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The Cuban Microbiome: Insights into Health, Disease, and the Role of Diet in Microbiome Recovery

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ABSTRACT

Introduction: Dietary patterns play a significant role in shaping the gut microbiome, where dysbiosis is closely associated with the development of diseases like type 2 diabetes and other health outcomes. The Cuban diet provides a distinct and valuable framework for advancing microbiome research. **Objectives:** To characterize the Cuban microbiome within the Cuban healthy population and highlight the potential of therapeutic strategies targeting specific microbial taxa to restore gut homeostasis in patients with type 2 diabetes mellitus. **Methods:** A total of 92 fecal samples were analyzed to characterize the healthy Cuban microbiome and in parallel a cohort of 20 participants of individuals with type 2 diabetes. Microbiome analysis was conducted by metagenomic 16S rRNA. Statistical comparisons were performed employing the Mann-Whitney U test. All statistical analyses were performed with a significance threshold of $p < 0.05$. **Results:** At baseline, the 16S rRNA gene sequencing an average of 2,174 ± 122 total taxa were identified, suggesting a diverse and healthy gut microbiome among healthy participants. The analysis of the Cuban microbiome at the phylum level revealed a distinctive microbial composition reflective of the traditional Cuban diet. The comparison between the healthy microbiome with that of individuals with diabetes showed that while no taxa showed statistically significant differences between the two cohorts, certain patterns suggest shifts in microbial communities that align with diabetes-associated dysbiosis observed in other populations. **Conclusion:** This work will contribute to the global understanding of how diet and environment influence the human microbiome. By focusing on the Cuban population, this research bridges a critical gap in microbiome science, highlighting the importance of context-specific studies to improve health outcomes globally.

Keywords: microbiome; alpha diversity; Cuban population; diabetes

El microbioma cubano: Perspectivas sobre la salud, la enfermedad, y el papel de la dieta en la recuperación del microbioma

RESUMEN

Introducción: Los patrones dietéticos desempeñan un papel significativo en la configuración del microbioma intestinal, donde la disbiosis está estrechamente relacionada con el desarrollo de enfermedades como la diabetes mellitus tipo 2 y otros resultados de salud. La dieta cubana proporciona un marco distinto y valioso para avanzar en la investigación del microbioma. **Objetivos:** Caracterizar el microbioma de la población cubana sana. De esta manera, resaltar estrategias terapéuticas potenciales dirigidas a taxones microbianos específicos que podrían restaurar la homeostasis intestinal en pacientes con diabetes mellitus tipo 2. **Métodos:** Se analizaron 92 muestras fecales para caracterizar el microbioma cubano saludable y en paralelo una cohorte de 20 individuos con diabetes tipo 2. El análisis del microbioma se realizó mediante secuencia de ARNr 16S metagenómico. Las comparaciones estadísticas se realizaron empleando la prueba U de Mann-Whitney para una significación de $p < 0{,}05$. **Resultados:** La secuenciación identificó un promedio de 2174 ± 122 taxones totales, lo que sugiere un microbioma intestinal diverso y saludable entre los participantes sanos. El análisis filogenético reveló una composición microbiana distintiva que refleja la dieta cubana tradicional. La comparación entre el microbioma saludable con el de individuos con diabetes mostró que, si bien ningún taxón evidenció diferencias estadísticamente significativas entre las 2 cohortes, ciertos patrones sugieren cambios en las comunidades microbianas que se alinean con la disbiosis asociada a la diabetes observada en otras poblaciones. **Conclusiones:** Este trabajo permitirá trazar estrategias de salud a través de intervenciones dietéticas personalizadas y contribuir a la comprensión global de cómo la dieta y el medio ambiente influyen en el microbioma humano. Al centrarse en la población cubana, esta investigación cierra una brecha crítica en la ciencia del microbioma, destacando la importancia de los estudios específicos del contexto para mejorar los resultados de salud a nivel mundial.

Palabras clave: microbioma; diversidad alfa; población cubana; diabetes

INTRODUCTION

The gut microbiome, a dynamic and diverse community of microorganisms residing in the human gastrointestinal tract, has a profound influence on host health. (1) It plays a critical role in maintaining metabolic balance, modulating immune responses, and protecting against pathogenic infections. Recent research has illuminated the central role of the microbiome in a variety of diseases, with particular emphasis on metabolic disorders such as type 2 diabetes (T2D). (2,3) Alterations in the gut microbiome, a condition known as dysbiosis, have been implicated in the pathogenesis of T2D, affecting gut permeability, immune function, and systemic inflammation. (4)

Dietary habits are one of the most critical factors shaping the composition and diversity of gut microbial communities. (5) Distinct dietary patterns across populations –such as the fiber-rich Oriental and Vegetarian diets, the Mediterranean diet, and the fat- and protein-heavy Western diet– have been shown to produce characteristic microbiome profiles. These profiles are associated with varying health outcomes, including risks for obesity, T2D, and chronic inflammation. (6)

The Cuban diet, deeply rooted in traditional and minimally processed foods, offers a unique opportunity to study a microbiome influenced by a blend of plant-based staples and moderate animal protein intake. (7) However, comprehensive evaluations of the Cuban gut microbiome are limited, and its comparative positioning relative to other global diets remains unclear.

In contrast, the typical Western diet, which is low in fiber and high in fat and refined sugars, has been linked to the development of dysbiosis, an imbalance in the gut microbiome characterized by a reduction in beneficial microbes and an overgrowth of pro-inflammatory taxa. (8) This dysbiotic state is particularly evident in individuals with T2D, who often exhibit a microbiome dominated by opportunistic pathogens such as *Escherichia coli* and members of the *Enterobacteriaceae* family. These taxa contribute to systemic inflammation and impaired glucose metabolism, hallmarks of T2D.

This study aims to characterize the Cuban microbiome across multiple taxonomic levels—phylum, family, and species. We will also explore the species-level composition to identify potential biomarkers of gut health and diet-specific adaptations within the Cuban microbiota. Finally, this study aims to provide a comprehensive analysis to elucidate, at least in part, the significant changes in gut microbiome composition associated with T2D and highlight the potential of therapeutic strategies targeting specific microbial taxa to restore gut homeostasis.

METHODS

Sample collection

Fecal samples were collected from participants enrolled in two distinct clinical trials. The first trial evaluated the Fucoidan supplement in healthy adult volunteers, registered in the Cuban Public Registry of Clinical Trials (RPCEC) under the identifier RPCEC00000443, and conducted from June 2024 to December 2024. The second trial investigated the symbiotic formulation Sugar Shift in patients with type 2 diabetes mellitus, registered in the RPCEC under the identifier RPCEC00000413, and carried out from June 2021 to April 2022. (2,3) For this work we analyzed the fecal samples collected at baseline, prior to the use of the respective formulations. Both trials adhered to international ethical standards and received approval from the Ethics Committee of Hermanos Ameijeiras Clinical and Surgical Hospital. Written informed consent was obtained from all participants before their inclusion in the studies. The first cohort comprised healthy individuals participating in a safety study investigating a prebiotic formulation, while the second cohort included individuals diagnosed with T2D enrolled in a separate study evaluating the efficacy of a probiotic formulation. Baseline fecal samples were collected from all participants prior to the initiation of any interventions, ensuring that the data accurately reflected their pre-treatment microbiome composition.

Participants were instructed to collect fecal material from the center of the stool using a sterile collection kit provided by the study team. The samples were deposited on clean paper to prevent contact with water or external contaminants. To preserve microbial DNA and RNA integrity, the collected samples were immediately transferred into tubes containing DNA/RNA Shield (Zymo Research, Irvine, CA, USA). This solution stabilizes and preserves the microbiome's DNA, allowing for storage and shipment at room temperature without compromising the quality of the nucleic acids, although samples were ultimately stored at −80°C to ensure maximum preservation for sample processing and analysis (EzBiome, Gaithersburg, MD, USA)

Microbiome analysis

The 16S rRNA gene metagenomic sequencing

Metagenomic 16S rRNA sequencing was conducted by EzBiome (Gaithersburg, MD, USA). DNA concentration was measured using the QuantiFluor dsDNA System on a Quantus Fluorometer (Promega, Madison, WI, USA). The V3-V4 region of the 16S rRNA gene was amplified using primers IlluminaF: CCTACGGGNGGCWGCAG and IlluminaR: GACTACHVGGGTATCTAATCC.

Amplicon PCR was performed with 12.5 ng of input DNA, KAPA HiFi HotStart ReadyMix, and primers under standard cycling conditions, followed by purification with Mag-Bind RxnPure Plus beads (Omega Biotek, Norcross, GA, USA). A second PCR added barcodes and sequencing adapters. Libraries were normalized with the Mag-Bind® EquiPure Library Normalization Kit, pooled, quality-checked on an Agilent 2200 TapeStation, and sequenced using 2×300 bp paired-end reads on the Illumina MiSeq platform.

Sequence processing and quality control

Raw sequencing data were analyzed using the QIIME 2 bioinformatics pipeline (version qiime2-amplicon-2023.9). Paired-end demultiplexed reads were processed with the DADA2 plugin to correct Illumina sequencing errors, ensuring accurate differentiation of microbial taxa at the strain level. (9) Chimeric sequences were identified and removed using DADA2's built-in algorithm.

The output of this workflow included a feature table containing the frequency of each amplicon sequence variant (ASV) across samples and a set of representative sequences. These outputs, including a features table, were further used in downstream analyses, for taxonomic assignment and diversity assessments. Sequence quality metrics, including read length distributions and per-base quality scores, were visualized using QIIME 2's interactive visualization tools, enabling detailed quality assurance prior to further analyses. For statistical analyses and ecological interpretation, ASVs were preferred over traditional operational taxonomic units (OTUs) to retain fine-scale ecological information without arbitrary clustering thresholds.

Alpha diversity analyses

To compare alpha diversity, we calculated species richness, diversity, and phylogenetic metrics using the qiime's diversity alpha command. The analysis included the Chao1 index, Shannon index, Simpson index, Faith's phylogenetic diversity (PD), and Pielou's evenness. Rarefaction at 25,000 sequences per sample ensured comparability and minimized biases from unequal sequencing depths.

The Chao1 index was used to estimate species richness to incorporate the number of observed ASVs and account for rare taxa to predict undetected diversity. The Shannon diversity index combined richness and evenness, reflecting both the number of ASVs and their relative abundances, with higher values indicating more diverse and evenly distributed communities. The Simpson index was calculated to evaluate community dominance, quantifying the probability that two randomly selected individuals belong to the same ASV, with higher values indicating less diversity. Faith's phylogenetic diversity measured the evolutionary diversity of microbial communities by calculating the total branch length of a phylogenetic tree encompassing all ASVs within a sample and offering insights into the phylogenetic breadth of the microbiome. Pielou's evenness assessed the uniformity of ASV abundances by normalizing Shannon diversity by the logarithm of observed ASV richness, with values ranging from 0 to 1, where 1 represents complete evenness. Statistical comparisons of alpha diversity between groups (e.g., baseline and treated) were performed using non-parametric Wilcoxon rank-sum tests, while group-level comparisons were evaluated using Kruskal-Wallis tests.

Taxonomic analysis

Taxonomic classification was performed using QIIME 2 (version qiime2-amplicon-2023.9) to assign taxonomy to amplicon sequence variants (ASVs) for each cohort. The Greengenes2 2024.09 database was employed as the reference taxonomy. Initially, a Naive Bayes classifier was trained on the Greengenes2 2024.09 reference sequences using the feature-classifier fit-classifier-naive-bayes method in QIIME 2. Subsequently, the trained classifier was applied to the representative ASV sequences from each cohort using the feature-classifier classify-sklearn method to assign taxonomic labels. ASV feature tables were collapsed to the desired taxonomic level using the qiime *taxa collapse command*, and relative abundances were normalized to account for differences in sequencing depth.

Statistical analyses were conducted to identify differentially abundant taxa across cohorts. The Linear Discriminant Analysis (LDA) Effect Size (LEfSe) algorithm (10) was used to detect significant taxonomic enrichments, with taxa displaying LDA scores greater than 2 and p-values less than 0.05 considered statistically significant. Kruskal-Wallis tests were applied to assess differences across multiple cohorts, with p-values adjusted using the Benjamini-Hochberg correction to control for false discovery rates. Taxonomic profiles were further visualized and refined in R (version 3.6.3) using the ggplot2 and ggpubr packages. These methods allowed for a comprehensive analysis of taxonomic distributions and enabling comparisons of microbial communities.

Statistical analysis

Statistical comparisons were performed to assess significant differences in taxonomic composition and diversity between cohorts. For taxonomic differences, we used Mann-Whitney U tests to compare the relative abundance of species between the two groups, adjusting for multiple comparisons using the Benjamini-Hochberg correction. Alpha diversity metrics were compared using the Mann-Whitney U test, to test for significant differences in microbial community composition between the groups. All statistical analyses were performed with a significance threshold of $p < 0.05$.

RESULTS

Sampling and processing

Subjects were randomly assigned to the treatment groups using a simple randomization method with an equal allocation ratio among the three study groups. In Study 1, a total of 92 baseline samples were analyzed to characterize the healthy Cuban microbiome. In parallel, the baseline data for individuals with T2D consisted of a cohort of 20 participants was analyzed to explore the microbiome composition associated with diabetes.

Metagenome quality

The 16S rRNA gene sequencing yielded an average of 34,624 ± 8,820 high-quality reads per sample, with 89.9% of total raw reads having Phred scores greater than or equal to 30. Rarefaction curves indicated sufficient sequencing depth, showing saturation across all samples and ensuring comprehensive microbial community coverage. Alpha diversity metrics confirmed that sequencing depth did not significantly affect richness or diversity indices, validating the adequacy of read counts. At baseline, an average of 2,174 ± 122 total taxa were identified, suggesting a diverse and healthy gut microbiome among participants.

Phylum level composition of the healthy Cuban microbiome

The analysis of the Cuban microbiome at the phylum level revealed a distinctive microbial composition reflective of the traditional Cuban diet, which emphasizes minimally processed foods, plant-based staples, and moderate animal protein intake. (11) To place these findings in a global context, the

Cuban microbiome was compared to those associated with other major dietary patterns, including the high-fat, low-fiber Western diet (American and European), the plant-rich Oriental diet, and the fiber-dense Vegetarian diet.

Key microbial indicators, such as the Bacillota-to-Bacteroidota (F/B) ratio and microbial diversity metrics, were evaluated across these diets to highlight dietary influences on microbial composition and overall gut health. (12) The Cuban microbiome exhibits a moderate Bacillota-to-Bacteroidota (F/B) ratio, averaging 2.02. This intermediate value lies between the higher ratios associated with Western diets and the lower ratios linked to fiber-rich diets. This suggests that the Cuban diet maintains a balance between plant-based carbohydrates and moderate amounts of animal-based fats and proteins. (12) Figure 1 summarizes the salient features of each diet type.

Genus level composition of the Cuban microbiome

Figure 2 illustrates a heatmap representing the distribution of taxa in the healthy Cuban microbiome. Each row corresponds to a microbial taxon, while each column represents an individual participant from the healthy cohort. The intensity of color reflects the relative abundance of each taxon, with darker shades indicating higher abundance and lighter shades indicating lower abundance. This visual summary highlights the diversity and variability of the healthy Cuban microbiome, offering insights into the taxa that dominate and those that are present at lower levels.

Alpha diversity of the healthy human microbiome

The alpha diversity metrics presented in this dataset provide insights into the richness, evenness, and phylogenetic breadth of the microbial communities. Figure 3 summarizes the Chao1 index values, estimating species richness, which range from approximately 481.96 to 1,230.87 across samples.

The Cuban microbiome dysbiosis in diabetes

Figure 4 displays the $\log_{10}$-transformed relative abundances of six key bacterial phyla –Actinomycetota, Bacillota, Bacteroidota, Verrucomicrobiota, Fusobacteriota, and Pseudomonadota– across two cohorts: healthy and diabetes. Boxplots represent the variability within each cohort, while gray lines with dots connect mean abundances, highlighting cohort-level trends. Statistical significance was assessed using independent t-tests, and *p*-values are displayed in the figure.

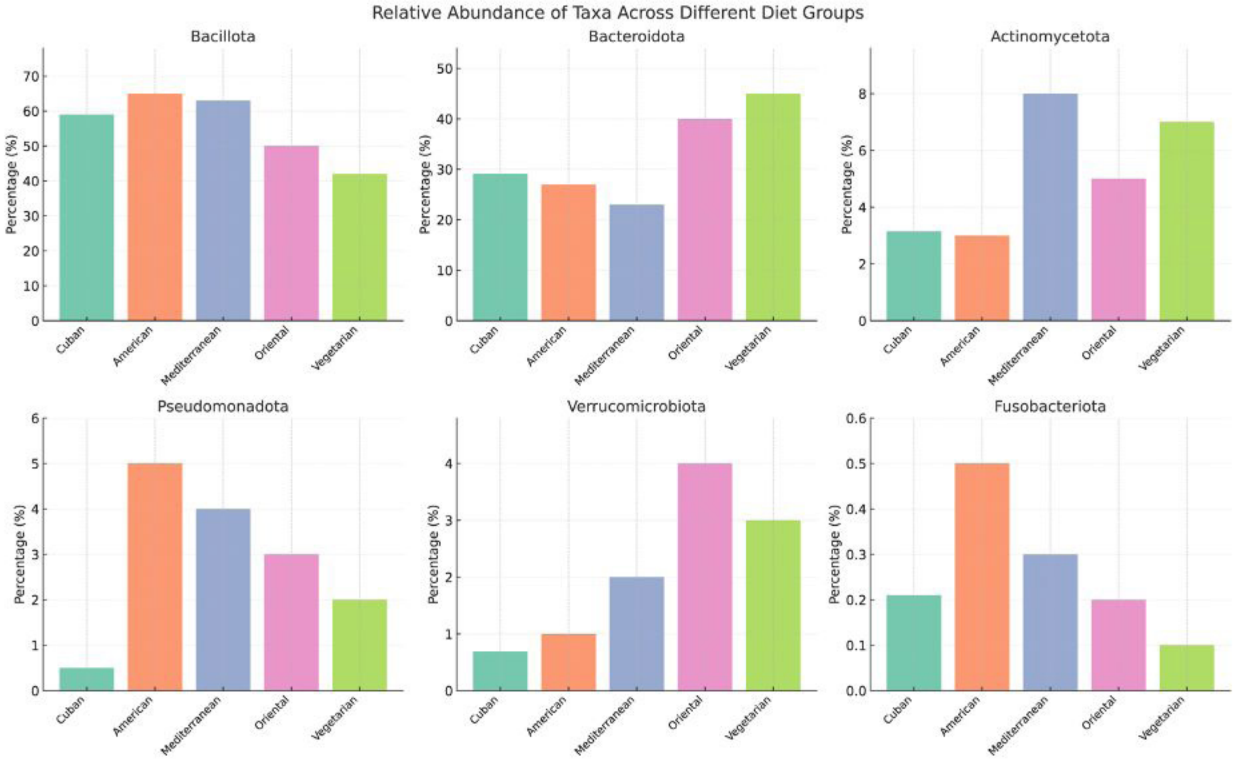

Fig. 1. Relative abundance of phyla across different diet groups. This figure displays the relative abundance percentages of six major taxa (Bacillota, Bacteroidota, Actinomycetota, Pseudomonadota, Verrucomicrobiota, and Fusobacteriota) in microbiomes associated with five diet groups: Cuban, American, Mediterranean, Oriental, and Vegetarian. Each panel represents a separate taxon, with bars indicating the percentage composition in each group

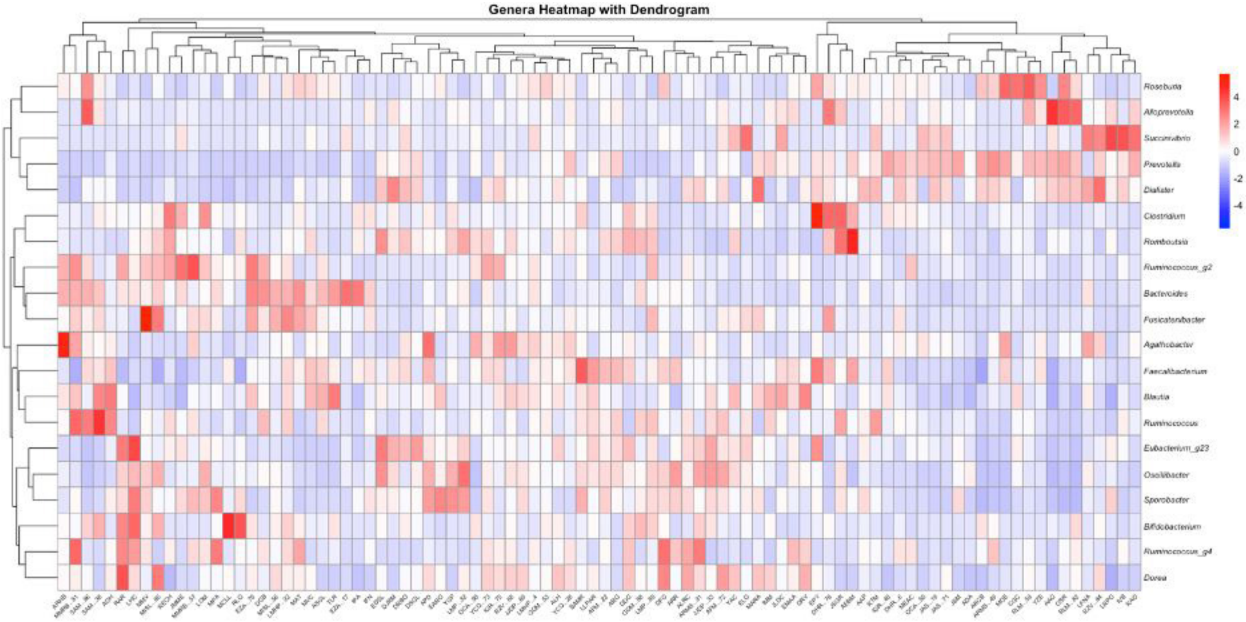

Fig. 2. Heatmap representing then relative abundance of principal genera in the adult Cuban microbiome

In the diabetes cohort, the relative abundance of Bacillota was significantly higher compared to the Healthy cohort ($p < 0.05$). Conversely, Bacteroidota levels were significantly lower in the diabetes cohort ($p < 0.05$). Pseudomonadota abundance was significantly elevated in the diabetes cohort ($p < 0.01$). In contrast, Verrucomicrobiota were more abundant in the healthy cohort ($p < 0.01$). For Actinomycetota and Fusobacteriota, no significant differences were observed between the cohorts ($p > 0.05$), although a slight trend toward higher Actinomycetota levels in the diabetes cohort was noted. Fusobacteriota had negligible abundance in both groups.

The analysis of the biomarkers Bacillota to Bacteroidota ratio (FB ratio) and Lachnospiraceae to Enterobacteriaceae ratio (LE ratio) provides insights into differences in the microbiome composition of healthy and diabetic cohorts within the Cuban population. The FB ratio, commonly used as an indicator of gut microbial balance, reveals a higher mean in the diabetic cohort (mean: 3.08 ± 2.14) compared to the healthy cohort (mean: 2.04 ± 1.22). The LE, interpreted as an indicator of gut inflammation, demonstrates stark differences between the cohorts. The healthy cohort exhibits a much higher mean LE (mean: 1231.87 ± 1542.73) compared to the diabetic cohort

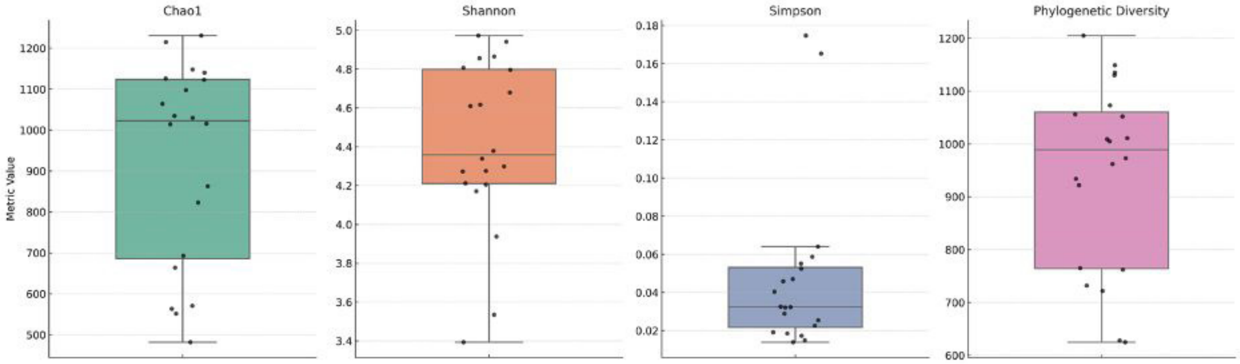

Fig. 3. Boxplots of alpha diversity metrics with jittered points for microbial communities

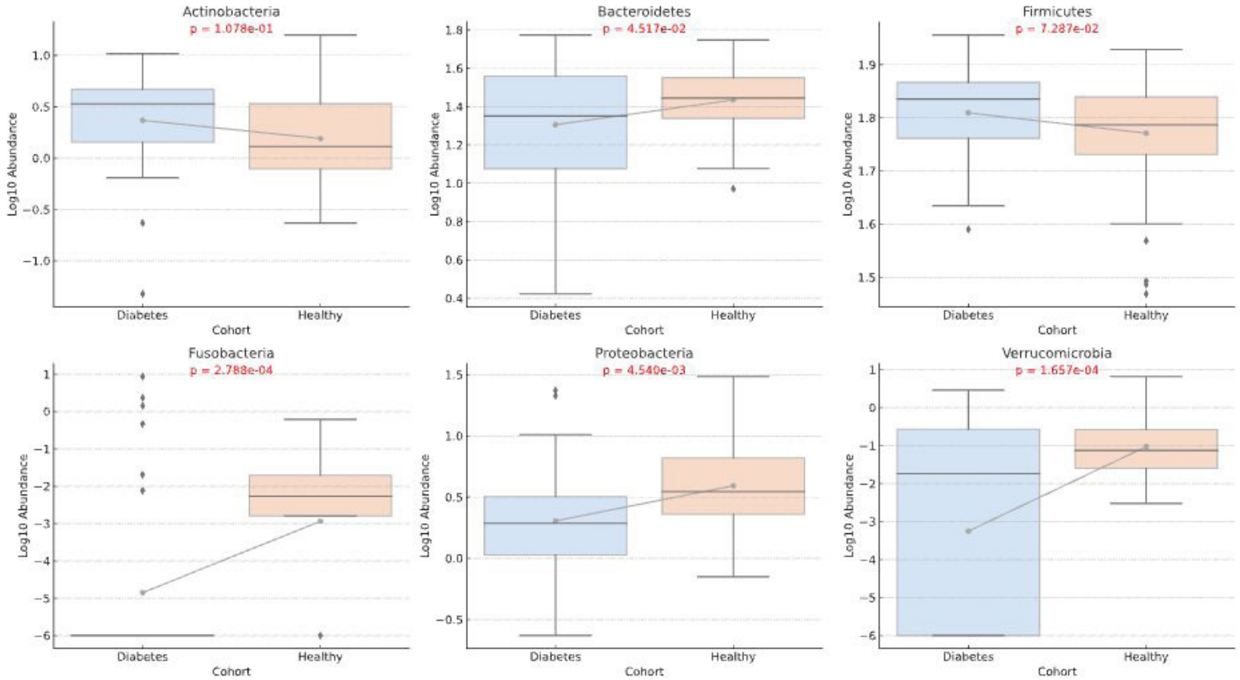

Fig. 4. Relative abundance of key bacterial phyla in healthy and diabetes cohorts

(mean: 192.62 ± 264.91). The analysis of the Cuban microbiome in individuals with diabetes was compared to healthy individuals to assess distinct trends in microbial composition (table 1).

DISCUSSION

The human gut microbiome plays a pivotal role in maintaining health, influencing metabolic, immune, and inflammatory pathways. Diversity metrics, revealed that the Cuban microbiome exhibited higher microbial diversity than Western diets but did not reach the levels observed in Oriental or Vegetarian diets. (13) These findings reflect the traditional Cuban diet's role in promoting microbial diversity, while also suggesting areas for potential improvement, such as increasing the intake of diverse plant-based fibers or prebiotics consisting of soluble fiber or resistant starch. The comparative nature of this analysis provides insights into how the Cuban microbiome aligns with or diverges from microbiomes shaped by other dietary patterns. (14)

Phylum level composition of the healthy Cuban microbiome

The Cuban microbiome demonstrates a relative abundance of Bacillota (formerly Firmicutes) at 59.0%, which is lower than the levels observed in Western diets (American: 65%, European: 63%) but higher than those in Oriental (50%) and Vegetarian (42%) diets. This reduced abundance compared to Western diets may reflect the Cuban diet's lower fat and animal protein content, as Bacillota, particularly the class Clostridia, tend to thrive in such conditions.

Clostridia, a dominant class within Bacillota that is closely associated with protein and fat metabolism, accounts for 40% of the Cuban microbiome. This is lower than levels observed in Western diets but comparable to levels observed in Vegetarian and Oriental dietary patterns. These findings highlight the Cuban diet's balance between plant -and animal- based food sources, which influence the composition and metabolic functions of Bacillota in the gut microbiome.

The relative abundance of Bacteroidota particularly the class Bacteroidia in the Cuban microbiome, which is slightly higher than levels observed in Western diets but lower than in Oriental and Vegetarian diets, reflects the inclusion of sufficient fiber and complex carbohydrates in the Cuban diet, as these nutrients are known to support the growth and metabolic activity of this phylum in the production of beneficial metabolites like short-chain fatty acids. (15,16) This suggests that while the Cuban diet promotes a relatively diverse and balanced microbiome, increasing the intake of diverse plant-based fibers could further optimize gut microbial health.

The relative abundance of Actinomycetota in the Cuban microbiome (3.14%,) showed comparable levels to Western

Table 1. Principal taxa trends in the diabetic microbiome as compared to the healthy microbiome

Taxon	Healthy cohort		Diabetes cohort		*p*-value
	Mean	St. Deviation	Mean	St. Deviation	
Agathobacter	1.977	1.854	4.045	6.220	0.088
Akkermansia	0.353	1.026	0.234	0.588	0.453
Bacteroides	6.756	7.131	5.235	7.279	0.336
Bifidobacterium	2.186	2.986	1.449	2.291	0.177
Blautia	6.812	3.689	6.740	4.444	0.938
Catenibacterium	0.693	1.357	2.844	3.980	0.008
Clostridium	1.852	1.903	1.398	2.933	0.442
Collinsella	0.150	0.255	1.522	1.504	0.002
Dialister	1.427	1.220	1.869	1.553	0.174
Dorea	1.523	0.734	1.794	1.097	0.224
Eubacterium	1.059	0.602	2.142	1.676	0.002
Faecalibacterium	5.316	2.692	5.172	3.017	0.822
Fusicatenibacter	1.274	1.417	1.034	0.704	0.244
Oscillibacter	1.982	1.311	2.708	2.520	0.148
Prevotella	16.789	14.453	15.480	17.434	0.719
Romboutsia	1.614	1.445	0.727	1.211	0.002
Roseburia	1.786	1.475	2.586	3.185	0.202
Ruminococcus_g2	2.117	2.434	2.997	3.779	0.249
Ruminococcus_g4	1.537	1.350	1.965	1.787	0.247
Succinivibrio	2.867	5.346	1.763	5.477	0.353

diets (3%-4% in American populations and 6%-8% in European populations). However, this is markedly lower than the levels observed in fiber-rich diets such as Oriental (5%) and Vegetarian (7%) dietary patterns. (17) Within this phylum, the Actinomycetota class, which includes health-promoting taxa like Bifidobacterium spp., accounted for 2.51% in the analyzed Cuban cohorts. This lower proportion of Bifidobacterium spp. reflects reduced consumption of prebiotic fibers, such as resistant starch, inulin, and fructooligosaccharides, compared to populations consuming Mediterranean or Vegetarian diets, which are known to enhance the growth of this beneficial genus. (18) *Bifidobacterium* spp. play a critical role in gut health by producing short-chain fatty acids (SCFAs), such as acetate and butyrate, and by modulating immune responses, contributing to overall microbial balance and protection against pathogens. (5) These findings highlight an opportunity to enhance gut health in Cuban populations through increased consumption of fiber-rich and prebiotic-containing foods. (19)

Pseudomonadota, often associated with inflammation and dysbiosis, is present at a very low level in the Cuban microbiome (5.4%). Pseudomonadota (formerly Proteobacteria), which includes genera such as Enterobacter and Escherichia/Shigella, is often associated with moderate to high fat and protein consumption. The intermediate levels observed in the Cuban microbiome suggest a diet that incorporates animal proteins but in balanced amounts compared to Western diets, which typically exhibit higher Pseudomonadota levels. (15,20) However, this level may also indicate susceptibility to low-grade inflammation or exposure to environmental stressors, such as contaminants or agricultural pesticides, which are known to favor the growth of Pseudomonadota. These findings suggest that increasing the consumption of soluble fibers and prebiotics, could help reduce Pseudomonadota levels and promote a healthier gut microbiome. (18)

The Cuban microbiome exhibits a relatively low abundance of Verrucomicrobiota at 0.69%, which is significantly lower than levels observed in Oriental diets (4.0%), Vegetarian diets (3.0%) and Western diets (American: 1.0%, European: 2.0%). The dominant class within this phylum, Verrucomicrobiota, which includes the well-known species *Akkermansia muciniphila,* comprises a small fraction (0.13%) of the Cuban microbiome.

Akkermansia muciniphila is a mucin-degrading bacterium that plays a crucial role in maintaining gut barrier integrity and modulating host metabolism. It produces SCFAs which contribute to intestinal health and systemic metabolic benefits, including reduced inflammation and improved insulin sensitivity. (21) The significantly lower abundance of Verrucomicrobiota in the Cuban microbiome may reflect dietary patterns that lack sufficient prebiotic fibers, which support the growth of A. muciniphila. (22) The low presence of Verrucomicrobiota in the Cuban microbiome may also serve as a marker for dietary optimization strategies aimed at promoting a more balanced and resilient gut microbiome. (23)

The relative abundance of Fusobacteriota in the Cuban microbiome was 0.21%, which is notably low compared to levels typically associated with colorectal cancer (CRC) in other populations. Fusobacteriota, particularly species like *Fusobacterium nucleatum,* have been implicated in CRC pathogenesis (24). Recently, the American Cancer Society reported in 2024 that during 2023, approximately 153,020 individuals were diagnosed with CRC in the United States. Similarly, in Europe, studies have shown that the rise in CRC incidence is largely confined to younger age groups (25–49) years in 14 primarily high-income, Western countries. (25)

In contrast, our study on the Cuban population revealed an average age of diagnosis of 70.18 years, with a minimum age of 58 years and a maximum of 86 years. These findings align with data from the Cuban Health Statistics, which report a similar age profile for CRC diagnosis. The difference in age-related CRC patterns between Cuba and Western populations could provide valuable insights into the potential interplay between microbial, dietary, and lifestyle factors contributing to disease onset and progression. (24)

Cuban diet: a unique mix

The Cuban microbiome's balanced Bacillota-to-Bacteroidota (F/B) ratio reflects the dietary diversity inherent in traditional Cuban cuisine, which incorporates both plant-based and animal-based foods. This balance is crucial, as an excessively high F/B ratio is associated with adverse health outcomes such as obesity, metabolic syndrome, and chronic inflammation. (26) This balance is likely influenced by the inclusion of moderate amounts of fiber-rich staples such as beans, rice, and root vegetables (e.g., yuca and malanga), alongside proteins and fats derived from pork, chicken, and fish. Together, these components foster a diverse microbial ecosystem capable of maintaining metabolic stability and gut health. The Shannon diversity index, as shown in Figure 3, further highlights the health-promoting potential of the Cuban microbiome. Higher diversity is closely linked to microbial resilience, improved metabolic function, and reduced susceptibility to dysbiosis. (27) The consumption of fermented beverages, such as guarapo, might also contribute beneficial metabolites that help maintain microbial balance.

Genus level composition of the Cuban microbiome

Patterns observed in the heatmap may reflect dietary influences, environmental factors, and the overall stability of the healthy microbiome in this unique population. These genera have been well-documented in the literature for their critical contributions to gut homeostasis and metabolic functions. For instance, the genus *Bacteroides* is renowned for its versatile metabolic capabilities, particularly in the breakdown of complex polysaccharides, which supports host nutrition and microbial growth. (28) Its prominence in the Cuban cohort aligns with dietary patterns that are likely high in plant-based fibers and complex carbohydrates, essential components of the traditional Cuban diet.

The genus *Faecalibacterium* stood out as another dominant genus, known for its production of butyrate, a key SCFA with anti-inflammatory properties to support intestinal barrier integrity and immune regulation. (29) The detection of *Alistipes* spp as a significant genus in the cohort underscores its emerging role in gut health. While *Alistipes* has been associated with anti-inflammatory effects, it has also been linked to dysbiosis in some pathological conditions. (30) Its dual role suggests that it may serve as a keystone taxon, balancing microbial communities under favorable conditions but potentially contributing to pathology when disturbed. The moderate abundance of *A. muciniphila*, a genus known for its ability to degrade mucin and maintain gut barrier integrity, aligns with the hypothesis that the Cuban microbiome reflects the beneficial effects of a fiber-rich diet.

Alpha diversity of the healthy human microbiome

Healthy human gut microbiomes typically exhibit Shannon indices in this range, further supporting the notion of a stable microbial ecosystem. (31) The high Shannon diversity in the Cuban microbiome highlights the potential benefits of traditional dietary patterns that support microbial richness and evenness. This finding underscores the importance of dietary diversity and whole, minimally processed foods in maintaining a healthy gut microbiome. Further studies could explore the specific dietary components driving this diversity and their implications for overall health. The observed range in this dataset suggests an absence of over-dominance by specific taxa, reflecting an overall healthy microbial balance, where no single species dominates excessively.

The phylogenetic diversity (PD), which considers evolutionary relationships among species, ranges from approximately 625 to 1205. This metric highlights the evolutionary breadth of the microbial communities, with higher PD values indicating more phylogenetically diverse communities. The dataset's PD values align with what is typically observed in healthy human microbiomes, suggesting a broad phylogenetic representation. When compared to other microbiomes, the metrics in this dataset suggest a relatively healthy microbial ecosystem. However, without detailed metadata on diet, geography, or health status, the broader implications remain speculative.

Overall, the diversity metrics reveal a stable and diverse microbial community, with richness and evenness values consistent with those observed in healthy human gut microbiomes. (32,33) The alteration in microbial diversity and composition in T2D is not merely a reflection of the disease but may also actively contribute to its progression by affecting host metabolism, immune function, and gut barrier integrity.

Biomarkers of dysbiosis

The analysis of the biomarkers Bacillota to Bacteroidota ratio (FB ratio) and *Lachnospiraceae* to *Enterobacteriaceae* ratio (LE ratio) provides insights into differences in the microbiome composition of healthy and diabetic cohorts within the Cuban population. The higher mean of the FB ratio in the diabetic cohort, commonly used as an indicator of gut microbial balance, suggests that individuals with diabetes in the Cuban population exhibit an increased prevalence of Bacillota relative to Bacteroidota. Elevated FB ratio values have been associated with metabolic imbalances, including obesity and insulin resistance, both of which are often comorbid with diabetes. While the mean FB ratio is higher in diabetics, the relatively wide standard deviation within this group suggests substantial inter-individual variability, potentially reflecting differences in diet, medication use (e.g., metformin), or diabetes severity.

In the healthy cohort, a lower FB ratio reflects a balanced gut microbiome, likely shaped by the traditional Cuban diet, which supports Bacteroidota proliferation, aiding carbohydrate metabolism and gut health. A higher LE reflects a microbiome with a greater relative abundance of *Lachnospiraceae*, a family of SCFA producers, relative to *Enterobacteriaceae*, which are often associated with gut inflammation and dysbiosis. (34)

In the Diabetic cohort, the significantly lower LE suggests a microbial shift favoring *Enterobacteriaceae* over *Lachnospiraceae*. *Enterobacteriaceae* are known to thrive in inflammatory conditions and have been implicated in promoting low-grade chronic inflammation, a hallmark of diabetes and related metabolic disorders. (35) Conversely, the healthy cohort's higher LE highlights a microbial community to maintain gut homeostasis and produce beneficial metabolites, further underscoring the protective role of the traditional Cuban diet in shaping a resilient microbiome.

Key taxonomic shifts

Increased relative abundance and variability of *Agathobacter* in diabetes may reflect individualized responses to metabolic or inflammatory changes, though not statistically significant. (36) Another interesting finding is the reduced abundance of *Akkermansia*, a taxon often linked to gut health and metabolic regulation. While the difference is small and not statistically significant (p = 0.453), aligns with findings from other studies where reduced *Akkermansia* levels are associated with metabolic disorders. (37)

Bacteroides also demonstrates a slight reduction in the diabetes cohort compared to the healthy cohort. This genus is typically associated with carbohydrate metabolism and short-chain fatty acid production. Its reduced abundance in diabetes may indicate a shift away from taxa that contribute to gut homeostasis and energy regulation. (38)

The genus *Bifidobacterium*, known for its anti-inflammatory properties and role in maintaining gut health, shows a lower mean in diabetes compared to healthy individuals. This reduction could signify an altered microbiota unable to counteract the low-grade inflammation often observed in diabetes. (39)

Broader implications and limitations of the study

These trends suggest that the Cuban microbiome in diabetes exhibits features of dysbiosis. While the traditional Cuban diet, rich in plant-based fiber and legumes, may contribute to preserving some beneficial microbial taxa, the observed shifts still align with global patterns of microbiome changes in metabolic diseases. The lack of statistical significance in these comparisons underscores the importance of increasing the sample size to improve statistical power and conducting functional analyses to explore the metabolic consequences of these shifts.

Conclusion

The Cuban microbiome exemplifies the impact of dietary diversity and traditional practices on gut health. By promoting microbial diversity, balancing key taxa, and suppressing pro-inflammatory microbes, the Cuban diet supports a gut environment that is both metabolically stable and anti-inflammatory. These findings position the Cuban diet as a midpoint between the processed Western diet and fiber-rich Oriental and Vegetarian diets, offering valuable insights for public health interventions aimed at preserving traditional dietary practices. Future efforts to further enhance the Cuban microbiome could focus on increasing the variety of plant-based fibers in the diet, fostering even greater microbial diversity and resilience. This study underscores the importance of understanding the interplay between diet, the microbiome, and health outcomes, providing a framework for microbiome-targeted interventions in diverse populations

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