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SHORT COMMUNICATION

Enzymatic Activity of *Prototheca zopfii* Strains Isolated from Cows with Mastitis

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Abstract

Bovine mastitis caused by *Prototheca* spp. can be a disease of high significance because of economic losses and the potential risk to public health. The aim of our study was to evaluate enzymatic activity of *Prototheca zopfii*. For this study, we used 15 *P. zopfii* strains previously isolated from cows with clinical and subclinical mastitis in Poland. We determined enzymatic profile of *Prototheca* species using the API ZYM system. Of the enzymatic activities detected during the study, acid phosphatase, leucine arylamidase, naphthol-as-bi-phosphohydrolase, esterase, lipase esterase, valine arylamidase, alkaline phosphatase, and lipase C14 were found in high percentage of strains.

Key words: *Prototheca zopfii*, mastitis, API ZYM

The genus *Prototheca* includes unicellular achlorophyllous microalgae that belong to the family *Chlorellaceae*. These organisms reproduce asexually by formation of variable numbers of sporangiospores within a sporangium (Marques *et al*., 2010). *Prototheca* spp. can be found in the environment as ubiquitous detritus inhabitants and contaminants of various substrates (Lass-Flörl and Mayr, 2007). *Prototheca* spp. are often associated with wet areas containing decaying manure and plant matter. The increasing number of isolations of *Prototheca* spp. from 5–14% of bovine mastitis cases indicates the need of a detailed evaluation of this problem (Bueno *et al*., 2006; Costa *et al*., 1996). Prototheca mastitis in cows is mostly caused by *Prototheca zopfii* (Costa *et al*., 1996; Cunha *et al*., 2010; Cunha *et al*., 2006; Jagielski *et al*., 2010; Jagielski *et al*., 2011; Lassa *et al*., 2011; Roesler *et al*., 2006) and sometimes by *Prototheca wickerhamii* (Marques *et al*., 2006) although *P. wickerhamii* is isolated primarily from humans (Galan *et al*., 2006; Lass-Flörl and Mayr, 2007).

The enzymatic activity of the yeast, molds and dermatophytes can be a symptom of their virulence, as well as a factor facilitating their invasion (Brasch and Zaldua, 1994; Krajewska-Kułak *et al*., 1998). For this reason, the aim of the present study was to evaluate enzymatic activity of *P. zopfii* isolated from the cows with clinical and subclinical mastitis in Poland.

For this study, 15 *P. zopfii* isolates previously obtained from clinical and subclinical bovine mastitis were subcultured on Sabouraud 4% dextrose agar (bioMe'rieux, Poland) and incubated for 48 to 72 h at 37°C. Identification was performed using routine culture, macro- and microscopic morphological characterization, and API 20CAUX (bioMe'rieux, Poland) methods (Marques *et al*., 2006; Padhye *et al*., 1979; Costa *et al*., 1996; Costa *et al*., 1997). We also used test that differentiates of each species of algae based on their susceptibility to clotrimazole according to Casal and Gutierrez (1983). The disks with antibiotic used in this study contained 50 μg of clotrimazole, susceptibility was defined as a zone of inhibition of 10 mm or more. Based upon morphologic features, resistance to clotrimazole and ability to assimilate glucose and glycerol all isolates were identified as *P. zopfii*.

In this study the enzymatic profile of *Prototheca* species was determined using the API ZYM system. The API ZYM system (bioMe'rieux, Poland) is a semiquantitative micromethod which allows rapid determination of 19 enzymatic reactions.

Investigated strains were characterized by the activity of enzymes listed in Table I. We detected enzymatic activities of alkaline phosphatase, esterase, esterase lipase, lipase C14, leucine arylamidase, valine arylamidase, acid phosphatase and naphthol-as-biphosphohydrolase. These enzymes were produced by majority of strains, with the exception of lipase C14, where only 5 strains were producing this enzyme.

None of the strains exhibited activity of trypsin, α-chymotrypsin, α-galactosidase, β-glucuronidase, N-acetyl-β-glucosaminidase, α-mannosidase, α-fucosidase,

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Table I
Enzyme activity of 15 strains of *Prototheca zopfii* in API ZYM test

Enzyme test		Number of strains	% of strains
I	check	0	0
II	alkaline phosphatase	11	73,33
III	esterase	15	100
IV	esteraze lipase	15	100
V	lipase C14	5	33,33
VI	leucine arylamidase	15	100
VII	valine arylamidase	11	73,33
VIII	cystine arylamidase	0	0
IX	trypsin	0	0
X	α-chymotrypsine	0	0
XI	acid phosphatase	15	100
XII	naphthol-as-biphosphohydrolase	15	100
XIII	α-galactosidase	0	0
XIV	β-galactosidase	0	0
XV	β-glucuronidase	0	0
XVI	α-glucosidase	0	0
XVII	β- glucosidase	0	0
XVIII	n-acetyl-b-glucoseaminidase	0	0
XIX	α-manosidase	0	0
XX	α-fucosidase	0	0

cystine arylamidase, β-galactosidase, α-glucosidase, β-glucosidase, α-fucosidase, and N-acetyl-β-glucosaminidase.

Enzymatic activity of *P. zopfii* isolates was in close similarity to those stated by Malinowski *et al*. (2002) and Casal *et al*. (1985).

P. zopfii is one of the most responsible for bovine prototheca mastitis. The agent is widely disseminated in the environment and its occurrence in herds is generally associated with the presence of mud, use of contaminated water during milking and flaws in milking routines (Bueno *et al.*, 2006). Milk from cows with prototheca mastitis usually has elevated somatic cell counts to the point that it can be elevated in bulk tank milk, as well as the standard plate count for the herd may also be elevated. Due to the detrimental impact of prototheca infections on milk quality and lack of response to treatment, culling is advised for known infected cows (Kirk and Mellenberge, http://www.uwex.edu/milkquality/PDF/prototheca.pdf).

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