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Research Article

Combined analysis of cross-population healthy adult human microbiome reveals consistent differences in gut microbial characteristics between Western and non-Western countries

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ABSTRACT

Despite extensive research on the gut microbiome of healthy individuals from a single country, there are still a limited number of population-level comparative studies. Moreover, the sequencing approach used in most related studies involves 16 S ribosomal RNA (rRNA) sequencing with a limited resolution, which cannot provide detailed functional profiles. In the present study, we applied a combined analysis approach to analyze whole metagenomic shotgun sequencing data from 2035 healthy adult samples from six countries across four continents. Analysis of core species revealed that 13 species were present in more than 90 % of all investigated individuals, the majority of which produced short-chain fatty acids (SCFA)-producing bacteria. Our analysis revealed consistently significant differences in gut microbial species and pathways between Western and non-Western countries, such as *Escherichia coli* and the relation of MetaCyc pathways to the TCA cycle. Specific changes in microbial species and pathways are potentially related to lifestyle and diet. Furthermore, we identified several noteworthy microbial species and pathways that exhibit distinct characteristics specific to China. Interestingly, we observed that China (CHN) was more similar to the United States (USA) and United Kingdom (GBR) in terms of the taxonomic and functional composition of the gut microbiome than India (IND) and Madagascar (MDG), which were more similar to the China (CHN) diet. The current study identified consistent microbial features associated with population and geography, which will inspire further clinical translations that consider paying attention to differences in microbiota backgrounds and confounding factors.

1. Introduction

The human gut harbors a wide, complex, and diverse community of microorganisms that play a fundamental role in human health and disease [1,2]. Each person harbors approximately 150–400 bacterial species in the gut, representing the largest number and concentration of microorganisms in the human body [2–4].

The microbiome is extensively involved in physiology and metabolism [3]. Various studies have revealed that changes in the microbiota are significantly associated with various diseases, such as obesity [5], type 2 diabetes [6], inflammatory bowel disease [7], and hypertension [8]. Moreover, emerging research has highlighted the association between microbial dysbiosis and a range of diseases, including colorectal cancer [9], oral diseases [10], rheumatoid arthritis [11] and cardiovascular diseases [12]. Therefore, an important step in restoring a healthy microbiota and intervening in microbial dysbiosis in patients is

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to understand the characteristics of a healthy human microbiome.

Recently, several studies have investigated the characteristics of healthy microbiomes in various countries such as the United States [13], China [14], South Korea[15], Japan[16], the Netherlands [17] and Belgium [18]. These studies provide a better understanding of the relationship between microbiota variation and the covariates of an average healthy population and demonstrate the variability in composition and function of the gut microbiota associated with host and environmental factors, such as age, sex, and geographical region. However, the majority of these studies only considered individuals from a single country and focused on assessing the association between gut microbiota and host covariates. For instance, two US-based gut microbiota datasets demonstrated recurrent associations between specific taxa in the gut microbiota and ethnicity [19]. Another large-scale Chinese cohort study emphasized that geography has the largest explanatory power for the composition of the gut microbiota [14]. Otherwise, few population-level comparative studies are currently available [16,18, 20]. Therefore, conducting a comparative analysis to investigate gut microbiota in different countries is of great value.

The sequencing approach used in most of these studies involved 16 S ribosomal RNA (rRNA) gene sequencing. The major limitations of this method are as follows. First, taxon annotation is based on only a single region of the bacterial genome, which has limited resolution [21]. More importantly, 16 S rRNA sequencing does not provide information about the metabolic potential of communities [21,22]. An alternative approach to 16 S rRNA gene sequencing is shotgun metagenomic sequencing, which can be defined more accurately at the species level and provides detailed functional profiles.

With the development of technology and decreases in sequencing costs, a growing number of metagenomic datasets around the world are available. Conducting a combined analysis by combining these datasets can offer comprehensive insights beyond those provided by individual studies. Comparative analysis of the composition and function of the human microbiota across countries with diverse lifestyles and geographic locations can offer profound insights and a comprehensive understanding of the gut microbiome in a healthy state. In this study, we performed a large-scale combined analysis of 14 publicly available shotgun metagenomics-based studies to explore population-level variations in the gut microbiome. Our study has several key objectives. First, we aimed to explore the overall characteristics of the gut microbiome in all individuals and to identify its distinctive features within six specific populations, while also investigating the relationship between diet and gut microbiota. Second, we sought to identify the core microbial features specific to each population as well as those shared among all individuals. Furthermore, we aimed to consistently identify the different gut microbial species and pathways in Western and non-Western countries. Finally, considering China's recent rapid industrialization and economic development, we compared the differences in gut microbiota between China and other Western countries, as well as between populations in Asia and Africa.

2. Materials and methods

2.1. Data set and metadata collection

We collected shotgun metagenomic sequence data of individuals from the curated Metagenomic Data (https://github.com/waldronlab/curatedMetagenomicData/, version 3.0.1, accessed on September 30, 2021) repository [23]. CuratedMetagenomicData provides standardized, curated human microbiome data for downstream analyses that include species relative abundance and MetaCyc pathway abundance for samples. The taxonomic abundances for each sample were calculated using MetaPhlAn3 [24], and HUMAnN3 [24] was used to analyze the MetaCyc pathway abundance. Briefly, MetaPhlAn3was run onpreprocessed readsusing default parametersto generatemicrobialcommunity profiles. HUMAnN3 is a pipeline that efficiently and accurately profiles both the presence/absence and abundance of microbial pathways in a community by utilizing MetaCyc pathway definitions. HUMAnN3 maps the preprocessed reads to the UniRef90 catalogue and reconstructs the metabolic pathway profile based on gene family outputs, which are annotated to MetaCyc reactions.

Of the 20,282 samples from 86 available studies, we selected those that had samples with the following characteristics: (1) no antibiotic or probiotic use (antibiotics_current_use = no). (2) stool samples were collected from healthy adults (age category = adult; disease = healthy). Of note, referring to recently published literature [25], healthy individuals, regardless of whether they had been determined as healthy in the original studies, were considered to be part of the nonhealthy group if their reported BMI fell within the range of underweight (BMI <18.5), overweight (BMI ≥ 25 and < 30), or obese (BMI ≥ 30). Moreover, female participants who were pregnant or not reported as nonpregnant in the original study were excluded from our analysis. Only countries with at least 50 samples meeting the above criteria were considered. In total, 2035 healthy adult metagenomic samples from 14 studies fulfilled our selection criteria. These samples, gathered using the returnSamples function, were subsequently utilized for further analyses. (Supplementary Fig. S1, Supplementary Table S1). These 14 metagenomic studies [17,26–38] encompassed six countries across four continents: America, Europe, Asia, and Africa. They included four studies from China (CHN) with 269 individuals, three from the United States (USA) with 151 individuals, including those from a study jointly conducted with the United Kingdom (GBR), three from India (IND) with 97 individuals, two from the Netherlands (NLD) with 904 individuals, and one from Madagascar (MDG) with 97 individuals. The joint USA-GBR study included 517 individuals from the GBR.

The Food and Agriculture Organization Corporate Statistical (FAOSTAT) database is a collection of online databases containing time-series records that cover international agricultural statistics for 210 countries [39]. Data were provided by national governments or extrapolated by the Food and Agriculture Organization of the United Nations. Dietary intake data (kg/capita/year) for 93 food categories were collected from the FAOSTAT database (https://www.fao.org/faostat/, accessed on November 18, 2021). The food categorizations used in our study were provided directly by the FAOSTAT database, and we did not perform any additional categorizations or adjustments.

2.2. Microbiome data analysis

All alpha and beta diversity analyses were conducted in the R environment (http://www.r-project.org/) using phyloseq [40], microeco [41] and vegan packages [42], except for permutational multivariate analysis of variance (PERMANOVA) analysis using Primer7 with the PERMANOVA+ plugin.

Alpha diversity was calculated using the Shannon diversity index. To compare samples with different sequences, all the samples were rarefied to the same number of reads. Bacteroidetes/Firmicutes (B/F) ratio was calculated using the ratio of the relative abundances of Bacteroidetes and Firmicutes. Beta diversity was assessed based on Bray-Curtis distances calculated from the relative abundances of microbial taxonomic and functional features. Principal coordinate analysis was performed using the Bray-Curtis dissimilarity matrix. To test the statistical significance of differences in beta diversity, PERMANOVA was performed using type III sums of squares and 999 permutations of residuals under a reduced model. The random factor "study" and the fixed factors "country," "age" and "sex" were specified for the PERMANOVA model. Intergroup differences in taxonomic and functional compositions of the gut microbiome were also assessed using PERMANOVA.

With the exception of *A. butyriciproducens* [43], the Short-chain fatty acids (SCFA) capabilities of the species were evaluated based on the findings of Frolova et al. [44]. Frolova et al. utilized genomic signatures to reconstruct pathways and categorized genomes based on simplified binary phenotypes, indicating the ability ("1") or inability ("0") of a given

human gut microbiota (>800 known human gut microbiota species) to produce SCFAs.

2.3. Statistical analysis

All statistical analyses were performed using the R software (version 4.1). The Mann-Whitney-Wilcoxon (MWW) test was used to compare intra-population variations in microbial functional composition and microbial taxa composition. The MaAsLin 2 package was used to perform linear mixed-effects models (analysis_method = "LM" in the MaAsLin2 function). To control for all potential confounding variables, we added two covariates to the model for population–microbiota association: age, and sex. To control for potential study effects resulting from different isolation methods, DNA extraction protocols, and sequencing platforms, the study accession was set as a random effect in MaAsLin2 [27]. The formula of the linear mixed regression model was as follows: Feature abundance ~ country + age + sex + country:age + country:sex + country:age:sex + (1|study), where feature abundance was the log-transformed relative abundance of species and MetaCyc pathways. MaAsLin2 adds a pseudo-count of half of the minimum species or MetaCyc pathway level detected before the log transformation of relative abundances. Statistical differences among population alpha diversity and B/F ratios were also tested using MaAsLin2 with linear mixed-effects models (analysis_method = "LM" in the MaAsLin2 function) to control for age, sex, and study effects. Multiple tests were controlled with FDR correction calculated by the Benjamini-Hochberg method. Adjusted P values < 0.1 were deemed significant in all differential abundance analyses. Hierarchical clustering with Euclidean distance was used for the cluster analysis of the six countries based on the profiles of different food categories.

3. Results

3.1. Overall population-level gut microbiome characteristics

First, we investigated the abundance distribution of the microbial taxa. The predominant bacterial genera in individuals from CHN, USA, and GBR were *Bacteroides*, whereas IND and MDG were dominated by *Prevotella* (Supplementary Fig. S2A, B). Intriguingly, the relative abundance of *Bacteroides* in CHN did not differ significantly from that in the USA, GBR and NLD (MaAsLin, $p > 0.05$). Moreover, the included studies from NLD harbored *Bifidobacterium* as the most abundant genus and the genus *Ruminococcus* had the second largest mean abundance. Furthermore, *Escherichia* had the second-largest mean abundance in the MDG and exhibited a significantly higher abundance than that in other countries (MaAsLin2, $p < 0.05$, Supplementary Table S2). Additionally , *Euryarchaeota* was the only detected phylum within the Archaea domain, with average relative abundance of 0.899 % (standard deviation: 0.026) and a prevalence of 38.5 % (Supplementary Table S3). *Euryarchaeota* was dominated by *Methanobrevibacter smithii* (relative abundance: 0.851 %, standard deviation: 0.025) and identified in 754 individuals across all 14 studies healthy adults (Supplementary Fig. S2C), whereas the abundance and prevalence of other species belonging to *Euryarchaeota* were very small. The relative abundance of *Methanobrevibacter smithii* highly varied across individuals, ranged from 0 % to 35 %. Interestingly, we observed that *Methanobrevibacter smithii* was more prevalent in MDG than in other countries and the least common in CHN, and its prevalence was higher in western countries than Asian countries, with the exception of USA.

As shown in Supplementary Fig. S2D, the ratio of *Bacteroidetes* to *Firmicutes* (B/F ratio) showed high inter-individual variation within the populations. After controlling for age, sex, and batch effect, the CHN population had a significantly higher B/F ratio than the other populations (MaAsLin2 [45], $p < 0.05$), whereas the MDG and NLD populations had the lowest B/F ratios.

3.2. Microbial compositions shared among populations

To evaluate the microbial species that are potentially critical to the gut ecosystem, we calculated the prevalence of bacterial taxa to explore the taxonomic compositions shared between individuals. We found that more than half of the species (432/835) appeared in only 1 % of the samples, and 649 out of 835 species were low prevalent species that presented in less than 10 % of the entire population (Supplementary Fig. S3, Supplementary Table S4). Additionally, by comparing the number of shared species under different species prevalent thresholds of 50 % with 117 species, 60 % with 104 species, 70 % with 80 species, 80 % with 56 species, and 90 % with 38 species across various countries (Supplementary Fig. S4), we found that the species prevalent in CHN showed a higher degree of similarity to those in the USA, GBR, and NLD.

Next, we identified 13 species (*Collinsella aerofaciens, Agathobaculum butyriciproducens, Faecalibacterium prausnitzii, Anaerostipes hadrus, Fusicatenibacter saccharivorans, Blautia wexlerae, Blautia obeum, Dorea longicatena, Dorea formicigenerans, Eubacterium hallii, Roseburia inulinivorans, Eubacterium rectale, Bacteroides uniformis*) that were present in more than 90 % of all investigated individuals, indicating that they may play critical roles in the gut microbiome ecosystem of healthy individuals. They accounted for 28.87 % of relative abundance. Only *Faecalibacterium prausnitzii* appeared in more than 95 % of all individuals, and none was detectable in all individuals. Interestingly, most of the core species (12 of 13) were SCFA-producing bacteria, with the exception of *Fusicatenibacter saccharivorans*.

Furthermore, we identified the core microbiota (shared by more than 90 % of individuals) of each population at different taxonomic ranks (Supplementary Fig. S5 A-C). At the phylum level, the core species in all countries were *Firmicutes, Bacteroidetes, Actinobacteria,* and *Proteobacteria*, but their abundances varied among individuals (Supplementary Fig. S5A). At lower taxonomic levels, such as genus, IND had the lowest number of core genera (five genera), whereas GBR had the highest (25 genera) and had highly similar core genera with NLD. There were 16 core genera in China and 12 in the United States. Notably, CHN and USA shared 11 core genera (Supplementary Fig. S6A). Interestingly, *Prevotella* was not a core genus in IND; however, its average abundance was very high. At the species level, this situation was similar to that observed at the genus level (Supplementary Fig. S6B). The core species of CHN were more similar to those in the USA.

We also identified core pathways (shared by more than 90 % of individuals) to investigate the functional compositions shared by most individuals. Overall, nearly half of the pathways (230/543) appeared in more than 90 % of individuals, irrespective of their country, whereas 152 pathways were shared by less than 10 % of the entire population (Supplementary Fig. S7A, Supplementary Table S5). Consistent with the results of previous studies, these results suggest that different species of unrelated healthy individuals can activate similar functions by utilizing common pathways.

3.3. Selected studies indicate microbial community structures in China show greater similarity to USA and GBR than to other countries

National populations have been associated with gut microbiome alpha diversity in previous findings [46]. Therefore, we assessed the alpha diversity in six populations using the Shannon diversity index and performed multivariate association with linear model (MaAsLin) analyses to control for age, sex, and batch effects (treating batch effects as random effects) [45]. The results demonstrated that the CHN population was not significantly different from the other five populations, except for the NLD (MaAsLin, $p < 0.05$). Moreover, the Shannon diversity indices of Western countries, the USA, GBR, and NLD did not differ significantly (MaAsLin, $p > 0.05$, Supplementary Fig. S8A).

Furthermore, we calculated Bray - Curtis distances based on the microbial species to compare the microbial community structures in the six study populations. We observed that the majority of intra-population

Bray-Curtis distances were greater than 0.5, indicating a significant variation in the gut microbiome among individuals within each population. (Supplementary Fig. S8B). We further calculated intra-population variations based on their relative MetaCyc pathway abundances (Supplementary Fig. S7B) and observed that the degree of intra-population variation in microbial functional composition was significantly lower than the intra-population variations in microbial taxa composition.

Next, we performed principal coordinate analysis (PCoA) based on the Bray-Curtis dissimilarity matrix for microbial species, which demonstrated that individuals from the same country tended to cluster together (Fig. 1A). A permutational multivariate analysis of variance (PERMANOVA) test confirmed a significant segregation between the distributions of these countries after accounting for sex, age, and study effects ($p < 0.001$, Supplementary Table S6). Using the square-root transformed estimated components of variation (sq. root ECV), our analysis determined that the study effect accounted for the largest proportion of the variation (square root ECV 13.8, $p < 0.001$, Supplementary Fig. S9). This aligns with previous estimates of the impact of study effects on the taxonomic composition of the gut microbiome. Despite the significant contribution of the study effects, country of origin still played an important role in the variance between samples (square root ECV 6.7, $p < 0.001$). Beyond the country of origin, age and sex were also significant contributors to microbial variance, with square root ECV values of 5.1 ($p < 0.001$) for age, and 4.0 ($p < 0.001$) for sex.

We also investigated the degree of compositional differences between CHN and other countries based on the pairwise PERMANOVA test of Bray-Curtis distances of microbial species and MetaCyc pathway abundance (Fig. 1B). The results showed significant differences in the community composition between CHN and other countries (all $p < 0.05$). Surprisingly, the largest average similarity values were observed among CHN, USA, and GBR. Among the pairs of countries, the CHN and MDG had the lowest average similarity values.

3.4. Exploring the complex relationship between diet and abundances of *Bacteroides/Prevotella*

It is well known that diet is an important factor influencing gut microbiomes [47–49]. A high abundance of *Bacteroides* has been associated with Western diets and animal-based diets rich in animal proteins and saturated fats [50]. High levels of *Prevotella* are associated with non-Western diets, carbohydrate-based diets rich in fiber[50,51]. This trend was also observed in this study. *Bacteroides* were found to be more abundant in Western populations, whereas non-Western populations harbored a higher abundance of Prevotella. Although *Prevotella* was the core genus in CHN (Supplementary Fig. S2A) and its relative abundance was large, the relative abundance of Bacteroides in CHN was nevertheless comparable to USA and GBR and was significantly higher than NLD. To investigate the association between diet and the gut microbiome, we collected dietary intake data (kg/capita/year) for 93 food items from the FAOSTAT database. This approach was selected because of the limited availability of detailed dietary data. Although dietary data may not capture the diverse dietary patterns within a specific country, they can still provide insights into general trends. Cluster analysis of the six countries based on the profiles of different food categories showed that CHN had a close relationship with IND and MDG because of the high similarity in dietary components (Fig. 2A). This result suggests that the abundance of Bacteroides and Prevotella might not be solely attributable to diet, indicating that factors other than diet may also influence the abundance of these microbial groups. This observation provides a perspective different from the traditional view in previous literature that the abundance of *Bacteroides/Prevotella* is often associated with diet. Interestingly, we observed that CHN was more similar to USA and GBR in terms of overall microbial composition, instead of IND and MDG, which have diets that are more similar to CHN. It is important to note that, while this cluster analysis effectively captured the distinct dietary characteristics of each country, there remains an inherent bias due to the use of general dietary trends and the potential discrepancy between reported dietary data and actual consumption. Despite this, the high similarity in the dietary components among CHN, IND, and MDG, particularly the high vegetable, rice, and wheat levels and relatively low animal product (such as poultry and bovine meat) levels and alcohol consumption, still provides meaningful insights into the diet-gut microbiota relationship in different population groups.

3.5. Consistent differences in gut microbial characteristics were observed between Western and non-Western countries

Given the strong differences in industrialization, economic development, and lifestyles between Western and non-Western countries, we performed multivariate association with linear model analyses (MaAsLin2) to test for significantly differentially abundant taxa at the species

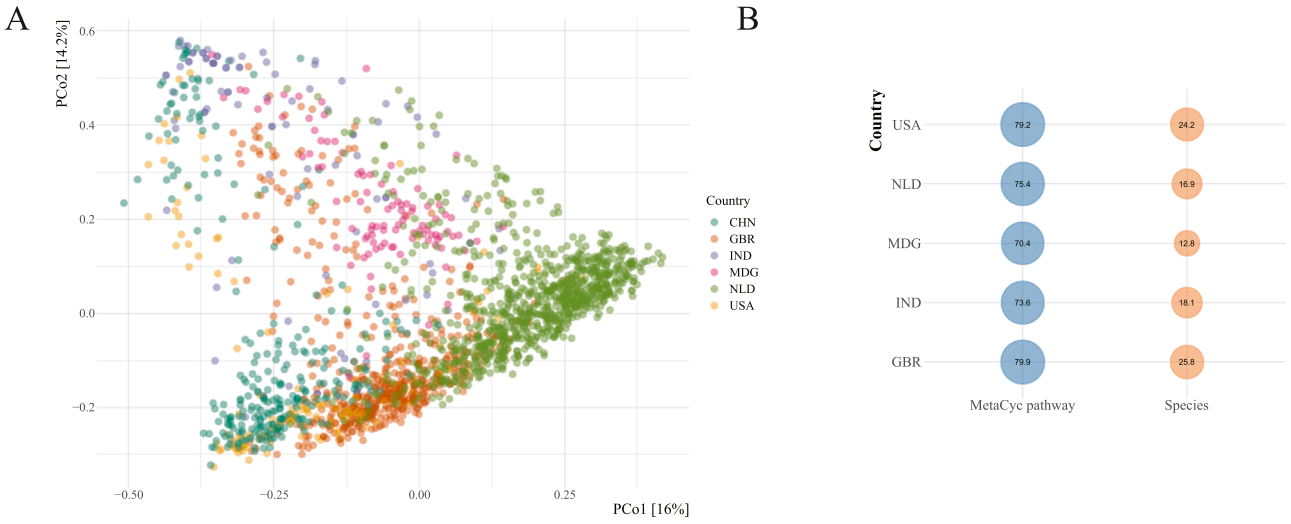

Fig. 1. Population-level diversity in healthy adult gut microbiomes among the six countries. A Principal coordinate analysis (PCoA) for Bray-Curtis distances. Each point represents an individual and each color represents a country. B Bubble plots showing the average similarity between CHN and others countries. The values displayed in each circle represent the average similarity values derived from the pairwise PERMANOVA based on Bray-Curtis distances of microbial species and MetaCyc pathway in Primer7. USA, United States; GBR, United Kingdom; IND, India; NLD, Netherlands; CHN, China; MDG, Madagascar. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

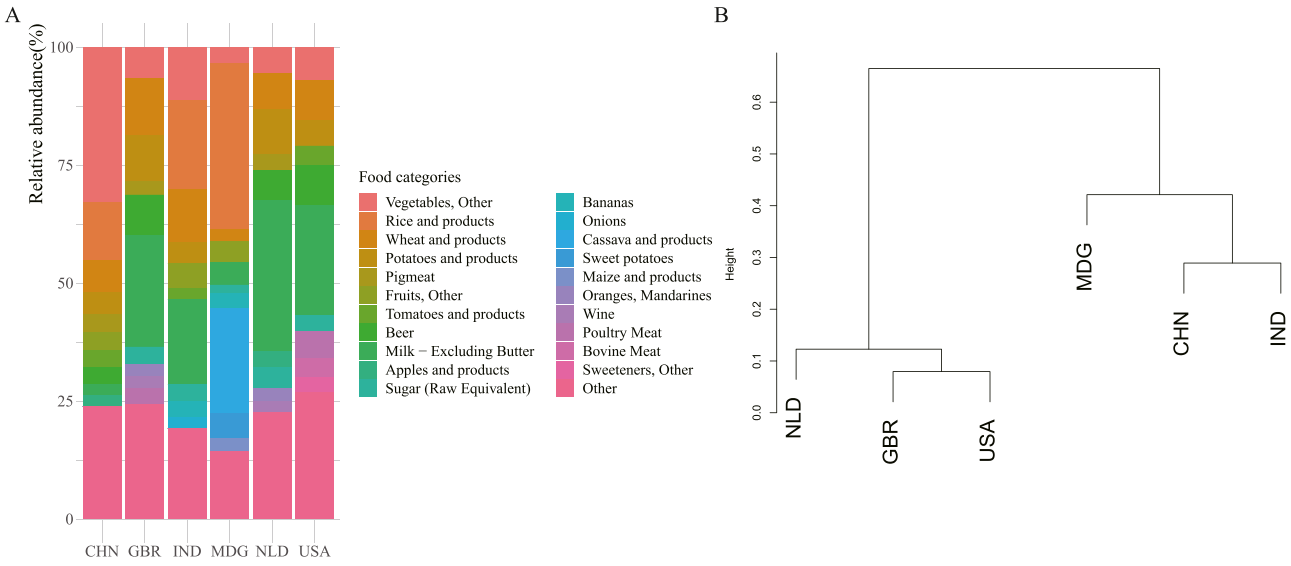

Fig. 2. Hierarchical clustering of the 6 countries based on average dietary intake data A Overall distribution of dietary intake in each country, only top 10 food categories are shown B The dendrogarm of the 6 countries based on the dietary intake data (kg/capita/yr) for 93 food items. USA, United States; GBR, United Kingdom; IND, India; NLD, Netherlands; CHN, China; MDG, Madagascar.

level and MetaCyc metabolic pathways between Western and non-Western countries.

Two approaches were employed to merge the samples. The first involves dividing the sample into two groups based on whether they belong to Western or non-Western countries. We then analyzed the overall differences in microbial species and pathways between the two groups. The second approach entails first determining the differences in microbial species and pathways among different countries and then identifying the consistent differences between Western and non-Western countries based on the variations among the countries.

Using the first approach, disparities in the gut microbial species have been observed between Western and non-Western countries. Our analysis revealed 10 species (Supplementary Table S7), including *Akkermansia muciniphila, Anaerostipes hadrus, Clostridium leptum, Clostridium sp. CAG 167, Barnesiella intestinihominis*, and *Streptococcus thermophilus*, that were more abundant in Western countries than in non-Western countries. Notably, three of these species (*Akkermansia muciniphila, Clostridium leptum*, and *Streptococcus thermophilus*) are considered potentially beneficial. Conversely, eight species, including *Escherichia coli*, *Prevotella copri*, and *Prevotella stercorea* were less abundant in Western countries. We identified 69 MetaCyc pathways that showed significant differences in abundance between non-Western and Western countries (Supplementary Table S8). Specifically, 64 pathways were more abundant in non-Western countries than in Western countries. Among these 64 pathways, 11 were associated with amino acid biosynthesis and degradation, whereas 10 were associated with Cofactor, Carrier, and Vitamin Biosynthesis.

Using the second approach (Supplementary Tables S9, 10), we observed 10 microbial features (including one species, *Escherichia coli*, and nine Metacyc pathways: superpathway of glycolysis, pyruvate dehydrogenase, TCA (Tricarboxylic Acid), and glyoxylate bypass; glyoxylate cycle; TCA cycle IV (2-oxoglutarate decarboxylase); superpathway of glyoxylate bypass and TCA; TCA cycle VII (acetate-producers); phytol degradation; TCA cycle VIII (helicobacter); and superpathway of glyoxylate cycle and fatty acid degradation) that consistently exhibited higher abundance in non-Western countries compared to Western countries. Interestingly, most of the consistently altered MetaCyc pathways were related to energy metabolism functions, such as the TCA cycle.

3.6. *Differentially abundant bacterial species and metabolic pathways between China and other countries*

Over the past decade, China's economy has developed rapidly, resulting in an increasingly Westernized lifestyle. However, significant differences remain between China and Western countries in terms of eating habits. Therefore, it is valuable to compare the differences in the gut microbiota between China and other countries with different diets and levels of development. Fig. 3.

After adjusting for covariates, we identified 97 species that exhibited significantly different abundances in China and other countries. (Fig. 4A and Supplementary Table S11). Most of these species belonged to the phyla Firmicutes, Bacteroidetes and Actinobacteria. The significantly differential distributions between CHN and NLD predominantly belong to the Actinobacteria, Bacilli, and Bacteroidia classes. The significantly differential distributions between the CHN and USA are mainly in the Bacteroidia and Clostridia classes, whereas the distributions in other countries are relatively evenly spread across all classes. *Bacteroides finegoldii* is the only species that shows significant differences when compared with all other countries. Apart from being notably less abundant than in the United States, it is significantly enriched compared to any other country. The disparity in the number of differential species between China and other countries was minimal. Notably, each country had a larger number of species significantly enriched than significantly depleted when compared to China (Fig. 4B). Furthermore, we conducted an assessment on species displaying significant abundance variations across three or more populations (Fig. 4C). We observed that some species belonging to Bacteroides, such as *Bacteroides plebeius*, *Bacteroides stercoris* and *Bacteroides finegoldii*, were more abundant in CHN, whereas *Roseburia sp CAG 182*, *Bifidobacterium adolescentis* and *Slackia isoflavoniconvertens* decreased in CHN relative to other countries.

We then explored the functional alterations between the CHN and other countries. At the pathway level, we found 92 pathways which were differentially abundant. (Supplementary Table S12) Fourteen pathways exhibited significant changes in abundance in three or more populations (Fig. 5). Interestingly, we found that there are five pathways related to the TCA cycle. Moreover, we found that eight pathways involved in menaquinone (vitamin K2) biosynthesis were significantly enriched in CHN compared to those in NLD. A trend towards higher enrichment was also observed when compared to the USA and GBR, although this was not statistically significant. Vitamin K is a series of

Fig. 3. The heat map illustrates pathways that demonstrate a greater abundance in non-Western countries than in Western countries. Each facet of the heat map represents the differential pathways between a specific non-Western country (CHN, IND, and MDG) and Western countries (USA, GBR, and NLD). The Coef values displayed in each cell represent the coefficient values derived from the MaAsLin2 analysis. USA, United States; GBR, United Kingdom; IND, India; NLD, Netherlands; CHN, China; MDG, Madagascar.

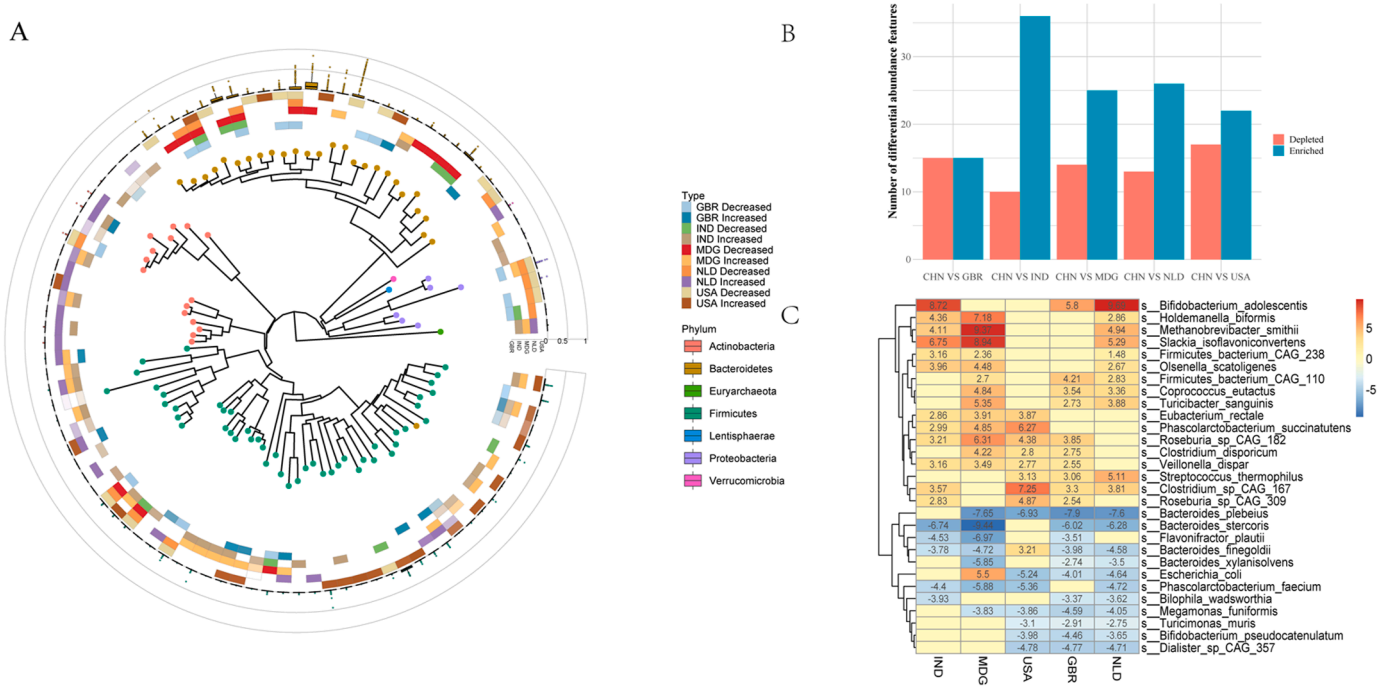

Fig. 4. Differential species between China (CHN) and other countries A Phylogenetic tree showing the evolutionary relationships among 97 species found to be statistically significant between CHN and other countries. The color of each node is consistent with the color of the corresponding phylum node located in the lower right corner. The outer heatmap is marked for significant differential species compared to each country. The color scale represents the degree of difference, the lighter the color, the smaller the difference, and vice versa. The outermost boxplots show the relative abundance of species in CHN. B The number of significant differential species between CHN and other countries **C** heatmap showing species with significant abundance changes between CHN and other countries. Only species with significant changes in three or more populations are shown. Values in heatmap cells indicate the beta coefficient from multivariate linear association testing with MaAslin2. USA, United States; GBR, United Kingdom; IND, India; NLD, Netherlands; MDG, Madagascar. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

structurally related compounds that share a common naphthoquinone ring structure but differ in the length and saturation of an attached lipophilic side chain. Menaquinones (MKn) are a form of Vitamin K and play an important role in human health. MKn is obtained from two important sources, first from certain animal-based foods and fermented foods, and second through production by gut microbiota [52,53].

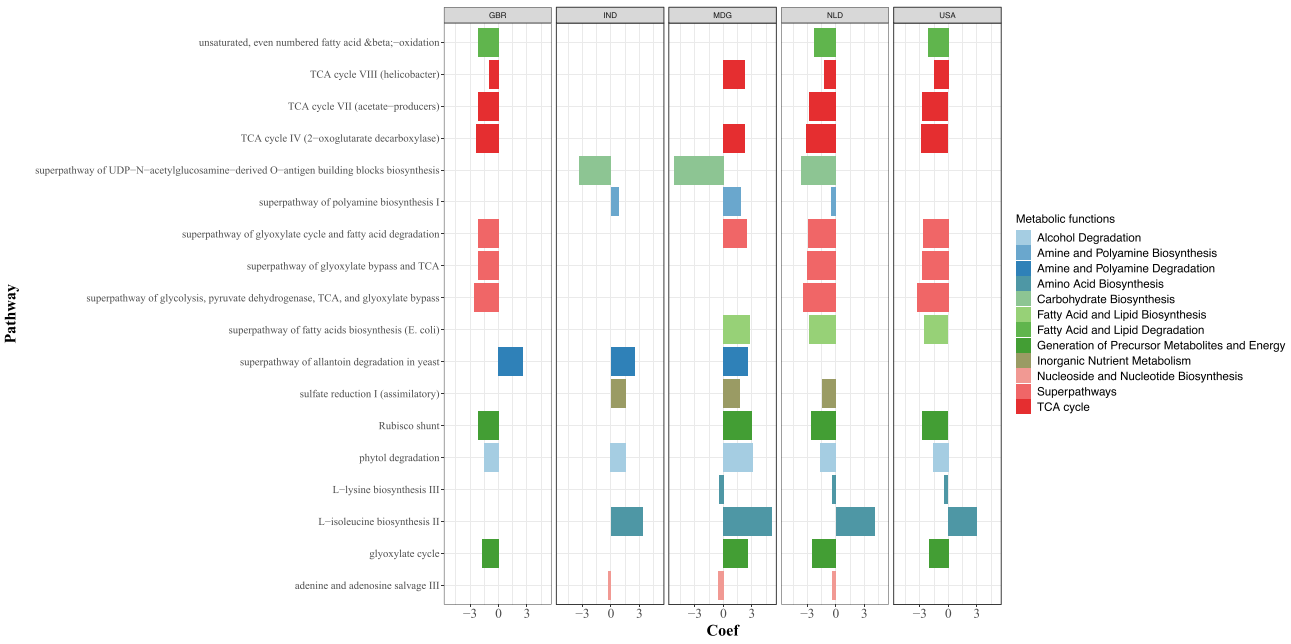

Fig. 5. Significant abundance pathway changes in three or more populations compared to China (CHN). Metabolic pathways are colored based on the category of metabolic function. The coefficient values on the x-axis represent the Coef values obtained from the MaAsLin2 analysis. USA, United States; GBR, United Kingdom; IND, India; NLD, Netherlands; MDG, Madagascar. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

Chinese dietary habits are associated with a relatively low intake of MKn-rich foods, such as cheese, milk, and yogurt. Cheese, milk, and yogurt are staple foods commonly found in the NLD diet. It is plausible that this dietary pattern could affect the composition of the gut microbiota in the CHN and NLD groups, which might lead to alterations in the abundance of pathways involved in menaquinone synthesis. Therefore, the results should be interpreted with caution, and additional studies are required to validate this interpretation.

4. Discussion

In this study, we analyzed shotgun metagenomic data from 2035 healthy individuals from six countries across four continents to explore population-level variations in gut microbiomes. The integration of shotgun metagenomic data allowed us to compare the overall taxonomic and functional composition of the gut microbiome across the six populations. A comparative analysis revealed consistently different gut microbial species and pathways in Western and non-Western countries. Furthermore, we identified several remarkable microbial species and their pathway characteristics in China.

In both taxonomic and functional composition of the gut microbiome, significant differences among the six populations based on Bray-Curtis dissimilarity measures were observed, indicating that the country specifies and influences the overall community structure of the gut microbiomes of healthy adults. Surprisingly, CHN was found to be more similar to USA and GBR in terms of overall microbial taxonomic and functional composition, as opposed to IND and MDG, which have diets that are more similar to those in China. Food plays a crucial role in shaping and maintaining the gut microbiota. Moreover, industrialization and economic development have had a profound impact on the composition of the gut microbiota. Given the rapid pace of industrialization and economic growth witnessed in China over the past decade, it is possible that alterations in both diet and industrialization have collectively contributed to changes in the gut microbiota. However, we currently lack sufficient data to conduct more in-depth research on this phenomenon. It is necessary to gather more comprehensive data in the future to study this phenomenon. Moreover, our results revealed that the degree of intra-population variation in microbial functional composition was relatively low compared to that in microbial taxa composition, indicating that the functional profile is relatively stable despite the high variability in the gut microbiota of individuals.

Despite striking differences across populations, the gut microbiomes of healthy adults share certain essential features. Therefore, we characterized the core gut microbiota and pathways prevalent in unrelated individuals to explore the critical features of the gut ecosystem. *Faecalibacterium prausnitzii* was the only species that appeared in more than 95 % of all individuals. *F. prausnitzii*, an important butyrate-producing bacterium found in the intestine, enhances gut barrier protection, exhibits anti-inflammatory effects [54,55] and strain-level genomic diversity, and SCFA metabolism may differ among strain clusters [56]. We found 13 species shared by more than 90 % of the investigated individuals (core species). It is worth mentioning that the majority of core species were SCFA-producing. SCFA-producing bacteria with elevated fecal SCFA concentrations may promote energy intake from fibers, inhibit opportunistic pathogens, and protect hosts against inflammation and colonic diseases [57]. The high prevalence of SCFA-producing bacteria across heterogeneous populations indicates that these bacteria are essential for the maintenance of host health. In terms of metabolic functions, we found nearly half of the pathways appeared in more than 90 % of individuals, irrespective of their country, diet, and lifestyle, demonstrating that metabolic function is more conserved and that core functions are essential for the entire microbial community. Considering that the function of the microbial community was less varied, this suggests that this function may be superior to taxonomy in defining a healthy gut microbiome.

Significant differences in gut microbial characteristics were observed between Western and non-Western countries. We found that *Prevotella stercorea* was more abundant in non-Western countries than in Western countries. Interestingly, recent research has found steady development of a trophic network centered around *Prevotella stercorea* in the gut microbiota of non-Western countries [58]. This trophic network may be important for maintaining a healthy gut microbiome. Moreover, we identified 69 pathways that were more abundant in non-Western countries than in Western countries. Notably, the superpathway of

L-tryptophan biosynthesis was more abundant in non-Western countries, consistent with previous studies showing that tryptophan produced by gut microbiota acts as a peripheral signal that influences host diet selection. It is plausible that the relatively lower meat intake in these countries might be a factor in the increased tryptophan biosynthesis. However, this assumption requires additional validation through metabolomic studies. Our findings provide some support for this hypothesis, suggesting a way in which the human gut microbiota adapts to varying dietary compositions and intakes, as inferred from an analysis of a comprehensive cross-population dataset.

The present study also highlights several remarkable microbial features that are characteristic of China. For instance, we observed that some species belonging to *Bacteroides*, such as *Bacteroides plebeius*, *Bacteroides stercoris* and *Bacteroides finegoldii*, were more abundant in CHN than in other countries. Interestingly, a previous study showed that *B. plebeius* was more enriched in the gut microbiomes of individuals from Japan than in those from North America, and that *B. plebeius* was associated with seaweeds [59]. Seaweeds and sea tangles are the main maricultural products in China and are a common food in people's daily meals. These findings suggest a potential role of dietary intake in influencing the composition of the gut microbiota.

This study had several limitations. First, owing to the absence of physiological variables, we were only able to investigate the effects of some available factors, such as age and sex, on the gut microbiota. Data concerning factors such as alcohol consumption [60,61], Bristol Stool Scale (BSS) [18], urban/rural lifestyles, and detailed dietary patterns would have contributed to a more comprehensive analysis. This study was conducted at the species level. Future studies between microbes and populations should be conducted at the strain level, as strains are the basic functional units that communicate with hosts, and different strains in the same species may exert different functions [56]. Another noteworthy aspect that requires further investigation is the abundance of bacteriocin biosynthetic gene clusters in the gut microbiome. Given their significant influence on community structure, composition, and diversity, the importance of bacteriocin biosynthetic gene clusters is undeniable. Therefore, further studies in this area are imperative for future research. Furthermore, the selecting criteria for healthy individuals to investigate the 'healthy microbiome,' there is a potential limitation for the selection/collider bias, where associations may be influenced by the selection criteria rather than solely based on real biological factors, and the outcome obtained in our study should be carefully interpreted. A major limitation is that the dietary intake data from FAOSTAT used in this study may not accurately represent the food habits of the cohort taken from those countries. It is possible that some of the Chinese individuals in our study might have adopted a more US-like diet, and the potential influence of Westernization on the dietary habits of the Chinese individuals included in our sample could have contributed to the observed similarities in the gut microbiota between the Chinese and US individuals. Despite these limitations, our findings revealed a noteworthy pattern in the gut microbiota of healthy individuals from both populations, suggesting that factors other than diet may play a significant role in shaping the gut microbiota. Our study highlights the diet-gut microbiota relationship in healthy individuals but emphasizes the need for more precise dietary data in future research. Comprehensive studies with detailed dietary assessments and microbiota analyses will help clarify the factors influencing gut microbiota composition and improve the accuracy and generalizability of our findings.

5. Conclusions

Collectively, significant differences were observed among the six populations in both the taxonomic and functional compositions of the gut microbiome, suggesting that country-specific factors play a role in shaping the overall community structure of the gut microbiome in healthy adults. Notably, when compared to other countries, China exhibited a similar overall microbial taxonomic and functional composition as the United States and the United Kingdom. Furthermore, we found that most core species in all individuals were SCFA-producing bacteria. Furthermore, our findings suggest that diet may partially account for variations in the gut microbiome, including some microbes and pathways; however, it is important to note that diet might not be the sole explanatory factor. We discovered consistent differences in the gut microbial characteristics between Western and non-Western countries. The results of the current study will inspire further clinical translations that consider paying attention to differences in the microbiota background and confounding factors.

Ethics approval and consent to participate

Not applicable.

Consent for publication

This manuscript has not been previously published. All authors have consented to the publication of the manuscript in this journal.

CRediT authorship contribution statement

Yanghao Sheng: Conceptualization, Methodology, Software, Writing – original draft. **Jue Wang:** Methodology, Software. **Yongchao Gao:** Formal analysis. **Yilei Peng:** Formal analysis. **Xiong Li:** Project administration. **Weihua Huang:** Investigation. **Honghao Zhou:** Project administration. **Rong Liu:** Methodology, Writing – review & editing. **Wei Zhang:** Conceptualization, Supervision, Funding acquisition, Writing – review & editing.

Declaration of Competing Interest

The authors declare that the publication of this paper has no conflict of interest.

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Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.csbj.2023.11.047.

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