

Exploring the diversity of yeast-like *Prototheca* microalgae in aquatic environments: comments on the taxonomy and description of three new species

Tomasz Jagielski, Mateusz Iskra

Department of Medical Microbiology, Institute of Microbiology, Faculty of Biology,
University of Warsaw, I. Miecznikowa 1, 02-096 Warsaw, Poland

Objectives:

The *Prototheca* genus (Trebouxiophyceae) comprises unicellular, nonphotosynthetic, yeast-like microalgae, associated with rare yet potentially very serious infections of animals and humans, collectively referred to as protothecosis. In animals, the disease most commonly affects dairy cattle, resulting in (sub-)clinical mastitis, while in humans the predominant manifestations are linked to cutaneous, articular, and systemic involvement. The taxonomy of the *Prototheca* genus has long been controversial and frequently revised. Recent studies based on the phylogenetic analysis of the apocytochrome B-coding sequence data have established a new taxonomic classification system of the *Prototheca* algae, installing within the genus a total of 15 species.

Although the *Prototheca* algae are believed to be ubiquitous in nature, since the mid-1980s, no studies have investigated in depth their environmental habitat. The purpose of this study was to explore the occurrence of Protothecae in a wide range of natural and artificial aquatic environments and demonstrate the species diversity of the algae by using a molecular taxonomic profiling approach.

Materials & Methods: In total, 362 samples were collected from freshwater and artificial water reservoirs across Poland over a 2-year period (2018-2020). Liquid and semisolid samples were spread on the *Prototheca* Isolation Medium (PIM) plates, either directly or after 48-hour pre-incubation in liquid PIM. Plates were incubated at 30°C for 2-5 days. Colonies suspected of being *Prototheca* spp., upon macro- and micromorphology observations, were subjected to species-level identification, by using PCR-sequencing of the *CYTB* gene. To

better recognize the phylogenetic relatedness, ribosomal DNA sequencing was performed on selected *Prototheca* isolates. The assimilation profiles of new species were examined using API®20C AUX system (bioMérieux, France).

Results:

Of the samples collected, 51 (14%) yielded *Prototheca* growth with *P. wickerhamii* being the most frequently isolated species (17 or 33.3%), followed by *P. pringsheimii* (12; 23.5%), *P. cerasi* (7; 13.7%), *P. bovis* (5; 9.8%), *P. ciferrii* (3; 5.8%), *P. cookei* (2; 3.9%), and *P. zopfii* (1; 1.9%). Based on the *CYTB* gene and rDNA phylogenies, four isolates, designated PK1, PK2, PK6, and W3, were conspicuously different from all other *Prototheca* species described so far. Their *CYTB* gene sequences showed less than 88% similarity to each other and less than 96% to all other *Prototheca* species. Colonies of those strains were creamy-white, slightly raised, with a smooth surface and even margins. Each strain was of butyrous consistency except W3, whose colonies were clearly slimy. Upon auxanography, all strains utilized glucose, galactose, trehalose, whereas glycerol was assimilated only by W3 and PK2. Furthermore, PK1 grew much more slowly than the remaining isolates. The combined geno- and phenotyping concluded in the proposal of three new *Prototheca* species, namely *Prototheca lentecrescens*, *Prototheca fontanea*, and *Prototheca vistulensis*.

Conclusions:

After four decades, this is the first study to explore thoroughly the occurrence of *Prototheca* spp. in water sources. Unexpectedly, *Prototheca* were isolated at a relatively low rate. This is in contrast to a common belief of environmental ubiquity of the algae. However, finding three novel species among so few strains recovered speaks of important species diversity of the *Prototheca* algae.