# Occurrence of *Prototheca* spp. in aquatic environments in Poland

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**Background:** *Prototheca* species are saprophytic, and occasionally pathogenic, unicellular, achlorophyllous algae ubiquitously distributed in nature. Aquatic niches contaminated with the *Prototheca* algae may provide a source of infection for vertebrates either through direct contact with or traumatic inoculation. The aim of this study was to cross-sectionally examine the occurrence of the *Prototheca* microalgae in water environments in Poland.

**Materials:** A total of 362 samples were collected from freshwater and artificial water reservoirs across Poland between October 2018 and March 2020. Liquid and semisolid samples were spread on the *Prototheca* Isolation Medium (PIM) plates, after either concentration through centrifugation and resuspension in sterile water or 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-RFLP and/or sequence analysis of the *cytb* gene. For selected *Prototheca* spp. strains, three rDNA loci (LSU, SSU, and ITS) were PCR-amplified and sequenced. The assimilation profiles of novel species were examined using API®20C AUX system (bioMérieux, France).

**Results:** Of the samples collected, 51 (14%) yielded *Prototheca* growth with species isolation rates of 33.3%, 23.5%, 13.7%, 9.8%, 5.8%, 3.9%, and 1.9% for *P. wickerhamii*, *P pringsheimii*, *P. cerasi, P. bovis, P. ciferrii, P. cookei*, and *P zopfii*, respectively. Upon *cytb*- and rDNA-derived phylogenies, four strains named PK1, PK2, PK6, and W3 were clearly distant from all *Prototheca* species described so far. Their *cytb* gene sequences of those strains showed less than 87.9% similarity to each other and less than 95.7% to all other *Prototheca* species. Colonies morphology of mentioned strains were similar to *P. moriformis*, with exception for butyrous consistency for PK1, PK2 and PK6. Capsule was present for W3 strain.

**Conclusions:** Since the mid-1980s, this study provides the first extensive investigation into the occurrence of *Prototheca* spp. in water sources. A relatively low isolation rate of

*Prototheca* algae may be somewhat surprising. It seems inconsistent with a belief of the environmental ubiquity of the algae, that has long lingered in the literature. Still, given a low recovery rate, the species diversity of the Protothecae can be considered high.