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STUDIES ON THE ISOLATION AND IDENTIFICATION OF BACTERIA FROM HOME MADE CURD OF TWO DIFFERENT MILK AND ITS ANTIMICROBIAL ACTIVITY

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Abstract

Curd is a fermented milk product traditionally consumed for its high nutritional value and health-promoting properties. It is produced by the action of lactic acid bacteria such as *Lactobacillus*, *Lactococcus*, *Streptococcus*, and *Bifidobacterium*, which are generally regarded as safe microorganisms (Fuller, 1989). Cow milk curd exhibits significant antibacterial activity against *Escherichia coli* due to the presence of lactic acid bacteria such as *Lactobacillus* and *Streptococcus* species. During fermentation, LAB produce lactic acid, which lowers the pH of the curd and creates an unfavorable environment for the growth of *E. coli* (Ray & Sandine, 1992). Fresh homemade curd samples prepared using two different milk types such as cow milk and buffalo milk and Serial dilutions were carried out up to 10^{-6} to reduce bacterial load for proper isolation. Mueller–Hinton agar plates were prepared and sterilized. The test bacterial cultures were evenly spread on the agar surface using a sterile cotton swab. Wells of approximately 6 mm diameter were punched aseptically using a sterile cork borer. 100 µl of buffalo curd extract was added to one well and 100 µl of cow curd extract was added to another well. The plates were incubated at 37°C for 24 hours. After incubation, the zones of inhibition around each well were measured in millimeters using a ruler. The antibacterial activity of buffalo and cow milk curd was evaluated using the agar well diffusion method against three pathogenic bacteria, namely *Escherichia coli*, *Staphylococcus aureus*, and *Bacillus subtilis*. Both buffalo milk curd and cow milk curd exhibited noticeable antibacterial activity against all the tested organisms. However, buffalo milk curd showed consistently higher zones of inhibition compared to cow milk curd. The present study titled "Studies on the Isolation and Identification of Bacteria from Homemade Curd of Two Different Milk and Its Antimicrobial Activity" concludes that homemade curd prepared from both buffalo milk and cow milk is a rich source of beneficial lactic acid bacteria (LAB). Overall, the study confirms that homemade curd is a natural and effective source of antimicrobial and probiotic bacteria, with buffalo milk curd showing superior efficacy over cow milk curd. These findings support the traditional use of curd in diet and health and highlight its potential application in functional foods, probiotics, and natural antimicrobial agents.

Keywords: Curd, *antimicrobial activity, Escherichia coli*, *Staphylococcus aureus*, *Bacillus subtilis*

Introduction

Curd is a fermented milk product formed by the action of lactic acid bacteria (LAB), mainly belonging to the genera *Lactobacillus*, *Lactococcus*, *Streptococcus*, and *Leuconostoc* (Tamime & Robinson, 2007). Homemade

curd is widely consumed due to its nutritional value, probiotic properties, and antimicrobial activity (Panesar, 2011).

Curd is a fermented milk product traditionally consumed for its high nutritional value and health-promoting properties. It is produced by the action of lactic acid bacteria (LAB) such as *Lactobacillus*, *Lactococcus*, *Streptococcus*, and *Bifidobacterium*, which are generally regarded as safe (GRAS) microorganisms (Fuller, 1989).

One of the significant functional properties of curd is its antimicrobial activity. During milk fermentation, LAB produce various antimicrobial substances including lactic acid, acetic acid, hydrogen peroxide, carbon dioxide, diacetyl, and bacteriocins, which inhibit the growth of pathogenic bacteria (Holzapfel et al., 2001). The accumulation of organic acids lowers the pH of curd, creating an unfavorable environment for many food-borne pathogens (Shah, 2007).

Several studies have reported that curd and LAB isolated from curd exhibit inhibitory effects against pathogenic microorganisms such as *Escherichia coli*, *Staphylococcus aureus*, *Salmonella spp.*, *Shigella spp.*, and *Listeria monocytogenes* (Vinderola and Reinheimer, 2003). Among the antimicrobial compounds, bacteriocins produced by *Lactobacillus* species are proteinaceous substances that specifically inhibit closely related and pathogenic bacteria (Todorov and Dicks, 2008).

Cow milk curd exhibits significant antibacterial activity against *Escherichia coli* due to the presence of lactic acid bacteria (LAB) such as *Lactobacillus* and *Streptococcus* species. During fermentation, LAB produce lactic acid, which lowers the pH of the curd and creates an unfavorable environment for the growth of *E. coli* (Ray & Sandine, 1992).

In addition to organic acids, LAB secrete antimicrobial substances such as bacteriocins and hydrogen peroxide, which disrupt the cell membrane integrity of *E. coli* and inhibit its proliferation (Servin, 2004). Several studies using agar well diffusion assays have reported clear zones of inhibition of *E. coli* when exposed to curd whey or filtrates prepared from cow milk curd (Tambekar & Bhutada, 2010).

Salmonella typhi, a major causative agent of typhoid fever, is also inhibited by cow milk curd. The acidic pH generated during milk fermentation plays a crucial role in suppressing the growth of *S. typhi*, as this pathogen is sensitive to low pH conditions (Adams & Moss, 2008). Moreover, bacteriocins produced by LAB interfere with protein synthesis and membrane permeability of *Salmonella* species, leading to growth inhibition (Cleveland et al., 2001). Experimental studies have demonstrated that fresh cow milk curd shows stronger antagonistic activity against *S. typhi* compared to stored curd, indicating the importance of active LAB populations (Soomro et al., 2002). In recent years, there has been growing interest in natural antimicrobial agents due to the increasing problem of antibiotic resistance. Fermented foods like curd are gaining attention as potential sources of safe and effective antimicrobial compounds (Cleveland et al., 2001).

The type of milk used such as cow milk and buffalo milk significantly influences the microbial composition, texture, and acidity of curd because of differences in fat, protein, and lactose content (Walstra et al., 2006). Therefore, the present study concentrate isolation and identification of bacteria from curd of two different milk sources and its antimicrobial activity is important for understanding their microbial diversity and health benefits.

Materials and Methods

Sample Collection

Fresh homemade curd samples prepared using two different milk types such as cow milk and buffalo milk were collected in sterile containers and stored at 4°C until analysis (Harrigan, 1998).

Serial Dilution

One gram of curd sample was mixed with 9 ml of sterile distilled water to prepare a 10^{-1} dilution. Serial dilutions were carried out up to 10^{-6} to reduce bacterial load for proper isolation (Cappuccino & Sherman, 2014).

Isolation of Bacteria

Aliquots from appropriate dilutions were inoculated onto MRS agar plates using the spread plate technique. Plates were incubated at 37°C for 24–48 hours under aerobic or microaerophilic conditions (De Man et al., 1960). After incubation, colonies were observed for Shape, Size, Color, Margin, Elevation. Distinct colonies were sub cultured to obtain pure cultures (Pelczar et al., 2005). The morphological and biochemical characteristics observed are typical of lactic acid bacteria, which are known to be Gram-positive, catalase-negative, and fermentative organisms (Prescott et al., 2011).

Antimicrobial activity Activity of Buffalo Curd and Cow Curd

Preparation of Curd Extract

Ten grams of buffalo curd and cow curd were separately homogenized with 10 ml of sterile distilled water. The mixtures were centrifuged at 5000 rpm for 10 minutes, and the clear supernatant was collected as curd extract method done by Panesar, 2011.

Preparation of Test Bacterial Cultures

Test organisms such as *Escherichia coli, Staphylococcus aureus, Bacillus subtilis* were inoculated into nutrient broth and incubated at 37°C for 18–24 hours and to maintain uniform bacterial concentration during testing done by Cappuccino & Sherman, 2014.

Agar Well Diffusion Method

Mueller–Hinton agar plates were prepared and sterilized. The test bacterial cultures were evenly spread on the agar surface using a sterile cotton swab. Wells of approximately 6 mm diameter were punched aseptically using a sterile cork borer. 100 µl of buffalo curd extract was added to one well and 100 µl of cow curd extract was added to another well. Sterile distilled water was used as negative control (Perez et al., 1990). The plates were incubated at 37°C for 24 hours. After incubation, the zones of inhibition around each well were measured in millimeters using a ruler. The antibacterial activity of buffalo curd and cow curd extracts was compared based on the size of the inhibition zones (CLSI, 2020).

Results and Discussion

Isolation of bacteria from homemade curd prepared using buffalo milk and cow milk showed good growth on MRS agar, which is selective for lactic acid bacteria (De Man et al., 1960). The isolates were identified based on colony morphology, Gram staining, and biochemical characteristics. The results were shown in the table 1.

Table:1 Identification of LAB Isolates from Buffalo and Cow Milk Curd

Characteristic	Buffalo Milk Curd Isolate	Cow Milk Curd Isolate
Colony color	Creamy white	White
Colony shape	Circular	Circular
Margin	Entire	Entire
Elevation	Convex	Raised
Gram reaction	Gram-positive	Gram-positive
Cell shape	Rod-shaped	Cocci / short rods
Catalase test	Negative	Negative
Oxidase test	Negative	Negative
Sugar fermentation	Positive	Positive
Probable LAB group	*Lactobacillus* sp.	*Lactococcus Streptococcus* sp.

The results confirm that both buffalo milk and cow milk curd contain a rich population of lactic acid bacteria, which play a key role in milk fermentation and curd formation (Tamime & Robinson, 2007). Growth on MRS agar and the absence of catalase activity strongly support the identification of the isolates as LAB (Cappuccino & Sherman, 2014).

In buffalo milk curd, the dominant isolates were rod-shaped LAB, indicating the predominance of *Lactobacillus* species. Buffalo milk is known to contain higher fat and protein content, which enhances the growth of lactobacilli and results in thicker and more acidic curd (Walstra et al., 2006).

In contrast, cow milk curd showed mainly cocci or short rod-shaped LAB, suggesting the presence of *Lactococcus* or *Streptococcus* species. These bacteria are commonly associated with cow milk fermentation and are efficient lactose fermenters (Jay et al., 2005).

Both isolates were catalase and oxidase negative, which differentiates LAB from other aerobic bacteria and confirms their fermentative metabolism (Harrigan, 1998). Positive sugar fermentation further indicates their ability to convert lactose into lactic acid, leading to milk coagulation.

The observed variation in LAB types between buffalo and cow milk curd suggests that milk composition significantly influences LAB diversity and dominance. These LAB are beneficial due to their probiotic properties, antimicrobial activity, and role in improving gut health (Panesar, 2011).

The antibacterial activity of buffalo milk curd and cow milk curd was evaluated using the agar well diffusion method against three pathogenic bacteria, namely *Escherichia coli*, *Staphylococcus aureus*, and *Bacillus subtilis*. Antibacterial activity was measured by the diameter of the zone of inhibition (mm) formed around the wells and shown in the table 2.

Table 2. Antibacterial Activity of Curd Samples

Test Organism	Zone of Inhibition (mm)Buffalo Milk Curd	Zone of Inhibition (mm)Cow Milk Curd
Escherichia coli	14 ± 0.5	11 ± 0.4
Staphylococcus aureus	16 ± 0.6	13 ± 0.5
Bacillus subtilis	18 ± 0.7	15 ± 0.6

Both buffalo milk curd and cow milk curd exhibited noticeable antibacterial activity against all the tested organisms. However, buffalo milk curd showed consistently higher zones of inhibition compared to cow milk curd. Against *Escherichia coli*, buffalo milk curd produced a zone of inhibition of 14 ± 0.5 mm, whereas cow milk curd showed a smaller zone of 11 ± 0.4 mm. This indicates a stronger inhibitory effect of buffalo milk curd on Gram-negative bacteria.

In the case of *Staphylococcus aureus*, a Gram-positive pathogen, buffalo milk curd showed a zone of 16 ± 0.6 mm, while cow milk curd exhibited 13 ± 0.5 mm. The highest antibacterial activity was observed against *Bacillus subtilis*, where buffalo milk curd produced a zone of 18 ± 0.7 mm, compared to 15 ± 0.6 mm for cow milk curd.

The enhanced antibacterial activity of buffalo milk curd may be attributed to its higher total solids, protein content, and fat concentration, which support better growth and metabolic activity of lactic acid bacteria (LAB). LAB are known to produce antimicrobial substances such as organic acids mainly lactic acid, hydrogen peroxide, diacetyl, and bacteriocins, which inhibit pathogenic microorganisms (Tambekar and Bhutada, 2010). The greater sensitivity of Gram-positive bacteria (*Staphylococcus aureus* and *Bacillus subtilis*) compared to Gram-negative *Escherichia coli* can be explained by structural differences in their cell walls. Gram-negative bacteria possess an outer membrane that restricts the penetration of antimicrobial compounds, making them relatively more resistant Ray and Bhunia, (2014).

Similar observations have been reported by Tharmaraj and Shah (2003), who demonstrated that fermented dairy products exhibit significant antimicrobial activity due to LAB metabolites. Additionally, Kumar et al. (2015) reported stronger antibacterial effects in buffalo milk curd compared to cow milk curd, supporting the present findings. Thus, the results of this study clearly indicate that buffalo milk curd possesses superior antibacterial activity compared to cow milk curd, highlighting its potential role as a functional food with natural antimicrobial properties.

Conclusion

The present study titled “Studies on the Isolation and Identification of Bacteria from Homemade Curd of Two Different Milk and Its Antimicrobial Activity” concludes that homemade curd prepared from both buffalo milk and cow milk is a rich source of beneficial lactic acid bacteria (LAB). The isolated bacteria were successfully identified based on their morphological, cultural, and biochemical characteristics, confirming the predominance of LAB such as *Lactobacillus* spp., which are known for their probiotic properties. The antimicrobial assay demonstrated that isolates from both curd samples showed significant inhibitory activity against pathogenic bacteria, namely *Escherichia coli*, *Staphylococcus aureus*, and *Bacillus subtilis*. However, buffalo milk curd isolates showed comparatively higher zones of inhibition, indicating stronger antimicrobial

potential. Overall, the study confirms that homemade curd is a natural and effective source of antimicrobial and probiotic bacteria, with buffalo milk curd showing superior efficacy over cow milk curd. These findings support the traditional use of curd in diet and health and highlight its potential application in functional foods, probiotics, and natural antimicrobial agents. Further molecular characterization and in vivo studies are recommended to explore the full therapeutic potential of these bacterial isolates.

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