



ARTICLE

## Occurrence and pathogenicity of *Candida* spp. in unpasteurized cheese

Liliane Wanderley<sup>1</sup>, Andréia Bianchin<sup>1</sup>,  
Carla Rosane Paz Arruda Teo<sup>1</sup> and Alexandre Meneghello Fuentefria<sup>2\*</sup>

Received: April 9 2012 Received after revision: January 25 2013 Accepted: March 12 2013  
Available online at <http://www.ufrgs.br/seerbio/ojs/index.php/rbb/article/view/2196>

**ABSTRACT:** (Occurrence and Pathogenicity of *Candida* spp. in Unpasteurized Cheese). The aim of this study was to assess the prevalence and pathogenicity of the most common species of the genus *Candida* isolated from artisanal cheese produced in a city, in Santa Catarina countryside. A total of 251 strains of *Candida* were isolated, with growth capacity at 37 °C, from the analysis of 45 artisanal cheese samples. Of these, 2.4% were identified as *C. albicans* and 97.6% as *Candida non-albicans*, which were distributed in 79.3% of *C. krusei*, 12.3% of *C. glabrata* and 6.0% *C. tropicalis*. Regarding to the activity of the proteinase enzyme, the production was observed in 0.8% of the samples. About 0.4% of yeast isolates showed strong production of biofilm on polystyrene screening test. According to this data, it was found a large prevalence of non-*albicans Candida* isolated in artisanal cheese with potential capacity for development and colonization due to the human body temperature.

**Key words:** cheese, *Candida*, *Candida non-albicans*, prevalence, biofilm.

**RESUMO:** (Ocorrência e patogenicidade de *Candida* spp. em queijos não pasteurizados). O objetivo deste estudo foi avaliar a prevalência e patogenicidade das espécies mais comuns do gênero *Candida* provenientes de queijos coloniais produzidos em um município do interior de Santa Catarina. Um total de 251 isolados de *Candida*, com capacidade de crescimento a 37 °C, foram obtidos a partir de 45 amostras de queijos coloniais analisados. Destes, 4,4% foram identificados como *C. albicans* e 97,6% como *Candida não-albicans*, distribuídos em 79,3% de *C. krusei*, 13,3% de *C. glabrata* e 6% de *C. tropicalis*. Em relação à atividade da enzima proteinase, a produção foi observada em 0,8% das amostras. Cerca de 0,4% das leveduras isoladas apresentaram forte produção de biofilme no teste de triagem sobre poliestireno para adesão microbiana. De acordo com os dados deste estudo, foi observada uma prevalência de *Candida não-albicans* em queijos coloniais com potencial capacidade de desenvolvimento e colonização na temperatura corporal humana.

**Palavras-chave:** queijos, *Candida*, *Candida não-albicans*, prevalência, biofilme.

### INTRODUCTION

The *Candida* yeasts are constituents in the oral, intestinal and vaginal human mucosa microbiota and remain colonizing these habitats until they find appropriate opportunistic conditions to express virulence factors, to invade the mucosa and to cause the infection. The most prevalent species of clinical interest in this genus are *Candida albicans*, *C. tropicalis*, *C. glabrata*, *C. krusei*, *C. parapsilosis*, *C. dubliniensis*, *C. guilliermondi* e *C. kefyr* (Colombo *et al.* 2006, Lewis *et al.* 2009, Falagas *et al.* 2010, Miceli *et al.* 2011).

The pathogenic species of this genus have also been isolated from milk obtained from animals affected by mastitis, as well as artisanal cheeses, accompanied by emerging genus such as yeast *Rhodotorula*, *Trichosporon*, *Geotrichum*, *Sporobolomyces* among others (Santos & Marin 2005, Spanemberg *et al.* 2009, Lima *et al.* 2009, Melville *et al.* 2011).

When found in high concentration in food and the host gastrointestinal tract presents appropriate conditions for opportunistic colonization, *Candida* species can propagate secreting toxic metabolites causing wide

variety of symptoms (Colombo *et al.* 2006, Silva 2010). In the case of the gastrointestinal candidiasis the predisposing factors for its manifestation include immune system deficiency, hypothyroidism, diabetes, oral contraceptive use, alcoholism, a rich diet in simple carbohydrates and the continuous use of antibiotics (Menezes *et al.* 2004).

The pathogenicity of *Candida* sp. in the gastrointestinal tract is related to a combination of factors that contribute to its virulence. The process of infection includes adhesion factors that mediate the binding of yeast to the cell surface. These factors of aggression develop the capacity to adhere to mucous membranes and the production of enzymes such as protease, lipase and phospholipase, which contribute to their proliferation and invasion (Schulze & Sonnenborn 2009).

Although there are numerous reports on the occurrence of pathogenic yeasts in dairy products in the world, the literature provides only limited data on the diversity of these products, as well as, about their health risks due to the potential virulence of these isolates. It is known that contamination can occur through lack of attention

1. Curso de Nutrição, Universidade Comunitária da Região de Chapecó, UNOCHAPECÓ. Chapecó, SC, Brazil.

2. Departamento de Análises, Faculdade de Farmácia, Universidade Federal do Rio Grande do Sul (UFRGS). Av. Ipiranga 2752, CEP 90.610-000, Porto Alegre, RS, Brazil.

\* Author for correspondence. E-mail: alexmf77@gmail.com

to proper hands hygiene in manufacturing milk, or even by incorrect tools cleaning for milk and its derivatives processing (El-Sharoud *et al.* 2009, Fadda *et al.* 2010, Melville *et al.* 2011, Lavoie *et al.* 2012).

Due to the lack of information on food safety and hygiene of dairy products against pathogenic yeasts, especially among populations of small villages, this study aimed to determine the prevalence and pathogenicity factors of the most common pathogenic species of the genus *Candida* (*C. albicans*, *C. tropicalis*, *C. krusei* e *C. glabrata*) in artisanal cheeses produced in a city in Santa Catarina countryside

## MATERIALS AND METHODS

### Samples

Samples were collected in Arvoredo City, located in western Santa Catarina, Brazil. The 45 samples of artisanal cheese were conditioned individually in sterile plastic bags and taken to the microbiology laboratory for analysis in less than 24 hours.

### Evaluation of the prevalence of *Candida* species

Initially 25 g of cheese samples were weighed and 225 mL of 0.1% peptone solution were added. They were homogenized in a stomacher at a speed of 3000 rpm for 2 minutes and the dilutions were preceded.

Each dilution was applied in Petri dishes containing Sabouraud dextrose agar with chloramphenicol by Pour-Plate. The plates were incubated at a temperature between 25 and 30 °C for 72 to 120 hours. Later, we performed streaking in chromogenic medium CHRO-Magar *Candida*<sup>®</sup>, incubated at a temperature of 30 °C for 4 days for a later reading according to the manufacturer. Microculture, assimilation of carbohydrates and nitrogen sources and also carbohydrate fermentation tests were used as confirmatory techniques of presumptive identification by chromogenic medium, according to Kurtzman & Fell (1998).

All isolates proved to be enabled to develop at 37 °C.

### Proteinase activity determination

To evaluate the potential pathogenicity of the tissue, the proteinase activity of *Candida* was analyzed in terms of bovine serum albumin (BSA) degradation according to the technique described by Tamura *et al.* (2007). In brief, a 36 hours yeast suspension was inoculated into a 1% BSA plate. The plate was incubated for 5 days at 37 °C. The presence of proteinase was determined by the formation of a transparent halo around the yeast colonies and enzyme activity was measured.

The proteinase activity (Pz) was determined in terms of the ratio of the diameter of the well to the diameter of the proteolytic unstained zone. When the Pz equaled 1, no proteinase activity was detected in the strain. Thus, the results were classified as negative (Pz = 1 cm) and positive (Pz = 0.64 cm and 0.99 cm).

### Evaluation of adhesion ability of *Candida* strains

To evaluate the potential ability of adhesion of the isolates, the profile of adherence of strains was determined by testing the biofilm formation in polystyrene tubes as the specimen, according to the technique used by Stepanovic *et al.* (2000) and Shin *et al.* (2002) with modifications, by a spectrophotometer, in triplicate.

The *Candida* strains were first cultivated on SDA (Sabouraud dextrose agar) for 24 hours at 35 °C. Later, aliquots of 100 µL of standard cell suspensions (10<sup>3</sup> CFU/mL) were transferred to polystyrene bowls and completed with 400 µL of tryptose soy broth containing 8% glucose.

After an incubation period of 24 hours at 37 °C, the culture was removed carefully with a Pasteur pipette and each tube was washed with distilled water to remove the non-adherent or weakly adhered yeasts.

The adherent biofilm layer was measured by spectrophotometry at 450 nm and quantified as non-forming, weakly forming and strong biofilm-forming. According to the propositions (Stepanovic *et al.* 2000, Shin *et al.* 2002) that absorbance < 0.070 indicates non-biofilm formation and absorbance > 0.280 indicates a strong biofilm formation.

## RESULTS

A total of 251 strains of *Candida* were isolated, with growth capacity at 37 °C, from the analysis of 45 samples of artisanal cheese. Of these, 2.4% were identified as *C. albicans* and 97.6% as non-*albicans* *Candida*, which were distributed in 79.3% of *C. krusei*, 12.3% of *C. glabrata* and 6.0% *C. tropicalis*. The remaining percentage of *Candida* non-*albicans* isolates are distributed among *C. parapsilosis*, *C. guilliermondi*, *C. dubliniensis*, *C. kefyr* and *C. lusitaniae*.

According to the objectives of the study, only 2 of these 251 isolates of four *Candida* species analyzed showed proteinase activity, *C. glabrata* and *C. tropicalis*, with Pz between 0.71 and 0.76.

There was prevalence in the species *C. krusei* (74.5%), *C. glabrata* (11.9%), *C. tropicalis* (5.6%) and *C. albicans* (1.6%) as not forming biofilms. Thus, as shown in Table 1, most strains analyzed showed no adherence ability.

## DISCUSSION

Dairy products produced from raw milk may carry a wide variety of potentially pathogenic agents, including yeasts (Melville *et al.* 2011, Lavoie *et al.* 2012). The study by Crawshaw *et al.* (2005) indicates the presence of non-*albicans* species increasingly linked to diseases of the mammary gland of the animal, which is the main source of these microorganisms. According to a study conducted by Spanamberg *et al.* (2009), the identification of the species *C. aaseri*, *C. caseynolitica*, *C. saitoana* and *C. sorbofilha* demonstrates a variety of species

**Table 1.** Distribution of phenotypic adhesion profile of *Candida* spp. isolated from artisanal cheese through the technique of biofilm formation.

OD Range	<i>C. albicans</i>		<i>C. glabrata</i>		<i>C. krusei</i>		<i>C. tropicalis</i>	
	n <sup>o</sup>	%	n <sup>o</sup>	%	n <sup>o</sup>	%	n <sup>o</sup>	%
> 0.280					1	0.4		
0.170 – 0.279					1	0.4		
0.070 – 0.170	2	0.8	1	0.4	10	4	1	0.4

OD Range, adherence values by absorbance in percent.

present in artisanal cheese, which corroborates with the assumption of non-*albicans* species in these products. Aalbaek *et al.* (1994) and Santos and Marin (2005) found in their studies species of non-*albicans* *Candida* isolates from milk, among them *C. krusei*, *C. tropicalis* and *C. guilliermondii*, confirming the results of our study, in which were also prevalent non-*albicans* species such as *C. krusei* followed by *C. glabrata*. Lately, Lavoie *et al.* (2012) and Melville *et al.* (2011) in studies of microbial ecosystems in cheese and raw milk reported that the presence of non-starter adventitious yeasts may have an influence on the organoleptic characteristics of dairy products or represent a risk to public health as they may cause diseases in consumers.

Species of non-*albicans* *Candida* have been responsible for increasing the proportion of cases of fungemia and other complex cases of candidiasis (Colombo *et al.* 2006, Lewis *et al.* 2009, Falagas *et al.* 2010, Miceli *et al.* 2011). In terms of virulence and pathogenicity, these species behave with equal or greater virulence compared with *C. albicans* (Abbas *et al.* 2000, Kremery & Barnes 2002). One of the most common emerging species of the genus, *C. krusei*, has an intrinsic resistance to antifungal fluconazole, and is now responsible for increasing numbers of recurrence cases of various forms of candidiasis in the population (Sanguinetti *et al.* 2005, Miceli *et al.* 2011).

*Candida krusei* was the most prevalent species representing 79.3% of the isolates obtained from the artisanal cheese. Isolates of this species are considered pathogenic potential of colonization in immunocompromised or immunosuppressed patients. Some studies report that the colonization of the gastrointestinal mucosa by *C. krusei* has been a common mechanism in these patient groups, involving a chronic inflammatory response and, consequently, tissue necrosis (Schulze & Sonnenborn 2009). The gastrointestinal tract is claimed to be the largest source of candidiasis in compromised hosts, because while in healthy individuals, colonization occurs in 20 to 30% of cases, in debilitated patients may reach 85% (Neto *et al.* (1997).

The proteinase production is indicated as one of the factors that are related to successful colonization in the host mucosa and subsequent dissemination of *Candida* (Komiya *et al.* 2007). In this study of 2007, the *Candida* strains isolated from cheeses showed little production of the enzyme protease (0.80%) when compared with studies of other authors, like de Menezes *et al.* (2004), where 44% of the isolates expressed the enzyme. Proteolytic activity was showed in 52% of *Candida* isolates according to Souza *et al.* (1990). Recently,

Melville *et al.* (2011) observed in yeasts isolated from bovine raw milk samples the proteinase production in about 20% of the strains at 37 °C, the favorable growing condition for pathogenicity in the host. These comparative studies indicate a potential capacity of the yeasts isolated from our study to cause a disseminated candidiasis, even in higher infectious doses.

The microbial adherence, in turn, is also an important factor in the pathogenesis of various infectious diseases such as candidiasis. It is not just restricted to the mechanism of adherence to tissues, but also the contamination and biofilm formation in human prostheses (Seneviratne *et al.* 2008). According to Schulze & Sonnenborn (2009), the biofilm surrounding the cells of yeast act as a protective cocoon and thus protects the yeast cell of the attacks originated from the environment, including the host immune system. In this study, only 0.4% of the strains isolated from artisanal cheese showed strong biofilm production, and 93.6% did not form biofilm, which was identified a percentage of 5.2% for biofilm formation by non-*albicans* species. Studies by Tamura *et al.* (2007) and Shin *et al.* (2002) also showed higher production of biofilm on the species to the genus *Candida*, and 88.2% and 61% respectively.

Spanamberg *et al.* (2009) in their study, concluded that the proper hygiene of the milker is the key point in the process of cheese artisanal production, because the hands act as a microorganism transmitter, including pathogenic yeasts, which can contaminate the udder, milk and all the material used.

Carelessness with Good Manufacturing Practices is the determinant factor in the large number of pathogenic species of *Candida* found in the samples analyzed. The study by Lima *et al.* (2009) reports that no yeast is usually found when the ingredients are added in the cheese preparation. Probably the contamination by pathogenic yeasts is due to the contact of ingredients with equipment, clothing or food handler hands .

## CONCLUSION

In artisanal cheese samples (type) who had pathogenic *Candida* isolates, there were a smaller number of strains that showed adhesion capacity and proteinase activity.

Through these data it was found a large prevalence of non-*albicans* *Candida* with potential capacity for development in the human body due to its temperature, even with low virulence enzyme expression, representing a risk to consumers of artisanal cheeses.

Brazilian Sanitary Surveillance Agency should en-



courage the producers of artisanal cheese to develop a better quality product. The development of informative booklets with appropriate techniques for the manufacture of cheese, offering workshops on Good Manufacturing Practices are actions that should be developed, as well as, strengthen supervision in small communities, regarding hygiene practices that should be followed.

### ACKNOWLEDGEMENTS

The financial support of the Chapecó Region Community University is gratefully acknowledged.

### REFERENCES

- AALBAEK, B., STENDERUP, J., JENSEN, H.E., VALBAK, J., NYLIN, B. & HUDA, A. 1994. Mycotic and algal bovine mastitis in Denmark. *Acta pathologica, microbiologica, et immunologica Scandinavica*, 102: 451-456.
- ABBAS, J., BODEY, G.P., HANNA, H.A., MARDANI, M., GIRGAWY, E., ABI-SAID, D., WHIMBE, E., HACHEM, R. & RAAD, I. 2000. *Candida krusei* fungemia an escalating serious infection in immunocompromised patients. *Arch of Internal Medicine*, 160: 2659-2664.
- COLOMBO, A.L., NUCCI, M., PARK, B.J., NOUËR, A.S., ARTHINGTON-SKAGGS, B., DA MATTA, D.A., WARNOCK, D. & MORGAN J. 2006. Epidemiology of candidemia in Brazil: a nationwide sentinel surveillance of candidemia in eleven medical centers. *Journal of Clinical Microbiology*, 44: 2816-2823.
- CRAWSHAW, W.M., MACDONALD, N.R. & DUNCAN, G. 2005. Outbreak of *Candida rugosa* mastitis in a dairy herd after intramammary antibiotic treatment. *Veterinary Research*, 156: 812-813.
- EL-SHAROUH, W.M., BELLOCH, C., PERIS, D. & QUEROL, A. 2009. Molecular identification of yeasts associated with traditional Egyptian dairy products. *Journal of Food Science*, 74: 341-346.
- FADDA, M.E., VIALE, S., DEPLANO, M., PISANO, M.B. & COSENTINO, S. 2010. Characterization of yeast population and molecular fingerprinting of *Candida* next term zeylanoides isolated from goat's milk collected in Sardinia. *International Journal Food Microbiology*, 136: 376-380.
- FALAGAS, M.E., ROUSSOS, N. & VARDAKAS, K.Z. 2010. Relative frequency of *albicans* and the various non-*albicans* *Candida* spp. among candidemia isolates from inpatients in various parts of the world: a systematic review. *International Journal Infectious Diseases*, 14: 954-966.
- KOMIYAMA, E.Y., SANTOS, S.S.F., JORGE, A.O.C., MARTINS, C.A.P. & KOGA-ITO C.Y. 2007. Produção de exoenzimas por amostras de *Candida albicans* isoladas de pacientes com periodontite crônica e indivíduos-controle. *Revista de Odontologia da Universidade de São Paulo*, 19: 288-292.
- KREMERY, V. & BARNES, A.J. 2002. Non-*albicans* *Candida* spp. causing fungemia: pathogenicity and antifungal resistance. *The Journal of Hospital Infection*, 50: 243-60.
- KURTZMAN, C.P. & FELL, J.W. 1998. Classification of yeasts. In: KURTZMAN, C.P. & FELL, J.W. (Eds.) *The yeasts. A taxonomic study*. Amsterdam: Elsevier. p. 891-947.
- LEWIS RE. 2009. Overview of the changing epidemiology of candidemia. *Current Medical Research and Opinion*, 25: 1732-1740.
- LAVOIE, K., TOUCHETTE, M., ST-GELAIS, D. & LABRIE, S. 2012. Characterization of the fungal microflora in raw milk and specialty cheeses of the province of Quebec. *Dairy Science Technology*, 92: 455-468.
- LIMA, C.C., LIMA, L.A., CERQUEIRA, M.P., FERREIRA, E.G. & ROSA C.A. 2009. Bactérias do ácido láctico e leveduras associadas com o queijo-de-minas artesanal produzido na região da Serra do Salitre. *Arquivos Brasileiros de Medicina Veterinária e Zootecnia*, 61: 266-272.
- MELVILLE, P.A., BENITES, N.R., RUZ-PERES, M., YOKOYA, E. 2011. Proteinase and phospholipase activities and development at different temperatures of yeasts isolated from bovine milk. *Journal of Dairy Research*, 78: 385-390.
- MENEZES, E.A., GUERRA, A.C.P., RODRIGUES, R.C.B., PEIXOTO, M.M.V., LIMA, L.S. & CUNHA, F.A. 2004. Isolamento de *Candida* spp. no mamilo de lactantes do Banco de Leite Humano da Universidade Federal do Ceará e teste de susceptibilidade a antifúngicos. *Jornal Brasileiro de Patologia e Medicina Laboratorial*, 40: 299-305.
- MICELI M.H., DÍAZ J.A. & LEE S.A. 2011. Emerging opportunistic yeast infections. *Lancet Infectious Diseases*, 11: 142-151.
- NETO, M.M., COSTA, R.S., REIS, M.A., GARCIA, T.M.P., FERRAZ, A.S., SABER, L.T.S., BATISTA, M.N., MUGLIA, V. & FIGUEIREDO, J.C. 1997. Candidíase em pacientes transplantados renais. *Revista da Sociedade Brasileira de Medicina Tropical*, 30: 485-491.
- SANGUINETTI, M., POSTERARO, B., FIORI, B., RANNO, S., TORELLI, R. & FADDA, G. 2005. Mechanisms of azole resistance in clinical isolates of *Candida glabrata* collected during a hospital survey of antifungal resistance. *Antimicrobial Agents and Chemotherapy*. 49: 668-679.
- SANTOS, R.C. & MARIN, J.M. 2005. Isolation of *Candida* spp. from mastitic bovine milk in Brazil. *Mycopathology*, 159: 251-253.
- SCHULZE, J. & SONNENBORN, U. 2009. Yeasts in the gut: from commensals to infectious agents. *Deutsches Ärzteblatt International*, 106: 51-52.
- SENEVIRATNE, C.J., SAMARANAYAKE, L.P. & JIN, L. 2008. Biofilm lifestyle of *Candida*: a mini review. *Oral Diseases*, 14: 82-90.
- SHIN, J.H., KEE S.J., SHIN, M.G., KIM, S.H., SHIN, D.H., LEE, S.K., SUH, S.P. & RYANG, D.W. 2002. Biofilm production by isolates of *Candida* species recovered from nonneutropenic patients: comparison of bloodstream isolates with isolates from other sources. *Journal of Clinical Microbiology*, 40: 1244-1248.
- SILVA R.F. 2010. Infecções fúngicas em imunocomprometidos. *Jornal Brasileiro de Pneumologia*, 36: 142-147.
- SOUZA, E.B., PAULA, C.R., PURCHIO, A., GAMBALE, W., CORRÊA, B. & CURY A.E. 1990. Aspectos morfo-fisiológicos, fatores de virulência e sensibilidade a antifúngicos de amostras de *Candida albicans*, sorotipos A e B, isoladas em São Paulo, Brasil. *Revista de Microbiologia*, 21: 247-253.
- SPANAMBERG, A., SANCHES, E.M.C. & SANTURIO, J.J.M. 2009. Mastite micótica em ruminantes causada por leveduras. *Ciência Rural*, 39: 282-290.
- STEPANOVIC, S., VUKOVIC, D., DAKIC, I., SAVIC, B. & SVABIC-VLAHOVIC, M. 2000. A modified microtiter-plate test for quantification of staphylococcal biofilm formation. *Journal of Microbiology Methods*, 40: 175-179.
- TAMURA, N.K., NEGRI, M.F.N., BONASSOLI, L.A. & SVIDZINSKI, T.I.E. 2007. Fatores de virulência de *Candida* spp. isoladas de cateteres venosos e mãos de servidores hospitalares. *Revista da Sociedade Brasileira de Medicina Tropical*, 40: 91-93.