification of some components of the "desiccome" (*i.e.*, a set of genes, proteins, and metabolites involved in an organism's ability to tolerate desiccation) that can be used as xeroprotectants developed by nature [2]. Studies found that the presence of unique intrinsically disordered proteins, the termed tardigrade disordered proteins (TDPs), is linked to the ability to tolerate desiccation [2, 3]. Interestingly, only 3 classes of TDPs seem sufficient to mediate natural and/or induced tolerance to desiccation stress: cytosolic (CAHS), secretory (SAHS), and mitochondrial (MAHS) abundant heat soluble proteins. The evolutionary origin of TDPs is unknown (SAHS have low sequence homology to fatty acid binding proteins, MAHS and CAHS have no homology to other proteins) and the presence of TDPs has been investigated only on few species of the almost 1400 composing the phylum Tardigrada and thus their diffusion is poorly understood [3].
The phylum is composed of two classes (Heterotardigrada and Eutardigrada) and within Eutardigrada there are two orders: Apochela and Parachela. While anhydrobiosis can be observed within all the above-mentioned taxa, preliminary results on the few analyzed species so far have shown that TDPs are present only in those belonging to the order Parachela [4]. Nevertheless, exploratory analyses found signals of CAHS transcripts also in apochelans [5], highlighting the need to investigate more thoroughly the diffusion and phylogenetic origin of TDPs.

Activities and Objectives Several tardigrade species with different phylogenetic positions, ecological needs, and anhydrobiotic capabilities will be used to carry out parallel experiments on the anhydrobiotic gene expression of TDPs and on the phylogeny and evolution of these proteins within the phylum. Specimens belonging to different species will be dried with established protocols and their RNA will be extracted and analysed from dry and hydrated (controls) animals. For each candidate species, both the initial and terminal phases of drying, as well as the rehydration process will be considered. The TDP expression will also be evaluated in relation to different temperatures. The synergistic effect of temperature and dehydration will provide a better understanding of tardigrades response to the influence of climate change. Real-time PCR will be used with designed specific probes to verify the transcription and dynamics of gene expression under different conditions

and to monitor at the same time the expression of multiple TDPs. TDP genes of species representative of tardigrade biodiversity and from different habitats (*e.g.* moss, lichen, leaf-litter, and freshwater) will be sequenced to identify their variability among and within different water bear taxa, to reconstruct their phylogeny within the phylum and to link biological abilities of species to TDPs evolution.

Thanks to these studies, it will be possible to obtain useful data for the better understanding of the processes and molecular factors involved in anhydrobiosis in the different evolutionary lines of tardigrades; therefore, this project will allow to contribute to the basic biology studies. Moreover, I will be able to contribute to develop the applications resulting from the use of xeroprotectans to exploit their technological potential on fields where elective or enforced (by climate change) drying is dealt with. One application could be the development of long-term storage options for desiccation-sensitive cells/gametes. This will be a viable alternative to the current cryo-storage strategy, which has a high CO_2 production, and will help in counter the ongoing loss of biodiversity and erosion of genetic diversity.

Bibliography

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