GENOTYPING OF BOVINE MASTITIS ISOLATES OF Prototheca Zopfii IN POLAND

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The aim of the study was to employ the newly-devised molecular approach to investigate the genotypic composition of the population of P. zopfii bovine MASTITIS isolates according to that threefold classification. The study included 44 isolates of Prototheca sp. from the culture collection of the Department of Pathophysiology of Reproduction and Mammary Gland of the National Veterinary Research Institute (Bydgoszcz, Poland). The isolates were originally retrieved from milk of 38 cows with clinical or subclinical MASTITIS.

Genomic DNA was extracted with the DNeasy Plant Mini Kit (Qiagen), following the manufacturer's instructions. The determination of P. zopfii genotypes (1 & 2) and P. blaschkeae was performed by 18S rDNA-based genotype specific PCR and PCR-RFLP assay, as described by Roesler et al. All but one isolate were identified as P. zopfii genotype 2, based on the presence of a genotype 2-specific band (165 bp) in their amplification profiles. One isolate yielded a pattern that contained the P. blaschkeae-specific amplicon (126 bp).

The results obtained by genotype-specific PCR analysis were entirely confirmed by using the PCR-RFLP typing system. The 409-bp fragment of the 18S rDNA gene, amplified from all but one MASTITIS isolates tested, were digested with the P. zopfii genotype 2-specific restriction enzyme SmaI, generating two fragments of 230 bp and 179 bp. The 450-bp fragment of the 18S rDNA obtained from only one isolate was cut by the BclI restriction enzyme, resulting in two fragments of 315 bp and 135 bp. This isolate was thus determined to be P. blaschkeae.

In conclusion the findings from this study clearly show the predominance of the P. zopfii genotype 2 in the etiology of bovine mammary protothecosis in Poland.

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