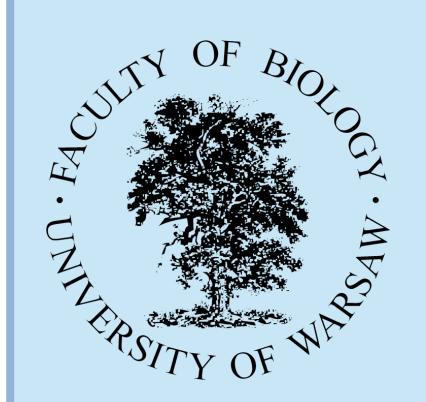
Identification of *Prototheca* species isolated from mastitis cases in Poland



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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*).

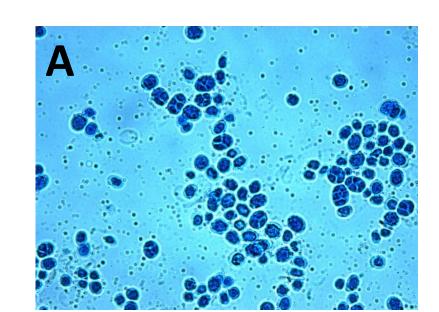
The aim of this study was to investigate the distribution of *Prototheca* species/genotypes among strains isolated from protothecal mastitis cases in dairy cows in Poland.

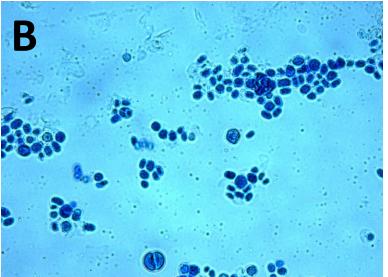
Materials and Methods

One hundred of *Prototheca sp.* 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, including micromorphological criteria (**FIG. 1**). 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 the 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 with a pattern specific for *P. blaschkeae* (formerly *P. zopfii* genotype 3) (**FIG. 2**).





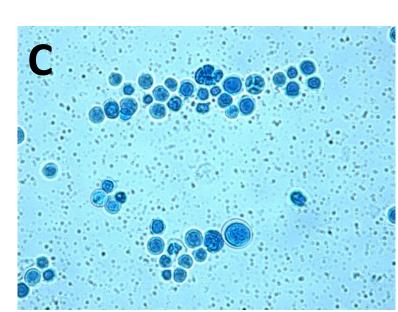


FIG. 1. *P. zopfii* genotype 1 (A), *P. zopfii* genotype 2 (B) and *P. blaschkeae* (C) stained with Lactophenole cotton blue (LCB).

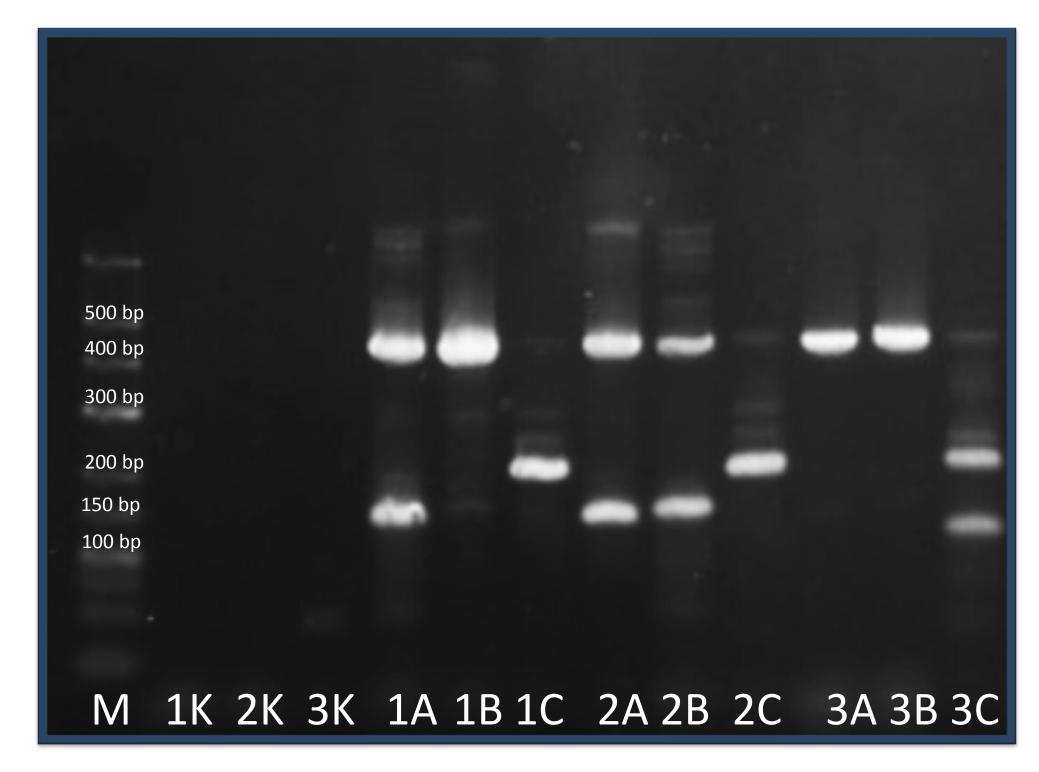


FIG. 2. Differentiation of *Prototheca* strains by PCR. Amplified products were visualised on agarose gel. Lanes A for *P. zopfii* genotype 1 (BO-4), B for *P. zopfii* genotype 2 (POL-1), C for *P. blaschkeae* (POL-20). Lane K – control lanes; M – molecular weight marker.

Conclusions

This study demonstrates that *Prototheca* strains isolated from mastitis cases in dairy cows from 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.

References

- 1. Jagielski T, Lagneau PE (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.