



Diversity of yeasts from bovine mastitis in Southern Brazil

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Summary

Mastitis is one of the most serious problems in the dairy cattle farms. The great majority of the cases are caused by bacteria, but lately there have been an increasing number of reports about cases of mycotic etiology. The objective of this work was to characterize the yeasts and yeast-like fungi associated with milk of cows with mastitis. Milk samples (n = 248) from a dairy belt situated around the region of Passo Fundo, hinterland of the state of Rio Grande do Sul, Southern Brazil, were analyzed. Aliquots of 0.1 ml of milk were inoculated on yeast extract-malta agar with chloramphenicol. After a period of incubation of 3-5 days at 22-25 °C, the counting of the morphologically distinct colonies was performed, as well as the isolation and identification through phenotypical and physiological criteria. It was possible to isolate 68 yeast species from 43 (17.3%) of the samples. The most frequent genera were *Candida* (37.9%), *Pichia* (19.1%), *Cryptococcus* (10.3%) and *Rhodotorula* (10.3%).

Key words

Bovine mastitis, Yeast, Yeast-like fungi, Cows, Milk, *Candida*

Etiología de la mastitis bovina producida por levaduras en el sur de Brasil

Resumen

La mastitis es uno de los problemas más graves en los rebaños lecheros. La gran mayoría de los casos son causados por bacterias, aunque últimamente la literatura está registrando un aumento de los casos de etiología micológica. El objetivo de este trabajo fue caracterizar levaduras y otros hongos levaduriformes aislados de leche de vacas con mastitis. Fueron analizadas 248 muestras de leche del cinturón lechero situado alrededor de la región de Passo Fundo, interior del estado de Rio Grande do Sul, sur de Brasil. Alicuotas de 0,1 ml de leche fueron inoculadas en agar extracto de levadura-malta con cloranfenicol. Después de un período de incubación de 3-5 días a 22-25 °C fue realizado el recuento de las colonias diferenciadas morfológicamente, así como el aislamiento e identificación a través de criterios fenotípicos y fisiológicos. En 43 (17,3%) de las muestras fue posible aislar 68 especies. Los géneros más frecuentes fueron *Candida* (37,9%), *Pichia* (19,1%), *Cryptococcus* (10,3%) y *Rhodotorula* (10,3%).

Palabras clave

Mastitis bovina, Levaduras, Hongos levaduriformes, Vacas, Leche, *Candida*

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Mastitis is a disease that causes enormous losses to the dairy cattle activity, both in the quantity and in the quality, with consequences for the production of the milk derivatives. The great majority of the cases are caused by bacteria, but reports dealing with fungal etiology, which are sometimes under the form of outbreaks [5-7] and commonly related with post-treatment with antimicrobial agents [12], have become more frequent.

Though moulds and yeasts are widely distributed in nature, only the yeasts are usually implicated as the cause of mycotic mastitis infections in dairy animals [9,17]. The most frequent genus is *Candida* [2,19]. Other genera like *Cryptococcus*, *Pichia* and *Trichosporon*, albeit found in low prevalence, have also been isolated from clinical cases with reports of 1-12% of prevalence [11,18,22,23,25,26].

The objective of this work was to characterize the yeasts and yeast-like fungi associated with milk of cows with mastitis in southern region of Brazil.

Materials and methods

Sampled animals. The study was carried out with multiparous cows from 28 dairy farms, under the intensive system of husbandry, distributed in a dairy belt around the region of Passo Fundo, hinterland of the state of Rio Grande do Sul, Southern Brazil, from November/2005 to March/2006. The cows did not receive any treatment before sampling.

Criteria for clinical or subclinical mastitis. The clinical mastitis was characterized by clinical signs and/or abnormal milk secretion detected by the strip cup test. Subclinical mastitis was identified by the conventional CMT (California Mastitis Test).

Milk samples. A total of 248 milk samples were obtained, being 54 from cows with clinical mastitis and 194 from cows with subclinical mastitis. The samples (10 ml) were aseptically collected in sterile glass bottles after disinfection of the teats with alcohol 70 °C and kept at a temperature of 0-4 °C.

Isolation and identification of yeasts and yeast-like fungi. Aliquots of 0.1 ml from milk samples were spread in triplicate on acidified YM agar (0.3% yeast extract, 0.3% malt extract, 1% glucose, 0.5% peptone, 2% agar, 400 mg/l of chloramphenicol, pH 4.5). The plates were kept for 3 to 5 days at 22-25 °C (temperature that allows the development of pathogenic and non-pathogenic yeasts). Representative colonies of each morphological type were isolated and purified in Petri dishes containing YM agar. The strains were maintained in Sabouraud Dextrose Agar (Oxoid) slants covered with a layer of sterile mineral oil and kept in the refrigerator. The yeasts were phenotypically characterized by standard tests [3,21,38]. Identification was performed according to Barnett et al. (3) and the computer program Yeastcompare (C. Cirello and M.A. Lachance, Copyright© 1999-2001) that allows comparison of physiological characteristics, such as growth in different temperatures and assimilation of specific carbon and nitrogen sources, using as control already classified species. The yeast-like strains that produced arthroconidia were classified in the genera *Geotrichum* or *Trichosporon*. Species that were urease and diazonium blue B (DBB) negative were identified as *Geotrichum*, while those that were urease and DBB positive as *Trichosporon* [3].

Results

A total of 68 fungal species were isolated from 43 (22.1%) out of 194 milk samples from cows with subclinical mastitis. They were classified into the following genera: *Candida* (37.9%), *Pichia* (19.1%), *Rhodotorula* (10.3%), *Cryptococcus* (10.3%), *Geotrichum* (5.8%), *Debaryomyces* (5.8%), *Trichosporon* (4.4%), and the remaining (1.4%) into *Kluyveromyces*, *Galactomyces* and *Rhodospiridium* (Table). Yeasts and yeast-like fungi were not isolated from cases of clinical mastitis.

Discussion

There are reports in the literature about occurrence of yeasts in milk and cheese from bovine, ovine and goat species [10,11]. Fungi can be found in a great variety of substrates, such as mammary gland, hands, soil and water [32]. The environmental contamination associated with lack of hygiene during the milking and poor equipment cleaning leads to the development of mastitis.

The use of antimicrobials for a long period is pointed out as the main factor that propitiates the occurrence of mycotic mastitis because they affect the microflora of

the mammary glands, which acts as an animal natural defense [1]. It is well established that the yeasts of the genus *Candida*, for example, are capable of utilizing antibiotics like penicillin and tetracycline as nitrogen sources [27]. In addition, large doses of antibiotics may cause reduction in the vitamin A, leading to injury to the udder's epithelium, facilitating the invasion of fungi [22]. Other sources of yeast infection have been traced to the use of yeast contaminated antimicrobial preparations [4].

On the other hand, the diversity of the mycobiota present in raw milk [36] can be explained by the chemical composition of the milk, which favours the growth of various species of yeasts with different biochemical and physiological profiles such as the ability of the species *Kluyveromyces marxianus* and *Candida catenulata* to metabolize the milk constituents [16,33].

The great majority of yeasts are considered saprobic, though in some cases they are considered as potentially pathogenic [9]. In a study carried out using 2078 milk samples from healthy and infected animals, 10% (208) corresponded to yeasts, being 3.2% (66) of the genus *Candida* [11]. In our results, though the species of the genus *Candida* represented 37.9% of the total isolates, interestingly, there was no isolation of *Candida albicans*, a normal member of gastrointestinal mycobiota of mammals [30]. In a previous work [15] performed in another part of this survey (RS), *Candida* spp. was isolated in 1.3% out of 896 samples of infected animals, among which 0.9% were *C. albicans*. In the State of São Paulo, *C. albicans* was isolated in 8.9% amongst 260 milk samples from cows with mastitis [35].

In the past decades, non-*C. albicans* species have been reported as triggers of several kinds of infections [31]. Various studies report the presence and isolation of yeasts and yeast-like fungi in milk samples from animals with

Table. Diversity of yeasts and yeast-like fungi.

Genera	%	Species	Number		
<i>Candida</i>	37.90	<i>Candida aaseri</i>	1		
		<i>Candida caseynolitica</i>	1		
		<i>Candida catenulata</i>	2		
		<i>Candida Glabrata</i>	1		
		<i>Candida membranaefaciens</i>	2		
		<i>Candida pseudoglebosa</i>	1		
		<i>Candida robusta</i>	1		
		<i>Candida rugosa</i>	2		
		<i>Candida saitoana</i>	4		
		<i>Candida sorbophila</i>	2		
		<i>Candida zeylanoides</i>	4		
		<i>Candida</i> spp.	6		
		<i>Pichia</i>	19.10	<i>Pichia castillae</i>	1
				<i>Pichia deserticola</i>	2
<i>Pichia guilliermondii</i>	5				
<i>Pichia membranaefaciens</i>	1				
<i>Pichia kluyveri</i>	2				
<i>Pichia kluyveri-like</i>	1				
<i>Pichia</i> sp.	1				
<i>Cryptococcus</i>	10.30	<i>Cryptococcus curvatus</i>	2		
		<i>Cryptococcus laurentii</i>	2		
		<i>Cryptococcus</i> spp.	4		
<i>Rhodotorula</i>	10.30	<i>Rhodotorula glutinis</i>	3		
		<i>Rhodotorula minuta</i>	3		
		<i>Rhodotorula mucilaginosa</i>	1		
<i>Debaryomyces</i>	5.80	<i>Debaryomyces hansenii</i>	4		
<i>Geotrichum</i>	5.80	<i>Geotrichum capitatum</i>	1		
		<i>Geotrichum flagrans</i>	1		
		<i>Geotrichum</i> spp.	2		
<i>Trichosporon</i>	4.40	<i>Trichosporon</i> spp.	3		
<i>Galactomyces</i>	1.47	<i>Galactomyces geotrichum</i>	1		
<i>Kluyveromyces</i>	1.47	<i>Kluyveromyces marxianus</i>	1		
<i>Rhodospiridium</i>	1.47	<i>Rhodospiridium toruloides</i>	1		
TOTAL	100		68		

mastitis [19,24,35] and also in the tanks of milk storage [34,36]. In relation to outbreaks of bovine mastitis, some reports point a non-*albicans* yeast cause, represented by species such as *Candida tropicalis*, *Candida guilliermondii*, *Candida lusitanae*, *Candida kefyr*, *Candida rugosa*, *Candida catenulata*, *Candida zeylanoides*, *Candida lambica* and *Candida inconspicua* [5,11,25,28,29,35,37]. The presence of *Candida aaseri*, *Candida caseynolitica*, *Candida saitoana* and *C. sorbophila* in this work, which are not commonly isolated in cases of subclinical mastitis, support the hypothesis that species that are normally considered non-pathogenic might cause harm to the mammary gland, even though in this survey, no fungi were isolated from cases of clinical mastitis.

In this survey, *Cryptococcus* spp. represented 10.3% of the isolates. *Cryptococcus neoformans*, which is considered the most dangerous species, was not identified. This species has previously been isolated in several cases of clinical and subclinical mastitis as well as from the milk of healthy cows [13,14]. Other authors reported the pre-

sence of *Cryptococcus laurentii* and *Cryptococcus curvatus* in cases of mastitis [11,20] and in tanks of milk storage [37]. *Prototheca* spp. which have been isolated from mixed infections with yeasts from affected quarters [24], were not found in the analyzed samples from subclinical mastitis.

The presence of yeasts and yeast-like fungi may trigger alterations in the milk and dairy products due to the release of extracellular enzymes such as lipases and proteinases [8], which affect the quality and organoleptic characteristics influencing the shelf-life of the product.

The yeasts found in bovine milk may be part of the normal microbiota or might cause damage to the mammary gland [11,25,36]. The diversity of yeasts and yeast-like fungi found in the present study might be influenced by the type of management system employed in the milk farm studied. Although it is not expected that these microorganisms can survive the thermal treatment, milk may be a carrier for a great diversity of agents that could be harmful to public health.

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