

**"Let's dry up and survive together":
is anhydrobiosis in water bears (Tardigrades) modulated by a specific microbiome community
and does it depend on bacteria that survive desiccation together with them?**

Tardigrades, commonly called water bears, and measuring from 0.01 to 1.2 mm, are considered the most resistant animals on Earth. These cosmopolitan, microscopic organisms inhabit all ecosystems on all continents. Tardigrades arouse widespread interest of scientists all over the world, especially due to their unusual ability of cryptobiosis, thanks to which they can survive very low and very high temperatures (from absolute zero to over 150°C), high doses of UV and ionizing radiation (up to 9,000 Gy), very low and very high atmospheric pressure (up to 74,000 atmospheres), or exposure to a space vacuum. In a state of complete dehydration, i.e. anhydrobiosis, they can survive up to several years. Thus the question arises, what makes tardigrades almost indestructible? Can they owe these extraordinary abilities to specific microbiome inhabiting their organism? If so, which bacteria play an important role in this process?

It is commonly known that the composition of the microbiome in various animals depends on many factors, including diet, ecological niche occupied or reproduction mode. In turn, bacteria that form this microbiome affect various aspects of the life of their host, such as metabolism or toxin inactivation. Important functions in the biology and microevolutionary processes of their hosts are played primarily by symbiotic bacteria. There are numerous studies focusing on the analysis of microbiome profiles and identification of symbiotic bacteria in invertebrates, especially in Arthropoda. On the other hand, knowledge on the Tardigrada microbiome is limited to just a few articles, in which its species-specificity and differences from bacteria found in habitats of the studied Tardigrada species have been demonstrated. Currently, molecular biology tools, including metagenomic sequencing technology, are sufficiently developed and can be used for precise analysis of microbiome profiles in water bears. The pilot study of the microbiome of several species of tardigrades carried out by our team, using new bioinformatics tools, enabled pioneering discovery of the endosymbiotic bacterium of the genus *Wolbachia*. This bacterium is horizontally and/or vertically transmitted from parents to offspring by infected females and can significantly interfere with the host biology, mainly through manipulation of the reproduction process.

The purpose of this project is a comprehensive analysis of the microbiome profiles of different species of tardigrades using metagenomic sequencing. The microbiome in each tardigrade species will be examined at various developmental stages, i.e. eggs, adults (before and after anhydrobiosis), as well as individuals in the stage of anhydrobiosis. Research will cover species inhabiting various ecosystems (freshwater, moist terrestrial and terrestrial xerothermic), with a different diet (herbivores and predators) and characterized by different reproductive modes (parthenogenesis, gonochorism). Metagenomic sequencing will involve the V3-V4 fragment of the bacterial 16S rRNA gene. The obtained operational taxonomic units (OTUs) of various bacteria will be subjected to appropriate bioinformatic analysis based on available databases of reference sequences. As a result, it will be possible to identify bacteria specific for a given species of tardigrades and in the given developmental stage. In this manner, we will also obtain information on the differences in the microbiome composition in species with different diet, characterized by different reproduction modes and inhabiting different microenvironments. And most importantly, we will obtain the answer, which bacteria occur in anhydrobiotic tardigrade specimens, and thus which ones may be responsible for the ability of tardigrades to anhydrobiosis. To confirm the role of microbiome in Tardigrada anhydrobiosis, we will also carry out an experiment that will allow the analysis of changes occurring in the microbiome and the ability of tardigrades to anhydrobiosis. To this end, we will replace the microbiome of tardigrades with anhydrobiosis ability with the one derived from tardigrades, which do not show this behaviour by appropriately conducted experimental cultures. Subsequently, the original microbiome will be recreated experimentally. Thus, we will investigate whether these are the bacteria composing the microbiome that are responsible for successful anhydrobiosis in tardigrades. We will also analyze the metabolic profiles of the microbiome and how they are altered at each stage of the experiment.

If some bacteria play an important role in anhydrobiosis, our research will be groundbreaking in understanding this phenomenon, not only in tardigrades, but also in other invertebrates such as nematodes, rotifers and some crustaceans.