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DETERMINATION OF ANTAGONISTIC ACTIVITY OF YEAST IN MILK AND MILK-BASED PRODUCTS

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Abstract:

Yeasts are widely regarded by consumers as a safe and reliable source of ingredients and additives for food production. They also serve as an excellent model for studying natural barriers against harmful or spoilage-causing bacteria. This study aimed to identify native yeasts with antagonistic activity against food-relevant pathogenic bacteria and evaluate their potential both qualitatively and quantitatively. Ten yeast strains were isolated from fifteen milk and dairy product samples collected from various sources. Biofilm formation, a key indicator of antimicrobial potential, was assessed using the Congo red test, revealing that five out of ten isolates produced biofilms. These biofilm-forming yeasts were tested for antagonistic activity against *Pseudomonas aeruginosa*, *Escherichia coli*, and *Staphylococcus aureus* using the agar diffusion method. Results showed that all five isolates inhibited *S. aureus* and *E. coli*, but none exhibited activity against *P. aeruginosa*. These findings suggest that these yeast strains could serve as biocontrol agents against specific pathogens, though further screening and characterization are needed to fully explore their potential in the food industry.

Keywords: *Yeast, Antimicrobial Activity, Biofilm Formation, Pathogenic Bacteria, Food Industry*

Introduction:

Yeast antagonistic activity refers to the ability of certain yeast species to inhibit the growth of other microorganisms, including pathogenic bacteria and fungi, through

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mechanisms such as the production of antimicrobial compounds, competition for nutrients, or biofilm formation (Hatoum *et al.,* 2012; Liu & Sui, 2014). This natural defense mechanism has attracted significant attention in various industries, particularly in food production, where beneficial microorganisms can enhance food quality, safety, and shelf life. While yeasts are already recognized for their role in dairy fermentation and spoilage prevention, ongoing research is exploring their potential antagonistic effects against pathogens in milk and milk-based products (Sánchez *et al.,* 2014; Zhai *et al.,* 2019).

Milk and its derivatives, such as yogurt, cheese, and kefir, provide nutrient-rich environments that can support the growth of diverse microorganisms. The presence of beneficial yeasts in these products can be advantageous, as they may inhibit pathogenic bacteria like *Salmonella spp.*, *Staphylococcus aureus*, *Escherichia coli*, and *Listeria monocytogenes*, which are responsible for foodborne illnesses (Buyuksirit & Kuleasan, 2014). Identifying yeast species with strong antagonistic activity in dairy products is essential not only for enhancing food safety but also for improving fermentation properties and extending shelf life (Sigler & Höfer, 1991).

Recent studies have focused on the antagonistic properties of yeasts isolated from dairy products and their potential use as natural biopreservatives. Research by Sánchez *et al.,* (2014) and Zhai *et al.,* (2019) highlights the importance of evaluating yeast strains from diverse sources and investigating their antimicrobial mechanisms, such as the production of organic acids, hydrogen peroxide, or antifungal peptides. Several mechanisms have been proposed to explain this antagonistic activity, including parasitism of pathogens, induction of host resistance, and biofilm formation (Liu & Sui, 2013). Additionally, the release of antimicrobial substances like killer toxins has been explored as a natural biocontrol strategy with industrial applications (Hatoum *et al.,* 2012).

A deeper understanding of the interactions between yeasts and other microorganisms in milk-based products could lead to the development of new preservation methods, reduced reliance on chemical preservatives, and the creation of functional foods. The current study aims to evaluate the antagonistic potential of various yeast strains against microbial contaminants in milk and milk-based products. This research could provide valuable insights into the use of promising yeast species with antimicrobial properties, ultimately improving the quality and safety of dairy products.

Methodology

Collection of samples:

Fifteen samples of milk and milk-based products, including raw milk, homemade curd, commercial curd, ghee, and commercial wine, were collected from various sources. Homemade curd, ghee, and raw milk samples were gathered using sterile stainless-steel containers, while commercially available curd packets were collected similarly. To ensure cleanliness, the sample packets were thoroughly cleaned to remove surface contamination and external dirt. The curd samples were then transferred to sterile test tubes and stored at 4°C for further analysis.

Processing of the sample:

Fatty acids and other impurities that could disrupt the organisms must be eliminated from the curd samples using centrifugation. Additionally, the organisms in the pellet should be concentrated.

Isolation of Yeast from various Milk and Milk - based products:

One gram of each sample was introduced into Glucose Yeast Extract Broth (GYEB) and incubated at 27°C for 24 hours. A loopful of the inoculated broth was then streaked onto Sabouraud dextrose agar (SDA) plates, which were incubated at 37°C for 48 hours. Distinct colonies suspected to be yeast were isolated and streaked onto fresh SDA plates. These plates were incubated at 27°C for another 48 hours to obtain pure cultures, which were subsequently stored at 4°C for further analysis.

Procedure:

For the experiment, 24-hour fresh cultures were utilized. A loopful of the test organisms was inoculated into 10 ml of trypticase soy broth supplemented with 1% glucose in a test tube. The tubes were then incubated at 37°C for 24 hours. After incubation, the broth was decanted, and the tubes were washed with phosphate buffer. The tubes were stained with

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0.1% crystal violet, and any excess stain was rinsed off using deionized water. The tubes were dried by inverting them, and biofilm activity was assessed through visual inspection.

Antagonistic activity of Yeast against pathogens:

A loopful of fresh yeast culture was inoculated into 15 mL of Glucose Yeast Extract Broth (GYEB) and incubated at 27°C for 48 hours. The yeast cultures were then streaked onto Sabouraud Dextrose Agar (SDA) plates and incubated for another 48 hours to achieve robust growth.

Separately, a loopful of test pathogens *Staphylococcus aureus*, *Escherichia coli*, and *Pseudomonas aeruginosa* was subcultured into 15 mL of Nutrient Broth (NB) and incubated at 37°C for 24 hours. Nutrient Agar (NA) plates were uniformly inoculated with the broth cultures of *Staphylococcus aureus*, *Escherichia coli*, and *Pseudomonas aeruginosa* using sterile swabs.

Using sterile forceps or a needle, yeast agar plugs were aseptically transferred and placed onto each NA plate. The plates were then incubated at 37°C for 24 hours. After incubation, the diameter of the clear zones around the yeast plugs was measured to assess antimicrobial activity.

Results:

A total of fifteen milk and milk-based product samples were collected from different sources; ten of these were yeast cultures, which were then identified using both macroscopic and microscopic characteristics.

Characterization of the Yeast strains: -

Gram-positive non-sporulating oval budding cells were further identified and described as yeast using sugar assimilation, fermentation, and urease. Five of the ten isolates displayed assimilation patterns, and seven of them demonstrated positive sugar fermentation. All ten were positive towards urease.

Biofilm production:

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Biofilm formation, a crucial initial step in identifying potential candidates with antagonistic properties against pathogens, was assessed for the yeast strains to evaluate their antagonistic potential. Using the Congo red assay, five out of the ten yeast isolates exhibited complete biofilm formation. (Table 1)

Table 1. Biofilm production by yeast

S.No	Sample	Biofilm Formation
1.	Isolate 1	Complete biofilm formation
2.	Isolate 2	Incomplete biofilm formation
3.	Isolate 3	Incomplete biofilm formation
4.	Isolate 4	Complete biofilm formation
5.	Isolate 5	Complete biofilm formation
6.	Isolate 6	Complete biofilm formation
7.	Isolate 7	Incomplete biofilm formation
8.	Isolate 8	Complete biofilm formation
9.	Isolate 9	Incomplete biofilm formation
10.	Isolate 10	Incomplete biofilm formation

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Antagonistic activity of Yeast against pathogens:

The five yeast isolates that demonstrated strong biofilm production were tested for antagonistic activity against the pathogens *Staphylococcus aureus, E. coli, and Pseudomonas aeruginosa* using the agar diffusion method. Results indicated that all five isolates exhibited zones of inhibition against *Staphylococcus aureus and E. coli*, confirming their antagonistic activity. However, none of the isolates showed antagonistic properties against Pseudomonas spp., as no zones of inhibition were observed. Further screening is necessary to identify isolates with potential antagonistic activity against Pseudomonas spp. and to explore their application in biocontrol strategies.

Discussion

In this study, 15 samples of milk and milk-based products were collected from various sources, leading to the isolation and identification of 10 yeast cultures. The isolates exhibited typical yeast morphology, characterized as Gram-positive, non-sporulating, oval budding cells, consistent with findings from previous studies on dairy-associated yeasts (Tremblay *et al.,* 2018). Biochemical characterization, including urease production, sugar fermentation, and assimilation, revealed that all ten isolates were urease-positive, a trait commonly associated with species such as *Candida* and *Debaryomyces* (Deschamps *et al.,* 2016). Urease activity is particularly significant in dairy environments, as it enables yeasts to utilize urea as a nitrogen source. Additionally, seven isolates demonstrated sugar fermentation, while five exhibited sugar assimilation, reflecting their metabolic versatility. These results align with earlier research on dairy yeasts, which often display diverse metabolic capabilities (Marques *et al.,* 2014).

Biofilm formation, a key indicator of microbial antagonism, was evaluated using the Congo red assay. Five out of the ten isolates formed complete biofilms, suggesting their potential to inhibit pathogens through physical interactions or the production of antimicrobial compounds. Biofilm-forming yeasts are known to enhance microbial resistance to environmental stresses and outcompete pathogens by limiting access to nutrients and space (Lee *et al.,* 2019). This aligns with previous studies demonstrating the antagonistic effects of biofilm-forming yeasts, such as *Saccharomyces cerevisiae* and *Candida* species, against foodborne pathogens like *Listeria monocytogenes* and *Escherichia coli* (Parvez *et al.,* 2020).

The antagonistic activity of the five biofilm-forming isolates was tested against *Pseudomonas aeruginosa*, *Escherichia coli*, and *Staphylococcus aureus* using the agar diffusion method. All five isolates inhibited *E. coli* and *S. aureus*, likely due to the production of antimicrobial compounds such as organic acids, peptides, or volatile substances (Nampoothiri *et al.,* 2012). However, none of the isolates showed activity against *P. aeruginosa*, possibly due to its inherent resistance mechanisms, such as efflux pumps or biofilm tolerance (Nunez *et al.,* 2019). These findings underscore the need for further research to elucidate the specific mechanisms of yeast-pathogen interactions and optimize conditions for enhancing antagonistic activity (Høiby *et al.,* 2010).

The study highlights the potential of yeast isolates from dairy products as natural biocontrol agents. Their ability to form biofilms, produce antimicrobial compounds, and inhibit pathogens like *E. coli* and *S. aureus* makes them promising candidates for applications in food safety and preservation. However, further characterization of these isolates, including species identification, safety assessment, and optimization of growth conditions, is essential to fully harness their potential.

Conclusion:

This study provides valuable insights into the antagonistic potential of yeast isolates from milk and milk-based products. The ability of these yeasts to form biofilms and inhibit pathogens such as *E. coli* and *S. aureus* underscores their potential as natural biocontrol agents in the dairy industry. However, the lack of activity against *P. aeruginosa* highlights the need for further research to explore additional mechanisms and optimize their application. These findings pave the way for developing yeast-based strategies to enhance the safety, quality, and shelf life of dairy products, reducing reliance on chemical preservatives and addressing the growing challenge of antibiotic resistance.

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