

# New frontiers in the microbiological research of extreme marine environments: The antioxidant system of Antarctic protozoa

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Ciliated protozoa represent an important component of the aquatic environment and have an important function in bridging the flow of oligo- and macro-elements to higher trophic levels (1). Among them, ciliated protozoa, found throughout the planet including polar regions, offer a unique perspective on basic biological processes.

These microbial eukaryotes acquired great value as experimental model systems due to the facility with which they can be handled, their structural and functional differentiations, their accessibility to genetic and molecular approaches, their large evolutionary distance from other commonly used eukaryotic genetic model systems, and its potential for biotechnological applications coupled with its biosafety. In fact, their complexity rivals human cells, making them a good alternative to human tissues. The cells are inexpensive to grow, requiring little more than a shaker, and they grow rapidly to high density in a variety of media and conditions. Protozoa, although are unicellular, possess many core processes conserved across a wide diversity of eukaryotes (including humans) that are not found in other single-celled model systems (such as yeasts *Saccharomyces cerevisiae* and *Schizosaccharomyces pombe*). Conventional tools for genetic analysis and molecular genetic tools for experimental analysis of gene function have been developed for use in protozoa. Using these techniques homologues of human genes can be characterized, knocked out, and overexpressed among many other things.

This has been impressively demonstrated by two Nobel prizes awarded for work on the ciliated protozoan *T. thermophila*, for the discoveries of catalytic RNA in 1989 (2) and of how chromosomes are protected by telomeres and telomerase in 2009 (3,4). In each case, the phenomena and the mechanisms first described in ciliates have proven to be of widespread occurrence and of fundamental importance for the biology of eukaryotes. Tetrahymena is also a promising system for biotechnological applications. It is used for quick, reliable, sensitive and inexpensive bioassays. For example, it is used for determining the protein nutritional value of human foods, the toxicity of compounds of chemical or biological origin, and for monitoring water quality. It has potential for industrial synthesis of pharmaceuticals. Its capacity for massive, synchronous regulated secretion confers the potential for industrial synthesis of useful proteins. Since mosquitoes and snails are parasitized by other Tetrahymena species and closely related genera, *T. thermophila* has the potential for the development of tools for the biological control of major world-wide human diseases for which those organisms are intermediate vectors: schistosomiasis, malaria, and yellow fever. Tetrahymena-based biotech strategies to fight fish hatchery and pet store epidemics of the closely related ciliate Ichthyophthirius are currently being explored.

In the last years, these microbial eukaryotes provided also potential for many studies in various fields of scientific research, such as biomedicine and ecotoxicology, which have shown that the basis of many phenomena (cell response to environmental contaminants and drugs) is the formation of reactive oxygen species (ROS). ROS are radical and non-radical molecules derived from the partial reduction of molecular oxygen and characterized by distinctive properties that define the level of induced damage. ROS are

continuously produced in cells as a by-product of aerobic metabolism (5), but it is well known that many factors, such as exposure to xenobiotics, drugs and UV rays, hypoxia and hyperoxia, and pathologies, contribute to the increase of ROS formation, also increasing the risk of oxidative stress (6). When the rate of ROS formation is excessive, it can overwhelm the antioxidant capacity of organisms, increasing the risk of oxidative stress and the irreversible damage of biological molecules. However, all oxygen-utilizing organisms face excessive ROS formation by increasing both the expression of antioxidant enzymes, such as superoxide dismutase, catalase, glutathione peroxidase, peroxiredoxin, and the concentration of other molecules (e.g. vitamins, carotenoids, ubiquinone, glutathione) involved in the defense against ROS.

To date the information on the antioxidant system of protozoa is still very limited, despite some research has been done in recent years, in order to characterize the molecular components of this cellular defense system and their kinetics of activation against the risk of oxidative stress. Little is known especially about how sea-ice organisms cope with oxidative stress, but isolated Antarctic ciliated protozoa seems to have an efficient antioxidant systems, with enzymes such as superoxide dismutase and methionine sulfoxide reductase (7,8), being evolved in an hyperoxic environment. In fact, the main characteristic of Antarctic seawater is its low constant temperature of about 11.8°C that determines a high concentration of dissolved gasses, O<sub>2</sub> in particular (9). However, at present, it is unclear whether the higher efficiency of Antarctic antioxidant systems is due to the presence of high performance proteins or an efficient regulation of their biosynthesis (10).

The Antarctic polar region is a natural laboratory for evolutionary ecology studies. The cold but thermally stable seawaters of the Southern Ocean home to a large amount microbial ecosystems, useful for exploring speciation and evolution with limited phenomena of gene flow from outside biota. This is due to the geographical isolation of the Antarctic continent, since its separation from Gondwanaland and the formation of the Antarctic Circumpolar Current, about 25 million years ago (11).

The Antarctic/polar marine coastal waters are astonishingly rich in life and harbour a huge variety of organisms with unique morphological, physiological and genetic evolutionary adaptations. It is a common belief that the rapid climate change that we are globally facing is destined to irreversibly damage all marine ecosystems. This damage will likely be more dramatic in Antarctic/polar regions, where the effects of the climate change are clearly revealed by the decline of the seasonal sea ice extent. Predictive theoretical models are not sufficient by themselves to assess how and to what extent the basic components of polar food webs may respond and adapt to climate change. Gene sequencing-based studies provide an important tool to gather a more comprehensive and integrated view of these responses. By elucidating basic aspects of the molecular and physiological bases of adaptation to cold, these studies can inform how past events have driven species diversification and extinction, and this information is essential to forecast the response of the Antarctic/polar marine life to environmental changes.

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In 2017 the Italian Program for Antarctic Research (PNRA) funded a research project, entitled “How key components of the coastal Antarctic food web respond to global change: an “omic” approach” (PNRA16\_00099). One of the main project objectives is to provide insights into the mechanisms underlying the genetic response of basic components of the Antarctic food web to global climate change, the results from these comparative analyses are expected to define a comprehensive reference ‘atlas’ of novel genetic signatures specific to Antarctic marine life. Through comparative analyses of transcriptome-based signatures/profiles, this project aims to better define the physiological limits of, and the repair mechanisms available to, multiple Antarctic species that occupy different levels of the Southern Ocean trophic chain/food web, in response to stressful environmental conditions. The organisms to be examined include photosynthetic and heterotrophic microorganisms, benthic invertebrates and notothenioid fish from selected locations of Terra Nova Bay and the Antarctic Peninsula: two Antarctic geographic areas that are facing markedly different dynamics of climate change and warming rates. Some species will be analysed under their native conditions to identify whether variations in gene expression distinguish these allopatric con-specific/congeneric, aimed to populations. Among the considered organisms there are ciliate protozoa. Ciliates (Ciliophora, Alveolata) represent a large fraction of eukaryotic microbes inhabiting the Antarctic coastal seawaters (Petz, 2005). They are unicellular eukaryotes occupying ecological niches in nearly all environments, where they play an essential role, at least according to the microbial loop hypothesis (12,13).

Antarctic ciliates include at least seven distinct morpho-species (three of which are still waiting for a definitive taxonomic designation) of the cosmopolitan and ubiquitous ciliate, Euplotes. Comparative gene-sequence analyses have already clearly established the various degrees of kinship that link the spatially distinct populations of these polar Euplotes species to one another, as well as to their non-polar relatives. Particularly important in this context is the knowledge of the ecological and biogeographic diversification that distinguishes four of the species that will be used in this project. One, *E. focardii*, is credited as a species endemic to, and ancient colonizer of Antarctic waters; the other three, *E. euryhalinus*, *E. nobilii* and *E. petzi*, have a bipolar distribution and are credited as much more recent colonizers of Antarctic waters, their Antarctic and Arctic populations showing various degrees of genetic connection.

Some species will be analysed under their native conditions to identify whether variations in gene expression distinguish these allopatric con-specific/congeneric, aimed to populations. Other species will be subjected to thermal challenge experiments.

The expected results of this research project will first implement the knowledge on the molecular evolution of these proteins, but they may also have physiological and ecotoxicological applications. So far, only single and functionally unrelated genes, such as those coding for the heat-shock protein hsp70 and the methionine sulfoxide reductase, have been analysed for their expression in stressed Antarctic Euplotes cells. The project plans to obtain whole transcriptomes from the available Euplotes species, and characterize other genes responsive to thermal stress, in particular those belonging to the antioxidant defense systems. These data, along with those already available for other Antarctic organisms (14,15), will allow to paint a picture more and more clear how evolutionary processes have allowed these organisms to develop adaptations that have made them suitable to live in an extreme environment like Antarctica.

The possible applications in physiology and ecotoxicology will relate to the homeostatic potential of these organisms in relation to environmental changes and the development of tests for the evaluation of quality in marine ecosystems using ciliated protozoa as bioindicator organisms and antioxidant proteins as stress and/or exposure biomarkers.

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