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

Mateusz Iskra, Joanna Rudna, Zofia Bakuła, and Tomasz Jagielski*

Department of Medical Microbiology, Institute of Microbiology, Faculty of Biology,
University of Warsaw, I. Miecznikowa 1, 02-096 Warsaw, 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 the most popular.**

Recently, the mitochondrial *cytb* gene (its partial sequence) 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.

Conclusions

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

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 amplified by PCR 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).

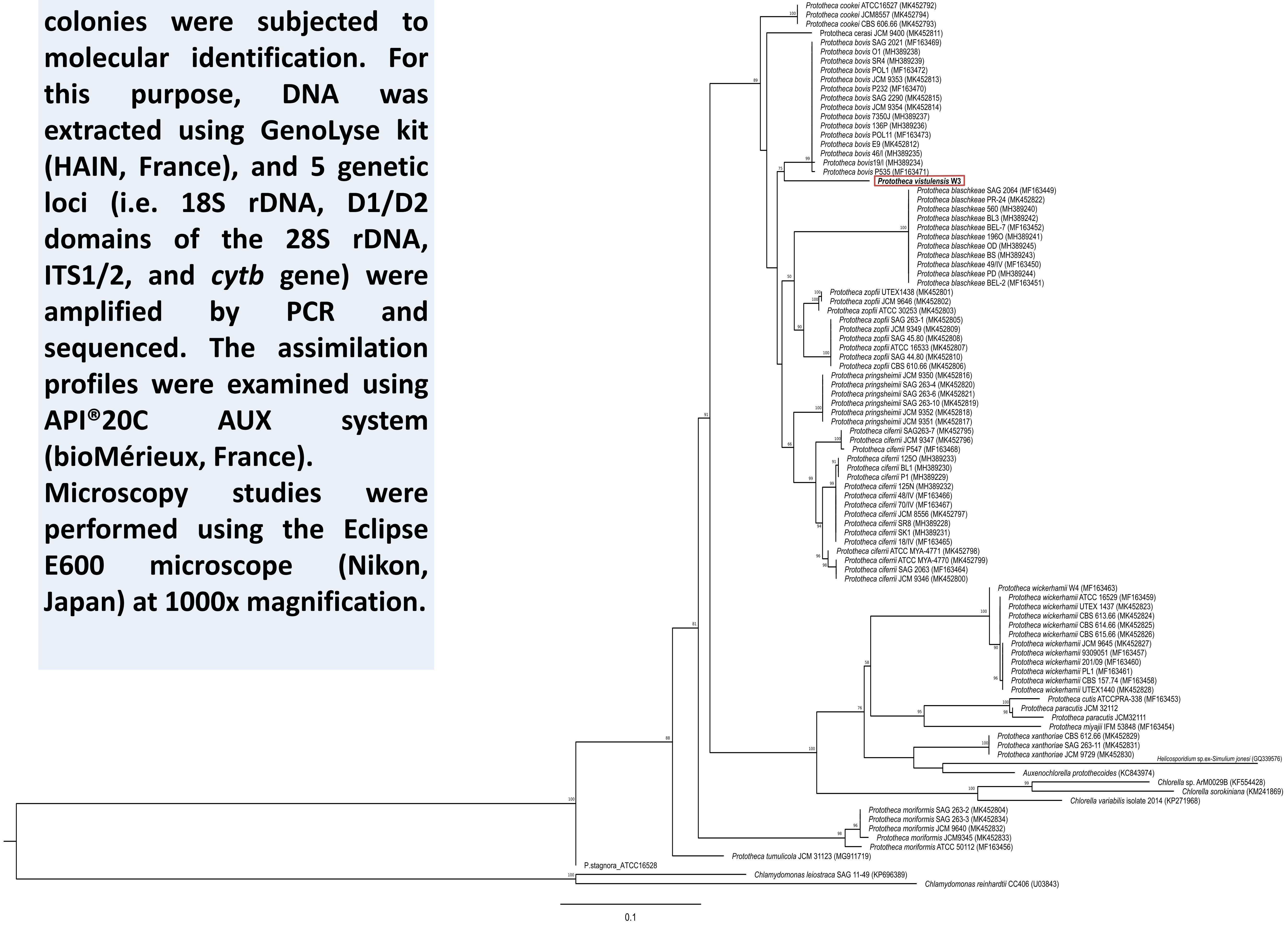

FIG. 1. **Maximum likelihood (ML) phylogram based on the partial *cytb* gene sequences of 86 *Prototheca* and 5 related taxa. Numbers at the nodes are ML bootstrap values (bs) above 50%. The phylogram was rooted to *Chlamydomonas leiostraca* (SAG 11-49) and *Chlamydomonas reinhardtii* (CC 406). Numbers in parentheses are GenBank accession numbers of the partial *cytb* gene sequences. Scale bar indicates one substitution per 10 nucleotide positions.**

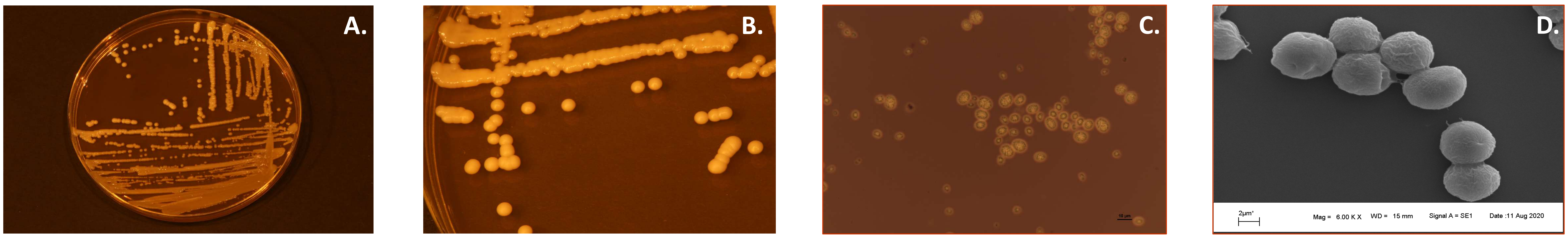

FIG. 2. ***Prototheca vistulensis*** **sp. nov. colonies on SDA medium after 78 hrs at 30°C – A, B: details of cell morphology upon nigrosine staining (x400) – C, and SEM (6000) – D.**

Correspondence to: Dr. Tomasz JAGIELSKI
Head of Department of Medical Microbiology
Institute of Microbiology, Faculty of Biology, University of Warsaw. E-mail: t.jagielski@biol.uw.edu.pl