A comprehensive analysis of the structure and composition of the cell wall of pathogenic and saprophytic algae of the *Prototheca* genus – in a *s*earch of pathogenicity factors of *Prototheca* algae

Prototheca species are unicellular, achlorophyllous, yeast-like microalgae, ubiquitously distributed in nature. Although normally saprophytic, these organisms can act as opportunistic pathogens and cause a variety of pathologies in both animals and humans, collectively referred to as protothecosis. In fact, *Prototheca* algae are the only known plants that have repeatedly caused infections in humans and other vertebrates.
Historically, when identification relied exclusively on phenotypic criteria, the *Prototheca* organisms would be classified as fungi due to their fungus-like morphology and the lack of chlorophyll. Since then, several substantial revisions to *Prototheca* taxonomy have been made, with the increasing availability of chemotaxonomic and molecular data. Of 18 *Prototheca* species thus far described, six cause protothecosis in animals (*P. blaschkeae*, *P. bovis*, *P. ciferrii*, *P. cutis*, *P. wickerhamii*, *P. zopfii*), and six – in humans (*P. blaschkeae*, *P. bovis*, *P. cutis*, *P. miyajii*, *P. wickerhamii*, *P. zopfii*).

The most common form of animal protothecosis is bovine mastitis, which represents a serious veterinary problem and incurs heavy economic losses to the dairy industry worldwide due to reduced milk production, increased costs of veterinary treatment, and early culling. Human protothecosis occurs worldwide and has recently been on the rise, especially among senile patients and patients suffering from different forms of immunosuppression. Of particular threat is disseminated protothecosis, associated with poor prognosis and high mortality rate (>50%). The situation is compounded by the paucity of effective treatment options. There is also a lack of consistency in the clinical response. *Prototheca* algae have often been demonstrated to exhibit resistance to multiple antimicrobial agents currently available, with virtually no mechanisms behind this resistance disclosed. Therefore, protothecosis represents a diagnostic and clinical challenge.

Prototheca algae have long remained on the margin of scientific interest. Consequently, there is a huge gap of knowledge regarding their biology and pathogenicity. This knowledge deficiency also applies to structure and composition of cell coverings, which are considered a key component responsible for algal survival in different harsh environmental conditions. The biochemistry and structural biology of the *Prototheca* cell wall had not been approached since the 1970s. The few major studies on this topic were carried out using methods now outdated and inaccurate, which produced somewhat conflicting results.

The aim of the project is to perform a comprehensive analysis of the structure and composition of the *Prototheca* sp. cell coverings, and cell wall in particular. The project is conceived as a two-pronged study, coupling the high-resolution imaging of cell wall structure and ultrasensitive methods of biochemical algal cell wall profiling.

The project will provide answers to pivotal questions regarding the *Prototheca* cell wall including: (i) what is the exact structure and chemical composition of the cell wall, depending on the species and its life strategy (pathogenic *vs* saprophytic)? (ii) How (and if) does the cell wall structure and chemical composition change over time (at different developmental stages)? (iii) What are the key molecules that might be potentially involved in the pathogenesis of *Prototheca* disease? The research project represents the most extensive study ever performed on the structure and composition of the *Prototheca* cell coverings. The results of the project will substantially expand the current knowledge on the biology of *Prototheca* spp.

Since the cell wall is usually a structure critical for determining the infection capacity of a pathogen, the project aims at identification and characterization of potential pathogenicity factors of *Prototheca* algae that might serve as molecular targets for new anti-*Prototheca* drugs or be used for immunization or immunotherapy.