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DIVERSITY AND ABUNDANCE OF LACTIC ACID BACTERIA PRESENT IN PROBIOTIC YOGURTS PRODUCED LOCALLY IN ACCRA, GHANA

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Abstract

Yogurt is a dairy product produced by the souring of pasteurized milk by the lactic acid-producing bacteria Lactobacillus delbrueckii and Streptococcus thermophilus, leading to its characteristic taste and texture. It is categorized as a probiotic food because of the health benefits it reportedly provides its consumers. However, for yogurt to confer added probiotic benefit beyond fermentation, well-characterized probiotic strains should be incorporated into the starter cultures used. Our study sought to delineate the lactic acid bacteria (LAB) present in Ghanaian yogurts labelled as probiotic. Twelve locally produced yogurts were obtained from random supermarkets within the Accra Metropolis. Total genomic DNA was extracted from the yogurt matrix using the DNeasy Mericon Food Kit. Multiplex PCR on extracted DNA was performed using eleven primers comprising universal Lactobacillus primers and species-specific probiotic LAB primers. Next-generation sequencing of the 16S rRNA gene from samples with multiple distinct bands was carried out. The Simpson and Inverse Simpson indices were used to compute alpha diversity and determine species richness and evenness in the locally produced yogurt samples. Seven out of the twelve locally produced yogurts passed sequencing QC and were taxonomically classified. The data showed low Simpson and Inverse Simpson indices for all yogurt samples, which is consistent with the dominance of defined starter cultures expected in yogurt fermentation. Lactobacillus delbrueckii was the dominant taxon in all seven sequenced samples, accounting for a mean of 86.6 $\pm$ 10.2% of reads per sample (range 69.1–97.2%). Apart from Lactobacillus delbrueckii, other strains of Lactobacillus, such as L. fermentum and L. reuteri, were detected in some samples. Reads matching potentially pathogenic taxa, including the Bacillus cereus group and Clostridioides difficile, were also identified, although their taxonomic reliability requires confirmation by culture-based or species-specific methods. Although there was variability in species abundance between the different yogurts, Lactobacillus delbrueckii was consistently dominant. Since yogurts are classified as a probiotic food due to the health benefits they confer, our study highlights the need to incorporate well-characterized probiotic strains into the starter cultures and to ensure microbiological safety, rather than relying on spontaneous taxonomic diversity for probiotic benefit.

Keywords
Lactic acid bacteria, Probiotics, yogurt, Next Generation Sequencing

Introduction

Yogurt is a concentrated fermented milk product produced by a symbiotic bacterial culture of *Lactobacillus delbrueckii* subsp. *bulgaricus* and *Streptococcus thermophilus*, as defined by the Codex Alimentarius (Yamamoto et al., 2021). These two specific bacterial strains associated with the fermentation of yogurt are known to contribute to its acidic taste, consistency, and the production of compounds responsible for its aroma (Borresen et al., 2012). However, other species of *Lactobacillus*, such as *L. acidophilus*, *L. fermentum*, *L. casei*, as well as strains of *Bifidobacterium*, can be added to increase the diversity of microbes in yogurt and confer specific health benefits to the consumer (Plaza-Diaz et al., 2019).

Although believed to have originated from Western Asia and the Middle East, the production and consumption of yogurt have spread around the world, serving as a regular part of the diet in some countries and a delicacy in others (Saleem et al., 2024). The start of the 20th Century saw the discovery of *Bacillus bulgaricus* by Stamen Grigorov, a Bulgarian medical student, as the lactic acid bacteria present in yogurt culture. In 1909, Ilya Metchnikoff linked the longevity of Bulgarian peasants to their consumption of yogurt. Consumption of yogurt helps to meet nutritional needs such as protein, calcium, and several B vitamins (Petrova et al., 2021). It is also known to be beneficial to the human gut microbiota and is associated with reduced lactose intolerance due to the presence of live strains of lactic acid bacteria in yogurt (Nyanzi et al., 2021). Studies have shown that yogurt consumption varies among populations, with a lower consumption in developing countries, which is also an indicator of economic change. Studies have also indicated the consumption of yogurt among healthier, highly educated individuals, thus creating the presupposition that most consumers only take yogurt for its health benefits (Ates and Ceylan, 2010). Fermented foods containing live bacteria are usually referred to as probiotic foods. Probiotics are defined as live microorganisms that, when consumed in the right quantities, confer health benefits to the consumer (Dahiya and Nigam, 2022). Although yogurts contain live

microorganisms, not all yogurts are probiotic. For yogurt to be probiotic, it must contain other beneficial bacteria apart from the two fermenting bacteria (Meybodi et al., 2020).

The diversity in gut microbiota has been linked with gut health, and a reduced diversity is implicated in an unhealthy gut microbiome. Some chronic disease conditions, such as type 2 diabetes, asthma, and obesity, have been linked with decreased diversity and dysbiosis of the gut (Deng et al., 2019). Yogurt, being a popular microbe-containing food around the world, could contribute to the improvement of the gut microbiome diversity by increasing the diversity of probiotic bacteria in the product (Le Roy et al., 2022).

In Ghana, although yogurt is a popular and well-liked food, it is not consumed by many Ghanaians due to its high cost (Achaglinkame et al., 2023; Adubofuor et al., 2014). In this study, we investigated the relative abundance and diversity of *Lactobacillus* present in yogurts labelled as "probiotic" in supermarkets in the city of Accra in Ghana.

Materials and Methods

Sample acquisition

A total of twelve (12) different commercial brands of yogurt were obtained from supermarkets within the Accra Metropolitan Area. These were brands that were labeled as "probiotic yogurt." They were labeled B, C, E, G, J, L, N, X1, X2, X3, X4, and X5. The samples were transported on ice to the laboratory for further analysis.

Sample preparation and DNA extraction

The yogurt samples were diluted in sterilized Ringer solution (Merck, Germany) in a 1:10 proportion, then centrifuged at 13,000 rpm for 10 min as described by Abdulamir et al. (2010). The pellet was resuspended in 500 μl of TAE Buffer pH 8.0 (ThermoFisher Scientific, Waltham, MA, USA). Total genomic DNA was extracted from the pellet obtained using DNeasy Mericon Food Kit (Qiagen GmbH, Hilden, Germany) according to the manufacturer's instructions. Briefly, 1 ml of Food Lysis Buffer and 2.5 μL of proteinase K were added to 200 μL of the homogenized pellet. The resulting solution was vortexed (Thermo Scientific, Korea) and incubated in a thermomixer (Vorwerk International, Europe) for 30 minutes at 60 °C with constant shaking at 1000 rpm. After incubation, the solution was cooled on ice and centrifuged at 2,500 g for 5 mins. 700 μL of the clear supernatant was transferred to a microcentrifuge tube containing 500 μL of chloroform (Park Scientific Limited, Northampton, UK). This was vortexed vigorously for 15 s and centrifuged at 14,000 g for 15 mins. 350 μL of the upper aqueous phase was then pipetted into a microcentrifuge tube containing 350 μL of Buffer PB and vortexed. The solution was pipetted into the QIAquick spin column and centrifuged at 17,900 g for 1 min and the flow-through discarded. 500 μL of Buffer AW2 was added to the spin column and centrifuged at 17,900 g for 1 min, and the flow-through discarded. The collection tube was centrifuged again under the same conditions to dry the membrane. The QIAquick spin column was transferred to a 2 ml microcentrifuge tube, and a volume of 150 μL Buffer EB was pipetted directly onto the membrane. The resulting solution was then incubated for 1 min at 25 °C and centrifuged at 17,900 g for 1 min. The eluted DNA was stored at –20 °C. The quality and yield of the extracted DNA were determined using the NanoDrop Microvolume Spectrophotometer (ThermoFisher Scientific, USA), A260/A280 ratio value.

Multiplex PCR Reaction

The multiplex PCR contained a total of 11 primers (Table 1) and was carried out in a final volume of 25 μL with the reaction mixture containing One Taq 2X master mix (ThermoFisher Scientific, Waltham, MA, USA), 15 μM forward and reverse primers, 10 ng genomic DNA, and sterile nuclease free water. The multiplex PCR was performed on a SureCycler 8800 (Agilent Technologies, USA) under the following conditions: 3 min at 95 °C for initial denaturation, 35 cycles consisting of denaturation for 30 s at 95 °C, annealing for 30 s at 57 °C, and elongation at 73 °C for 60 s. The final extension step was performed at 73 °C for 5 min, and the holding temperature was 4 °C. For the negative control, the master mix was devoid of genomic DNA, and *Lactobacillus casei* was used as a positive control to validate the multiplex PCR.

Gel Electrophoresis

The PCR amplification was analyzed by gel electrophoresis on a 1.5% agarose gel (LE analytical grade, Promega, Madison, USA) run in 1 × TBE (Promega, USA) at 80 V for 50 min. The molecular size marker GeneRuler 100 bp DNA Ladder (Thermo Fisher Scientific) was used. The gel was stained with 0.5 μg mL^{-1} ethidium bromide (Sigma, USA) for 5 min, and PCR amplification patterns were documented with Amersham™ Imager 600 (GE HealthCare Technologies Inc., Chicago, Illinois, USA).

Next Generation Sequencing

The amplicons were diluted to 10.5 ng/μL. End preparation for the diluted samples was carried out using the Ultra II End Prep kit (NEB) as per the manufacturer's instructions. The individual samples were then barcoded using the ONT Native Barcoding Expansion kit (EXP-NBD-196) as per the manufacturer's native barcoding protocol. The barcoded samples were pooled in equal volumes into one DNA LoBind tube and cleaned using Ampure XP beads (Beckman Coulter). Quality control of the pooled library was done using the Qubit 4.0 fluorimeter (Invitrogen) to determine the concentration of the sample. Adapter ligation of the library was done using the AMII adapter mix (ONT) as per the manufacturer's native barcoding protocol. The adapter ligated library was then purified using Ampure XP beads (Beckman Coulter) and SFB (ONT) as per the ONT adapter-ligated purification protocol. The final concentration of the library was determined using the Qubit 4.0 fluorimeter (Invitrogen). A total of 200 fM of the final library was then loaded on a FLO-MIN106 flow

Table 1. Universal and species-specific primers used for the multiplex PCR detection of *Lactobacillus* species in yogurt samples

LAB species Targeted	Primer	Sequence (5' – 3')	Annealing Temp. (°C)	Size (bp)	References
All *Lactobacillus*	IDL03R	CCACCTTCCTCCGGTTTGTCA	56.31	–	Kwon et al. (2004)
All *Lactobacillus*	IDL04F	AGGGTGAAGTCGTAACAAGTAGCC	57.38	–	Kwon et al. (2004)
L. acidophilus	IDL22R	AACTATCGCTTACGCTACCACTTTGC	57.96	606	Kwon et al. (2004)
L. delbrueckii	IDL31F	CTGTGCTACACCTAGAGATAGGTGG	59.32	184	Kwon et al. (2004)
L. gasseri	IDL42R	ATTTCAAGTTGAGAGTCTCTCTCTC	54.4	272	Kwon et al. (2004)
L. reuteri	IDL52F	ACCTGATTGACGATGGATCACCAGT	57.68	1105	Kwon et al. (2004)
L. rhamnosus	IDL73R	GCCAACAAGCTATGTGTTCGCTTGC	59.32	448	Kwon et al. (2004)
L. fermentum	Fermenti-F	GACCAGCGCACCAAGTGATA	53.83	–	Bamgbose et al. (2022)
L. fermentum	Fermenti-R	AGCCTAGCGTTCGTGGTAAT	51.78	–	Bamgbose et al. (2022)
L. casei	Casei-F	CCACAATCCTTGGCTGTTCT	51.78	727	Kwon et al. (2004)
L. casei	Casei-R	GCTTGAGGCGATTGTAATCC	51.78	727	Kwon et al. (2004)

Note: F = forward primer; R = reverse primer; bp = base pairs. The primers and sequences used for identifying lactic acid bacteria strains targeted conserved genetic regions, ensuring specific and efficient amplification. Their details, including annealing temperatures and expected amplicon sizes, were documented for accuracy and reproducibility.

cell (ONT) and sequenced on the MinION MK1B sequencing device (ONT).

Sequencing Quality Control

Following sequencing, raw reads obtained from each barcoded sample were evaluated for quality using FASTQC prior to inclusion in downstream analysis (Andrews, 2010). Samples were retained for taxonomic classification if the post-PCR DNA concentration exceeded 10 ng/μL, as determined by Qubit fluorimetry, and if enough reads were obtained after demultiplexing to permit reliable taxonomic assignment. Of the twelve samples sequenced, seven (B, C, E, G, J, L, and N) satisfied these criteria and were retained for further analysis. The remaining five samples (X1, X2, X3, X4, and X5) failed to meet the DNA concentration threshold or yielded insufficient sequencing depth and were therefore excluded from subsequent taxonomic and diversity analyses.

Bioinformatics Analysis

Raw reads generated by the MinION MK1B sequencing device were basecalled and demultiplexed to produce per-sample FASTQ files, from which adapter and barcode sequences were trimmed using Trimmomatic prior to downstream genome assembly using SPAdes (Bankevich et al., 2012). Demultiplexed reads from each sample were queried against the NCBI 16S ribosomal RNA reference database using the Basic Local Alignment Search Tool for nucleotides (blastn) executed at the command line through the standalone BLAST+ suite (Camacho et al., 2009). For each read, the top-scoring hit was taken as the species-level taxonomic assignment.

For each sample, reads sharing the same species assignment were tallied, and the ten most abundant species were retained for downstream analysis. Per-sample relative abundance was computed by expressing each species' read count as a percentage of the total reads assigned within the top ten taxa of that sample. Aggregate (pooled) relative abundance across all seven sequenced samples was obtained by summing read counts per species and dividing by the corresponding grand total.

Alpha-diversity metrics, comprising Simpson, Inverse Simpson and Fisher's α indices, were computed from the species-level read-count, to estimate species richness and evenness within each sample. The taxonomic and relative abundance outputs of this pipeline were used to generate the community composition visualizations presented in Figure 2 and the alpha-diversity visualizations presented in Figure 3.

Phylogenetic Analysis

The evolutionary history was inferred by using the Maximum Likelihood method based on the Tamura 3-parameter model. The tree with the highest log likelihood (–4366.69) is shown. The percentage of trees in which the associated taxa clustered together is shown next to the branches. Initial tree(s) for the heuristic search were obtained automatically by applying Neighbor-Join and BioNJ algorithms to a matrix of pairwise distances estimated using the Maximum Composite Likelihood (MCL) approach and then selecting the topology with superior log likelihood value. A discrete Gamma distribution was used to model evolutionary rate differences among sites (5 categories (+G, parameter = 0.2012)). The tree is drawn to scale, with branch lengths measured in the number of substitutions per site. The analysis involved 19 nucleotide sequences. Codon positions included were 1st+2nd+3rd+Noncoding. All positions with less than 90% site coverage were eliminated. That is, fewer than 10% alignment gaps, missing data, and ambiguous bases were allowed at any position. There were a total of 1314 positions in the final dataset. Evolutionary analyses were conducted in MEGA7 (Kumar et al., 2016).

Results and Discussion

Results

Multiplex PCR of lactic acid bacteria strains

In identifying probiotic strains in locally manufactured yogurts, we employed a multiplex assay using eleven *Lactobacillus* primers (Table 1). Before the investigation, it was confirmed that all these samples had Lactobacilli by the appearance of a *Lactobacillus* genus-specific PCR product (data not shown). Furthermore, six strains distributed among the samples were identified. *L. delbrueckii* was found in all samples except X1 and G. *L. gasseri* was also detected in samples B, G, J, and L. *L. rhamnosus* was found in only samples E

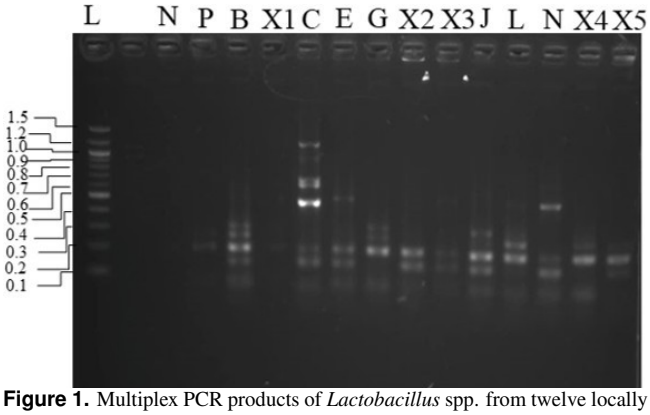

Figure 1. Multiplex PCR products of *Lactobacillus* spp. from twelve locally produced yogurt samples (B, C, E, G, J, L, N, X1, X2, X3, X4 and X5) and reference controls, resolved on a 2% agarose gel and visualized under ultraviolet light after ethidium bromide staining. L: 100 bp DNA ladder. N: negative (no-template) control. P: positive control (genomic DNA from a reference *Lactobacillus* strain). Lanes B–X5: PCR products from individual yogurt samples. Diffuse bands below approximately 100 bp correspond to primer dimers and were not interpreted as positive species-specific products.

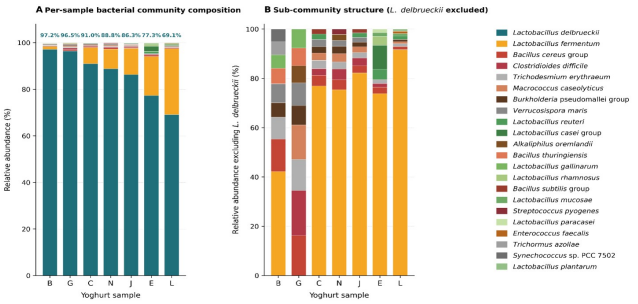

Figure 2. Bacterial community composition of seven probiotic yoghurt samples profiled by 16S rRNA Nanopore sequencing. (A) Per-sample relative abundance of all twenty-two bacterial taxa detected. Each bar sums up 100% of the reads assigned to that sample's top-ten taxa. Numbers above each bar indicate the relative abundance of *Lactobacillus delbrueckii* in that sample. Samples are ordered by *L. delbrueckii* dominance, from highest (B, 97.2%) to lowest (L, 69.1%). (B) The same data with *L. delbrueckii* excluded and the remaining taxa rescaled to 100%, to make the sub-community structure visible. In most samples the non-*L. delbrueckii* fraction was dominated by *L. fermentum*, except in sample G, in which *L. fermentum* was not detected. Read-count data and detection frequencies for each species are provided in Supplementary Table S1.

and N. Furthermore, *L. acidophilus*, *L. casei*, and *L. reuteri* were only found in sample C (Figure 1; Table 1).

Relative Frequency and Abundance

Out of the twelve (12) samples, seven (7) passed sequencing quality control and were taxonomically classified. The relative abundance of bacterial species identified in each yogurt sample varied, with species distributed among *L. delbrueckii*, *L. fermentum*, *Bacillus cereus* group, *Trichodesmium erythraeum*, *Verrucosispora maris*, *Bacillus thuringiensis*, *L. gallinarum*, *Trichormus azollae*, *L. reuteri*, *Bacillus subtilis* group, *Macrococcus caseolyticus*, *Clostridioides difficile*, *Enterococcus faecalis*, *L. plantarum*, *Streptococcus pyogenes*, *Burkholderia pseudomallei* group, *Synechococcus* sp. PCC 7502, *L. casei* group, *L. rhamnosus*, *L. paracasei*, *L. mucosae*, and *Alkaliphilus oremlandii* (Figure 2).

At the per-sample level, *Lactobacillus delbrueckii* was the most abundant taxon in all seven samples, with a mean relative abundance of 86.6 $\pm$ 10.2% and a range of 69.1–97.2%. Percentagewise, *L. delbrueckii* was highest in sample B (97.2%) and G (96.5%), followed by C (91.0%), N (88.8%), J (86.3%), E (77.3%), and lowest in L (69.1%) (Figure 2). This was followed by *Lactobacillus fermentum*, which was the second most abundant taxon in six of the seven samples, reaching 28.3% in sample L but undetected in sample G.

To better resolve the minor taxa, *L. delbrueckii* was excluded from the dataset and the remaining reads were rescaled, exposing the underlying sub-community structure of each sample (Figure 2B). In six of the seven samples, *L. fermentum* dominated the non-*L. delbrueckii* fraction, accounting for between 42.2% (sample B) and 91.7% (sample L) of those reads. In sample G and in the absence of *L. fermentum*, its sub-community was dominated by *Clostridioides difficile* (18.3%), *Bacillus cereus* group (16.3%), *Macrococcus caseolyticus* (14.0%), and *Trichodesmium erythraeum* (12.6%).

When reads were pooled across all seven samples, *L. delbrueckii* accounted for 89.1% of total reads, followed by *L. fermentum* (8.1%), with all remaining taxa together contributing less than 3% (Supplementary Table S1b). Among these minor taxa, the most abundant were *Bacillus cereus* group (0.45%), *Clostridioides difficile* (0.34%), *Trichodesmium erythraeum* (0.33%), *Macrococcus caseolyticus* (0.27%), *Burkholderia pseudomallei* group (0.24%), and *Verrucosispora maris* (0.23%).

Alpha Diversity Measures

The alpha diversity of bacterial communities of different yogurt samples was assessed using three diversity indices: Simpson's Diversity Index, Inverse Simpson's Index, and Fisher's Alpha Index (Figure 3). Each index provides insight into species richness and evenness within individual yogurt samples (B, N, E, L, J, C, and G) collected from six locations (Accra, East Legon, Legon, Madina, Marina Mall, and Okpon-glo).

The highest Simpson index value ($\sim$0.5) among the yogurt samples was observed for sample B, suggesting a more even distribution of bacterial species. This was followed by samples N, E, L, J, and C. In contrast, the lowest diversity was recorded in sample G, indicating the predominance of a single or few bacterial taxa. Similarly, the inverse Simpson's index shows the same trend. Moreover, the highest Fisher index ($\sim$1.8) was observed for sample N, suggesting that this yogurt sample harbors a relatively rich bacterial community. This was followed by samples B, E, C, L, J, and G. Sample G recorded the lowest Fisher index value, indicating the lower microbial richness in the sample (Figure 3).

Phylogenetic Analysis

The size of the nodes (circles) represents the relative abundance of each bacterial taxon, with larger circles indicating

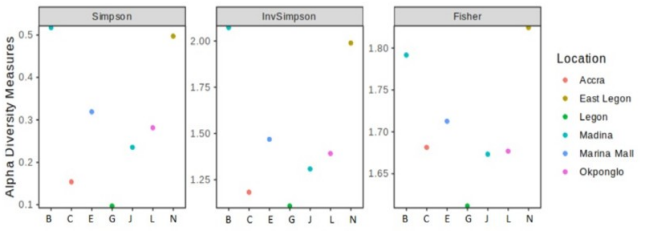

Figure 3. Alpha-diversity indices for the seven probiotic yoghurt samples that passed sequencing quality control (B, C, E, G, J, L and N). (Left) Simpson's index. (Middle) Inverse Simpson's index. (Right) Fisher's α index. Indices were calculated from species-level read-count tables. Low values across all three metrics reflect dominance of *Lactobacillus delbrueckii* in each sample, consistent with the design intent of yogurt fermentation.

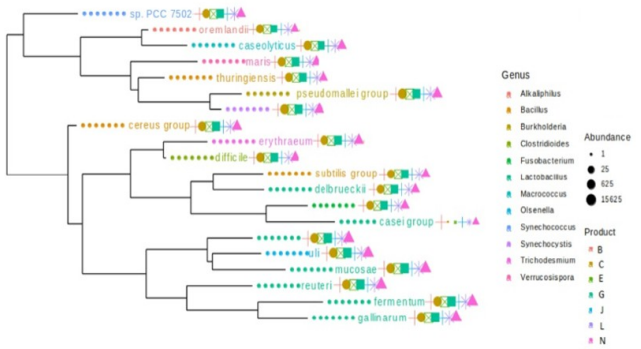

Figure 4. Maximum likelihood phylogenetic tree showing the evolutionary relationships among the bacterial taxa detected in the seven sequenced yoghurt samples. The phylogenetic tree above illustrates the evolutionary relationships among bacterial taxa at the genus level in locally produced yogurt samples. The tree includes multiple genera, with species represented by different-colored labels corresponding to the taxonomic groups defined in the legend. The tree structure reflects the hierarchical clustering of taxa based on sequence similarity, with closely related species branching from common nodes.

higher sequence read counts. The genera *Lactobacillus*, *Synechococcus*, *Bacillus*, and *Clostridioides* are the dominant taxa, showing higher abundance across multiple clades. Moreover, *Trichodesmium* and *Verrucosispora* are less abundant.

Some distinct clustering patterns show evolutionary similarities between species. *Lactobacillus delbrueckii* and *Bacillus subtilis* are both members of the class Bacilli but belong to different orders, Lactobacillales and Bacillales respectively.

Discussion

Yogurt is popular worldwide and enjoyed due to its health benefits and taste. It is rich in probiotics, calcium, phosphorus, vitamins, and essential proteins (Hadjimbei et al., 2022). Yogurt consumption has been shown to improve gut health, manage diabetes, and reduce the likelihood of tumor formation and cancer (Aryana and Olson, 2017). However, although appreciated in Ghana, its consumption remains limited due to its relatively high cost. In response, local, small-scale production by independent producers has increased, making yogurt more affordable (Achaglinkame et al., 2023; Adubofuor et al., 2014). In this study, we investigated the relative abundance and diversity of *Lactobacillus* species present in yogurt products labeled as "probiotic" and sold in supermarkets within Accra, Ghana.

For the initial identification of probiotic strains, a multiplex PCR was employed. Results confirmed the presence of Lactobacilli in all locally manufactured yogurt samples, with six distinct strains detected. The absence of *L. delbrueckii* in samples X1 and G, along with the sample-specific presence of other species such as *L. gasseri*, *L. rhamnosus*, and the exclusive detection of *L. acidophilus*, *L. casei*, and *L. reuteri* in sample C, indicates variability in microbial composition across products. This variability may reflect differences in production practices and raw material quality among local producers, potentially affecting the probiotic efficacy of the products (Bankole et al., 2023).

Several studies have documented *L. acidophilus*, *S. thermophilus*, and *L. plantarum* as widely used probiotic strains in yogurt production. Comparatively, the relative abundance analysis shows *L. delbrueckii* was the predominant species, followed by other lactic acid bacteria and several non-lactobacilli species (Figure 2b). Furthermore, the presence of these non-target species raises concerns about possible contamination and the overall hygienic conditions during production. As noted by Moh et al. (2017), locally made yogurt may have a high risk of health hazards to consumers. While probiotic claims in locally produced yogurt are centered around *Lactobacillus*, contamination by other taxa could influence the safety and functional properties of the yogurt (de Souza et al., 2024).

A few of the taxa detected in the dataset, however, warrant cautious interpretation. Several low-abundance species, including the aquatic cyanobacteria *Trichodesmium erythraeum*, *Trichormus azollae*, *Synechococcus* sp. PCC 7502, and the marine sediment actinobacterium *Verrucosispora maris*, are not ecologically compatible with a pasteurized fermented dairy product. Their appearance most plausibly reflects known limitations of species-level taxonomic assignment from long-read 16S rRNA sequencing, where the elevated per-read error rate of Nanopore data combined with a top-hit BLAST strategy can produce occasional misassignments to distantly related reference sequences. These taxa are therefore best regarded as classification artefacts rather than true members of the yoghurt community. A separate caution applies to the potentially pathogenic taxa detected, namely the *Bacillus cereus* group, *Clostridioides difficile*, *Enterococcus faecalis*, *Streptococcus pyogenes* and the *Burkholderia pseudomallei* group, which are ecologically plausible as dairy contaminants where post-pasteurization handling is inadequate. However, these assignments are based on the top-scoring BLAST hit of individual reads rather than on culture-based isolation or species-specific molecular confirmation, and species-level resolution within these genera, particularly the *Bacillus cereus* group, is known to be unreliable from 16S rRNA alone. Each was also detected at very low abundance, contributing less than 0.5% of pooled reads. These results should therefore be treated

as preliminary indications warranting follow-up by culture-based recovery and confirmation by species-specific qPCR or whole-genome sequencing, rather than as confirmed evidence of contamination. *Macrococcus caseolyticus*, by contrast, is a non-pathogenic Gram-positive coccus commonly associated with raw milk and dairy products, and its detection in five samples at low abundance is consistent with its known ecology. As noted by Moh et al. (2017), locally made yogurt may carry a higher risk of microbial hazards to consumers, and contamination by non-target taxa could influence both the safety and the functional properties of the product (de Souza et al., 2024). For the present dataset, however, this concern remains to be tested by confirmatory methods.

The low Simpson and Inverse Simpson indices observed across all yoghurt samples were expected, given that yoghurt fermentation is driven by a defined starter culture whose dominance is the intended outcome of the production process. In this context, low taxonomic diversity is not in itself indicative of poor product quality. What matters for probiotic efficacy is not how many taxa are present, but which taxa dominate, and whether the dominant taxa are the well-characterized probiotic strains intended by the producer. Within this context, alpha-diversity metrics nevertheless provide useful information about the community structure within and between samples. The values of Simpson's, Inverse Simpson's, and Fisher's α indices show heterogeneity in species evenness across the samples (Cassol et al., 2025). For instance, sample B's higher Simpson index reflects a slightly more balanced sub-community alongside the dominant *L. delbrueckii*, whereas sample G's lower indices reflect even stronger single-taxon dominance. Increased species richness is not necessarily beneficial if additional taxa consist predominantly of non-probiotic or potentially pathogenic strains. Such diversity may undermine the functional and health-promoting properties of yogurt. Conversely, decreased species richness can be advantageous when it reflects selective enrichment of well-characterized probiotic strains. The impact of microbial diversity on product quality and probiotic efficacy is therefore context-dependent, emphasizing the need for comprehensive microbial characterization in evaluating the safety and functional potential of these yogurts (Bourrie et al., 2016; Marco et al., 2017).

Phylogenetic analysis illustrated the evolutionary relationships among the detected bacterial taxa, with dominant genera such as *Lactobacillus*, *Synechococcus*, *Bacillus*, and *Clostridioides* forming distinct clusters. *Bacillus subtilis* and *Lactobacillus delbrueckii* are located on adjacent branches of the inferred tree, reflecting their shared placement within the class Bacilli, although they belong to different orders (Bacillales and Lactobacillales, respectively) and are not closely related at the genus level. The clustering of *L. gallinarum* with *L. fermentum* suggests possible shared metabolic or functional traits that could influence the overall probiotic potential of the yogurt (Zheng et al., 2015). However, the close association of certain taxa with potential pathogens calls for further investigation into the safety aspects of these locally produced products.

Conclusion

The various methods (multiplex PCR and 16S rRNA sequencing) employed detected six different *Lactobacillus* strains across the yogurt samples. The relative abundance and phylogenetic analyses revealed variability in the presence and proportions of probiotic and non-probiotic strains, with *Lactobacillus delbrueckii* being the most dominant. Alpha diversity metrics further highlighted heterogeneity among samples, suggesting that increased species richness may not necessarily equate to enhanced probiotic benefits, particularly if dominated by non-probiotic or potentially pathogenic taxa. The findings in our study emphasize the need for rigorous microbial profiling in locally manufactured yogurts to ensure both their functional efficacy and consumer safety. Future research should focus on correlating these microbial profiles with health outcomes, thereby guiding quality control measures and supporting the development of superior probiotic products in the region.

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