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COMPARATIVE ANALYSIS OF VIABLE LACTIC ACID BACTERIA IN YAKULT AND HOMEMADE CURD

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Abstract: **Lactic acid bacteria (LAB) are widely used as probiotic in fermented dairy products, including commercial probiotic drinks and homemade curd. This study compared the viable LAB present in a commercial probiotic drink (Yakult) and the homemade curd using the standard microbiological methods. Viable LAB were enumerated by serial dilution and plate count on MRS agar, followed by Gram staining, catalase testing, and the antibiotic susceptibility analysis. Yakult showed a higher viable count than the homemade curd, representing an approximately 6.7-folds difference. Isolates from both samples were Gram-positive, rod-shaped, and catalase-negative, consistent with LAB characteristics. Antibiotic susceptibility testing indicated resistance to ampicillin, highest sensitivity to tetracycline, and intermediate susceptibly to erythromycin and azithromycin. These results demonstrate that Yakult contains a higher viable LAB population than the homemade curd under the tested conditions, highlighting difference in microbial viability between commercial and traditionally fermented probiotic dairy products.**

Key Words: **Lactic acid bacteria; Probiotic; Yakult; Homemade curd; colony-forming unit (CFU); Antibiotic susceptibility**

I. INTRODUCTION

Probiotics are live, non-pathogenic microorganisms that when consumed in adequateamounts, provide health benefits to the host (FAO/WHO,2002). The most common probiotics are Lactobacillus and Bifidobacterium, along with the yeast Saccharomycesboulradii (Hill et al.,2014).These microorganisms contribute to maintaingut microbiota homeostasis by inhibiting pathogenic microorganisms, competing for nutrients and adhesion site, strengthening the intestinal barrier, and modulating host immune response, thereby improving digestive health and systematic immune function (Macro et al., 2017; Gibson,G.R et al., 2017).

Fermented dairy products such as yogurt/curd and commercial probiotic drinks are widely consumed dietary sources of probiotics (FAO/WHO,2002).Commercial probiotic beverages such as Yakult are manufactured under controlled industrial protocols to ensure a defined and stable concentration of viable microorganisms per serving, typically expressed as colony-forming units (CFU) (de Melo Pereira et al., 2018).In contrast homemade curd is traditionally fermented dairy product prepared using starter cultures under non-standardized domestic conditions, resulting in greater variability in microbial composition and viable counts (shah et al., 2023).Although both products are commonly perceived as comparable sources of probiotics, differences in production methods and process control may lead to significant variation in quantity and consistency of live microorganisms consumed.

Despite the widespread consumption of commercial probiotic drinks and traditional prepared fermented dairy products, limited studies have directly compared their viable lactic acid bacteria (LAB) counts using standardized microbiological methods. The probiotic efficacy of fermented foods is closely linked to the presence of an adequate number of viable microorganisms at the time of consumption, commonly expressed as colony-forming units per millilitre (CFU/mL) (FAO/WHO,2002). Variations in fermentation conditions, starter cultures, temperature, and storage practices can significantly influence LAB viability, particularly in non-standardized domestic preparations such as homemade curd (Tamang et al.,2016). However,

comparative data evaluating microbial viability between industrially produced probiotic beverages and homemade curd remain scarce, especially under controlled conditions.
The present study aimed to quantify and compare LAB in Yakult and homemade curd using standard microbiologicaltechniques. Viable LAB counts were determinedusing plate counts method on MRS agar, followed by Gram Staining and morphological analysis to confirm LAB identification (de Melo Pereira et al., 2018). It was hypothesized that the commercially produced probiotic beverage, Yakult would exhibit higher viableLAB count thanhomemade curd due to standard manufacturing and fermentation conditions. This studyprovides the baselinecomparative data on differences in LAB viability between the commercial and traditionally fermented dairy products, contributing to a better understanding of their relative probiotic potential.

II. MATERIALS AND METHODOLOGY

2.1 Enumeration of lactic acid bacteria

Viable Lactic acid bacteria(LAB) were enumerated from commercial Yakult and homemade curd using standard plate count techniques on MRS agar, which is selective for LAB. All procedures were performed under aseptic conditions to minimize contamination (FAO/WHO, 2002).

2.2 Media preparation and sterilization

MRS agar was prepared by dissolving the required amount of dehydrated medium in 100 mL of distilled water in a sterile conical flask. The medium was sterilized by autoclaving at 121°C for 15 minutes.Sterility of the medium was confirmed by incubating the autoclaved mediaat 37 °C for 24 hours to confirm the absence of contamination prior to use. The sterilized MRS media was then poured aseptically into the sterile petri dishes and allowed to solidify. Prepared plates were incubated again to reconfirm the absence of contamination before sample inoculation.

2.3 Sample collection and serial dilution

Commercial Yakult and homemade curd were selected as test samples for microbial quantification. Homemade curd was prepared from pasteurized cow's milk using a small amount of previously set curd as starter and incubated at room temperature until coagulation.
For microbial enumeration, 1 mL of each sample was aseptically transferred into sterile test tubes containing 9ml of sterile saline solution and serially diluted up to 10^{-6}.Appropriatedilutionsof 10^{-3} and 10^{-4} were selected for plating to obtain countable colonies within the recommendation range (30-300 CFU per plate)(Tamang et al., 2016).

2.4 Inoculation and incubation

From each selected dilution,1 mL was aseptically spread onto the surface of MRS agar plates using a sterile L-shaped glass spreader to ensure uniform distribution of the inoculum. The inoculated plates were incubated aerobically at 37 °C for 24 hours until well-defined, countable colonies developed.

2.5 Colony counting and CFU calculation

After incubation, visible bacterial colonies were counted using a colony counter. Colony-forming units per millilitre (CFU/mL) were calculated using the standard formula:

$$\frac{\text{CFU}}{\text{mL}} = \frac{\text{number of colonies}}{\text{dilution factor} \times \text{volume plated in mL}}$$

The CFU/mL values obtained for Yakult and homemade curd were compared to assess difference in viable LAB counts between the two samples.

2.6 Gram staining

Gram Staining was performed for the preliminary identification LAB isolated from Yakult and homemade curd. Representative bacterial colonies grown on MRS agar were sub cultured and heat-fixed bacterial smear were prepared on the clean glass slides. The smears were then subjected to gram stating using crystal violet, iodine solution, alcohol as decolourizer, and safranin as counter strain following standard protocols. Microscopic examination was performed under 100x magnification (oil immersion) to observe Gram reaction and cellular reaction morphology. (Holt et al.,1994).

2.7 Catalase test

Catalase activity of LAB isolated from both Yakult and homemade curd was determined using a slide method. Fresh, well-isolated colonies obtained from MRS agar plates of each sample were aseptically transferred onto clean, dry slide using sterile inoculating loop. One drop of 3% hydrogen peroxide (H_2O_2) was added to each bacterial smear and the reaction was observed immediately. The presence of rapid bubbles formation indicated a catalase-positive reaction whereas the absence of effervescence within 10-20 seconds indicated a catalase-negative reaction, consistent with the characteristic properties of lacticacid bacteria (Tamang et al.,2016)

2.8Antibiotic susceptibility testing

Antibiotic susceptibility testing of lactic acid bacteria isolated fromboth Yakult and homemade curd was evaluated using the disc diffusion method. Fresh bacterial cultures grown on MRS agar were used to prepare bacterial suspension in sterile saline, and the turbidity was adjusted to approximately 0.5McFarland standard. The standard suspension were lawn-inoculated onto Muller Hintonagar

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plates using sterile cotton swab to ensure uniform bacterial growth

Commercial antibiotic discs of ampicillin, erythromycin ,tetracycline and azithromycin were aseptically placed on the inoculated agar surface placed on the inoculated agar surface using sterile forceps, ensuring adequate spacing between discs. The plates were incubated inverted at 37 °C for 18–24 hours. Following incubation, zone of inhibition around each antibiotic disc were measured in millimetres using a ruler, according to standard laboratory procedures.

The recorded zone dimeters were used to categorize the isolates as susceptible, intermediate or resistant to each antibiotic. (CLSI,2020; Schuetz et al.,2025).

III. RESULT

3.1 Colony morphology on MRS agar

After incubation at 37 °C for 24 hours, both Yakult and homemade curd produced well-defined, creamy-white, circular colonies on MRS agar plates, characteristic of lactic acid bacteria. Under identical incubation and dilution conditions, Yakult plates exhibited a noticeably greater number of well-isolated colonies than curd**.**

Representative MRS agar plate showing colony growth of Yakult and curd after incubation are presented in FIGURE 1A AND 1B.

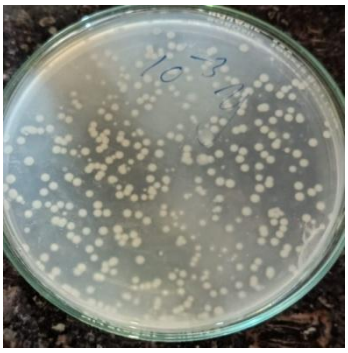

Figure 1A. Representative MRS agar plate showing colony growth of Yakult after incubation.

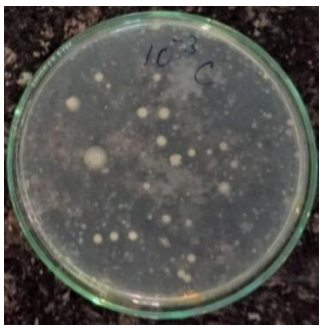

Figure 1B. Representative MRS agar plate showing colony growth of curd after incubation.

3.2 Colony counts and viable bacterial load

Colony enumeration was performed on plates from the 10^{-4} dilution. Yakult showed 362 colonies, corresponding to viable bacterial load of 3.62×10^{6} CFU/mL, whereas curd showed 54 colonies, corresponding to 5.4×10^{5} CFU/mL. Thus, Yakult contained approximately 6.7-fold higher viable lactic acid bacteria(LAB) count than homemade curd under experimental conditions.

SAMPLE	DILUTION FACTOR USED	COLONIES COUNTED	VOLUME PLATED	CALCULATED (CFU/mL)
CURD	10^{-4}	54	1.0	5.4×10^{5}
YAKULT	10^{-4}	362	1.0	3.62×10^{6}

Table 1. Colony counts and calculated CFU/mL values of Yakult and curd samples

3.3 Comparative Analysis of Yakult and Curd

Comparison of CFU values confirmed that Yakult exhibited approximately 6.7-fold higher viable LAB count than homemade curd, indicating a substantially greater probiotic load in commercial probiotic drink under the tested conditions.

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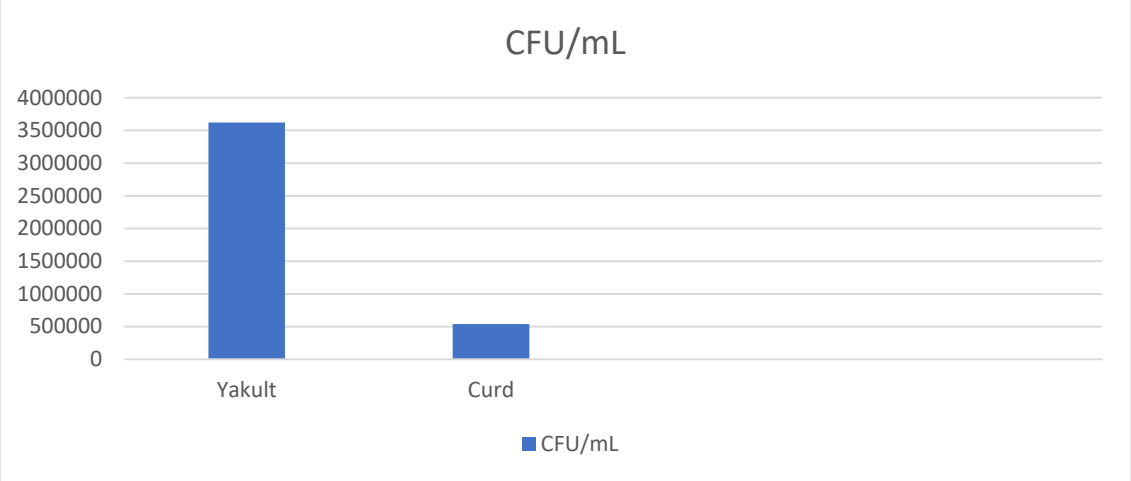

Figure 2. Comparison of viable counts (CFU/mL) of Yakult and curd.

3.4 Gram Staining

Gram Staining of isolates from both Yakult and homemade curd revealed Gram-Positive, rod-shaped bacterial cells, consistent with the morphology of lactic acid bacteria. Representative microscopic images for Yakult and curd are shown in figure 3A and 3B.

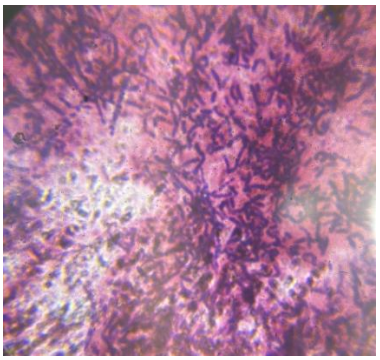

FIGURE 3A. Gram stained microscopic images of latic aid bacteria isolated from yakult under oil immersion (100x magnification)

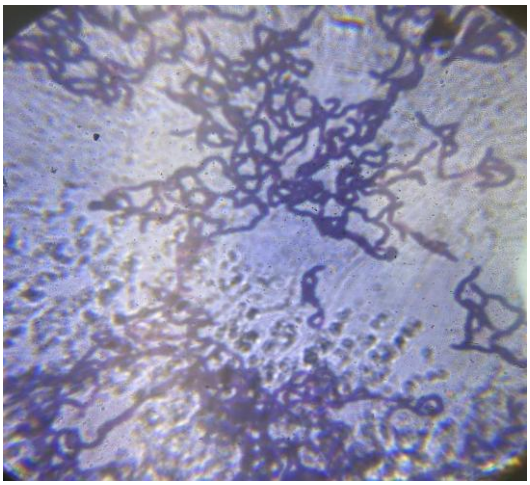

FIGURE 3B. Gram stained microscopic images of latic aid bacteria isolated from homemade curd under oil immersion (100x magnification)

3.5 Catalase activity of lactic acid Bacteria

Sample source	observation	Catalase reaction
Yakult	No immediate bubbling with 3% H_2O_2	Negative
curd	No immediate bubbling with 3% H_2O_2	Negative

Table 2. Catalase test for lactic acid bacteria from Yakult and curd

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Figure 4. Catalase slide test of lactic acid bacteria

Catalase activity of LAB isolated from Yakult and homemade curd was assessed using the slide catalase test with 3% hydrogen peroxide. Upon addition of hydrogen peroxide to fresh bacterial colonies, no immediate bubbles or effervescence was observed in isolates from either Yakult or curd.

Only a thin liquid film with occasional air bubbles was noted,which did not increase over time.

The absence of visible oxygen bubble formation was interpreted as a catalase-negative reaction, which is characteristic of lactic acid bacteria. These results further support the preliminary identification of hr isolates as lactic acid bacteria

3.6 Antibiotic susceptibility

Results for quantitive disc diffusion antibiotic susceptibility assay for lactic acid bacteria from Yakult and curd are presented in table 2, 3,4.

Antibiotic	Zone diameter		Mean(mm)	Interpretation	Categorization
	Sample 1	Sample 2			S/I/R
Ampicillin	0	0	0	Resistant	R
Erythromycin	13	11	12	Moderate sensitivity	I
Tetracycline	15	13	14	High sensitivity	S
Azithromycin	13	14	13.5	Moderate-high sensitivity	I

Table 2. Quantitative disc-diffusion antibiotic susceptibility of lactic acid bacteria from Yakult by disc diffusion

Antibiotic	Zone diameter(mm)		Mean (mm)	Interpretation	Categorisation
	Sample 1	Sample 2			S / I / R
Ampicillin	0	5	2.5	Resistant	R
Erythromycin	10	12	11	moderate sensitivity	I
Tetracycline	17	10	13.5	High sensitivity	S
Azithromycin	11	12	11.5	Moderate sensitivity	I

Table 3. Quantitative disc-diffusion antibiotic susceptibility of lactic acid bacteria from homemade curd by disc diffusion

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Antibiotic susceptibility was interpreted qualitatively as Sensitive (S), Intermediate (I), or Resistant (R) based on relative zone diameters, as standardized clinical breakpoints are not available for lactic acid bacteria.

Antibiotic	Yakult (mean zone, mm)	Curd (mean zone, mm)	Comparative observation
Ampicillin	0	2.5	Resistant in both; negligible inhabitation
Erythromycin	12	11	Comparable intermediate inhibition
Tetracycline	14	13.5	Highest inhibition in both samples
Azithromycin	13.5	11.5	Moderate inhibition; higher inhibition in Yakult

Table 4.comparison of antibiotic susceptibility patterns of LAB from Yakult and curd

LAB from Yakult and curd tested against these four antibiotics by disc diffusion. Both groups were resistant to ampicillin, showing negligible or very small inhibition zones(0–2.5 mm). Tetracycline produced the largest zones in Yakult(14 mm) and curd(13.5 mm), while erythromycin and azithromycin gave moderate inhibition (11–13.5 mm) in both samples (tables 2-4).

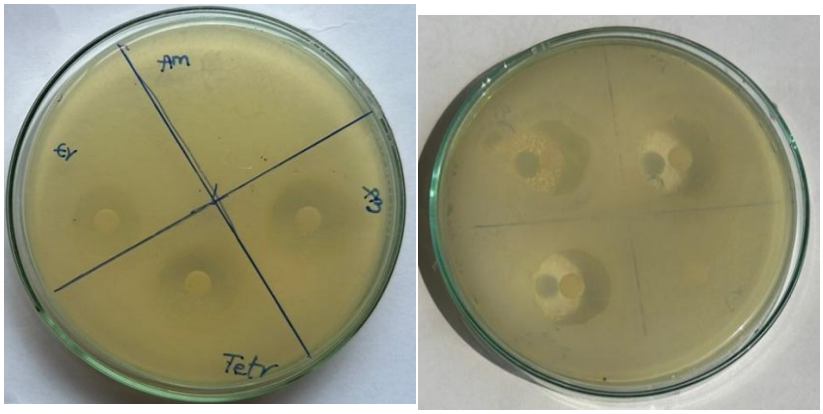

Figure 5. Antibiotic susceptibility of LAB isolated from Yakult.

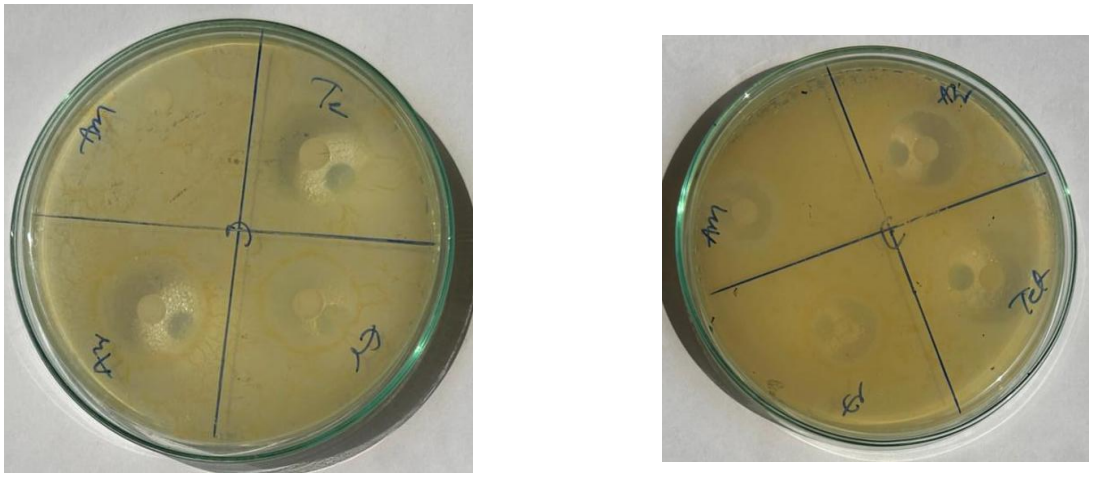

Figure 6. Antibiotic susceptibility of LAB isolated from curd.

IV. DISCUSSION AND CONCLUSION

The present study demonstrates that both Yakult and homemade curd are important dietary source of lactic acid bacteria (LAB) but with pronounced differences in viable counts and antibiotics susceptibility profiles that implications for their probiotic potential. Yakult showed a remarkable higher viable LAB loadcompared with curd, correspondingto approximately a 6.7-folddifference under the tested conditions. This finding isconsistent with industrial production of Yakult using defines starter cultures and tightly controlled fermentation parameters designed to deliver a standardized high dose of live lactobacillus casei strain Shirota per serving. In contrast, the lower and more variable counts observed in homemade curd likely reflect the influence of domestic preparation factors such as inoculum size,milk composition,fermentation, temperatures and incubation time,all which are known to modulate LAB growth dynamics in artisanal fermented dairy products. Nevertheless, the detection of substantial viable LAB populations in both products supports their role as accessible,food-based vehicles for probiotic organisms in every diets.

Morphological and biochemical characterization of the isolates further confirmed their identity as typical LAB,therebysupporting their suitability as probiotics from a safety perspective. Gram staining technique identified gram-positive, rod-shaped cells in both Yakult and curd, whilecatalase testing showed a negative reaction, indicating the absenceof catalase enzyme activity. These traits are characteristic of LAB, which are generally described as Gram-positive, non-sporing, catalase-negative bacteria that produce lactic acid as the major end product of carbohydrate fermentation. The concordance between the observed phenotype and classical descriptions of LAB suggests that isolates belong to genera such as lactobacillus and related groups commonly associated with fermented milk products and probiotic preparations. Such organisms are widely regarded as non-pathogenic and have a long history of safe use in food, which aligns with their proposed application as functional probiotics in human nutrition.

Antibiotic susceptibility testing revealed resistance to ampicillin in both Yakult and curd LAB, withtetracycline showing largest inhibition and erythromycin/azithromycin giving intermediate zones. These patterns reflect typical LAB traits-intrinsic beta-lactam resistance and variable macrolide/tetracycline susceptibility-and align with Yakult's strain selection for safety.

While phenotypic resistance raises horizontal gene transfer concerns, tetracycline sensitivity suggest a favourable probiotic profile. Yakult's higher CFU and consistency outperform curd's diversity, supporting both as dietary probiotics with future need for molecularvalidation.

In conclusion, Yakult provides superior, predictable probiotic delivery through higher CFU counts and strain consistency, while homemade curd offers accessible microbial diversity. Both contribute meaningfully to dietary LAB intake with acceptable safetyprofiles, through future molecular validation, speciesidentification, and functional assays are recommended to optimize their health applications. These findings guide informed consumer choices between commercial reliability and traditional accessibility in probiotic sourcing.

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