Microbial Compositions and Nutritional Properties of Commercial and Local Yoghurts in Bangladesh

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Abstract

Yogurt is a widely consumed traditional fermented food. The health benefits and shelf life of yogurt depend on the type and magnitude of fermenting microorganisms, the proportion of physicochemical parameters, and the presence of microbial and metal contaminants. This study aimed to investigate the physicochemical properties and bacterial diversity of sour and sweet yogurts, commercially and locally produced in Bangladesh. A total of 38 samples, 19 each for sour and sweet yogurts, randomly collected from several commercial and local brands in Bangladesh. The most standard AOAC methods were used to perform proximate analysis, AAS to determine minerals, and high-throughput Illumina sequencing of 16S rRNA genes to conduct metagenomic analysis. For statistical and bioinformatic analysis, R and QIIME 2 were used, respectively, to perform OTU extractions and rarefaction, alpha and beta diversity, and spearman correlation. Sweet yogurts contained significantly greater pH, fat, moisture, TS, SNF content (%, w/w) and *Streptococcus* spp.(%) than sour samples, whereas sour yogurts contained more moisture, ash, minerals content (Zn, Na, Ca and Mg) and *Lactobacillus* spp. (%). Sour samples had more bacterial diversity, along with probiotics and potentially harmful opportunistic path-ogens, including *Enterobacter, Lactococcus, Aeromonas* and *Acinetobacter*. Also, commercial brands exhibited higher abundance of some well-known probiotic strains than the local brands. The more the ash content, the more amount of Ca was exhibited. The relative abundance of most of the bacterial genera, except Lactobacillus, was positively correlated with each other. Except for *Lactobacillus*, fat (%) had negatively, whereas pH and moisture had positively influenced the growth of other bacterial genera. The presence of *Hafnia* in yogurt positively influenced the bioavailability of all minerals (Fe, Zn, Cu, Na, k, Ca and Mg). Nutritional parameters were varied based on the taste of yogurt samples with sour ones having greater nutritional values and bacterial diversity. These findings would help understand in consuming yogurts for health benefits.

1. Introduction

Bangladeshi yogurt, known as "dahi," is a creamy and tangy dairy product that plays a significant role in the country's culinary landscape. Often enjoyed as a refreshing accompaniment to spicy dishes or consumed on its own, Bangladesh's yogurt is renowned for its velvety texture and distinctively rich flavor. It has its nutritional advantages, probiotic content, sensory attributes, and prolonged shelf life[1]. With diverse varieties now available, featuring distinct fat contents, tastes, and flavors, sweet and sour constitute fundamental types characterized not only by taste but also by the microorganisms involved in their production. Typically, sour yogurt results from the fermentation of lactose in milk by lactic acid bacteria (LAB), leading to lactic acid production, pH reduction, and coagulation of the milk protein casein, imparting the characteristic sour taste [1, 2]. The acidic environment created serves as a preservation strategy, inhibiting the growth of pathogenic bacteria [3]. In contrast, sweet yogurt, achieved through the addition of flavors and sweeteners, involves curd produced from cow milk rennet, sweetened and rapidly boiled to achieve a substantial consistency [4, 5].

Beyond taste, yogurts serve as rich nutritional sources, containing 5–6% protein, 4.6–5.2% lactose, 0-3.5% fat, and various minerals and vitamins such as sodium, potassium, calcium, magnesium, copper, iron, phosphorus, zinc, and vitamins A, B6, B12, and C [6]. Moreover, yogurt functions as a probiotic carrier, offering numerous health benefits, including immune modulation, cholesterol metabolism, antimicrobial properties, and anti-cancer activity [7–9]. The key players in the yogurt microbiome, *Lactobacillus bulgaricus* and *Streptococcus thermophiles*, form the conventional starter culture, thriving even in low pH conditions [1]. These cultures foster the growth of other beneficial organisms, contributing to yogurt's viscosity, aroma, and flavor [3].

The expanding dairy industry in Bangladesh is intricately tied to the specific microbial composition in milk. Traditional yogurt cultures not only enhance flavor but also contribute to the bioavailability of minerals and vitamins through fermentation [10]. Recent advancements in metagenomic analysis, utilizing next-generation sequencing platforms and bioinformatics, have allowed a more comprehensive exploration of microbial communities in fermented foods, uncovering a diverse array of bacteria in Bangladeshi yogurt [11, 12]. While *Lactobacillus* and *Streptococcus* dominate as the most abundant genera, minor groups like *Acinetobacter, Lactococcus, Enterobacter, Aeromonas, Macrococcus, Staphylococcus*, and *Escherichia* have also been identified [13, 14]

As dairy production and marketing is rapidly growing in Bangladesh, understanding the microbial and nutritional aspects of yogurt becomes crucial. This study represents the first investigation into the microbial diversity and nutritional composition of Bangladeshi yogurt, with a focus on correlation analysis. Examining physicochemical parameters, mineral content, and bacterial communities through metagenomic sequencing, we aim to contribute valuable insights that can inform dietary choices, promote appreciation for probiotics, and impact the quality of yogurt in the Bangladeshi market. The outcomes of this research hold the potential to guide governmental efforts in making diverse and nutritious yogurt varieties more accessible to the general populace.

2. Methodology

2.1 Research Design and Sampling Site

A total of thirty-eight (n = 38) yogurt samples were analyzed in this study which includes eight (n = 8) newly collected samples, categorized as sour and sweet, belonging to 6 prominent local brands of Jashore (3 sour and 3 sweet), and 2 local brands of Satkhira (1 sour and 1 sweet) (Fig. 1). The samples were immediately placed in a cooling box with refrigerants (at 4°C), and then transferred (within 12 hours) to the General Laboratory at Jashore University of Science and Technology. Additionally, the 30 sequences data fileswere obtained from the National Center for Biotechnology Information (NCBI) under the BioProject accession number PRJNA733702 and nutritional properties of them were also collected from a publication conducted by Islam et al. 2021 [15]. These files belong to seven renowned commercial yogurt brands in Bangladesh, further categorized as sour (n = 15) and sweet (n = 15). In total, eleven sampling sites in Bangladesh were selected for this study based on the popularities of yogurt in the

selected area and its consumer demand (Location of sampling sites are included in Supplementary Data 1).

2.2 Nutritional, Biochemical, and Minerals Parameters Analysis

Biochemical parameters (ash, moisture, fat, solid-non-fat, total solid, and pH) and mineral contents of yogurt samples (n = 8) were determined using the Association of Official Analytical Chemists (AOAC) [16] method and procedure described in Hayet et al., 2021 [17]. Biochemical assays were done in triplicate and mean results were reported throughout the paper. Direct heating measured the percentage (%, w/w) of ash in samples burned in a muffle furnace at 550°C for 24 hours, while the AOAC oven technique measured moisture content (MC). After reaching a consistent weight, yogurt samples were oven-dried at 105°C for 3 h. Over-dried samples were put in a desiccator and weighed again. The dry weight was subtracted from the sample's initial weight to compute MC as a percentage. Total Solids (TS) % was calculated using AOAC gravimetric technique. Using Gerber technique, yogurt fat was measured [17]. After the 11.3g yogurt sample, the butyrometer received 10 ml of sulfuric acid with a specific gravity of 1.082 and 1 ml of isoamyl alcohol. The butyrometer was put in the 1100 rpm centrifuge. The butyrometer measured separated fat as a percentage. TS (%) minus fat generated SNF. The pH meter electrode was put into the sample at room temperature to measure pH. The mean of triplicate measurements was reported. For mineral analysis, yogurt was oven-dried to ashes. The inorganic residues left in the crucible were digested by adding HNO3 and diluting with ultrapure water to 100 mL, which was then introduced to the Flame Atomic Absorption Spectrophotometer (iCE-3000 FASS, Termofisher Scientific, USA) to determine minerals like Iron (Fe), Zinc (Zn), Copper (Cu), Sodium (Na), Potassium (K), Calcium (Ca), and Magnesium (Mg) at wavelengths of 589.0, 766.5, 422.7, 285.2, 248.3. Ca was 0.5–4.0 ppm [18, 19].

2.3 Total DNA Extraction and Metagenomic (16S rRNA) Sequencing

Using the DNeasy Powersoil Pro Kit (QIAGEN) and the manufacturer's instructions, yogurt samples were treated to extract all of the DNA content. To amplify the V3-V4 region of the bacterial 16S rRNA gene, sequencing libraries were created using primer pairs 341F (5'-CCTACGGGNGGCWGCAG-3') and 806R (5'-GAC-TACHVGGGTATCTAATCC-3') that included the Illumina overhang adapter sequence (Illumina, Inc., San Diego, CA, USA). 12.5 uL of 2X KAPA HiFi HotStart Ready Mix (Roche Diagnostic Corporation, Indianapolis, IN, USA), 5 uL of DNA extract, 2.5 uL of each primer, and 2.5 uL of HyClone water (Cytiva Life Sciences, Marlborough, MA, USA) were the ingredients of each PCR reaction. A T100TM heat cycler (Bio-Rad Laboratories, Inc., Hercules, CA, USA) was used for PCR amplifications. Following three minutes of initial denaturation at 95°C, there were 25 cycles of 30 s at 95°C, 30 s at 62°C, 30 s at 72°C, and 4 min at 72°C for the final extension in the PCR. The PCR products were purified using epMotion 5075 Liquid Handler (Eppendorf AG, Hamburg, Germany) and AMPure XP beads (Beckman Coulter, Inc., Brea, CA, USA). Following three minutes of initial denaturation at 95°C, 25 cycles of 30 s at 95°C, 30 s at 56°C, 30 s at 72°C, and 4 min at 72°C for final extension, the purified PCR products were then indexed using an

Illumina Nextera XT Index kit. AMPure XP beads were used to purify the indexed libraries. Following normalization and pooling using an epMotion liquid handler, the indexed and purified libraries were loaded into an Illumina NextSeq2000 v3 600 cycle-kit (P1 flowcell) reagent cartridge for 2 × 300 bp paired-end sequencing. The Georgia Genomics and Bioinformatics Core (GGBC), University of Georgia, Athens, GA, USA (RRID:SCR_010994), handled the preparation and sequencing of the whole library.

2.4 Taxonomic Profiling of the Amplicon Sequences

The sequences, consisting of 38 samples, were generated using a read length of 2 × 300 base pairs. This read length ensured that the 16S V3-V4 region was adequately covered for subsequent bioinformatics analysis. The quality of the FASTQ files was evaluated using FastQC v0.11[18]. Trimmomatic v0.39 was used with a sliding window size of 30, a minimum read length of 100 bp, and a minimum average quality score of 20 to remove low-quality ends and adapter sequences from each read [19]. After quality control, there were an average of 161019 pairs of reads for 16S samples (minimum = 10688 and maximum = 583515 pairs). QIIME 2v2022.2 was utilized as an integrated pipeline for OTU (Operational Taxonomic Unit) clustering, taxonomic assignment, and phylogenetic estimation [20]. QIIME 2's integrated VSEARCH metagenomics algorithm was utilized for read joining, dereplication of sequences, de novo clustering (OTU clustering with 99% identity), and de novo chimera checking (excluding chimeras and "borderline chimeras") [21]. Taxonomic assignment was done using the Greengenes database (v13_5), which included 99% OTU clustering and associated taxonomy information [22]. The 16S sequencing primer pairs and a naive-bayes classifier were used to train the reference database [23, 24]. Classify-sklearn algorithms were used to classify the assigned OTUs within the samples The sequences, consisting of 38 samples, were generated using a read length of 2 × 300 base pairs. This read length ensured that the 16S V3-V4 region was adequately covered for subsequent bioinformatics analysis. The quality of the produced FASTQ files was evaluated using FastQC v0.11[18]. Trimmomatic v0.39 was used with a sliding window size of 30, a minimum read length of 100 bp, and a minimum average quality score of 20 to remove low-quality ends and adapter sequences from each read [19]. After quality control, there were an average of 161019 pairs of reads for 16S samples (minimum = 10688 and maximum = 583515 pairs). QIIME 2 (version 2022.2) was utilized as an integrated pipeline for OTU (Operational Taxonomic Unit) clustering, taxonomic assignment, and phylogenetic estimation [20]. QIIME 2's integrated VSEARCH metagenomics algorithm was utilized for read joining, dereplication of sequences, de novo clustering (OTU clustering with 99% identity), and de novo chimera checking (excluding chimeras and "borderline chimeras") [21]. Taxonomic assignment was done using the Greengenes database (v13_5), which included 99% OTU clustering and associated taxonomy information [22]. The 16S sequencing primer pairs and a naive-bayes classifier were used to train the reference database [23, 24]. Classify-sklearn algorithms were used to classify the assigned OTUs within the samples [25, 26].

2.5 Statistical Analysis

Downstream analysis, encompassing alpha and beta diversity, microbial composition, and statistical comparison, was conducted using the “PHyloseq” package [27] in R software (version 4.2) [28]. For normalization, rarefaction of OTU counts was employed.Downstream analysis, encompassing alpha and

beta diversity, microbial composition, and statistical comparison, was conducted using the “PHyloseq” package [27] in R software (version 4.2) [28]. For normalization, rarefaction of OTU counts was employed.The "Vegan"[29], "ggpubr"[30], and "ggplot2"[31] packages of R were used to estimate the observed, Chao1, Shannon, Simpson, InvSimpson, and Fisher alpha diversity and then plotted accordingly. The "microbiomeutilities"[32] R package was used to identify distinctions in microbial abundance and diversity between two locations, utilizing the Wilcoxon rank sum test. The principal coordinate analysis (PCoA) with Bray-Curtis was utilized to measure Beta diversity. The P-value was measured using the permutational multivariate analysis of variance (PERMANOVA) with permutations for identifying the differences in samples. “Vegan”, “PHyloseq”, “Microbiome utilities”, “Tidyr”[33], and “ggplot2” packages were utilized for taxonomic comparison and generating heatmaps with dendrograms. The data were analyzed to measure the correlation between the physicochemical parameters and the relative abundance of bacteria utilizing the Statistical Package for the Social Sciences (SPSS) and R packages “Hmisc” and “corrplot” [34, 35]. Individual effects of the factors during the study utilizing the statistical analysis were conducted in duplicates, and the results were presented as the average of two values. The "Vegan"[29], "ggpubr"[30], and "ggplot2"[31] packages of R were used to estimate the observed, Chao1, Shannon, Simpson, InvSimpson, and Fisher alpha diversity and then plotted accordingly. The "microbiomeutilities"[32] R package was used to identify distinctions in microbial abundance and diversity between two locations, utilizing the Wilcoxon rank sum test. The principal coordinate analysis (PCoA) with Bray-Curtis was utilized to measure Beta diversity. The P-value was measured using the permutational multivariate analysis of variance (PERMANOVA) with permutations for identifying the differences in samples. “Vegan”, “PHyloseq”, “Microbiome utilities”, “Tidyr”[33], and “ggplot2” packages were utilized for taxonomic comparison and generating heatmaps with dendrograms. The data were analyzed to measure the correlation between the physicochemical parameters and the relative abundance of bacteria utilizing the Statistical Package for the Social Sciences (SPSS) and R packages “Hmisc” and “corrplot” [34, 35]. Individual effects of the factors during the study utilizing the statistical analysis were conducted in duplicates, and the results were presented as the average of two values.

3. Results

The quality and safety of yogurt are determined by its nutritional composition, microbial diversity, and the quality of the starting culture. There were a total of thirty-eight samples (n = 38), with half of them being sweet and the other half being sour. We obtained a total of fifteen (n = 15) commercial samples, which are produced and distributed nationwide, as well as twenty three (n = 23) locally produced samples, which are generated in various regions of Bangladesh and disseminated within those areas. We selected a total of eight districts (Bogura, Chittagong, Cox's Bazar, Dhaka, Gazipur, Jashore, Narsingdi, and Satkhira) for local and commercial brand selection that are well-known for their yogurt manufacturing in Bangladesh. This analysis contained a total of six samples of Commercial Sour and nine samples of Commercial Sweet. Additionally, there were thirteen samples of Local Sour and ten samples of Local Sweet included in the analysis (Table 1). We classified yoghurts with a sour and sweet taste as Taste-A, while Taste-B

was divided into two categories: Commercial (Sour and Sweet) and Local (Sour and Sweet). In order to identify brands, we designated commercial and local brands as Brand-A. To facilitate comparisons between commercial brands and local brands from different locations, we developed an additional category called Brand-B (Table 1).

Table 1. Study design and data source of this analysis.

Sample ID	Taste-A	Taste-B	Brand-A	Brand-B	Location	SRR ID	NCBI-ID	Year	References
D1	Sweet	Commercial Sweet	Commercial	Commercial Brand	Narsingdi	SRR17065927	B11	2021	Islam et. al., 2021
D2	Sweet	Commercial Sweet	Commercial	Commercial Brand	Narsingdi	SRR17065926	B12	2021	Islam et. al., 2021
D3	Sweet	Commercial Sweet	Commercial	Commercial Brand	Narsingdi	SRR17065915	B13	2021	Islam et. al., 2021
D4	Sweet	Commercial Sweet	Commercial	Commercial Brand	Dhaka	SRR17065904	B21	2021	Islam et. al., 2021
D5	Sweet	Commercial Sweet	Commercial	Commercial Brand	Dhaka	SRR17065893	B22	2021	Islam et. al., 2021
D6	Sweet	Commercial Sweet	Commercial	Commercial Brand	Dhaka	SRR17065882	B23	2021	Islam et. al., 2021
D7	Sour	Commercial Sour	Commercial	Commercial Brand	Dhaka	SRR17065871	B31	2021	Islam et. al., 2021
D8	Sour	Commercial Sour	Commercial	Commercial Brand	Dhaka	SRR17065870	B32	2021	Islam et. al., 2021
D9	Sour	Commercial Sour	Commercial	Commercial Brand	Dhaka	SRR17065869	B33	2021	Islam et. al., 2021
D10	Sweet	Commercial Sweet	Commercial	Commercial Brand	Gazipur	SRR17065868	B41	2021	Islam et. al., 2021
D11	Sweet	Commercial Sweet	Commercial	Commercial Brand	Gazipur	SRR17065925	B42	2021	Islam et. al., 2021
D12	Sweet	Commercial Sweet	Commercial	Commercial Brand	Gazipur	SRR17065924	B43	2021	Islam et. al., 2021
D13	Sour	Commercial Sour	Commercial	Commercial Brand	Gazipur	SRR17065923	B51	2021	Islam et. al., 2021
D14	Sour	Commercial Sour	Commercial	Commercial Brand	Gazipur	SRR17065922	B52	2021	Islam et. al., 2021
D15	Sour	Commercial Sour	Commercial	Commercial Brand	Gazipur	SRR17065921	B53	2021	Islam et. al., 2021
D16	Sweet	Local Sweet	Local	Local Chittagong	Chittagong	SRR17065920	B61	2021	Islam et. al., 2021
D17	Sweet	Local Sweet	Local	Local Chittagong	Chittagong	SRR17065919	B62	2021	Islam et. al., 2021
D18	Sweet	Local Sweet	Local	Local Chittagong	Chittagong	SRR17065918	B63	2021	Islam et. al., 2021
D19	Sour	Local Sour	Local	Local Chittagong	Chittagong	SRR17065917	B71	2021	Islam et. al., 2021
D20	Sour	Local Sour	Local	Local Chittagong	Chittagong	SRR17065916	B72	2021	Islam et. al., 2021
D21	Sour	Local Sour	Local	Local Chittagong	Chittagong	SRR17065914	B73	2021	Islam et. al., 2021
D22	Sour	Local Sour	Local	Local Cox's Bazar	Cox's bazar	SRR17065913	B81	2021	Islam et. al., 2021
D23	Sour	Local Sour	Local	Local Cox's Bazar	Cox's bazar	SRR17065912	B82	2021	Islam et. al., 2021
D24	Sour	Local Sour	Local	Local Cox's Bazar	Cox's bazar	SRR17065911	B83	2021	Islam et. al., 2021
D25	Sweet	Local Sweet	Local	Local Bogra	Bogura	SRR17065910	B91	2021	Islam et. al., 2021
D26	Sweet	Local Sweet	Local	Local Bogra	Bogura	SRR17065909	B92	2021	Islam et. al., 2021
D27	Sweet	Local Sweet	Local	Local Bogra	Bogura	SRR17065908	B93	2021	Islam et. al., 2021
D28	Sour	Local Sour	Local	Local Dhaka	Dhaka	SRR17065907	B101	2021	Islam et. al., 2021
D29	Sour	Local Sour	Local	Local Dhaka	Dhaka	SRR17065906	B102	2021	Islam et. al., 2021
D30	Sour	Local Sour	Local	Local Dhaka	Dhaka	SRR17065905	B103	2021	Islam et. al., 2021
D31	Sweet	Local Sweet	Local	Local Jashore	Jashore	SAMN39831121	Y2	2022	Studied Sample
D32	Sweet	Local Sweet	Local	Local Jashore	Jashore	SAMN39831122	Y4	2022	Studied Sample
D33	Sweet	Local Sweet	Local	Local Jashore	Jashore	SAMN39831123	Y8	2022	Studied Sample
D34	Sour	Local Sour	Local	Local Jashore	Jashore	SAMN39831124	Y9	2022	Studied Sample
D35	Sour	Local Sour	Local	Local Jashore	Jashore	SAMN39831125	Y13	2022	Studied Sample
D36	Sour	Local Sour	Local	Local Jashore	Jashore	SAMN39831126	Y14	2022	Studied Sample
D37	Sweet	Local Sweet	Local	Local Satkhira	Satkhira	SAMN39831127	Y18	2022	Studied Sample
D38	Sour	Local Sour	Local	Local Satkhira	Satkhira	SAMN39831128	Y19	2022	Studied Sample

3.1 Microbial Compositions and their Diversity

A total of 650 Operational Taxonomic Units (OTUs) were detected by analyzing yogurt samples using 16S rRNA amplicon sequencing with the V3-V4 amplicon region. The sour yogurt sample included 493 operational taxonomic units (OTUs), while the sweet yoghurt sample contained 441 OTUs. The research revealed the presence of 20 bacterial phyla and 1 archaeal phylum (Euryarchaeota) in all the samples. In Taste-A, both sweet and sour yoghurts included a total of 17 common phyla, with Firmicutes being the most prevalent in both sour and sweet samples (89.50% and 99.57%, respectively). In addition to Proteobacteria (10.12%), in sour samples, three phyla, namely Gracilibacteria, Gemmatimonadetes, and Spirochaetes, were found exclusively in sour samples. In contrast, two phyla, Euryarchaeota and Lentisphaerae, were solely present in sweet samples. Among the identified genera, 124 were present in both sour and sweet samples, with Lactobacillus and Streptococcus being the most prevalent (53.79%, 48.55%, and 31.40% and 50.46%, respectively). Additionally, Enterobacter and Lactococcus were

abundant specifically in sour samples. Moreover, 78 genera were exclusively found in sour samples, while 60 were unique to sweet samples (Supplementary Data 1). Notably, Cryseobacterium exhibited a significant difference ($p = 0.05$, Wilcoxon rank sum test) between the two taste types (Fig. 2A)

In relation to Taste-B, Firmicutes exhibited high abundance in commercial sour, commercial sweet, and local sweet samples, constituting 99.27%, 99.42%, and 99.70%, respectively. However, local sour samples displayed a comparatively lower Firmicutes abundance (84.98%), with 14.65% of unidentified organisms. Predominantly, *Streptococcus* and *Lactobacillus* were the most abundant genera in commercial sour (63.09%, 35.74%), commercial sweet (52.17%, 46.72%), and local sweet (50.21%, 48.93%), while local sour samples exhibited a distinct composition with *Lactobacillus* (62.12%), *Streptococcus* (16.78%), *Lactococcus* (5.70%), and 6.22% unassigned organisms. *Candidatus hepatoplasma* displayed a significant difference ($p = 0.005$, Wilcoxon rank-sum test) between commercial sweet and local sweet samples along with *Clostridium, Hafnia, Pseudomonas* and *Streptococcus* (Fig. 2B).

Among the 22 phyla present in both commercial and local samples (Brand-A), Firmicutes dominated in both cases (99.36% and 91.38%, respectively), with local samples also containing 8.36% Proteobacteria. Out of the 262 observed genera, 104 were shared between commercial and local samples, while 117 were exclusive to local and 41 exclusive to commercial samples. Commercial samples were characterized by high proportions of *Streptococcus* (56.53%) and *Lactobacillus* (42.33%), while local samples exhibited variability, including *Lactobacillus* (56.94%), *Streptococcus* (30.76%), *Lactococcus* (3.23%), and 3.75% unassigned organisms (Supplementary Data 1). Notably, *Candidatus hepatoplasma* and *Streptococcus* showed a significant difference ($p = 0.05$, Wilcoxon rank-sum test) between the two sample groups (Fig. 2C).

When comparing samples from distinct local regions with commercial ones (Brand-B), Firmicutes (> 99.35%) dominated both commercial and all local samples, except for local Cox's Bazar, where Proteobacteria (62.26%) was prevalent, accompanied by a lower count of Lactobacillus (37.08%). In terms of genus-level diversity, *Lactobacillus* and *Streptococcus* were prominent in commercial samples (42.33%, 56.53%) and local Chittagong (65.46%, 33.39%), local Dhaka (99.35%, 11.38%), local Jashore (83.67%), and local Satkhira (58.76%, 41.68%) samples. Notably, local Bogra showed a unique composition with 99.54% *Streptococcus* and 0.04% *Lactobacillus.* Conversely, local Cox's Bazar displayed a diverse array of organisms with varying abundances, including *Lactococcus* (24.52%), *Enterobacter* (26.62%), *Streptococcus* (11.38%), *Aeromonas (*6.07%), *Citrobacter (*1.22%), *Lactobacillus* (0.06%), and a substantial percentage of unknown organisms (25.51%) (Supplementary Data 1). *Kluyvera* and *Wautersiella* exhibited highly significant differences ($p = 0.001$, Wilcoxon rank-sum test) between commercial samples and local Cox's Bazar, while *Staphylococcus* showed high significance ($p = 0.001$, Wilcoxon rank-sum test) between commercial and local Bogra (Fig. 2D).

The main genera that showed varied presence in yogurt throughout various regions of Bangladesh were *Aeromonas, Lactobacillus, Lactococcus*, and *Streptococcus* (Fig. 3). With a few exceptions, the majority of the sour samples for commercial brands include *Lactobacillus*, whereas the majority of the sweet

samples have *Streptococcus* in significant quantity. However, in the Chittagong region, *Lactobacillus* and *Streptococcus* are about equally abundant in sweet yogurt, whereas *Lactobacillus* predominates in sour yogurt in Chittagong and Dhaka as well. Samples from Bogra, a renowned yogurt-producing area in Bangladesh, where Streptococcus bacteria are present in over 99.9% of the samples. Samples from the Jashore area mostly include *Lactobacillus* in both sweet and sour yoghurt, with a few outliers. In contrast to the local Chittagong samples, the local Satkhira samples include *Lactobacillus* in sweet yogurt and mostly *Streptococcus* in sour samples.

No significant difference ($p > 0.05$) was observed in bacterial diversity within the samples of Taste-A (Fig. 4A), Taste-B (Fig. 4B) and Brand-A (Fig. 4C). However, Cox's Bazar's local brands exhibited significantly higher diversity compared to other samples in Brand-B, as indicated by all indices tested. Observed and Chao1 indices highlighted lower bacterial richness ($p < 0.01$) in commercial brands versus local brands in Cox's Bazar, while local brands in Jashore showed lower richness ($p < 0.05$) than their commercial counterparts. Within local brands, Cox's Bazar samples demonstrated significantly higher bacterial richness ($p < 0.05$) than Chittagong samples. Moreover, Shannon, Simpson, and InvSimpson indices indicated significantly greater bacterial diversity ($p < 0.05$) in Cox's Bazar's local brands compared to those in Chittagong and Jashore. The Fisher index reflected a substantial difference ($p < 0.01$) in bacterial diversity, with commercial yogurt brands exhibiting lower diversity than local yogurt brands in Cox's Bazar and Bogra. Conversely, commercial yogurt brands displayed higher diversity ($p < 0.05$) than local brands in Jashore and Satkhira (Fig. 4D).

Bray Curtis distance and the Principal Coordinate Analysis (PCoA) exhibited significant difference (PERMANOVA, $p = 0.031$) between sweet and sour yogurt sample of Taste-A (Fig. 5A). However, there was no significant difference (Wilcoxon signed-rank test, $p > 0.05$) among the four different sample groups in Taste-B (Fig. 5B) and seven different sample groups in Brand-A was observed (Fig. 5C). Significant difference (PERMANOVA, $p = 0.001$) were also observed in bacteriome composition among the commercial brands and six local brands in Brand-B (Fig. 5D).

3.2 Effects of Environmental Conditions on the Composition of Microbial Communities

Various physicochemical factors, when assessed in relation to the relative abundance of bacterial communities in the samples, revealed significant positive and negative correlations. The majority of bacterial genera, excluding *Lactobacillus*, exhibited positive correlations with each other, indicated by the extended area of violet points (Fig. 6). Notably, *Lactobacillus* demonstrated significant negative correlations with several bacterial genera, including *Streptococcus* ($p < 0.001$), *Kurthia* ($p < 0.001$), *Enterococcus* ($p < 0.001$), *Enterobacter* ($p < 0.001$), *Lactococcus* ($p < 0.01$), and *Aeromonas* ($p < 0.01$). Conversely, *Lactococcus* displayed positive correlations with all bacterial genera except *Lactobacillus*. Negative correlations with *Lactobacillus* were also observed for *Enterobacter, Enhydrobacter, Acinetobacter, Macrococcus, Hafnia, Cetobacterium, Citrobacter, Wauterseilla, Enterococcus*, and *Kurthia*.

Except for *Lactobacillus* and *Thermus*, pH positively affected most other bacterial genera. *Lactobacillus* showed strong negative correlations with pH (coefficient = 0.57, p = 0.001) and moderate negative correlations with moisture (coefficient = 0.45, p = 0.01). Lower pH and moisture content were associated with higher *Lactobacillus* abundance (> 99%). Moisture content positively influenced the abundance of most bacterial genera, except *Lactobacillus, Staphylococcus, Cetobacterium*, and *Hafnia*. Conversely, these genera were negatively correlated with TS and SNF content in yogurt. Overall bacterial abundance was negatively influenced by the fat present in yogurt samples, with fat (%) negatively impacting the growth of bacterial genera where an exception to this is *Lactobacillus. Lactobacillus* exhibited a distinct correlation pattern with the studied bacterial genera and proximate parameters.

The presence of *Hafnia* in yogurt positively influenced the bioavailability of specific minerals; Fe, Zn, Cu, Na, K, Ca and Mg. However, the abundance of *Enhydrobacter* and *Thermus* negatively influenced the bioavailability of these minerals. Cu and Na content in yogurt showed a significant strong positive correlation with the abundance of *Hafnia*, while fat content exhibited the opposite trend. Notably, *Lactobacillus* in yogurt samples negatively influenced Fe, Zn, Cu, and Na content. With the exception of *Lactobacillus, Streptococcus, Staphylococcus, Hafnia*, and *Cetobacterium*, other bacteria showed a negative correlation with the mineral K.

Significant correlations were also observed among physicochemical and mineral properties themselves. Moisture and fat exhibited a moderate negative correlation with pH in yogurt (coefficient = 0.42, p = 0.01). Moisture in yogurt showed a highly significant strong negative correlation with TS and SNF content (coefficient = 0.85, p = 0.001; coefficient = 0.68, p = 0.001, respectively). Except for Ca and Mg, all minerals were positively correlated with pH. Fat content significantly and negatively influenced the concentration of Cu in yogurt (coefficient = 0.42, p = 0.01), while ash content positively influenced the concentration of Ca (coefficient = 0.38, p = 0.05). Thus, higher ash content in yogurt was associated with increased Ca content (Data available in Supplementary Data 2). A positive correlation was observed among all mineral content, except for Ca and Fe, which showed a significant moderate negative correlation (coefficient = 0.37, p = 0.05).

4. Discussion

Yogurt, a traditional dairy product, is rich in valuable nutrients and hosts a diverse range of microorganisms known for their probiotic effects. This study aimed to assess the nutritional quality, mineral content, and bacterial diversity of thirty-eight yogurt samples sourced from seven distinct commercial brands and local brands. The advent of next-generation sequencing technology has enabled the in-depth characterization of microbial communities in dairy products, such as yogurt, marking a dynamic area of research [36]. Through high-throughput sequencing, the study identified 650 Operational Taxonomic Units (OTUs), which were subsequently analyzed using the Greengenes database.

The biochemical tests found a discrepancy between high pH value (4.99–6.45) in the samples of the current study and pH value (4.0-4.4) of the earlier publication on yogurt [37]. According to the literature

Boukria et al., lactose fermentation into lactic acid by *Lactobacillus* (LAB) may cause a pH drop in sour yogurt [38]. The variations in pH across samples of two distinct taste groups and from ten brands indicate that acidification due to bacterial fermentation is probably a factor. Furthermore, insufficient incubation period and temperature, as well as regional and source variations, may all contribute to the increased range of pH found in this study across all yogurt samples.The biochemical tests found a discrepancy between high pH value (4.99–6.45) in the samples of the current study and pH value (4.0-4.4) of the earlier publication on yogurt [37]. According to the literature Boukria et al., lactose fermentation into lactic acid by *Lactobacillus* (LAB) may cause a pH drop in sour yogurt [38]. The variations in pH across samples of two distinct taste groups and from ten brands indicate that acidification due to bacterial fermentation is probably a factor. Furthermore, insufficient incubation period and temperature, as well as regional and source variations, may all contribute to the increased range of pH found in this study across all yogurt samples.

The average fat level of the samples used in this study varied from 0.2 to 5.7% (w/w), with notable variations in yogurt taste. According to Lucey et al., the fat level of yogurt ranges from 0 to 10% but is typically between 0.5 to 3.5% fat [39]. It has been reported that the quantity of fat in yogurt plays a crucial role in influencing its flavor, texture, appearance, and taste of the yogurts [40]. The findings of this research demonstrated that the quantity of fat of all thirty-eight samples met these criteria which negatively influenced the overall bacterial abundance. The average fat level of the samples used in this study varied from 0.2 to 5.7% (w/w), with notable variations in yogurt taste. According to Lucey et al., the fat level of yogurt ranges from 0 to 10% but is typically between 0.5 to 3.5% fat [39]. It has been reported that the quantity of fat in yogurt plays a crucial role in influencing its flavor, texture, appearance, and taste of the yogurts [40]. The findings of this research demonstrated that the quantity of fat of all thirty-eight samples met these criteria which negatively influenced the overall bacterial abundance.

It is recommended that yogurt maintains a moisture level below 84%, as higher moisture content can impact both texture and taste [41]. Notably, the sour yogurt samples in this study generally demonstrated higher moisture content than their sweet counterparts. Conversely, sweet yogurts exhibited higher average TS and SNF content compared to sour yogurts. The anticipated TS content ranges are 15.0–22.8% [42] and 18.4–21.41% for fruit yogurt [43]. While the sour samples aligned closely with these values, the sweet samples consistently displayed elevated TS levels, hinting at potential adulteration.It is recommended that yogurt maintains a moisture level below 84%, as higher moisture content can impact both texture and taste [41]. Notably, the sour yogurt samples in this study generally demonstrated higher moisture content than their sweet counterparts. Conversely, sweet yogurts exhibited higher average TS and SNF content compared to sour yogurts. The anticipated TS content ranges are 15.0–22.8% [42] and 18.4–21.41% for fruit yogurt [43]. While the sour samples aligned closely with these values, the sweet samples consistently displayed elevated TS levels, hinting at potential adulteration.

Interestingly, sour yogurt samples in this study showed higher concentrations of certain minerals such as Zn, Na, Ca, and Mg, while sweet yogurt samples had double the amount of Fe compared to sour ones. The concentration of Fe and Cu in the tested yogurt samples exceeded standard values set by the WHO in

1996. Furthermore, sweet yogurt exhibited twice the amount of Fe compared to sour yogurt in our study. Our results for Na, Ca, Mg, and K closely align with values reported in a study by Amellal-Chibane et al. [6] on various market yogurts. Higher Cu levels were observed in commercial yogurt samples, indicating potential migration from dairy animal feed, milk type, and handling contamination during transportation.

Human studies have indicated that calcium (Ca) can hinder iron (Fe) absorption, regardless of whether it is administered as Ca salts or in dairy products [44], emphasizing the intricate relationship between these minerals. Human studies have indicated that calcium (Ca) can hinder iron (Fe) absorption, regardless of whether it is administered as Ca salts or in dairy products [44], emphasizing the intricate relationship between these minerals. In our study, a negative correlation was observed between Ca and Fe, while a positive correlation was found between Ca and ash content. Apart from Ca and Fe, all minerals studied exhibited positive correlations.

Various food components, including sugars, fats, proteins, minerals, vitamins, flavorings, amino acids, and antioxidants, along with processing factors such as heat treatments, homogenization, and fermentation temperature, as well as microbiological factors like strain type and inoculum amount, collectively influence the stability of probiotics in yogurt [45]. The physicochemical characteristics of yogurt are greatly influenced by the microbiome, which impacts the product's quality and safety [46]. Various food components, including sugars, fats, proteins, minerals, vitamins, flavorings, amino acids, and antioxidants, along with processing factors such as heat treatments, homogenization, and fermentation temperature, as well as microbiological factors like strain type and inoculum amount, collectively influence the stability of probiotics in yogurt [45]. The physicochemical characteristics of yogurt are greatly influenced by the microbiome, which impacts the product's quality and safety [46].

In the present study, Sour yogurt samples had a more diverse and abundant microbiological signature and higher nutritional content than samples of sweet yogurt. Sweet yogurt samples displayed elevated levels of the genera *Streptococcus, Staphylococcus, Bacillus, Cetobacterium, Hafnia*, and *Pseudomonas*. In contrast, sour yogurt samples exhibited a higher abundance of *Enterobacter, Lactococcus, Aeromonas, Acinetobacter*, and *Citrobacter.* Local yogurt brands had a higher relative abundance of bacterial genera like *Lactobacillus, Streptococcus, Enterobacter*, and *Lactococcus* compared to commercial brands. The microbial ecology was predominantly influenced by the starter culture genera, potentially minimizing the growth of spoilage and pathogenic bacteria. This study also revealed that, a negative correlation exists between the pH values (4.99–6.45) and the relative abundance of *Lactobacillus* in thirty-eight yogurt samples. Literature has confirmed that the ideal pH and temperature ranges for *Lactobacillus* growth are 4.5–6.5 and 30–40°C, respectively [47]. Literature has confirmed that the ideal pH and temperature ranges for *Lactobacillus* growth are 4.5–6.5 and 30–40°C, respectively [47]. Certain spoilage-causing and pathogenic bacterial genera, including *Staphylococcus, Pseudomonas, Acinetobacter, Aeromonas, Shigella*, and *Enterobacter*, were detected in trace quantities in sour yogurt despite its acidic environment. This finding suggests the possibility of contamination occurring after production [48]. The less prevalent species of spoilage bacteria found in yogurt samples may come from utensils, raw milk, environment, and the manufacturing process. *Staphylococcus aureus*, a significant pathogen indicative of unhygienic

handling, processing, and packaging, was previously found in milk or its products (Incidence of *Staphylococcus aureus* and its enterotoxins in yoghurt). According to de Oliveira GB et al. 2015, psychotropic microorganisms, particularly *Pseudomonas* species, contribute to the deterioration of dairy products and reduce their shelf life [49]. *Pseudomonas*, along with *Acinetobacter*, is implicated in infections such as urinary tract and respiratory infections in humans [50]. While *Acinetobacter* is a part of the skin's microbial community, it can potentially lead to opportunistic infections. This finding suggests the possibility of contamination occurring after production Despite the acidic environment of sour yogurt, this study identified trace amounts of certain pathogenic bacterial genera, suggesting possible post-production contamination [48]. The less prevalent species of spoilage bacteria found in yogurt samples may come from utensils, raw milk, environment, and the manufacturing process. *Staphylococcus aureus*, a significant pathogen indicative of unhygienic handling, processing, and packaging, was previously found in milk or its products (Incidence of *Staphylococcus aureus* and its enterotoxins in yoghurt). According to de Oliveira GB et al. 2015, psychotropic microorganisms, particularly *Pseudomonas* species, contribute to the deterioration of dairy products and reduce their shelf life [49]. *Pseudomonas*, along with *Acinetobacter*, is implicated in infections such as urinary tract and respiratory infections in humans [50]. While *Acinetobacter* is a part of the skin's microbial community, it can potentially lead to opportunistic infections. Several bacterial genera, including *Aeromonas, Shigella*, and *Enterobacter*, are known to decrease the shelf life of dairy products [51]. Moreover, *Shigella*, a causative agent of foodborne bacterial infections, can be transmitted through contaminated water, food, or direct contact with an infected individual [52]. Several bacterial genera, including *Aeromonas, Shigella*, and *Enterobacter*, are known to decrease the shelf life of dairy products [51]. Moreover, *Shigella*, a causative agent of foodborne bacterial infections, can be transmitted through contaminated water, food, or direct contact with an infected individual [52].

Limitations of the present study include the lack of biochemical characterization of LAB species, the limited resolution of sequence data for identifying bacterial strains at a finer level, and the absence of exploration into the functional genomics of identified strains, among other factors. Despite these limitations, the study's findings hold significance for ensuring the safe production and preservation of yogurt quality. For future research on this traditional dairy product, emphasis should be placed on comprehensive mapping of all microbial consortia, exploring their functional implications in yogurt manufacturing, assessing the impact of yogurt consumption on gut health, and investigating adulteration using a larger sample size.

5. Conclusion

In conclusion, Bangladesh's yogurt, locally known as "dahi," stands as a delicious and integral part of the country's culinary heritage. Different brands and regions generate a wide variety of textures and flavors, with the majority of them being renowned for their origin. This study found that the abundance of microbes and mineral contents in yoghurt in Bangladesh varies depending on the location and brand. Furthermore, both sour and sweet yoghurt exhibited inconsistency in their microbial composition. To fully

understand and quantify yogurt's microbial flora spectrum, more extensive investigations with diverse samples from different places and improved analytical methods are needed.

Declarations

Conflict of Interest:

The authors declare no conflict of interest.

Data availability: The sequence data presented in this article are archived in the National Center for Biotechnology Information (NCBI) under the BioProject accession number PRJA1073473. Supplementary materials supporting the study's findings can be found in this article, including Data S1 and S2.

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Contributions:

MSR performed bioinformatics analysis, visualized figures, interpreted results, and drafted the original manuscript. SRC and TC carried out field experiments and curated the data. SMK and KTI assisted in data analysis and manuscript writing. MIUB performed 16S metagenomic sequencing. MTI and OKI conceived the study, availed the reagent support, critically reviewed the drafted manuscript, and supervised the research overall.

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Figures

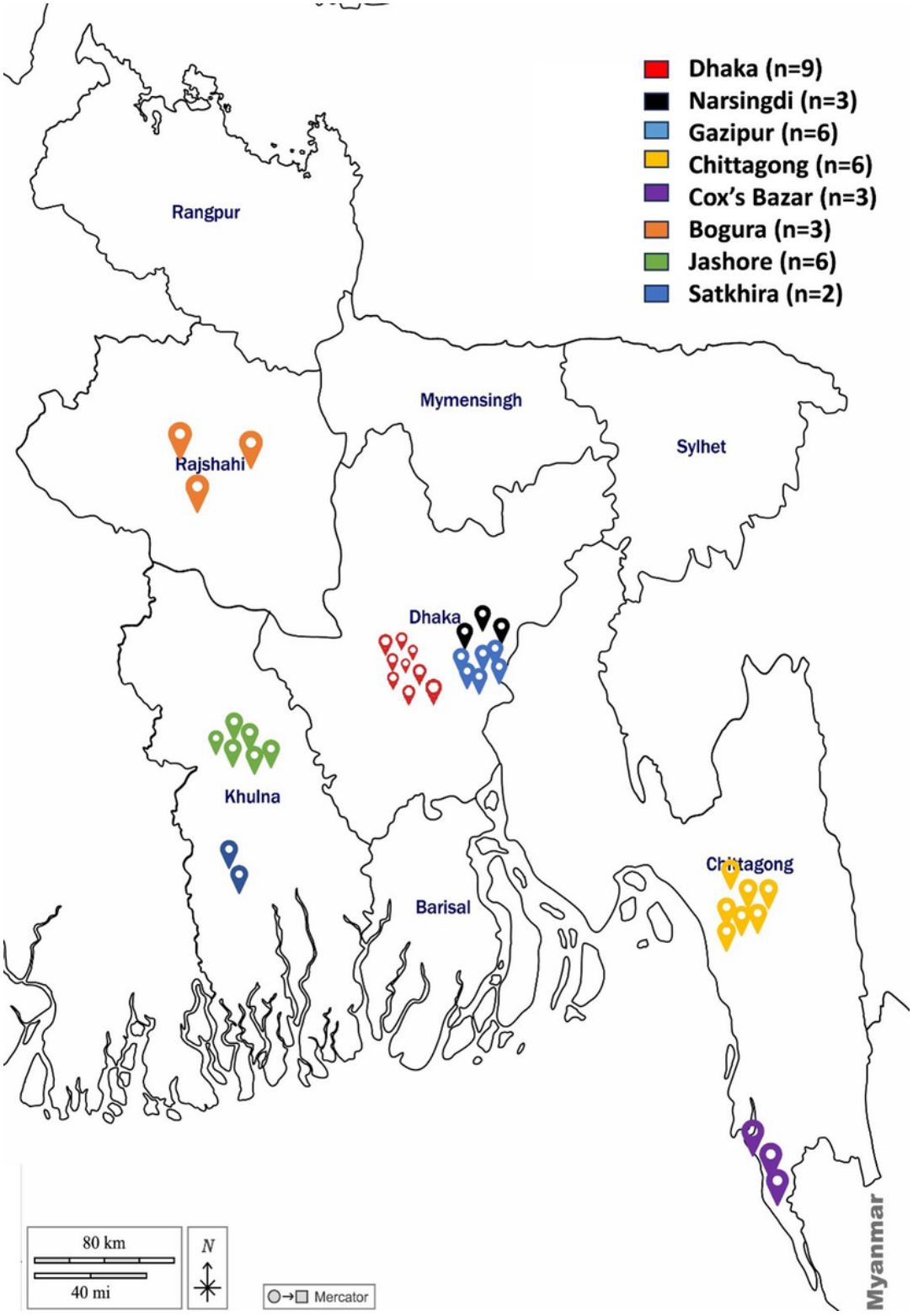

Figure 1

Map showing the different geographical locations of sampling sites in Bangladesh.

Samples collected from 8 different regions across the country summing up a total of 38 sweet and sour yoghurt (Image source: https://d-maps.com/).

Figure 2

The box plot illustrates the top twenty-five bacterial genera with different sampling criteria. Taste-A involves a comparison at the genus level between sour and sweet yogurt (A), whereas Taste-B represents a comparison at the genus level between Commercial and Local (sour and sweet) yogurt (B). Brand-A (C) and Brand-B (D) represent the comparison among Commercial and Local brands along with their regions, respectively. The diversity for each genus is plotted on boxplots and comparisons are made with pairwise

Wilcoxon test rank sum tests. Significance level (p-value) 0.001, 0.01, 0.05, and 0.1 are represented by the symbols "***", "**", "*", and "n.s", respectively.

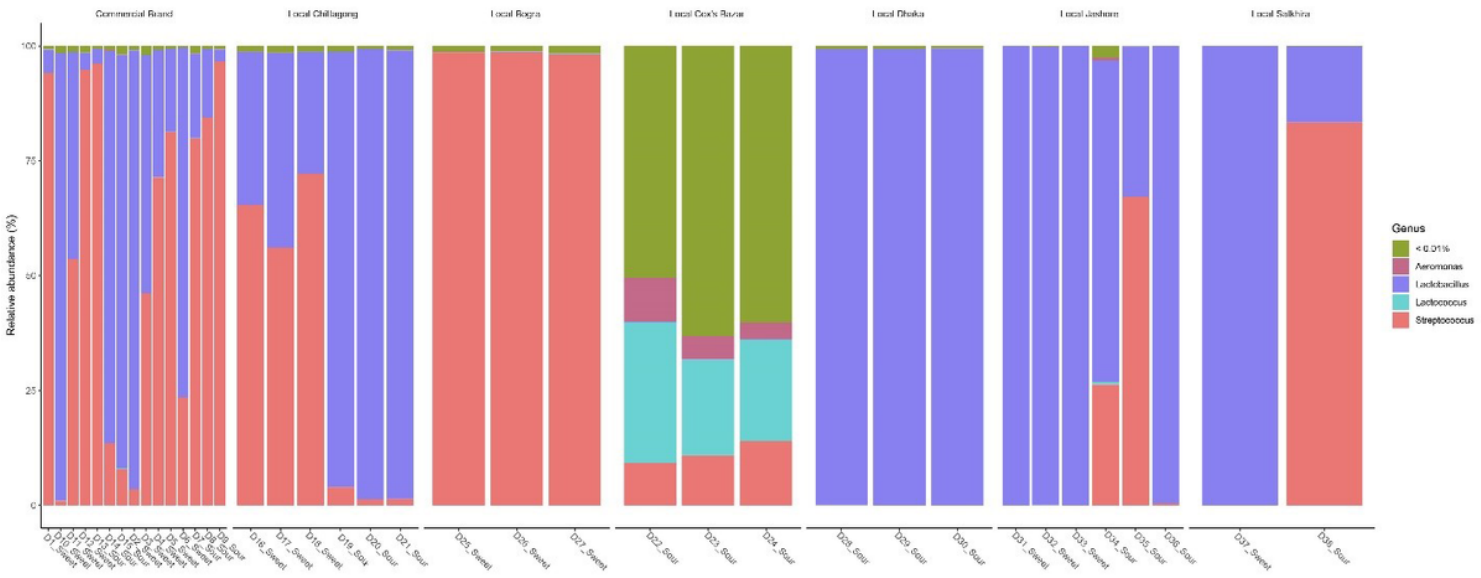

Figure 3

The taxonomic profiling of bacteria at the genus level in several samples taken from different regions of Bangladesh. The stacked bar graphs display the relative abundance and dispersion of the 5 most abundant bacterial genera, arranged in ascending order of their proportions. < 0.01% identifies the uncommon taxa with the median relative abundance in each category.

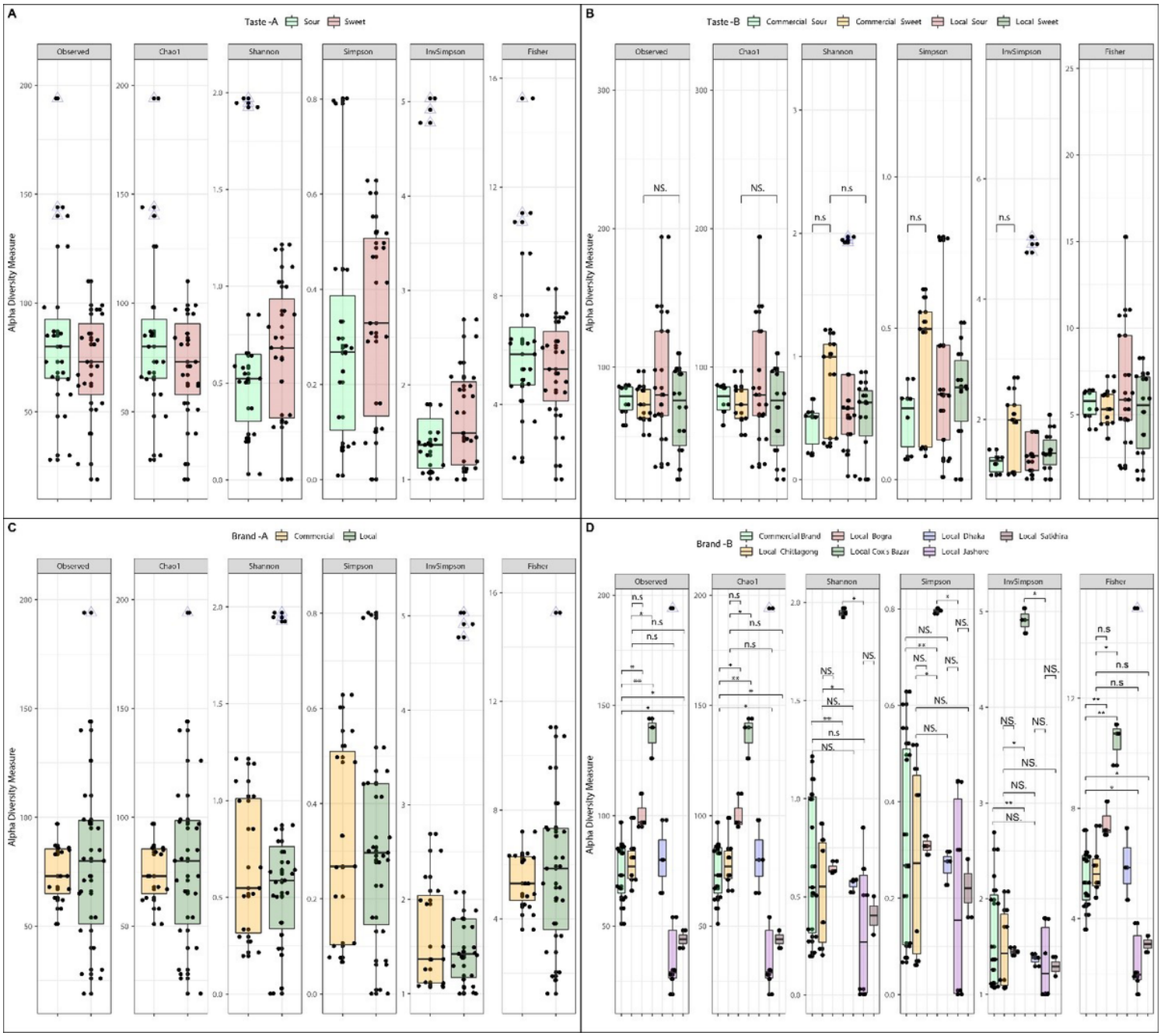

Figure 4

Comparative analysis of microbial alpha diversity measurement across several sample categories. Bacteriome diversity, (A) within sour and sweet yogurt samples defined as Taste-A; (B) commercial sweet, commercial sour, local sweet, and local sour yogurt samples defined as Taste-B; (C) commercial and local brands of yogurt samples defined as Brand-A; (D) commercial and area specific local brands of yogurt samples defined as Brand-B; measured by the indices: Observed, Chao1, Shannon, Simpson, InvSimpson and Fisher indices. X-axis represents the yogurt groups and the alpha diversity measure is shown on Y-axis. Pairwise Wilcoxon sum rank test and boxplot were applied to plot and compare diversity. Significance level (p-value) 0.001, 0.01, 0.05, and 0.1 are represented by the symbols "***", "**", "*" and "n.s" respectively.

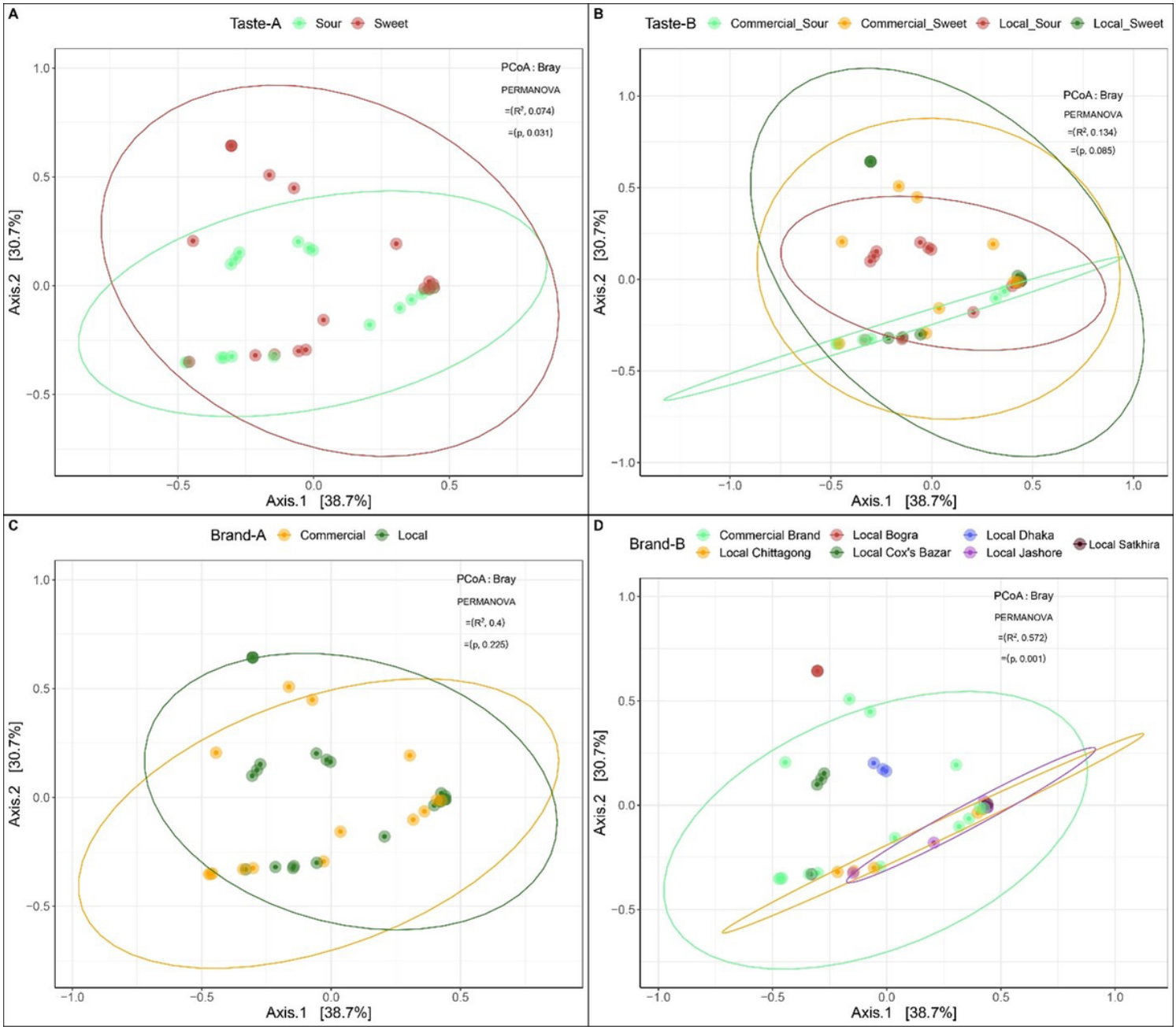

Figure 5

The various groups are segregated based on the beta diversity measure. The various colors correspond to the assigned samples from distinct sources.Principal coordinate analysis (PCoA) was conducted by applying Bray distance metrics between samples. Beta diversity in bacterial component of the yogurt microbiomes segregated yogurt samples according to (A) two different tastes (Taste-A); (B) commercial and local (sour and sweet) brands (Taste-B); (C) commercial and local brands (Brand-A); (D) commercial brands and seven different local brands (Brand-B). PERMANOVA was executed using 999 permutations to establish the significance (p-value) of differences between the groups.

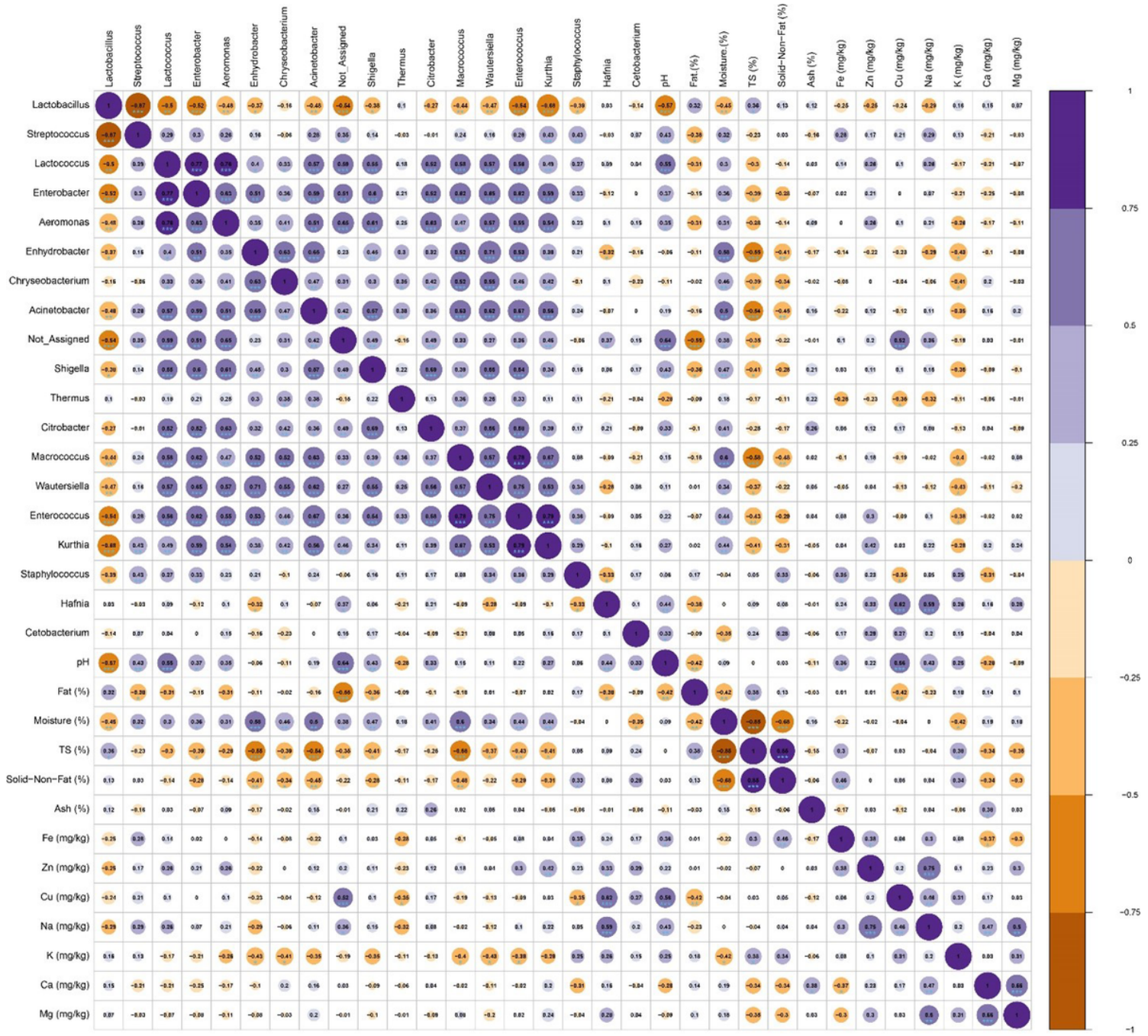

Figure 6

Pairwise Spearman's correlation between bacterial genera and physicochemical parameters. The circular points within the plot represent the correlation of 19 bacterial genera with the physicochemical parameters (pH, Fat, Moisture, TS, SNF, Ash, Fe, Zn, Cu, Na, K, Ca and Mg) tested in yogurt samples. The color of the points indicates whether the correlation is positive (violet) or negative (orange). The size of the points is proportional to the strength of the correlation. The numbers within the points represent the Spearman's correlation coefficient (rho). Significance level (p-value) 0.001, 0.01, 0.05, and 0.1 are represented by the symbols "***", "**", "*", and "n.s", respectively.

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

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