

DOI 10.2478/v10181-011-0009-8

Original article

Enzymatic activity of yeasts isolated from the inflamed mammary secretion in dairy cows

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Abstract

The aim of the study was to evaluate enzymatic activities of yeasts isolated from inflammatory mammary secretion. The yeasts isolated from cows with clinical and sub-clinical *mastitis* (134 strains) included: *Candida krusei* (62 strains), *Candida kefyr* (48 strains), *Candida lusitanae* (17 strains) and *Candida famata* (7 strains). The API ZYM system was used containing substrates to assess 19 hydrolytic enzymes. Substantial differences in the number and activity of hydrolyses were demonstrated in individual species. In *Candida krusei*, acid phosphatase showed the highest activity (4.36 points), in *Candida kefyr* and *Candida lusitanae* – leucine arylamidase (4.93 and 4.25 points, respectively), in *Candida famata* – α -glucosidase (4.75 points). No activity of trypsin, chymotrypsin, α -galactosidase, β -glucuronidase, α -mannosidase or α -fucosidase was observed in any of the yeasts examined.

Key words: cows, *mastitis* yeasts, enzymatic activity

Introduction

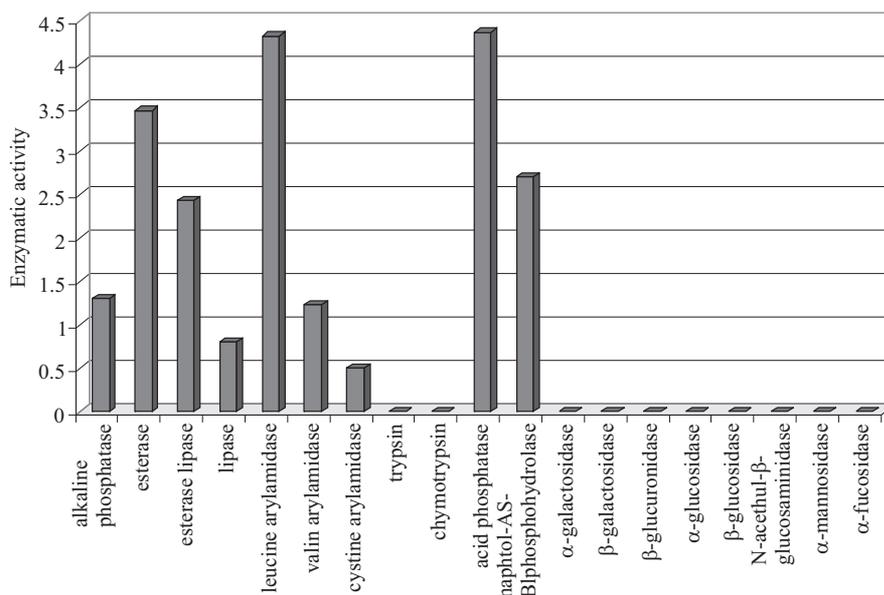
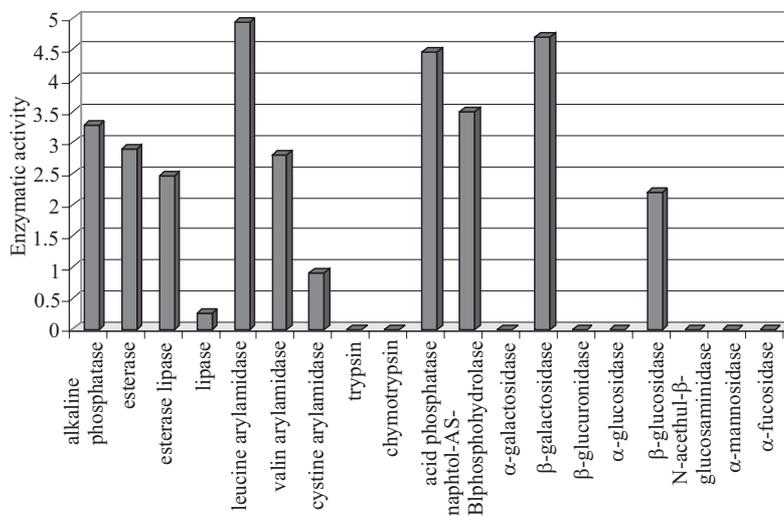
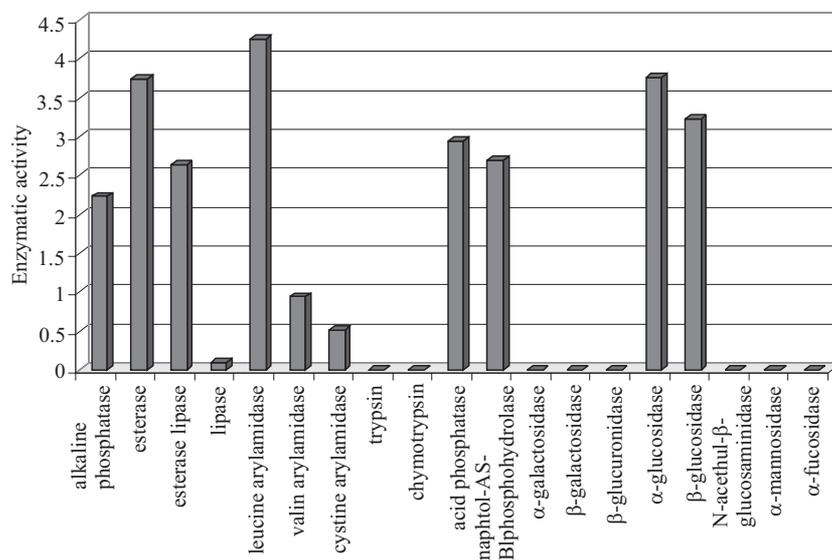
Yeasts are microorganisms widely spread in nature. Unlike bacteria, they get to the mammary gland only via the galactogenic route. The main source of infection is the environment of animals (Gedek 1970, Richard et al. 1980, Krukowski 2000). Moreover, yeast occur on the udder and teat skin, and the alimentary mucous membrane; they invade the teat canal or come from the infected milk yield cups or veterinary devices (Gedek 1970). The development of fungal mastitis depends on the predisposing factors, which include the long-term use of antibiotics and immunosuppression of animals. Another factor that facilitates the invasion of fungi to the tissues is the ability to secrete hydrolytic enzymes, which are responsible for hydrolysis of C-O, C-N and C-C bonds (Krajewska-Kulak et al. 1997, Plomer-Niezgoda and Baran 1997, Schaller et al. 2005, Dąbkowska 2007).

Due to their chemical and physical effects on the environment, they ensure their survival in tissues and are involved in the digestion of host's proteins (Krajewska-Kulak et al. 1998, Krajewska-Kulak et al. 2002). Enzymes produced by fungi facilitate the invasion to tissues and significantly affect the development of infections. The activity and character of the enzymes released varies with the kind of medium and is a relevant adaptive factor (Brasch and Zaldua 1994, Nawrocki and Korting 1995, Nowicki 1995, Plomer-Niezgoda and Baran 1997).

The aim of the study was to evaluate enzymatic activities of yeasts isolated from the inflammatory mammary secretion in cows.

Materials and Methods

The material consisted of 134 strains of yeasts isolated from the mammary secretion. Milk samples col-

Fig. 1. Enzymatic activity of *Candida krusei* (n=62)Fig. 2. Enzymatic activity of *Candida kefyr* (n=48)Fig. 3. Enzymatic activity of *Candida lusitanae* (n=17)

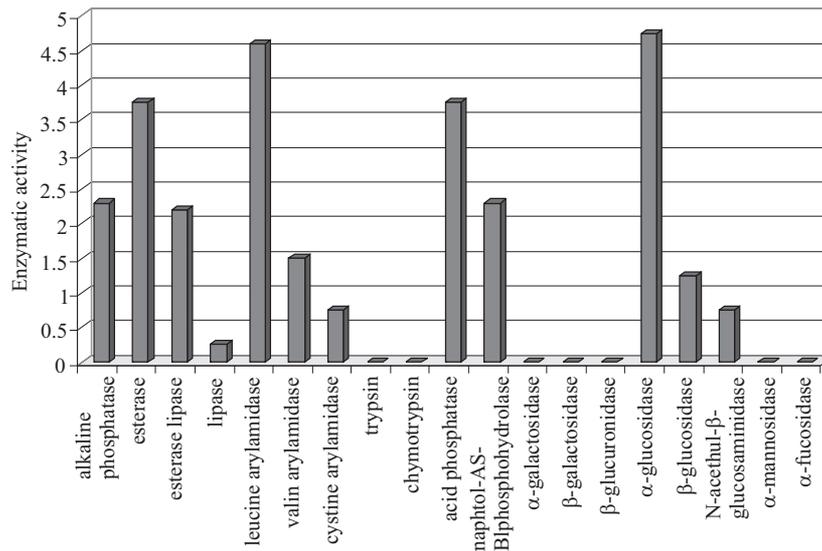


Fig. 4. Enzymatic activity of *Candida famata* (n=7)

lected from cows with clinical and subclinical *mastitis* treated at the Department and Clinic of Animal Reproduction in Lublin (Poland) in 2008. The diagnosis of udder inflammations was based on the anamnesis, clinical examinations of udders, macroscopic examinations of the secretion, the California Mastitis Test (CMT), somatic cell count in milk and bacteriological tests of the secretion. Milk samples were collected aseptically by the employers of the Department or local veterinary physicians, cooled and delivered to the laboratory. Microbiologic examinations were conducted according to standard methods (Malinowski and Kłossowska 2002). The genus and species of fungi were determined using the API 20 AUX test (BioMerieux, France).

The hydrolytic activity of fungi was examined using the API ZYM test (BioMerieux, France) containing substrates for the evaluation of 19 hydrolytic enzymes: alkaline phosphatase (1), esterase (2), esterase lipase (3), lipase (4), leucine arylamidase (5), valin arylamidase (6), cystine arylamidase (7), trypsin (8), chymotrypsin (9), acid phosphatase (10), naphthol-AS-BIphosphohydrolase (11), alpha-galactosidase (12), beta-galactosidase (13), beta-glucuronidase (14), alpha-glucosidase (15), beta-glucosidase (16), N-acethyl-beta-glucosaminidase (17), alpha-mannosidase (18) and alpha-fucosidase (19). From the 24-h culture with the Sabouraud medium, the suspension of fungus cells was prepared (density – 6° according to the McFarland scale) and placed in the microtubes on the API ZYM strip. Once the staining agents were added, the activity of enzymes of the hydrolysed substrate was determined (nanomoles) according to the intensity of colour reaction using the 5-point scale, where 0 denotes a negative reaction, 1 corresponds to the release of 5 nmol, 2 – up to 10 nmol, 3 – up to 20 nmol, 4 – up to 30 nmol and 5 – 40 nmol or more.

Results

The yeasts isolated from the mammary secretion (134 strains) included: *Candida krusei* (62 strains), *Candida kefir* (48 strains), *Candida lusitaniae* (17 strains) and *Candida famata* (7 strains).

The enzymatic activity of yeasts is presented in Figs. 1-4. The species examined showed significant differences in the number and activity of the released hydrolases. The activity of trypsin, chymotrypsin, alpha-galactosidase, beta-glucuronidase, alpha-mannosidase and alpha-fucosidase was not found in any of the species examined. In *Candida krusei* (Fig. 1), acid phosphatase and leucine arylamidase showed the highest activity (4.36 and 4.32 points, respectively). The *Candida kefir* strains (Fig. 2) were characterized by high activity of leucine arylamidase (4.93), beta-galactosidase (4.70), acid phosphatase (4.46) and phosphohydrolase (3.50). They were the only species to secrete beta-galactosidase. The isolated strains of *Candida lusitaniae* (Fig. 3) were characterized by high activity of leucine arylamidase (4.25) and slightly lower activity of alpha-glucosidase (3.76) and esterase (3.74). In *Candida famata* strains (Fig. 4), high activities of alpha-glucosidase (4.75) and leucine arylamidase (4.60) were demonstrated. Only this species secreted N-acethyl-beta-glucosaminidase.

Amongst the hydrolytic enzymes, leucine arylamidase showed high activity in all the species of yeasts examined (> 4.0) whereas lipase and cystine arylamidase the lowest one (< 1.0).

Discussion

There are not many literature reports concerning the enzymatic activity of yeasts isolated from *mastitis*

in cows (Malinowski et al. 2001, Lassa and Malinowski 2005). The majority of papers deal with human diseases. A few studies in animals were concerned with the enzymatic activity of *Malassezia* spp. (Król and Staroniewicz 2000) isolated from clinically healthy dogs and those with auditory meatus inflammation. It has been demonstrated that enzymes produced by fungi isolated from healthy animals were characterized by lower activities, although statistically significant differences were found only in relation to esterase lipase, cystine arylamidase and esterase. On the other hand, alkaline phosphatase and β -glucosidase showed lower activities in strains from affected animals. Similar results were reported by other authors (Mancianti et al. 2000). The study about the enzymatic activity of yeasts isolated from cows with mastitis (Lassa and Malinowski 2005) did not demonstrate the activity of trypsin, chymotrypsin, α -galactosidase, β -glucuronidase, α -mannosidase and α -fucosidase. Our findings confirmed those results. In our study, similarly to other reports (Białasiewicz et al. 1995, Lassa and Malinowski 2005) individual species of isolated fungi showed high differences in the number of activity of hydrolases. *Candida krusei* and *Candida kefyr* isolates were characterized by an extremely high activity of acid phosphatase whereas *Candida kefyr* was the only one to secrete β -galactosidase, which is consistent with other reports (Lassa and Malinowski 2005).

In our findings, the strains of yeasts isolated from the mammary secretion of cows with clinical and sub-clinical mastitis, were characterized by high activity of leucine arylamidase and acid phosphatase. Perhaps the activity and character of the released enzymes may be a relevant adaptation factor and manifestation of the fungus virulence facilitating tissue invasion (Krzemińska-Jaśkowiak et al. 1994, Munson et al. 2002). In conclusion, enzymatic properties may be used to assess the pathogenicity of yeasts (Moretti et al. 1998).

References

- Białasiewicz D, Głowacka A, Kurnatowska A (1995) Activity of selected hydrolytic enzymes of fungi from different families. *Medical Mycology* 2: 83-88.
- Brasch J, Zaldua M (1994) Enzyme patterns of dermatophytes. *Mycoses* 37: 11-16.
- Dąbkowska M (2007) Enzymatic activity of *Candida albicans* strains isolated from clinical specimens of a kidney transplant recipient. *Medical Mycology* 14: 73-74.
- Gedek B (1970) Mykosen in Rinderbeständen. *Zentralbl Veterinarmed B* 17: 234-237.
- Krajewska – Kułak E, Łukaszuk C, Niczyporuk W, Trybuła J (1998) Evaluation of activity of selected hydrolytic enzyme of the yeast-like fungi of *Candida* isolated from urether. *Medical Mycology* 5: 165-170.
- Krajewska-Kułak E, Łukaszuk C, Niczyporuk W, Trybuła J, Szczurzewski M (2002) Enzymatic biotypes of yeast-like fungi strains and their susceptibilities to antimicrobics isolated from ontocenosis of urogenital system. *Medical Mycology* 9: 67-74.
- Krajewska-Kułak E, Niczyporuk W, Karczewski J, Złotowski W (1997) Assessment of activity of selected hydrolytic enzymes in the yeast-like *Candida* fungi using API ZYM test. *Medical Mycology* 4: 147-152.
- Król J, Staroniewicz Z (2000) Assessment of the activity of egzoenzymes in *Malassezia pachydermatis* strains isolated from dogs. *Medical Mycology* 7: 7-12.
- Krukowski H (2000) Mycotic mastitis in cows. *Med Weter* 57: 18-20.
- Krzemińska-Jaśkowiak E, Pietkiewicz K, Ozdowska B (1994) Wykorzystanie enzymatycznej aktywności szczepów z rodzaju *Candida* izolowanych z pochwy do ich typowania i oceny chorobotwórczości. *Med Dosw Mikrobiol* 46: 225-231.
- Lassa H, Malinowski E (2005) Enzymatic activity of yeast and algae isolated from the inflamed udder secretion of cows. *Med Weter* 61: 673-675.
- Malinowski E, Kłossowska A (2002) Diagnostyka zakażeń i zapaleń wymienia. Państwowy Instytut Weterynaryjny, Pulawy, pp 44-72.
- Malinowski E, Lassa H, Kłossowska A, Kuźma K (2001) Enzymatic activity of yeast species isolated from bovine mastitis. *Bull Vet Inst Pulawy* 45: 289-295.
- Mancianti F, Rum A, Nardoni S, Corazza M (2001) Extracellular enzymatic activity of *Malassezia* spp. isolates. *Mycopathol* 149: 131-135.
- Moretti A, Pasquali P, Mencaroni G, Boncio L, Pergili Fioretti D (1998) Relationship between cell counts in bovine milk and presence of mastitis pathogens (yeasts and bacteria). *Zentralbl Veterinarmed B* 45: 129-132.
- Munson EL, Troy DR, Weber JK, Messer SA, Pfaller MA (2002) Presumptive identification of *Candida kefyr* on levine formulation of eosin methylene blue agar. *J Clin Microbiol* 40: 4281-4284.
- Nawrocki R, Korting HCH (1995) Differences in the hydrolytic activity of dermatophytes. *Medical Mycology* 2: 209-213.
- Nowicki R (1995) Enzyme release by dermatophytes dominating in the Gdańsk area. *Przegląd Dermatologiczny* 82: 38-48.
- Plomer-Niezgoda E, Baran E (1997) The estimation of the activity of hydrolytic egzoenzymes in selected moulds. *Medical Mycology* 4: 141-145.
- Richard J, Mc Donald JS, Fichtner RE, Anderson AJ (1980) Identification of yeasts from infected bovine mammary glands and their experimental infectivity in cattle. *Am J Vet Res* 41: 1991-1994.
- Schaller M, Borelli C, Korting HC, Hube B (2005) Hydrolytic enzymes as virulence factors of *Candida albicans*. *Mycoses* 48: 365-377.