

Identification of Candida Yeast in Stored Isolates from Subclinical Mastitis Cow Milk

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Abstract

Subclinical mastitis is a major issue in the dairy cattle industry, often caused by infections from microorganisms, including fungi of the genus *Candida*. This study aimed to identify *Candida* species in stored isolates from subclinical mastitis milk samples and analyze their characteristics. A total of 53 isolates derived from Stored Biological Materials (SBM) were tested using Sabouraud Dextrose Agar (SDA) and CHROMagar-Candida to identify species based on phenotypic characteristics and specific colony colors. The results showed that *Candida tropicalis* was predominant, accounting for 24 isolates (45.28%), followed by *Candida krusei* with 29 isolates (54.72%), while no isolates of *Candida albicans* or *Candida glabrata* were detected. These two *Candida* species are significant causative agents of subclinical mastitis, posing serious impacts on udder health, milk quality, and economic losses for farmers due to increased treatment costs.

Keyword: Subclinical mastitis; *Candida tropicalis*; *Candida krusei*; Sabouraud Dextrose Agar (SDA); CHROMagar-Candida

Introduction

Milk is one of the animal food products that plays an important role in meeting the community's nutritional needs. Dairy cow's milk contains protein, fat, vitamins, and minerals that are essential to support the growth and development of the body, especially in children (Wahyuningsih and Pazra, 2022). However, national milk production in Indonesia is only able to meet around 20% of the total domestic needs, while the remaining 80% must be met through imports (Ghandy, 2018; Wulandari, S. and Bowo, P.A., 2019). Based on data from the Ministry of Industry (2024), the national milk consumption rate in 2024 is estimated to reach 1,031,305 tons, while the average production only reaches 932,664 tons (BPS, 2024). This gap

is a major challenge in increasing the capacity of the Indonesian livestock sector.

One of the main obstacles in milk production is the high prevalence of mastitis in dairy cows. Mastitis is an inflammation of the mammary glands caused by pathogenic microorganisms, such as bacteria and yeast, as well as poor environmental conditions. In Indonesia, subclinical mastitis dominates with a prevalence of 97-98%, much higher than clinical mastitis, which is only 2-3% (Ningrum *et al.*, 2022). The impacts of subclinical mastitis include decreased milk quality and quantity, high animal care costs, and increased early culling rates (Anggraeni and Nurfuadi, 2021). Subclinical mastitis is difficult to detect because it does not show physical symptoms,

so it can only be identified through laboratory examination. This makes it a significant problem in dairy farm management.

Candida is one of the yeast genus that can cause mycotic mastitis, although its prevalence is relatively low compared to bacterial infections (Enany *et al.*, 2007; Ahmad and Glolib, 2016). The species that are often found in cases of mycotic mastitis in dairy cows are *Candida albicans*, *Candida krusei*, *Candida tropicalis* and *Candida grabata* (Dolgun *et al.*, 2022; Pal., 2023). SDA (*Sabouraud Dextrose Agar*) media is often used in previous studies to grow yeast colonies, but *Candida* has specificity towards *Candida* yeast species that can be distinguished based on the color of the colonies that grow. Therefore, it is necessary to identify the *Candida albicans* yeast species and through direct observation of *Candida* colonies grown in CHROMagar-*Candida* (CAC) media (Al-Abedi., 2020; Bayona *et al.*, 2022). Research on mastitis yeast in Indonesia is still limited, so further exploration is needed to identify *Candida* species found in subclinical mastitis cow's milk isolates (Rahmanita *et al.*, 2013).

This study aims to identify *Candida* in subclinical mastitis cow's milk isolates. The significance of this identification lies in its potential to offer theoretical insights through scientific data that enhances the understanding of yeast's role in mastitis, alongside practical contributions that can inform the development of strategies aimed at preventing and managing mastitis.

Materials and Methods

This study uses a descriptive method with a qualitative approach, because this study aims to determine the type of *Candida* yeast species found in stored biological materials originating from subclinical mastitis milk samples. This study was conducted by identifying the *Candida* genus from 53 stored biological materials from subclinical mastitis cow's milk from farms in the Tanjungsari area. Identification of the *Candida* genus will be carried out phenotypically. Each *Candida* genus that has been identified will be grown on CHROMagar-*Candida* media. The results of the study will be processed in the form of a table containing the species

Candida albicans, *C. tropicalis*, *C. krusei*, and *C. glabrata* which are identified based on the color of the colonies growing on CHROMagar-*Candida* media. The study was conducted at the Bacteria and Microbiology Laboratory, Faculty of Medicine, Padjadjaran University for a period of 2 weeks, starting from December 9 - December 20, 2024. This study has received approval from the Research Ethics Commission of Padjadjaran University number 2/UN6.KEP/EC/2025.

Table 1. Identification of *Candida* species color on CHROMagar (Nadeem *et al.*, 2010)

No.	<i>Candida</i> Types	Color on CHROMagar
1	<i>C. albicans</i>	Light Green
2	<i>C. tropicalis</i>	Metallic Blue
3	<i>C. krusei</i>	Pink
4	<i>C. glabrata</i>	White

Result and Discussion

Phenotypic Identification of Yeast Genus

The results of phenotypic re-identification of stored isolates in 53 samples were subjected to identification and characterization procedures. This phenotypic confirmation was carried out using the *Sabouraud Dextrose Agar* (SDA) method which had been incubated at 37°C for 48 hours. Re-identification of 53 stored isolates of the *Candida* genus yeast showed that the results of 53 stored isolates had characteristics of 53 Presumptive *Candida* yeast samples on SDA media showing creamy white, smooth, shiny and yeast-smelling colonies, these characteristics are in line with research conducted by Prayoga *et al.*, (2023) & Nuryati *et al.*, (2015).

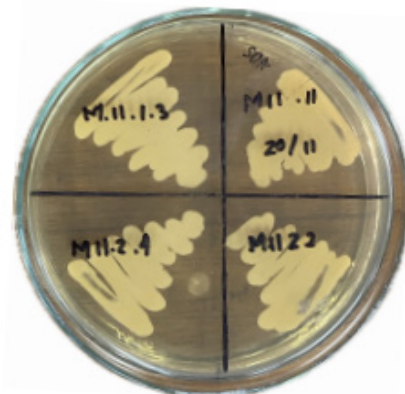


Figure 1. *Candida* colony results on *Sabouraud Dextrose Agar*

(SDA) media

The results of re-identification were carried out to confirm the yeast that led to Presumptive *Candida* based on its phenotypic characteristics in the media and tests. In 53 isolates showed the growth of creamy white colonies with a smooth and shiny texture, which is a characteristic of the *Candida* species. *Candida* is a yeast that grows in an oval or round shape, has a smooth surface, a slippery surface, is creamy to yellowish white, and smells of yeast (Qurrohman *et al.*, 2015; Prayoga *et al.*, 2023). According to Rosmania & Yanti (2020), SDA media is one of the media that can be used for yeast growth. SDA media contains glucose, dextrose, peptone, agar, and glucose content in the media. Thus, the growth of yeast in this SDA media can obtain a source of nutrition and has a good synthetic glucose content for its growth.

Research conducted by Ahmad and Gholib (2016) identified the cause of mastitis through the use of SDA media. In the identified samples, yeast from the *Candida* was found, which is one of the main agents causing subclinical mastitis. The results of this study indicate that SDA media is effective in supporting yeast growth and provides an early indication of the presence of the *Candida*. This finding confirms that the use of culture media such as SDA is very important in the process of identifying microorganisms. This media not only provides an optimal environment for *Candida* growth but also helps to visually distinguish colony characteristics, thus facilitating further analysis.

A less hygienic farm environment can increase the risk of yeast contamination in cow udders. Humid environmental conditions, unsterile milking equipment, or water used to clean the udder can be a medium for *Candida* contamination. Research shows that poor environmental conditions are the main factor in the spread of microorganisms such as *Candida* in dairy farms (Ahmad & Gholib, 2016; Gomes *et al.*, 2021). Research by Du *et al.*, (2018) shows that long-term antibiotic therapy can cause *Candida* overgrowth in udder tissue and in research by Yanuartono *et al.*, (2019) it was stated that cows in immunocompromised conditions are more susceptible to yeast infections.

Results of Identification of *Candida* Yeast Species Using CHROMagar-*Candida*

Identification of *Candida* species using CHROMagar *Candida* was performed on 53 stored isolate samples of *Candida* yeast species that had been previously confirmed as *Candida* genus yeast during phenotypic re-identification. Based on the test results using CHROMagar *Candida* based on the colony color that appeared on the media and the color identified using a chrometer, there was a Presumptive *C. tropicalis* species with a purplish metallic blue colony color identified in 24 isolates (45.28%), while Presumptive *C. krusei* with a pink colony color was found in 29 isolates (54.72%). No isolates showed the characteristics of light green (*C. albicans*) or white (*C. glabrata*) colony colors.

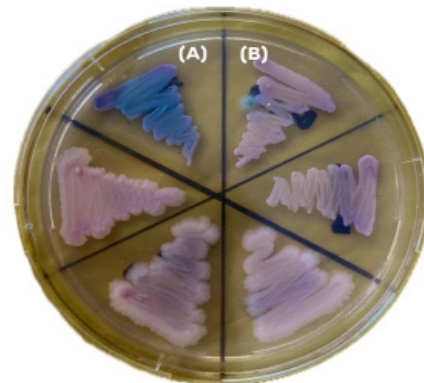


Figure 4. Colony results on CHROMagar *Candida* media show (A) Presumptive *Candida tropicalis*, (B) Presumptive *Candida krusei*.

This study is in line with that conducted by Singh *et al* (2013) and Jiang *et al.* (2024), where 37 samples of *Candida tropicalis* were successfully isolated and identified from the milk of mastitis cows. *Candida tropicalis* showed high adaptability, biofilm formation, and production of virulent enzymes such as protease and phospholipase. In addition, research conducted by Rahmanita and Wahyuni (2013) also showed similar results, where of the total 38 samples of cow's milk analyzed, 6.91% of them were successfully identified as *Candida krusei*. *Candida krusei* has natural resistance to fluconazole and tolerance to acidic environments.

This study shows that Presumptive *Candida tropicalis* and Presumptive *Candida krusei* species are more dominant. Presumptive

Candida tropicalis and Presumptive *Candida krusei* tend to be more frequently found in tropical areas with high temperatures and high humidity, such as the location of this study. Specific environmental conditions can affect the prevalence of certain Presumptive *Candida* species. This is different from studies in other areas that may have environmental conditions that are more supportive of the growth of *Candida albicans* such as in the study conducted by Dolgun et al, (2022).

Excessive use of antibiotics in dairy cows can disrupt the natural microbiota of the udder, allowing non-albicans species such as *Candida tropicalis* and *Candida krusei* to grow more dominantly. These species are known to have higher resistance to antibiotics than *Candida albicans* and *Candida grabata*. This is based on a study conducted by Sharifzadeh, A & Shokri, H. (2021), which emphasized the need to control the use of antibiotics wisely to prevent antimicrobial resistance.

Infection by *Candida tropicalis* causes a decrease in milk quality due to an increase in the number of somatic cells and changes in milk composition. In addition, this infection also reduces milk production due to damage to udder tissue (Du *et al.*, 2018). *Candida krusei* causes chronic inflammation of the udder tissue, reducing milk production, and increasing economic losses for farmers. In addition, *C. tropicalis* and *C. krusei* infections are resistant to treatment, making these infections difficult to treat, prolonging the duration of the disease (Jiang *et al.*, 2024). Therapeutic treatment used in cases of mastitis caused by yeast involves the administration of nystatin combined with prednisolone. In addition to nystatin, therapy may include the use of *clotrimazole*, *polymyxin*, *miconazole*, *amphotericin B*, or *fluorocytosine* (Milanov *et al.*, 2014).

Conclusion

Based on the results of the study were found Presumptive *Candida tropicalis* and Presumptive *Candida krusei*. Presumptive *Candida tropicalis* and Presumptive *Candida krusei* species dominated isolates from subclinical mastitis cow's milk, with proportions of 45.28% and 54.72%, respectively. The limitation of this study

lies in the minimal capability of CHROMagar to accurately identify specific *Candida* species. This limitation arises from the fact that CHROMagar serves as a general medium for cultivating *Candida*, and the assessment of color is highly subjective. To accurately determine specific *Candida* species, more precise testing methods are necessary, including biochemical tests (such as carbohydrate fermentation tests and germinal tests) as well as molecular identification.

Declaration of Gratitude

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