

Antibacterial, Antioxidant, and Biofilm-Forming Ability of Isolated and Identified Lactic Acid Bacteria Strains in Commercially Available Non-HACCP-Certified Cabbage (*Brassica oleracea*) Kimchi

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Abstract

Background: Probiotics are widely known for their role as dietary supplements in promoting gut health. Probiotics available in the Philippine and Korean markets do not align with their label claims. These discrepancies include misleading health claims and undisclosed probiotic effects of bacterial strains, including lactic acid bacteria (LAB).

Objective: This study determined the antibacterial, antioxidant, and biofilm-forming activities of lactic acid bacteria (LAB) strains isolated from non-HACCP-certified cabbage (*Brassica oleracea*) kimchi available in Davao City.

Methods: The researchers used a quantitative experimental design, isolating LAB strains on MRS agar and identifying them through biochemical tests (Gram stain, endospore stain, motility, and catalase tests). Antibacterial activity was tested against *Escherichia coli* and *Staphylococcus aureus* using the perpendicular streak method. Antioxidant activity was measured via the DPPH radical scavenging assay, while biofilm-forming activity was assessed using the Tissue Culture Plate method using an ELISA reader. Statistical analysis included one-way ANOVA, Tukey HSD, and Pearson correlation.

Results: The isolates showed no antibacterial activity but exhibited significant antioxidant activity, with Strain B showing the highest scavenging activity with significant differences. The strains also showed moderate biofilm-forming activity, but no significant differences were observed among them.

Conclusion: A weak, non-significant correlation was found between antioxidant and biofilm-forming activities.

Keywords: *Probiotics, Brassica oleracea, kimchi, antibacterial activity, Escherichia coli, Staphylococcus aureus*

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INTRODUCTION

Probiotics are well-known for supporting gut health, but a study by Dioso et al. (2020) highlights discrepancies in label claims for some probiotics sold in the Philippines and Korea, particularly concerning lactic acid bacteria (LAB) in cabbage kimchi. These discrepancies raise concerns about consumer trust and regulatory compliance, underscoring the importance of safety standards such as the HACCP system, which Korea's Ministry of Food and Drug Safety implements for cabbage kimchi. In the Philippines, the Pharmacy Law ensures accurate labeling of food products with health claims.

Further, Britton et al. (2021) identified significant challenges with probiotics, including regulatory gaps and high costs of clinical research, which hinder substantiation of health claims. Additionally, an interview with a distinguished expert conducted by the Philippine News Agency showed criticism on the misuse of the term "probiotics" by some manufacturers, which could jeopardize public health, and called for greater collaboration between regulatory bodies and academic institutions to establish guidelines for evaluating probiotic products (Adriano, 2023).

Furthermore, a study by Bayaga et al. (2021) revealed that urban Filipino mothers have limited knowledge of probiotics, primarily associating them with a specific brand. In Davao City, where probiotic consumption is rising, there is a notable lack of research on the labeling accuracy of probiotic products and the antibacterial, antioxidant, and biofilm-forming activities of LAB in non-HACCP-certified cabbage kimchi.

Based on previous studies, the researchers expect to isolate lactic acid bacteria (LAB) from the non-HACCP-certified cabbage kimchi and observe characteristics consistent with LAB. The isolated strains were expected to demonstrate varying degrees of antibacterial, antioxidant, and biofilm-forming activities, as these properties have been associated with the functional potential of LAB in fermented foods. However, differences in the activities among strains may indicate that the presence of LAB alone does not necessarily guarantee the probiotic benefits implied by product claims. Therefore, the results were expected to provide preliminary evidence that can be used to determine whether the functional properties observed in the isolated LAB support or contradict the expected probiotic benefits associated with the product. This may contribute to the assessment of label claims and emphasize the need for further verification of probiotic products available to consumers.

To fill this gap, researchers were to isolate and assess bacterial strains from non-HACCP-certified cabbage kimchi through biochemical identification and antibacterial, antioxidant, and biofilm-forming assays. The results were used to determine whether the observed functional properties of the isolated LAB support the probiotic-related benefits

associated with the product, thereby providing preliminary evidence for assessing the consistency of product claims with laboratory findings.

The study addressed the research question by examining the relationship between the presence of LAB in the selected product and the functional properties associated with probiotic activity. Biochemical screening established whether the isolated microorganisms exhibit characteristics consistent with LAB. The antibacterial, antioxidant, and biofilm-forming assays determined whether these isolates demonstrated functional activities commonly associated with probiotics. The results from these assessments provided a basis for determining whether the observed properties of the isolated LAB support the expected health-related benefits of the product. In this manner, the study does not only identify LAB from non-HACCP-certified cabbage kimchi but also provided laboratory-based evidence that may be used in evaluating the consistency of probiotic-related claims with the actual properties observed in the product.

MATERIALS AND METHODS

Research Design

This study utilized a quantitative experimental research design. This design allows researchers to conduct research objectively and in a controlled manner, maximizing precision and enabling significant conclusions about a hypothesis. It involves testing the relationship between experimental variables and includes hypotheses, controlled variables, independent and dependent variables, data collection, and statistical analysis.

Sample Collection and Preparation

In this experiment, a non-HACCP-certified Korean Cabbage Kimchi was purchased from NCCC Supermarket. Researchers inoculate lactic acid bacteria (LAB) from kimchi onto MRS agar plates containing 1% CaCO₃ to facilitate LAB identification by halo formation from acid production. The plates incubate for 24 hours at 30 degrees Celsius.

Afterward, colonies with halo zones were selected based on distinct morphology and purified using the streak plate method on MRS agar, which was then sealed with parafilm. An additional incubation at 37 degrees Celsius for 24-48 hours allows for further growth and morphological differentiation.

Biochemical Screening

Purified LAB colonies were tested for identity using several methods: Gram stain, endospore stain, motility test, and catalase test.

Gram Staining. Slides were prepared by adding a drop of sterile water containing a small amount of bacteria. A Gram stain was performed using crystal violet, iodine, and safranin. The stained slides were then examined under a microscope to identify gram-positive bacteria.

Endospore Staining. Heat-fixed bacterial smears on slides were stained with malachite green for 10 minutes, rinsed, and counterstained with safranin. The slides were examined for endospores; non-spore-forming bacteria appear as vegetative cells.

Motility Test. A single colony is stabbed into the SIM medium. After 48 hours at 35°C, motility was assessed; diffuse growth from the stab line indicates motility, while growth along the stab line suggests non-motility, typical of LAB.

Catalase Test. A small bacterial sample on a slide is combined with 3% hydrogen peroxide. Bubbling indicates catalase activity; the absence of bubbles indicates a negative result, characteristic of LAB.

Antibacterial Activity

The study tested *Escherichia coli* and *Staphylococcus aureus*, common bacteria linked to chronic diseases, using lactic acid bacteria (LAB) to assess their potential health benefits. The bacteria were sourced from Alsons Aquaculture Corporation in Sarangani Province. Using the perpendicular streak method (Girma et al., 2021), LAB isolates were streaked onto Mueller-Hinton agar plates and incubated at 37°C for 24 to 48 hours. *E. coli* and *S. aureus* were then streaked at a 90° angle. After incubation, plates were evaluated for zones of inhibition, and the following were recorded: Total Inhibition (TI), Growth Inhibition (GI), Growth Inhibition and Retardation (GIR), and No Inhibition of Growth (NIG).

Antioxidant Activity

The method by Park et al. (2024) involves transferring 3 mL of the sample to centrifuge tubes and centrifuging at 3000 x g for 10 minutes. The supernatant was mixed with an equal volume of phosphate-buffered saline (PBS). A 0.3 mM DPPH solution was prepared by dissolving 0.012 g of DPPH crystals in 100 mL of ethanol. Then, 2.5 mL of the supernatant was combined with 1 mL of DPPH solution and incubated at 31°C for 20 minutes. For the blank, 2.5 mL of supernatant was mixed with PBS and ethanol. Absorbance was measured at 517 nm using a UV-Vis spectrophotometer, and the percentage of radical scavenging activity was calculated.

Biofilm-Forming Ability

This test follows the Tissue Culture Plate (TCP) method described by Harika et al. (2020). *Lactobacillus* bacteria were inoculated in 5 mL of Trypticase soy broth (TSB) and incubated at 37°C for 24 hours. A 1:100 dilution is prepared by adding 0.1 mL of culture to 9.9 mL of sterile TSB. Next, 0.2 mL of this culture was added to each well of a 96-well TCP, with positive controls of *Escherichia coli* and negative controls of sterile TSB. The plates were incubated at 37°C for 24 hours to allow biofilm formation. After washing with phosphate-buffered saline (PBS), wells were stained with 0.2 mL of 0.1% crystal violet for 10 minutes, then washed and dried. To solubilize the stain, 0.2 mL of 33% glacial acetic acid is added, and the optical density (OD) at 570 nm was measured for biofilm classification: <0.17 (negative), 0.17-0.34 (weakly positive), 0.35-0.68 (moderately positive), and >0.68 (strongly positive).

The data gathered was analyzed using descriptive and inferential statistics to evaluate the antioxidant and biofilm-forming activities of lactic acid bacteria (LAB) from non-HACCP-certified cabbage kimchi. Descriptive statistics summarize antioxidant (DPPH scavenging activity) and biofilm-forming activities (optical density) across trials. A one-way ANOVA assesses significant differences among LAB strains, with Tukey's HSD post hoc test for further investigation.

Ethics Statement

This study was submitted to the Research Ethics Committee of the University of the Immaculate Conception with the protocol code UG-EX-12-24-0132. A certificate of exemption was issued having reviewed the study protocols and procedures. Furthermore, proper execution of microbial handling techniques, operational and individual safety practices, and environmental considerations were strictly followed at every stage of work done.

RESULTS

Three lactic acid bacteria (LAB) strains were isolated from the non-HACCP-certified cabbage kimchi and subjected to biochemical characterization. Figure 1 shows the purified LAB isolates grown on MRS agar, demonstrating the successful recovery of distinct bacterial colonies from the sample. The isolates were subsequently examined through Gram staining, endospore staining, motility testing, and catalase testing. As shown in Figure 2, all three strains exhibited purple-colored cells following Gram staining, indicating a Gram-positive reaction. Figure 3 shows the endospore staining results, in which no green-stained spores were observed, indicating that the isolates were non-spore-forming. The motility test results showed restricted growth along the stab line, indicating negative motility, while the catalase test produced no visible bubble formation, indicating a negative catalase reaction. The results of these biochemical tests were summarized in Table 1, where Strains A, B, and C all showed the same pattern: Gram-positive, non-spore-forming, non-motile, and catalase-negative reactions.



Figure 1. Purified isolated Lactic Acid Bacteria Strain from non-HACCP Cabbage Kimchi in MRS agar

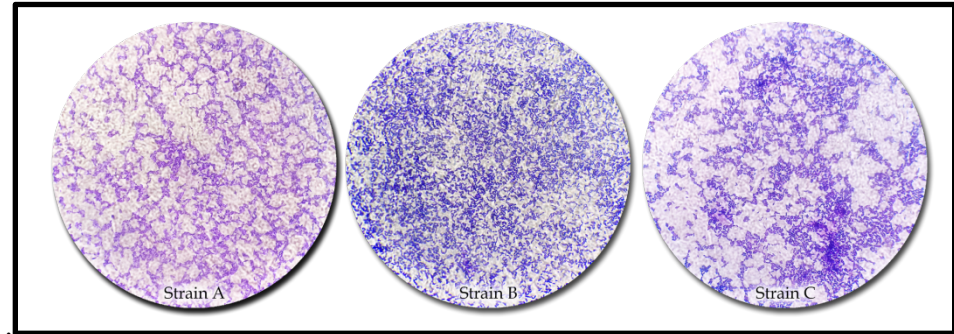


Figure 2. Gram staining result showing purple-colored gram-positive cells, indicating the presence of LAB, observed under oil immersion microscopy.

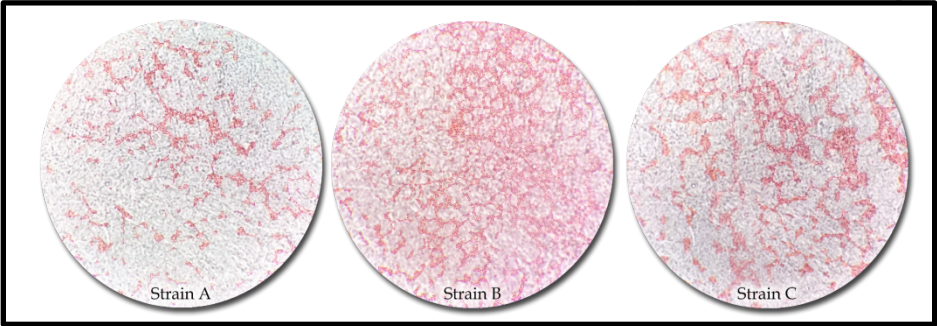


Figure 3. Endospore staining result showing vegetative cells without green-stained spores, confirming the non-spore-forming nature of LAB, observed under oil immersion microscopy

Table 1. Biochemical Characteristics of Isolated LAB Strains from Non-HACCP Certified Cabbage Kimchi

Isolated Strains	Gram Stain	Endospore Stain	Motility	Catalase
Strain A	+	-	-	-
Strain B	+	-	-	-
Strain C	+	-	-	-

The antibacterial activity of the three isolated LAB strains was evaluated against *Escherichia coli* and *Staphylococcus aureus*. Figure 8 presents the perpendicular streak assay used to observe the interaction between the LAB isolates and the test organisms. As shown in Table 2, Strains A, B, and C were classified as No Inhibition of Growth (NIG) in all three trials against both *E. coli* and *S. aureus*. Thus, no visible inhibition was observed for any of the three LAB strains against either test organism across the replicates.

Table 2. Antibacterial Activity of Isolated LAB Strains from Non-HACCP Certified Cabbage Kimchi as Measured by the Zone of Inhibition

Isolated Strains	<i>Escherichia coli</i>			<i>Staphylococcus aureus</i>		
	T1	T2	T3	T1	T2	T3
Strain A	NIG	NIG	NIG	NIG	NIG	NIG
Strain B	NIG	NIG	NIG	NIG	NIG	NIG
Strain C	NIG	NIG	NIG	NIG	NIG	NIG

*Classification based on inhibition: Total Inhibition (TI), Growth Inhibition (GI), Growth Inhibition and Retardation (GIR), and No Inhibition of Growth (NIG).

The antioxidant activity of the isolated LAB strains was determined using the DPPH radical scavenging assay. Figure 9 presents the DPPH radical scavenging activity of Strains A, B, and C, with *Bacillus clausii* included as the control. The corresponding values were presented in Table 3. Strain B recorded the highest mean DPPH scavenging activity at 42.80% ± 0.07, followed by Strain A at 42.18% ± 0.07 and Strain C at 41.85% ± 0.12. The relatively small standard deviations indicate limited variation among the three trials for each strain.

Table 3. Antioxidant Activity of Isolated LAB Strains from Non-HACCP Certified Cabbage Kimchi as Measured by the DPPH Assay

Isolated Strains	T1	T2	T3	Mean (±SD)
Strain A	42.22%	42.22%	42.10%	42.18% (±0.07)
Strain B	42.84%	42.84%	42.72%	42.80% (±0.07)
Strain C	41.98%	41.73%	41.85%	41.85% (±0.12)

The biofilm-forming activity of the isolated LAB strains was assessed based on their optical density (OD) values. As shown in Table 4, Strain A had a mean OD of 0.55 ± 0.20, while both Strain B and Strain C had a mean OD of 0.65 ± 0.16. Based on the specified OD classification, all three strains were categorized as Moderately Positive, with values within the 0.35–0.68 range. The individual OD values for Strain A were 0.778, 0.397, and 0.477; those for Strain B were 0.778, 0.698, and 0.477; and those for Strain C were 0.777, 0.697, and 0.476.

Table 4. Biofilm-Forming Ability of Isolated LAB Strains from Non-HACCP-Certified Cabbage Kimchi Based on Optical Density (OD) Values

Isolated Strains	R1	R2	R3	Mean (±SD)	Remarks*
Strain A	0.778	0.397	0.477	0.55 (±0.20)	Moderately Positive
Strain B	0.778	0.698	0.477	0.65 (±0.16)	Moderately Positive
Strain C	0.777	0.697	0.476	0.65 (±0.16)	Moderately Positive

*Classification based on OD thresholds: <0.17 = Negative, 0.17–0.34 = Weakly Positive, 0.35–0.68 = Moderately Positive, >0.68 = Strongly Positive.

A one-way ANOVA was conducted to determine whether the observed differences in antioxidant activity among the three LAB strains were statistically significant. As shown in Table 5.1, the analysis produced an F-value of 81.80 with $p < .001$, indicating a significant difference in DPPH radical scavenging activity among the strains. The Tukey HSD post hoc comparisons in Table 5.2 further showed significant differences between Strain A and Strain B ($p < .001$), Strain A and Strain C ($p = .011$), and Strain B and Strain C ($p < .001$). Based on the mean values, the antioxidant activity followed the order Strain B > Strain A > Strain C.

Table 5.1. One-Way ANOVA Summary Table for the Antioxidant Activity (% DPPH Radical Scavenging) of Isolated LAB Strains

Source	SS	df	MS	F	p
Between Groups	1.385	2	0.693	81.8	< .001
Within Groups	0.051	6	0.008		
Total	1.436	8			

Note. SS = Sum of Squares; df = degrees of freedom; MS = Mean Square; F = F-ratio; p = significance level.

Table 5.2. Tukey HSD Post Hoc Comparisons of LAB Strains on DPPH Assay

Comparison	Mean Difference	Std. Error	p	95% CI (Lower–Upper)
Strain A vs B	−0.61728*	0.07513	< .001	−0.8478, −0.3868
Strain A vs C	0.32922*	0.07513	0.011	0.0987, 0.5597
Strain B vs C	0.94650*	0.07513	< .001	0.7160, 1.1770

A one-way ANOVA was also performed to determine whether the biofilm-forming activity differed significantly among the three strains. Table 6 shows an F-value of 0.336 and p = .727. Since the p-value was greater than the 0.05 level of significance, no statistically significant difference was observed among the biofilm-forming activity of Strains A, B, and C based on their OD values.

Table 6. One-Way ANOVA Summary Table for the Biofilm-forming Activity (Optical Density Values) of Isolated LAB Strains

Source	SS	MS	F	p
Between Groups	0.02	0.01	0.336	0.727
Within Groups	0.178	0.03		
Total	0.198			

Note. SS = Sum of Squares; df = degrees of freedom; MS = Mean Square; F = F-ratio; p = significance level.

DISCUSSION

The biochemical profiles of the three isolates were consistent with characteristics commonly associated with LAB, including Gram-positive staining, absence of endospores, non-motility, and catalase-negative reactions (Odumosu et al., 2023). However, these phenotypic characteristics provide only a presumptive identification and were insufficient

to establish the isolates at the species or strain level. Consequently, the present study cannot assign a species-specific QPS status or conclude that the isolates were safe for consumption. EFSA's QPS framework requires confirmation of the taxonomic identity of the microorganism and consideration of the qualifications associated with the relevant taxonomic unit (EFSA, 2026). Molecular identification would therefore be necessary to determine whether the isolates belong to a QPS-recognized species and to support further evaluation of their safety and probiotic potential.

The results showed that none of the LAB strains tested exhibited antibacterial activity against *E.coli* or *S.aureus*, resulting in No Inhibition of Growth (NIG) classification. This conclusion was tentative due to the inability to identify the specific LAB strains. According to Metchnikoff's theory of probiotics, while these bacteria can support gut health, not all have antimicrobial properties, which can lead to unmet consumer health expectations. The absence of antibacterial activity in these strains highlights the need to verify claims for products lacking HACCP certification.

More importantly, the absence of antibacterial activity provides an important consideration in evaluating the probiotic-related claims associated with the product. Although the isolated strains exhibited characteristics consistent with LAB, none demonstrated inhibition against *E.coli* and *S.aureus* under the conditions of the present assay. This indicated that the presence of LAB in fermented kimchi should not automatically be interpreted as evidence that the product possesses all of the functional benefits commonly associated with probiotics. The present findings provide additional evidence for the importance of experimentally verifying functional claims rather than relying solely on the presence or designation of probiotic microorganisms. Thus, the results partially address the problem of labeling accuracy by showing that the functional property tested in this study does not uniformly correspond to the expected antibacterial benefit.

In terms of antioxidant capacity, the study assessed three LAB strains (A, B, and C) isolated from non-HACCP certified cabbage kimchi using the DPPH radical scavenging method. Strain B demonstrated the highest antioxidant activity at $42.80\% \pm 0.07$, followed closely by Strain A ($42.18\% \pm 0.07$) and Strain C ($41.85\% \pm 0.12$). Low standard deviations suggest reliable results. These findings aligned with Bryukhanov et al. (2022), who identified strong antioxidant mechanisms in LAB, and Uy et al. (2019), who confirmed the DPPH assay's effectiveness for evaluating antioxidant potential in probiotic strains.

The findings aligned with those of Park et al. (2024), who noted that certain LAB strains significantly contribute to the antioxidant benefits of traditionally fermented foods such as kimchi. These strains can neutralize free radicals, promoting health through their antioxidant properties. Bryukhanov et al. (2022) highlighted the importance of LAB's antioxidant capabilities in combating oxidative stress, which is linked to chronic diseases. This study underscores the potential health benefits of LAB strains, especially Strain B, as functional probiotics in fermented foods. Additionally, a one-way ANOVA found no significant differences in the biofilm-forming activity of LAB strains from non-HACCP-certified cabbage kimchi ($F(2, 6) = 0.336, p = .727$).

The null hypothesis is accepted, indicating no significant difference in the biofilm-forming activity of LAB strains in non-HACCP certified cabbage kimchi based on OD values. Although the LAB strains showed similar moderate biofilm-forming effects, they still contributed to the probiotic benefits of kimchi. This is in line with Shaaban et al. (2020), who noted that *Lactobacillus* strains with moderate activity retain essential probiotic traits, and with Salas-Jara et al. (2016), who highlighted the importance of biofilm formation for enhancing probiotic efficacy in fermented foods like kimchi.

The antioxidant findings further demonstrate why probiotic claims should be evaluated based on the functional characteristics of individual strains rather than on the general presence of LAB. Strain B demonstrated the highest DPPH radical scavenging activity at $42.80\% \pm 0.07$, while Strains A and C showed slightly lower activities. This finding was consistent with Park et al. (2024), who reported that LAB isolated from fermented foods such as kimchi may contribute to antioxidant activity. However, the significant difference among the strains indicates that the functional capacity of LAB is not necessarily uniform. Although the antioxidant results provide evidence supporting a potential functional benefit of the selected kimchi, they also demonstrated that the presence of LAB alone cannot establish that all probiotic-related benefits were present. This distinction is important in assessing the reliability of product claims because specific health-related properties require corresponding experimental evidence.

Overall, the findings provide preliminary evidence for evaluating the consistency of probiotic-related claims associated with non-HACCP-certified cabbage kimchi. The isolation of LAB confirms the presence of microorganisms consistent with those commonly associated with fermented foods, while the functional assays demonstrate that their properties were not uniformly expressed. The absence of antibacterial activity indicates that the expected antimicrobial benefit was not demonstrated under the conditions of this study, whereas the antioxidant and biofilm-forming assays demonstrated measurable functional activities. These findings suggest that the presence of LAB or the general classification of a product as probiotic should not be considered sufficient evidence for all associated health claims. Instead, individual functional properties should be experimentally verified. In this context, the study contributes to addressing the identified gap in labeling accuracy by providing laboratory-based evidence that can be used as a preliminary reference for determining whether specific probiotic-related claims were supported by the observed characteristics of LAB in commercially available products

CONCLUSION

Based on the data and findings presented, this research demonstrates that LAB can be isolated from commercially available non-HACCP-certified cabbage kimchi and that their functional properties vary depending on the activity evaluated. The isolated strains exhibited characteristics consistent with LAB, but none demonstrated antibacterial activity against *Escherichia coli* and *Staphylococcus aureus*. In contrast, all strains demonstrated antioxidant activity, with Strain B showing the highest DPPH radical scavenging activity, while all strains demonstrated moderate biofilm-forming activity. These findings indicate that the presence of LAB in the product does not necessarily guarantee the expression of all functional properties commonly associated with probiotics.

In relation to the identified problem of probiotic labeling accuracy, the findings provide preliminary laboratory-based evidence for assessing the consistency of probiotic-related claims with the actual functional properties observed in the product. The absence of antibacterial activity despite the presence of LAB demonstrates that specific probiotic benefits cannot be assumed solely from the presence of LAB, while the antioxidant and biofilm-forming activities provide evidence for selected functional properties. Thus, the study supports the need for product-specific and strain-specific verification of probiotic claims to promote accurate labeling, consumer awareness, and regulatory monitoring. However, because the study did not directly quantify or compare the microorganisms and

claims declared on the product label, the findings should be considered preliminary evidence rather than definitive proof of labeling accuracy.

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