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Title: *Prototheca vistulensis* sp. nov. – a new microalgal species isolated from the Vistula river, Poland

Background: *Prototheca* species are saprophytic, unicellular, yeast-like algae ubiquitously distributed in nature. Of the 15 species recognized, 6 can be pathogenic to humans and animals. Whereas *Prototheca wickerhamii* is a major causative agent of human protothecosis, *Prototheca bovis* is the commonest pathogen of animals. The most prevalent form of protothecal disease is bovine mastitis, which incurs severe economic losses to the dairy industry. Species identification of *Prototheca* algae has long relied upon phenotypic criteria. Since two decades, several molecular markers have been used, with rDNA markers being most popular. Recently, the mitochondrial *cytb* gene has been proposed as a new barcode for *Prototheca* speciation.

Objective: The aim of the study was to assess occurrence of *Prototheca* species in environmental samples.

Methods: Three 10-cm-deep water samples were collected from the Vistula river, at the height of the Vistula Boulevards in Kraków, Poland. One-mL volumes of each sample were inoculated into liquid *Prototheca* Isolation Medium (PIM) and incubated at 30°C for 48 hrs. Then, 0,1-mL culture volumes were spread onto PIM agar and incubated further for 48 hrs. *Prototheca* spp. suspected colonies were subjected to molecular identification. For this purpose, DNA was extracted using GenoLyse kit (HAIN, France), and 5 genetic loci (i.e. 18S rDNA, D1/D2 domains of the 28S rDNA, ITS1/2, and *cytb* gene) were PCR-amplified and sequenced. The assimilation profiles were examined using API®20C AUX system (bioMérieux, France). Microscopy studies were performed using the Eclipse E600 microscope (Nikon, Japan) at 1000x magnification.

Results: Only one out of three samples yielded growth of a strain that bore a clear morphological resemblance to *Prototheca* algae. It grew readily on PIM, Sabouraud-Dextrose, and Yeast-Extract-Peptone-Dextrose agar at 25°C and 30°C. Colonies were white, flat, glistening, and slimy, with smooth surface and margins. Microscopically, sporangia were globose to ovoid, variable in size (av. 11×9 µm), while sporangiospores were globose or angular, measuring 6×4 µm, on average. The presence of capsule was evident. The strain utilized glucose and glycerol, while galactose and trehalose were not assimilated. At the molecular level, the strain showed the closest affiliation to *P. bovis* (83-97% sequence similarity at all loci).

Conclusions: We provide a description of a new *Prototheca* species, for which we propose the name *Prototheca vistulensis* sp. nov.