BOVINE PROTOTHECAL MASTITIS IS CAUSED BY A PATHOGENIC SUBSPECIES OF *PROTOTHECA ZOPFII*

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Introduction

Colorless algae of the genus *Prototheca* of the family of *Chlorellaceae* are the only known plant causative agents of infections in humans and animals. The taxonomic status of *Prototheca* has been evolving since the last decades and four species are currently assigned to this genus: *P. zopfii*, *P. wickerhamii*, *P. stagnora*, and *P. ulmea*. A fifth not generally accepted species was assigned to *P. moriformis* (Krüger.W., 1894;Arnold and Ahearn, 1972;Pore, 1985;Pore, 1986).

World-wide, *P. zopfii* has been identified to induce a therapy-resistant endemic inflammation of the mammary gland in dairy cows which may lead to severe economic losses in an infected herd. Auxanographical and biochemical investigations of isolates from different habitats revealed that all bovine mastitis isolates showed a delayed assimilation of galactose and an increased assimilation of amino acids compared to isolates from cattle liquid manure (Blaschke-Hellmessen *et al.*, 1985;Roesler *et al.*, 2003). Therefore, existence of a particular mastitis associated biotype 2 of *P. zopfii* has been discussed.

The present study describes the investigation of 30 epidemiologically independent mastitis isolates of *P. zopfii* by biochemical investigation and by biotype-specific PCRs.

Material and methods

Based on sequence analyses of the 18S rDNA, which showed distinct differences between the former variants, specific oligonucleotides and restriction enzymes were chosen and used in biotype-specific PCR- and RFLP-assays. DNA preparations and the specific amplifications were performed as described (Roesler et al., 2005). Three reference strains representing the three biotypes of *P. zopfii* were used as internal biotype controls.

The biochemical identification of *Prototheca* species and the differentiation of the mastitis isolates into biotypes of *P. zopfii* was performed as described (Roesler *et al.*, 2001;Roesler *et al.*, 2003) by the microbial identification system (BBL Crystal®, Becton Dickinson, Detroit, MI, USA). Two kits of these identification system were utilized, BBL

Crystal Enteric/Nonfermenter® and BBL Crystal Gram positive®, respectively. In total, 50 different C- and N-sources were checked with these two kits for each strain. The test panels were incubated in a humid chamber at 37 °C for 48 hours. The discriminating biochemical characteristics are shown in table 1.

Results and conclusions

Both biotyping and genotyping revealed that all 30 epidemiologically independent mastitis isolates could be assigned to biotype 2 of *P. zopfii* and to one genotype of these algae species (figure 1). All clinical isolates were found to be different from reference strains of the other two biotypes 1 and 3.

Table 1: Discriminating characteristics of *Prototheca* zopfii biotypes. Nutrient utilization after 48 h using BBL Crystal® kits ([a], Enteric/NF™ or [b],Gram positive™) and is scored as: +, utilization; -, no utilization; -[C], utilization after 96 h.

Characteristic	*P. zopfii*, biotype 1	*P. zopfii*, biotype 2	*P. zopfii*, biotype 3
Galactose [a]	+	-[c]	+
Glycerol [a]	+	+	-
Arginine [a]	+	+	-
Lysine [a]	-	+	-
Fructose [b]	+	+	+
Trehalose [b]	-	-	-

Therefore, our results show that bovine Prototheca mastitis is caused by only one of the genotypes of *P. zopfii.* Based on sequence analyses this genotype should be reclassified as a subspecies *Prototheca zopfii* ssp. *bovimastitogenes*. The postulation of the different subspecies of *P. zopfii* is strongly supported by the disease-associated occurrence of the current biotype 2 at bovine mastitis which was not reported yet for the other biotypes (Schuster and Blaschke-Hellmessen, 1983). Based on our results we furthermore propose that the current biotype 3 of *P. zopfii* merits classification as a new *Prototheca* species.

So, the findings of earlier serological, biochemical, and morphological studies (Blaschke-Hellmessen *et al.*, 1985;Blaschke-Hellmessen *et al.*, 1987;Schmalreck *et al.*, 1998;Roesler *et al.*, 2003) could be confirmed by our biochemical and genetical results.

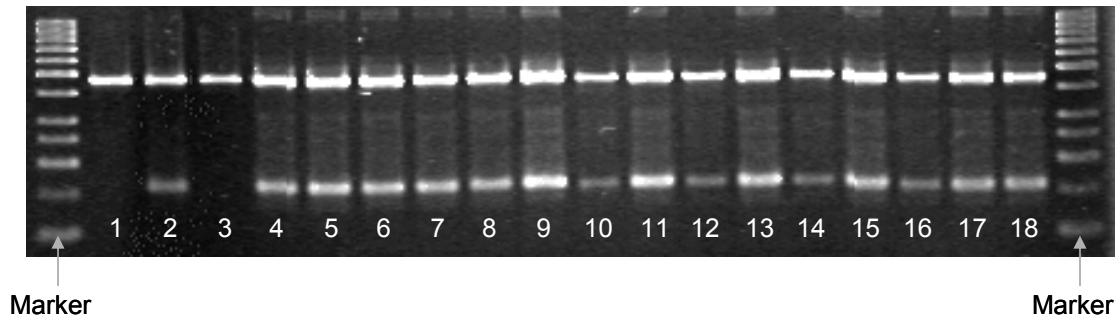

Figure 1: Results of biotype-specific PCRs for *P. zopfii* showing the specific products and the internal amplification controls. Lanes: M = Marker, 1 = *P. zopfii* biotype 1; SAG 2063^T, 2 = *P. zopfii* biotype 2; SAG 2021^T, 3 = *P. zopfii* biotype 3; SAG 2064^T, 4 -18 = different mastitis isolates.

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