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Isolation and preliminary analysis of *Bifidobacterium* strains from fecal samples of Mongolian individuals

Tuul Nyambal[1], Alexander Hübner[2], Khulan Lkhamsuren[3], Ser-Od Khuygaa[3], Raphaela Stahl[2], Anuujin Gantumur[4], Battogtokh Chimeddorj[4], Davaalkham Dambadarjaa[3], Khosbayar Tulgaa[5], Christina Warinner[2,6], Soninkhishig Tsolmon[7]

[1]Department of Nutrition, School of Public Health, Mongolian National University of Medical Sciences, Ulaanbaatar, Mongolia;
[2]Max Planck Institute for Evolutionary Anthropology, Federal Republic of Germany;
[3]School of Public Health, Mongolian National University of Medical Sciences, Ulaanbaatar, Mongolia;
[4]School of Biomedicine, Mongolian National University of Medical Sciences, Ulaanbaatar, Mongolia;
[5]Clinical Molecular Diagnostics Center, Mongolian National University of Medical Sciences, Ulaanbaatar, Mongolia;
[6]Department of Anthropology, Harvard University, Harvard, United States of America;
[7]School of Management, Mongolian University of Science and Technology, Ulaanbaatar, Mongolia.

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Corresponding Author:
Tuul Nyambal (MD., MBS.)
Department of Nutrition, School of Public Health, Mongolian National University of Medical Science, Ulaanbaatar, Mongolia
Email: tuul.n@mnums.edu.mn
ORCID: https://orcid.org/0000-0002-2747-9950

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Objective: This study aimed to characterize *Bifidobacterium* species in the gut microbiome of ethnic Mongolian individuals, determine their distribution based on geographic location, age, and sex, and isolate representative strains under laboratory conditions. **Methods:** A total of 100 stool samples were collected from 50 urban and 50 rural participants over the age of 18, with a balanced gender ratio. Rural participants were herders and maintained a traditional nomadic lifestyle. Data analysis was conducted using the R software and MetaPhlAn tool to profile microbial communities at the species level. **Results:** A total of 13 *Bifidobacterium* species were identified across the 100 samples. Species such as *B. adolescentis*, *B. angulatum*, *B. longum, B. bifidum, B. catenulatum, B. dentium*, and *B. breve* were predominantly found in rural participants, whereas *B. pseudocatenulatum*, *B. ruminantium*, *B. animalis*, *B. scardovii,* and *B. kashiwanohense* were more prevalent in urban participants. The overall species richness of *Bifidobacterium* was 2.9 times higher in rural participants than in urban ones. Culturable isolates frequently showed 2–3 morphologically distinct colony types per sample. **Conclusions:** *Bifidobacterium* species richness increased with age in rural participants, whereas it decreased with age in urban participants. Female participants had greater species diversity than males.
Keywords: Microbiome, *Bifidobacterium*, Urban, Rural, Species abundance

Introduction

Despite the widespread recognition of *Bifidobacteria* as a key component of the human gut microbiota, current understanding is largely based on Western populations, with limited data on underrepresented groups such as ethnic Mongolians.[1,2] Traditional Mongolian diets, rich in fermented animal-based dairy products and low in processed carbohydrates, differ substantially from Western dietary patterns and may influence the composition and diversity of gut *bifidobacteria*.[3] Moreover, environmental exposures, a nomadic lifestyle, and close contact

with livestock are unique factors that may shape the microbiota in this population.[4] Therefore, investigating the bifidobacterial composition in Mongolian individuals not only provides insights into the gut microbiota of an indigenous, minimally industrialized population but also contributes valuable data to understanding global microbiome variation and its relationship to diet, environment, and health.[5]

Bifidobacteria were first isolated in 1899 by Tissier from the feces of breastfed infants. It was later established that this genus accounts for up to 99% of the intestinal microbiota in newborns and represents a dominant genus in the adult human colon.[2,3] *Bifidobacterium* species have been isolated from various sources, including human blood, oral cavity, sewage, fermented dairy products, raw milk, and the intestines of birds, chimpanzees, gorillas, cattle, bats, pigs, certain rodents, and insects. To date, 106 distinct Bifidobacterium species have been identified, with over 50 species isolated from the human gut.[6]

Bifidobacteria account for approximately 3–17% of the human gut microbiome, making them among the most prevalent genera.[7,8] *B. adolescentis, B. bifidum, B. longum*, and *B. pseudolongum* are among the most common and widely distributed species. Phylogenetic analysis of a 169-amino-acid core-genome alignment classifies bifidobacteria into 10 distinct clusters based on their ecological origin. These clusters include *B. adolescentis, B. bifidum, B. asteroides, B. bombi, B. boum, B. longum, B. pseudolongum, B. pullorum, B. psychraerophilum*, and *B. tissieri*, with *B. asteroides* considered the most ancient lineage.[6]

The abundance and diversity of *Bifidobacterium* species vary significantly by age group, with distinct profiles observed in infants (0–1 year), adults (18–64 years), and the elderly (≥65 years), reflecting their importance as biomarkers of human health. These differences typically allow bifidobacteria to be categorized into clusters characteristic of either infants or adults.[9,10] Among the many species of *Bifidobacterium*, several are particularly recognized for their beneficial effects on human health: *Bifidobacterium longum* – Enhances immune function, supports digestive health, protects against gastric and intestinal inflammation, and helps reduce dermatological conditions. *Bifidobacterium bifidum* – Balances the intestinal microbiota, aids digestion, strengthens immune responses, and protects against infections. *Bifidobacterium infantis* – Promotes infant gut health, alleviates colic symptoms, supports immune system development, and reduces inflammation. *Bifidobacterium breve* – Contributes to digestive well-being, improves gut health, helps regulate body weight, and reduces blood lipid levels. *Bifidobacterium lactis* – Supports gastrointestinal health, boosts immunity, assists in lactose digestion, and helps mitigate lactose intolerance.[11]

Table 1. Common Bifidobacterium species found in the healthy human gut[9,12]

Infants	Adults	Elderly
B.breve		*B.catenulatum*
	B.longum	
B.longum ssp. Infants		*B.adolescentis*
B.bifidum	*B.angulatum*	*B.bifidum*
B.animalis	*B.scardovii*	*B.pseudocatenulatum*
B.lactis	*B.moukalabense*	
B.dentium	*B.ruminantium*	
B.kashiwanohense		

In conditions such as obesity and liver diseases, the composition and abundance of *Bifidobacteria* in the gut are significantly reduced. These alterations may reflect the important role of Bifidobacteria in maintaining gut health and systemic metabolic balance. However, data on *Bifidobacterium* diversity in non-Western populations, particularly in traditional lifestyles, remain limited. We aimed to determine the species diversity of *bifidobacteria* from ethnic Mongolians.

Table 2. Alterations in Bifidobacterium Composition in the Gut During Disease Conditions[9,13]

	Infants	Adults
Allergy	*B.longum* ↓	
	B.catenulatum ↑	
	B.pseudocatenulatum ↑	
	B.adolescentis ↑	
Celiac	*B.longum* ↓	
IBS		*B.adolescentis* ↑
		B.longum ↓
		B.catenulatum ↓
Hepatitis B virus		*B.dentium* ↑
		B.longum ↓
		B.catenulatum ↓
Obesity		*B.animalis* ↓
		B.adolescentis ↓
Long-time asthma		*B.adolescentis* ↑

↑ *indicates an increase, and* ↓ *indicates a decrease in the relative abundance of the Bifidobacterium species under the respective disease condition.*

Material and Methods

Sample Collection

We selected 50 urban and 50 rural participants aged 18 or older, in a gender-balanced ratio. Rural people were herders and had a nomadic culture. Stool samples were collected from 100 participants (Figure 1).

Figure 1. Locations included in the research.

Ethical Statement

This study, conducted with the highest ethical standards, was approved by the Medical Ethics Review Committee of the Ministry of Health, Mongolia (Project Number: NO.2020/155) and the Research Ethics Review Committee of the Mongolian National

University of Medical Sciences (Project Number: NO.2022/3-03). Informed consent, a cornerstone of our ethical approach, was obtained from all participants before the study commenced. Only partial medical examination data were referenced, and the study did not involve human experiments. The collection of fecal samples posed no foreseeable risks of harm or discomfort to the participants.

Stool Sample Collection

Participants were instructed to collect their stool samples at home using a sterile collection kit provided by the research team. The kit included a screw-cap sterile container labeled with a unique code, a disposable collection sheet designed to be affixed across the toilet opening, a pair of disposable gloves, 2–3 gel ice packs, and an insulated container for cold storage.

Participants were guided to:

- Secure the collection sheet over the toilet to avoid contamination.
- Defecate onto the sheet;
- Use the attached scoop on the container lid to transfer approximately 10–15 g (walnut-sized) of stool into the sterile container;
- Seal the sample and place it into the insulated box with ice packs until delivery.

They were also instructed to avoid contact with urine, toilet water, or any contaminated surfaces (e.g., the ground or toilet walls), and to wash their hands thoroughly with warm water and soap after collection. The sample was stored on ice and delivered to the researchers within two hours of collection. All necessary procedures and precautions were explained to participants in advance to ensure the integrity of the sample.[14,15]

Molecular Analysis of the Fecal Microbiome

From each fecal sample, nucleic acids were extracted, and the DNA yield was quantified. DNA libraries were prepared for downstream sequencing applications. Whole-genome sequencing was performed using next-generation sequencing (NGS) technologies. Bioinformatic analysis of sequencing data was conducted at the Department of Archaeogenetics, Max Planck Institute for Evolutionary Anthropology (Leipzig, Germany). Additional data processing and interpretation were carried out at the Mongolian National University of Medical Sciences (MNUMS).

DNA Extraction and Next-Generation Sequencing

Total genomic DNA was extracted from fecal samples using the QIAamp® DNA Microbiome Kit (Germany), following the manufacturer's protocol. DNA concentration and purity were assessed using a NanoDrop™ 1000 spectrophotometer (Thermo Scientific, USA). Microbiome sequencing was performed using the Illumina Paired-End 75 bp sequencing kit according to the manufacturer's protocol. Raw sequencing data were processed using a standardized bioinformatics pipeline for quality control, taxonomic assignment, and downstream microbiome analysis.[16–18]

Bifidobacteria Isolation and Cultivation Methods

Sample Preparation and Serial Dilution: Each sample was initially diluted using sterile physiological saline (0.9% NaCl). A tenfold serial dilution was performed by transferring 1 gram of the original sample into a sterile test tube containing 9 mL of saline, yielding a 1:10 dilution. From this, 1 mL was aseptically transferred to a second tube containing 9 mL of saline to achieve a $1:10^2$ dilution. This procedure was repeated successively up to the $1:10^{10}$ dilution.

Culturing and Incubation: From the higher dilutions, aliquots were inoculated onto Reinforced Clostridial Medium (RCM) agar plates supplemented with mupirocin to inhibit non-target microbial growth. The plates were incubated under anaerobic conditions at 37°C for 24–48 hours using an anaerobic jar system.

Morphological Identification: *Bifidobacterial* colonies were identified by their distinct morphological features. The cells are Gram-positive, non-motile, non-spore-forming, and do not produce capsules. Microscopically, they display pleomorphic, rod-shaped structures that may appear straight, curved, bifurcated, branched, or Y- or V-shaped.

Growth Characteristics: *Bifidobacteria* are obligate anaerobes at the time of initial isolation but can become oxygen-sensitive facultative anaerobes in laboratory conditions, especially when cultured under CO_2-enriched atmospheres. They grow optimally in nutrient-rich environments at temperatures between 37°C and 41°C and at a pH range of 6.0–7.0. Based on these criteria, *Bifidobacterium* strains were successfully cultivated and isolated.[19–21]

Statistical Analysis

Data were coded, entered, and analyzed using SPSS version 25.0 (SPSS Inc., Chicago, IL). For variables with high variability, geometric means and geometric standard deviations were calculated. Normality of continuous variables was assessed using the Kolmogorov–Smirnov test, with $p > 0.05$ indicating normality. For comparisons between two independent groups,

the Independent Samples t-test was applied for normally distributed data, and the Mann–Whitney U test for non-normally distributed data.

Alpha diversity of *Bifidobacterium* species was evaluated using Shannon and Simpson diversity indices to assess species richness and evenness within individual samples. Comparisons of diversity indices between groups (urban/rural, male/female, and age categories) were performed using independent t-tests and Mann–Whitney U tests. Differences were considered statistically significant at $p < 0.05$. Molecular biology data analysis was performed using the R software and the MetaPhlAn tool to profile microbial communities at the species level. [22–24]

Results

Research Participants' Data and Grouping Information

The participants ranged in age from 18 to 84 years, with a mean age of 42.4 ± 14.3 years. The rural group had a slightly higher average age compared to the urban group (43.8 vs. 41.0 years) (Table 3).

Table 3. Comparative analysis of age and BMI of the study participants

Parameters	Category	Urban (n=50)	Rural (n=50)	Total (n=100)
Age	Average (Mean±SD)	41.0±14.4	43.8±14.1	42.4±14.3
Number (n)	Min	18.0	19.0	18.0
	Max	73.0	84.0	84.0

SD-Standard deviation.

Results of the Relative abundance of *Bifidobacteria* within the total gut microbiome

Urban residents exhibited approximately 1.1 times lower total bacterial species richness compared to their rural counterparts. Among rural males, total species richness increased with age, reaching a maximum of 219 species in individuals aged 45–65, whereas the maximum observed in urban males of similar age was 202 species. In terms of bifidobacterial diversity, rural individuals showed an age-related increase in species richness, whereas urban dwellers showed the opposite trend (age-related decline; Figure 2).

Rural (Male)

Age	19	20	22	25	26	28	28	30	31	34	35	36	36	37	38	42	44	45	48	49	50	51	54	57	65	65	66	Avg
Number of total species	154	167	129	132	181	139	193	160	162	176	153	163	175	153	146	165	190	124	219	144	189	142	185	192	204	170	147	**165**
Number of *Bifidobacterium* species	5	6	7	6	9	3	3	3	7	5	9	7	9	8	9	8	6	1	6	4	1	9	6	6	8	11	0	**6**

Rural (Female)

Age	31	32	34	35	36	36	39	41	43	43	45	45	46	48	50	53	55	56	59	65	66	67	84	Avg
Number of total species	178	206	141	129	174	175	216	157	132	157	182	170	82	150	127	160	165	148	153	127	143	156	133	**155**
Number of *Bifidobacterium* species	6	9	8	5	4	6	10	6	11	6	7	12	4	10	1	11	10	12	7	7	9	7	7	**8**

Urban (Male)

Age	18	18	20	22	25	28	29	30	32	32	34	35	37	41	41	44	46	52	54	56	57	58	59	60	Avg
Number of total species	165	102	149	179	150	141	159	178	113	162	111	111	155	148	168	181	138	88	145	164	171	140	202	145	**149**
Number of *Bifidobacterium* species	7	4	7	7	7	8	7	7	1	9	5	0	7	6	6	6	6	1	10	5	5	8	7	7	**6**

Urban (Female)

Age	23	25	25	29	29	30	30	31	31	35	35	35	37	42	47	48	49	49	51	52	60	62	64	65	65	73	Avg
Number of total species	139	147	154	146	98	132	166	139	107	135	167	135	158	141	133	147	110	136	141	116	148	155	109	145	131	143	**138**
Number of *Bifidobacterium* species	4	4	5	8	5	8	4	7	6	8	5	4	6	10	7	4	0	4	6	4	9	5	1	7	3	8	**5**

Figure 2. Distribution of Bifidobacterium Species Among Individuals.

Among females, *bifidobacterial* species diversity in rural areas remained consistently high (≥10 species) between ages 39 and 56. In contrast, urban females aged 25 to 35 showed only 5 to 8 *bifidobacterial* species. Overall, both rural males and females exhibited greater *bifidobacterial* diversity and positive age-related trends, suggesting a potentially richer and more stable gut

microbiome compared to their urban counterparts. A statistically significant difference ($p < 0.05$) was observed among the four groups based on residence (rural/urban) and gender (male/female). This suggests that both place of residence and gender, when considered together, influence bifidobacterial diversity. A statistically significant difference was observed between the Rural Female and Urban Female groups ($p = 0.006$).

To further assess the variations in Bifidobacterium diversity across demographic groups, Shannon and Simpson diversity indices were calculated (Table 4).

Table 4. Comparison of diversity indices (Shannon and Simpson) among groups

Comparison	Index	Group 1 (Mean±SD)	Group 2 (Mean±SD)	*p*-value (t)	*p*-value (MW)
Rural vs Urban	Shannon	0.703 ± 0.418	0.701 ± 0.391	0.983	0.978
	Simpson	0.384 ± 0.227	0.396 ± 0.216	0.780	0.890
Male vs Female	Shannon	0.633 ± 0.432	0.773 ± 0.360	0.080	0.109
	Simpson	0.348 ± 0.238	0.433 ± 0.194	0.054	0.097
Mid-elderly vs Young	Shannon	0.682 ± 0.400	0.726 ± 0.413	0.600	0.779
	Simpson	0.381 ± 0.223	0.401 ± 0.222	0.653	0.747

Data are expressed as mean ± standard deviation (SD). p-values were calculated using the Independent Samples t-test and the Mann–Whitney U test. $p < 0.05$ was considered statistically significant.

As shown in Table 4, no statistically significant differences were found in Shannon or Simpson diversity indices between rural and urban participants, between males and females, or between age groups ($p > 0.05$). However, females tended to exhibit slightly higher diversity indices than males, suggesting potential gender-related differences in gut microbial composition. Despite the lack of significant differences at the community level, species-level comparisons revealed higher abundance of *B. angulatum* ($p = 0.0006$ (t-test), $p < 0.0001$ (M-W), *B. catenulatum* ($p = 0.0275$, $p = 0.0008$), and *B. pseudocatenulatum* ($p = 0.0100$, $p < 0.0001$) in rural participants, while *B. pseudocatenulatum* ($p = 0.0176$ (t-test), $p = 0.0145$ (M-W) was notably more dominant among females (Table 5).

Table 5. Comparison of *Bifidobacterium* species abundance among groups

Species	Rural vs Urban (p)	Male vs Female (p)	Young vs Mid-elderly (p)
B. adolescentis	0.897	0.396	-
B. angulatum	< 0.001	0.385	-
B. longum	0.221	0.041	-
B. bifidum	0.853	0.378	-
B. catenulatum	0.001	0.628	-
B. pseudocatenulatum	< 0.001	0.015	-
B. dentium	0.076	0.769	-
B. animalis	0.327	-	-
B. breve	0.695	-	-
B. moukalabense	0.159	-	-
B. kashiwanohense	0.327	-	-
B. scardovii	0.327	-	-
B. ruminantium	0.584	-	-

$p < 0.05$ indicates statistically significant difference based on t-test and Mann–Whitney U test results.

A total of 13 *Bifidobacterium* species were identified in the study. Among them, *B. adolescentis*, *B. angulatum*, *B. bifidum*, *B. catenulatum*, and *B. longum* were predominantly detected in individuals from rural areas, whereas *B. adolescentis*, *B. longum*, *B. pseudocatenulatum*, and *B. bifidum* were more common among urban residents. Notably, *B. angulatum* and *B. catenulatum* appear to reflect characteristics specific to the rural Mongolian population, while the abundance of *B. longum* may

be associated with the frequent consumption of traditional dairy products derived from yaks and native cattle in mountainous regions.

*B. adolescenti*s was identified as the predominant species, present in 87% of all samples. Regarding species diversity, an age-related augmentation in the number of *Bifidobacterium* species was observed among rural participants, whereas a declining trend was noted among urban residents.

Bifidobacterial Isolation and Cultivation

For isolation, fecal samples were diluted in sterile 0.9% NaCl solution to a final concentration of approximately 10^4 CFU/mL. One hundred microliters of the diluted sample were plated onto Bifidobacterium agar medium supplemented with 50 mg/L mupirocin. The medium was solidified in sterile Petri dishes and incubated under anaerobic conditions (AnaeroPack) at 37°C for 24–48 hours.

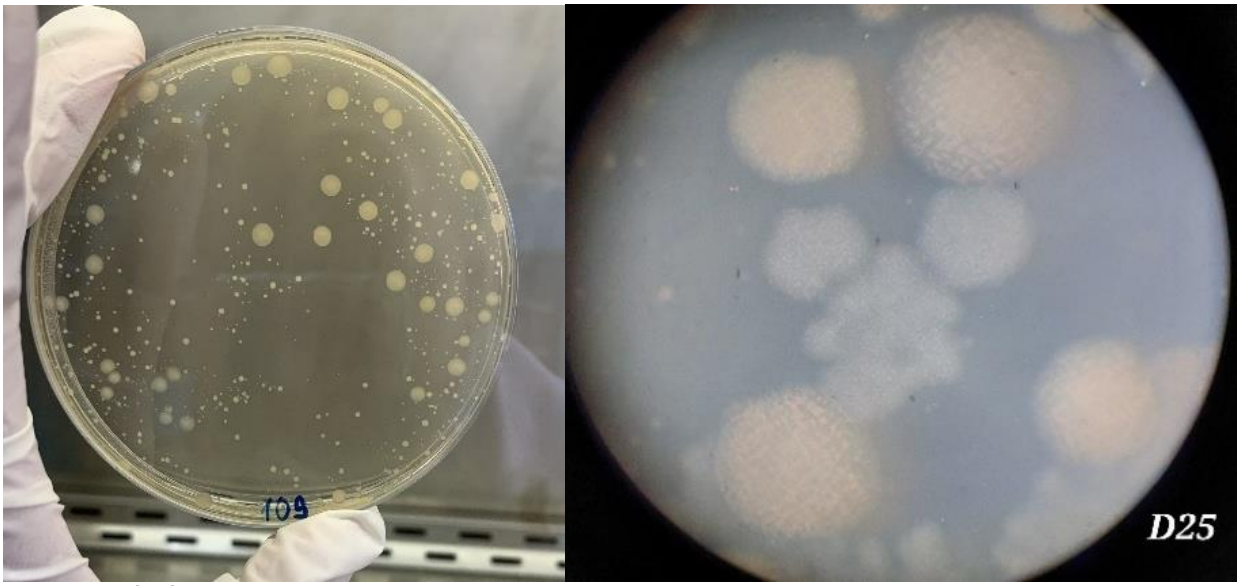

Figure 3.Colonies of *Bifidobacteria* grown on solid media.

A total of 116 *Bifidobacterium* isolates were obtained. Gram staining was performed, and Gram-positive isolates that were morphologically consistent with *Bifidobacteria* were selected for further analysis. These isolates were purified and preserved in 20% glycerol solution (Figure 4).

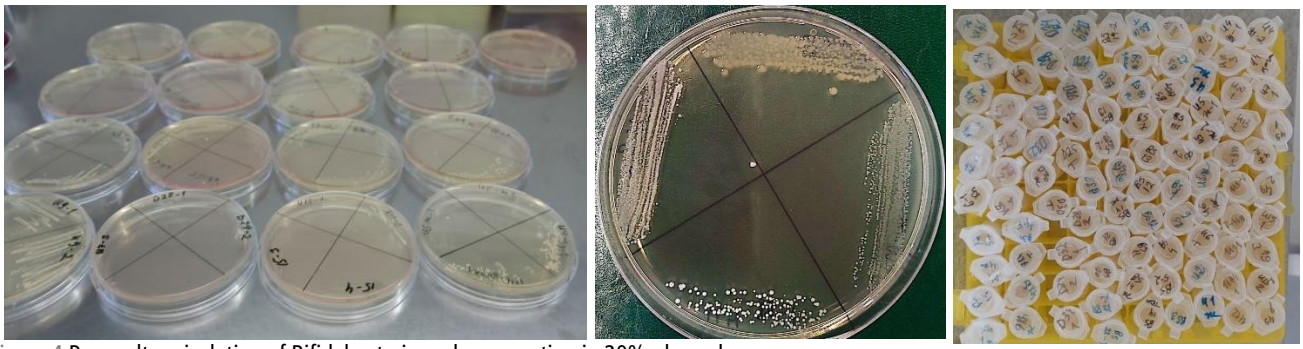

Figure 4.Pure culture isolation of Bifidobacteria and preservation in 20% glycerol.

The *Bifidobacterium* cell count in fecal samples ranged from 800,000 to 1.2 billion CFU/mL, with the highest counts observed in individuals from rural. In contrast, individuals from Ulaanbaatar showed lower counts. These quantitative results were consistent with the findings from microbiome sequencing (Figure 6). This boxplot illustrates the distribution of Bifidobacterium abundance across urban and rural participants. The central line in each box represents the median value, while the edges of the box indicate the interquartile range (IQR). Whiskers extend to 1.5 times the IQR, and individual points beyond this range are shown as outliers (Figure 5). Urban samples show a slightly higher median and more tightly clustered abundance values, whereas rural samples exhibit a wider spread with several extreme values, suggesting greater variability in *Bifidobacterium* abundance among rural individuals.

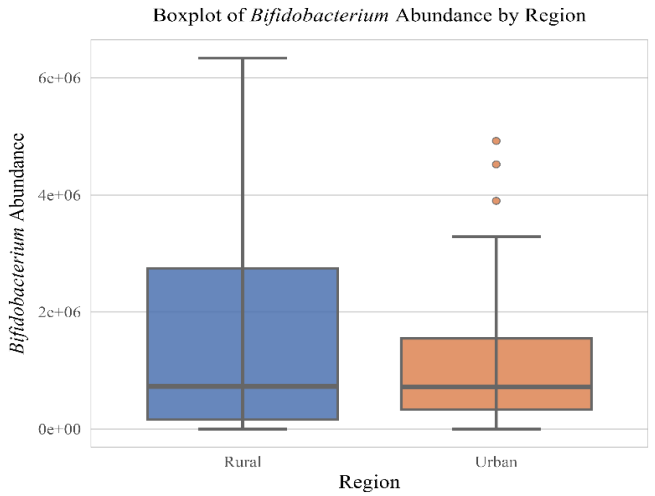

Figure 5. Boxplot of Bifidobacterium Abundance by Region.

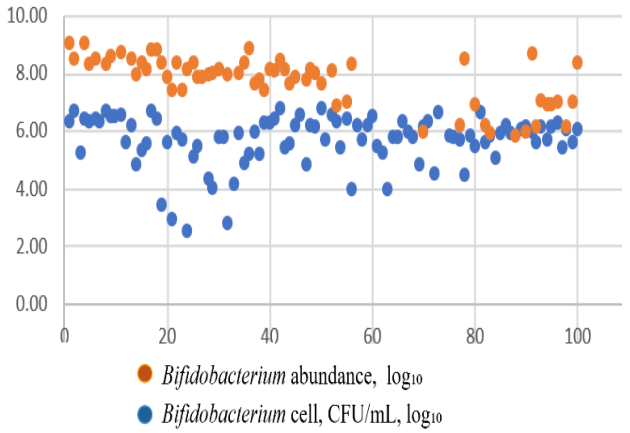

Figure 6. Agreement Between Sequencing and Culture Methods in Estimating *Bifidobacterium* Abundance. The *Bifidobacterium* abundance and *Bifidobacterium* CFU/mL values were transformed using the $\log_{10}(X)$ formula, and a scatter plot was generated to assess the correlation between the two variables.

A comparative analysis between metagenomic sequencing results and culture-based enumeration revealed a general consistency in individuals with high *Bifidobacterium* abundance. Specifically, fecal samples that exhibited high relative abundance of *Bifidobacterium* through sequencing also demonstrated elevated total colony counts when cultured on selective media. This concordance suggests that, for certain participants, the abundance of *Bifidobacterium* inferred from sequencing data may reflect viable and cultivable populations, supporting the reliability of both methods under appropriate conditions.

Cells were observed as single units, in pairs, or chains, with dimensions ranging from 0.5–1.3 μm in width to 1.5–8 μm in length (Figure 7).

Out of the 116 colonies characterized, most were pale white, circular, and soft in texture. All isolates were Gram-positive, catalase- and oxidase-negative, and exhibited anaerobic or facultative anaerobic growth (Figure 8).

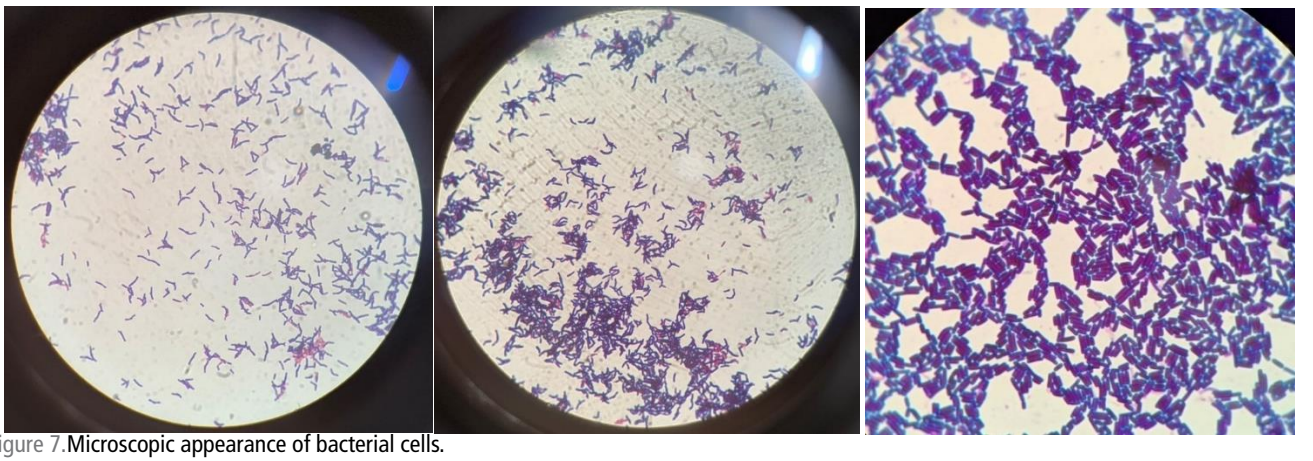

Figure 7.Microscopic appearance of bacterial cells.

The figure illustrates the aerotolerance properties of isolated *Bifidobacterium* strains. The isolates were classified as either strictly anaerobic or facultative anaerobic based on their ability to grow in the presence or absence of oxygen. A total of 116 *bifidobacteria* cultures were isolated and stored in 20% glycerol, and some physiological and biochemical activities of 8 cultures with different cell types were observed. 1 culture was facultative anaerobes and the others were anaerobes. 4 cultures were tolerated at pH 4. Two cultures produced gas and decomposed on lactose medium, while others precipitated. All cultures were active on maltose (Figure 9).

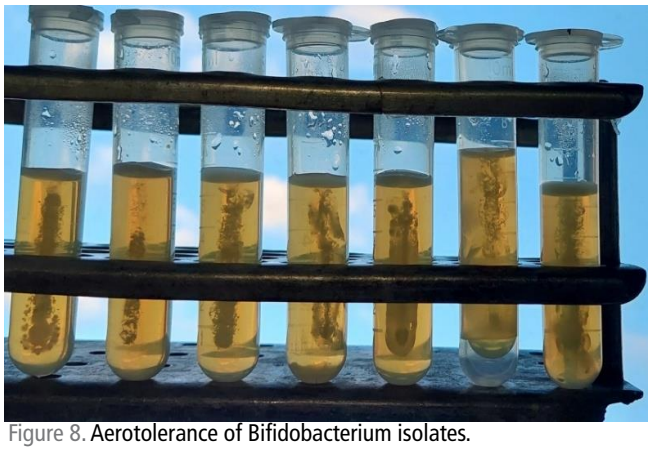

Figure 8. Aerotolerance of Bifidobacterium isolates.

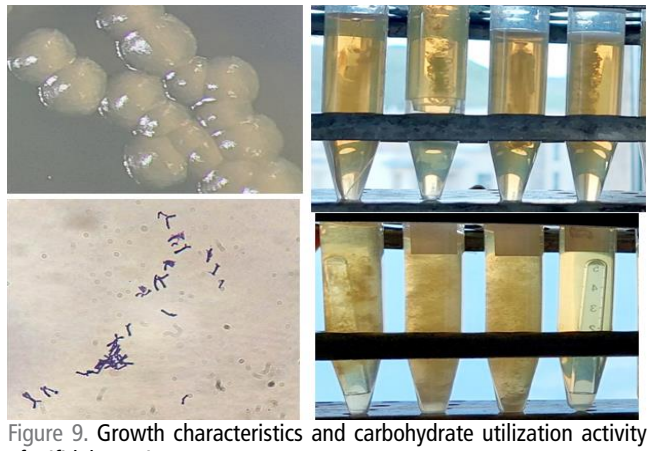

Figure 9. Growth characteristics and carbohydrate utilization activity of *Bifidobacteria*.

A total of seven bifidobacterial (B. longum-2 B. adolescentis-2, B. pseudocatenulatum-2, B. dentium) genomes were reconstructed from fecal samples collected across multiple regions. The majority of assemblies achieved high-quality (HQ) status based on completeness (>90%) and low contamination (<5%). Genome sizes ranged from 1.17 Mb to 2.43 Mb, with GC content values typical of Actinobacteria (56–60%). B. longum and B. adolescentis were the most commonly identified species, consistent with previous findings on their dominance in the human gut microbiota. One medium-quality (MQ) genome (B. pseudocatenulatum, BS3AL) showed lower completeness and a fragmented assembly, suggesting that additional sequencing depth may be required (Table 6).

Discussion

In our study, 13 bacterial phyla were identified, including *Firmicutes*, *Actinobacteria*, *Verrucomicrobia*, *Bacteroidetes*, *Proteobacteria*, and *Euryarchaeota*, of which *Firmicutes* and *Actinobacteria* together accounted for 95% of the total microbiota. Among the *Bifidobacterium* species analyzed, significant differences were observed in *B. angulatum*, *B. catenulatum*, and *B. pseudocatenulatum* between rural and urban participants, with higher abundance in rural adults. Moreover, *B. pseudocatenulatum* was more prevalent among female participants than among male participants. No statistically significant differences were observed between age groups.

The phylum *Actinobacteria* primarily consists of Gram-positive bacteria with high guanine and cytosine content in their DNA. These organisms are widespread in soil and aquatic environments and exhibit more complex, asymmetrical cellular morphology than those of other phyla. *Actinobacteria* are well known for producing secondary metabolites through their metabolic processes, including two-thirds of all naturally derived antibiotics, as well as many antitumor, antiparasitic, and antifungal compounds. Therefore, members of this phylum are of great importance in biotechnology, medicine, and agriculture.[25,26]

In terms of abundance, *B. angulatum* was 35.3 times more prevalent among rural residents than among urban residents, while *B. Catenulatum* was 44.8 times more abundant, reflecting the strong influence of environment and diet. The high prevalence of *B. angulatum* and *B. catenulatum* may represent the distinctive gut microbiota of rural Mongolian populations, whereas *B. longum* is likely associated with the traditional consumption of dairy products from cattle and yaks in mountainous regions.[27,28]

Probiotic strains detected in our study included *B. adolescentis*, *B. longum*, *B. bifidum*, B. *pseudocatenulatum*, *B. breve*, and *B. animalis*.[11]

Molecular research indicates that the average percentage of bifidobacteria in the human gastrointestinal (GI) tract is approximately 3% of the total microbiota, corresponding to a concentration of 10^9–10^10 CFU/g of feces.[9,27]

A study by Arboleya, et al. identified significant variations in the population of bifidobacteria within the human gut, influenced by lifestyle and maternal diet, with levels ranging from 2% to 14% in adulthood. In the 2017 study conducted by Francesca Turroni, et al. it was demonstrated that the quantity and diversity of bifidobacteria are affected by factors such as diet, age, mode of birth, and environmental conditions, with alterations in these factors impacting overall health. Turroni , et al. established that bifidobacteria constitute approximately 4.3 ± 4.4% of the total gut microbiome. A 2024 study conducted in

Hohhot revealed that the abundance of bifidobacteria in the gut is modulated by age, BMI, obesity, and environmental factors. It was observed that bifidobacteria comprised 60-70% of the gut microbiota during childhood, but this proportion declined to less than 5% among the elderly. The most influential factors affecting bifidobacteria were found to be living conditions and occupation. Although bifidobacteria typically account for 3% to 14% of the adult gut microbiome, our research demonstrated that, in Mongolian adults, their prevalence was notably higher, reaching up to 14%.

In 2012, Bat-Tsetseg S, et al. and colleagues isolated six different *Bifidobacterium* strains from the feces of over 100 urban residents and 20 individuals receiving mare's milk therapy near the city. They characterized these strains physiologically and biochemically, identifying their carbohydrate utilization profiles and antibiotic resistance. All strains were catalase-negative, oxidase-negative, Gram-positive, non-gas-producing from glucose, and capable of lactose fermentation. Similarly, our study isolated eight distinct *Bifidobacterium* strains that were catalase-negative, oxidase-negative, Gram-positive rods, and shared lactose utilization and a lack of gas production.[29]

In 2019, Saruuljavkhlan B, et al. reported that the average concentration of *Bifidobacterium* in the feces of 256 healthy adults from urban and rural areas was 4.66×10^6 CFU/mL. In comparison, our study identified an average of 2.06×10^9 CFU/mL.[30]

Although this study provides valuable insights into the *Bifidobacterium* composition of Mongolian adults, it has several limitations. The cross-sectional design allows only associative, not causal, interpretations. While age and BMI were considered, other confounding factors, such as detailed dietary intake, genetic background, physical activity, emotional state, and medication use, were not investigated. Therefore, the observed differences in bifidobacterial abundance and diversity may be influenced by these unmeasured variables.

In addition, our focus was limited to taxonomic identification and enumeration; the functional roles of the distinct *Bifidobacterium* communities remain to be explored. Future research should include multi-omic analyses (metagenomics, metabolomics, and transcriptomics) combined with dietary and lifestyle assessments to determine how environmental and host factors shape *Bifidobacterium* populations and their metabolic potential.[26,31] Longitudinal studies will be particularly important for understanding temporal dynamics and health implications among transitioning Mongolian populations.

Conflict of Interest

As the designated corresponding author, I attest that all other authors have seen and agreed to the submission. This paper has not been published anywhere and is not currently being considered for publication anywhere else. To our knowledge, there are no conflicts of interest. As the designated corresponding author, I attest that all other authors have seen and agreed to the submission.

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Authors Contribution

Tuul Nyambal: ORCID https://orcid.org/0000-0002-2747-9950, Conceptualization, data curation, formal analysis, funding acquisition, investigation, methodology, project administration, resources, software, supervision, validation, visualization, writing-original draft, writing-review and editing.

Alexander Hübner: ORCID https://orcid.org/0000-0003-3572-9996, Formal analysis, investigation, software, visualization, writing-original draft, writing-review and editing.

Khulan Lkhamsuren: ORCID https://orcid.org/0000-0003-3044-4764, Data curation, investigation, writing-original draft, writing-review and editing.

Ser-Od Khuygaa: ORCID https://orcid.org/0000-0002-8778-317X, Formal analysis, investigation, software, visualization, writing-original draft, writing-review and editing.

Raphaela Stahl: ORCID https://orcid.org/0000-0002-5915-9337, Formal analysis, investigation, visualization, writing-original draft, writing-review and editing.

Anuujin Gantumur: ORCID https://orcid.org/0000-0002-

5594-3597, Investigation.

Battogtokh Chimeddorj: ORCID https://orcid.org/0000-0002-5299-2406, Methodology, resources, supervision, writing-review and editing.

Davaalkham Dambadarjaa: ORCID https://orcid.org/0000-0001-6999-9367, Methodology, resources, validation, writing-review and editing.

Khosbayar Tulgaa: ORCID https://orcid.org/0000-0001-6288-9624, Methodology, resources, validation, writing-original draft , writing-review and editing.

Christina Warinner: ORCID https://orcid.org/0000-0002-4528-5877, Conceptualization, funding acquisition, investigation, methodology, project administration, resources, software, supervision, validation, writing-review and editing.

Soninkhishig Tsolmon: ORCID https://orcid.org/0000-0002-0730-5945, Conceptualization, funding acquisition, investigation, methodology, project administration, supervision, validation, writing-review and editing.

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Attachments 1

Table 6. Genomic Characteristics of Dominant Bifidobacterium Species from Mongolian Fecal Samples

N	Sample code	Name on FTA card	Classification	MIMAG	Number of contigs	Genome size (bp)	GC content (%)	N50 (bp)	Completeness [%]	Contamination [%]
1	BS1MB	B1-1	*d__Bacteria;p__Actinobacteriota;c__Actinomycetia;o__Actinomycetales;f__Bifidobacteriaceae;g__Bifidobacterium;s__Bifidobacterium longum*	HQ	25	2288883	60.1	198557	100	0
2	BS2DK	B13-2	*d__Bacteria;p__Actinobacteriota;c__Actinomycetia;o__Actinomycetales;f__Bifidobacteriaceae;g__Bifidobacterium;s__Bifidobacterium adolescentis*	HQ	15	2112621	59.3	150813	99.77	0.11
3	BS3AL	B110	*d__Bacteria;p__Actinobacteriota;c__Actinomycetia;o__Actinomycetales;f__Bifidobacteriaceae;g__Bifidobacterium;s__Bifidobacterium pseudocatenulatum*	MQ	375	1168393	57.5	3193	63.48	4.7
4	UT2BE	D28-2	*d__Bacteria;p__Actinobacteriota;c__Actinomycetia;o__Actinomycetales;f__Bifidobacteriaceae;g__Bifidobacterium;s__Bifidobacterium adolescentis*	HQ	15	2121363	59.3	1298068	99.55	0.7
5	UB1DE	U1	*d__Bacteria;p__Actinobacteriota;c__Actinomycetia;o__Actinomycetales;f__Bifidobacteriaceae;g__Bifidobacterium;s__Bifidobacterium dentium*	HQ	139	2431607	58.5	29032	92.77	0.68
6	UB1DE	U1-1	*d__Bacteria;p__Actinobacteriota;c__Actinomycetia;o__Actinomycetales;f__Bifidobacteriaceae;g__Bifidobacterium;s__Bifidobacterium pseudocatenulatum*	HQ	44	2231613	56.2	125772	99.05	1.02
7	UB2NS	U9-3	*d__Bacteria;p__Actinobacteriota;c__Actinomycetia;o__Actinomycetales;f__Bifidobacteriaceae;g__Bifidobacterium;s__Bifidobacterium longum*	HQ	20	2365926	59.7	217857	99.54	0.23

HQ – high-quality; MQ – medium-quality genome. Values for completeness and contamination were determined using CheckM.