

Identification of *Prototheca* species isolated from mastitis cases in Poland

Zofia Bakuła¹, Alicja Ratajczyk¹, Henryk Krukowski², Henryka Lassa³, Mariola Bochniarz⁴
Andrzej Lisowski², Władysław Wawron⁴, Jacek Bielecki¹ and Tomasz Jagielski¹

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

²Department of Animal and Environmental Hygiene, University of Life Sciences in Lublin,
Akademicka 13, 20-950 Lublin, Poland

³Milk Testing Laboratory, Chodkiewicza 15 Street, 85–065 Bydgoszcz, Poland

⁴Department and Clinic of Animal Reproduction, Faculty of Veterinary Medicine,
University of Life Sciences, Głęboka 30, 20-612 Lublin, Poland

Introduction and Aim. Algae of the genus *Prototheca* are unicellular, achlorophyllic yeast-like organisms, ubiquitously distributed in the environment [1]. Four of the six currently postulated species, namely *P. zopfii* (genotype 1 and genotype 2), *P. wickerhamii*, *P. blaschkeae*, and *P. cutis* are pathogenic to humans and animals, being the causative agents of protothecosis. The most prevalent form of animal protothecosis is bovine mastitis (mainly caused by *P. zopfii* genotype 2 and *P. blaschkeae*). The aim of this study was to investigate the distribution of *Prototheca* species/genotypes among strains isolated from protothecal mastitis cases in dairy cows in Poland.

Materials and Methods. One hundred of *Prototheca* strains isolated between 2004 and 2015 from bovine mastitis cases in Poland (originated from 29 different dairy herds and 9 voivodeships) were included in the study. All strains were collected and classified to the genus level using phenotype-based approaches. The investigated *Prototheca* strains were cultured aerobically on Sabouraud Dextrose Agar plates for 72 h at 25°C. Genomic DNA was extracted using Bacterial & Yeast Genomic DNA Purification Kit (EURx). Species identification was carried out using genotype-specific PCR assays for *P. zopfii* genotype 1, *P. zopfii* genotype 2, and *P. blaschkeae* (formerly *P. zopfii* genotype 3) as described previously by Roesler *et al.* [2].

Results. Among a hundred strains tested, 97 showed indistinguishable patterns characteristic for *P. zopfii* genotype 2. Two isolates exhibited the *P. zopfii* genotype 1 specific pattern. Only one isolate presented pattern specific for *P. blaschkeae* (formerly *P. zopfii* genotype 3).

Conclusion. This study demonstrates that *Prototheca* strains isolated from mastitis cases in dairy cows in Poland are almost exclusively *P. zopfii* genotype 2. The high detection rate of *P. zopfii* genotype 2 in clinical samples support previous observations that protothecal bovine mastitis is mainly caused by this genotype [3-5]. This is probably due to a particular ability of *P. zopfii* genotype 2 for colonization and/or infection of the dairy cows.

The study was financed by the National Science Centre «SONATA» Programme (2014/15/D/NZ7/01797) and by the Faculty of Biology, University of Warsaw intramural grant DSM (501/86-110103).

References

1. Jagielski T, Lagneau P-E (2007) Protothecosis. A pseudofungal infection. *J Mycol Méd*, 17:261-270.
2. Roesler U, Möller A, Hensel A, Baumann D, Truyen U (2006) Diversity within the current algal species *Prototheca zopfii*: a proposal for two *Prototheca zopfii* genotypes and description of a novel species, *Prototheca blaschkeae* sp. nov. *Int J Syst Evol Microbiol*, 56:1419-1425.
3. Möller A, Truyen U, Roesler U (2007) *Prototheca zopfii* genotype 2: the causative agent of bovine protothecal mastitis? *Vet Microbiol*, 120: 370-374.
4. Bozzo G, Bonerba E, Di Pinto A, Bolzoni G, Ceci E, Mottola A, Tantillo G, Terio V (2014) Occurrence of *Prototheca* spp. in cow milk samples. *New Microbiol*. 37:459-464.
5. Jagielski T, Lassa H, Ahrholdt J, Malinowski E, Roesler U (2011) Genotyping of bovine *Prototheca* mastitis isolates from Poland. *Vet Microbiol*, 149:283-287.