The diversity and composition of the human gut lactic acid bacteria and bifidobacterial microbiota vary depending on age

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Abstract
Aging is associated with gut microbiota alterations, characterized by changes in intestinal microbial diversity and composition. However, no study has yet focused on investigating age-related changes in the low-abundant but potentially beneficial subpopulations of gut lactic acid bacteria (LAB) and Bifidobacterium. Our study found that the subjects’ age correlated negatively with the alpha diversity of the gut bifidobacterial microbiota, and such correlation was not observed in the gut LAB subpopulation. Principal coordinate analysis (PCoA) and analysis of distribution of operational taxonomic units (OTUs) revealed that the structure and composition of the gut bifidobacterial subpopulation of the longevous elderly group were rather different from that of the other three age groups. The same analyses were applied to identify age-dependent characteristics of the gut LAB subpopulation, and the results revealed that the gut LAB subpopulation of young adults was significantly different from that of all three elderly groups. Our study identified several potentially beneficial bacteria (e.g., Bifidobacterium breve and Bifidobacterium longum) that were enriched in the longevous elderly group (P < 0.05), and the relative abundance of Bifidobacterium adolescentis decreased significantly with the increase in age (P < 0.05). Although both bifidobacteria and LAB are generally considered as health-promoting taxa, their age-dependent distribution varied from each other, suggesting their different life stage changes and potentially different functional roles. This study provided novel species-level gut bifidobacterial and LAB microbiota profiles of a large cohort of subjects and identified several age-or longevity-associated features and biomarkers.

Key points
• The alpha diversity of the gut bifidobacterial microbiota decreased with age, while LAB did not change.
• The structure and composition of the gut bifidobacterial subpopulation of the longevous elderly group were rather different from that of the other three age groups.
• Several potentially beneficial bacteria (e.g., Bifidobacterium breve and Bifidobacterium longum) that were enriched in the longevous elderly group.

Keywords Gut microbiota · Longevity · Lactic acid bacteria · Bifidobacterium · SMRT sequencing

Introduction
Advances in modern medicine and better health care have drastically transformed human life expectancy. Longevity is a complex and multifactorial phenotype determined by self-factors (including gender, genetics, epigenetics, and psychology (Ostan et al. 2016; Simpson et al. 2019)) and environmental factors (including geographic location, atmosphere, ethnicity, lifestyle, and diet (Li et al. 2017; Wu et al. 2019)). Recent studies have confirmed that most of these factors would influence the human gut microbiota, which is closely linked with human health (Conlon and Bird 2015; Gaulke and
The gut microbiota undergoes continuous changes from birth to death, and the gut microbiota of older adults has been found to be less stable and less diverse, which could be contributed by changes in lifestyle, nutrition, metabolism, and increases in incidence of disease due to advancing age and use of corresponding medication (Tuinhon et al. 2010). Gut dysbiosis is associated with multiple diseases, such as metabolic diseases, gastrointestinal diseases, autoimmune diseases, and mental/psychological diseases (Heintz and Mair 2014; Icaza-Chavez 2013; Wang et al. 2017); thus, gaining an in-depth knowledge of the human gut microbiota and its association with aging and longevity would be critical in maintaining gut homeostasis and potentially helping achieve health in elderly. Another good reason for studying the elderly gut microbiota is to reduce and postpone age-related morbidities; however, there has been inadequate research in such aspects.

Some studies have investigated the gut microbiota of centenarians, as they are the best model for understanding longevity due to the lower incidence of chronic diseases as well as longer and healthier life in centenarians (Biagi et al. 2017; Kim et al. 2019; Odamaki et al. 2016). Most of these studies were performed predominantly in South Korea, Japan, and Western countries, and studies based on the Chinese elderly population have been limited to local areas, such as Bama, Guangxi (Wang et al. 2015); Suzhou and Nantong, Jiangsu (Bian et al. 2017); and Dujianyang and Ya’an, Sichuan (Kong et al. 2016). The gut microbiota is known to be highly personalized, and remarkable variations have been found among subjects of different geographic regions and ethnicities (He et al. 2018). Therefore, it would be of interest to design studies that include a relatively large sample size and cover a wide geographical spread, which could potentially provide more information and expand our understanding of the association between the complex phenomenon of longevity and the gut microbiota.

Significant differences were also observed in the composition and diversity between gut microbiota of younger adults and the elderly (Claesson et al. 2012; Maukonen et al. 2008). Other studies have further reported that such differences were not limited to the gut microbiota composition but the metabolism of the gut-inhabiting microbes (Tuikhar et al. 2019; Woodmansey et al. 2004), influencing the physiological function of the host. As early as more than 100 years ago, Elie Metchnikoff, the father of lactic acid bacteria (LAB), attributed the longevity of Bulgarian peasants to their yogurt consumption. He further theorized that the gut microbiota played an important role in human health maintenance and that lactobacilli could prolong human lifespan (Cavaillon and Legout 2016). Previous animal studies have shown that the gut microbiota is involved in modulating the host lifespan (Kato et al. 2018; Smith et al. 2017), especially Bifidobacterium and Lactobacillus, which play a crucial role in prolonging the lifespan of animals (Kaushal and Kansal 2012; Komura et al. 2013; Park et al. 2018; Zanni et al. 2017). Although LAB and Bifidobacterium comprise a relatively small fraction of the total gut microbial community, both of them are common inhabitants of human intestine that facilitate important physiological functions like nutrient absorption and regulation of host metabolism (Turroni et al. 2014). Owing to the important physiological role of intestinal LAB and Bifidobacterium, it would be of great scientific interest to characterize their composition in the gut. However, up to now, there has been limited information and complete profiles of the gut LAB and Bifidobacterium subpopulations at a fine taxonomic level, particularly in centenarians. For example, a previous study found that Bifidobacterium accounted for approximately 4.4 ± 4.3% of the gut microbiota of 91 volunteers from five northern European countries (Lay et al. 2005). Louis et al. (2007) found that Lactobacillus comprised only 0.01–1.8% of healthy human intestinal gut microbiota. Both studies reported relatively low levels of gut bifidobacteria and lactobacilli, and no further resolution of these taxa on the species level was provided as most other published studies. The relatively low proportion of gut bifidobacteria and lactobacilli together with the limited taxonomic resolution of most sequencing technologies has hindered the fine profiling of gut LAB and bifidobacteria. Indeed, the gut LAB and bifidobacteria are often underestimated or even undetected in conventional metagenomic studies of healthy adults (Pasolli et al. 2020). One added difficulty for detecting gut LAB and bifidobacteria in elderly is that older adults tend to have a smaller population of these potentially beneficial bacteria in their colon (Silvia et al. 2016).

To investigate the composition of gut LAB and bifidobacteria in older adults in China, here a cross-sectional survey was conducted. Fecal samples from 105 participants were collected from eight different provinces or provincial-level municipalities in China (nine cities). To identify an association between longevity and the microbial composition, subjects of four age groups were recruited based on the definition of the World Health Organization (https://www.who.int/), i.e., longevous elderly (≥ 90), elderly (75–87), young elderly (65–74), and young adult (20–28). Third-generation sequencing technology, such as the Pacific Biosciences (PacBio) single-molecule real-time (SMRT) sequencing platform, has been used to describe full-length 16S rRNA microbiota profiles of environmental samples to the species-level precision due to its ability to produce long sequence reads (Mosher et al. 2013). The high taxonomic resolution of third-generation sequencing can not be achieved by other sequencing platforms. The PacBio SMRT technology (Pacific Biosciences Inc., CA, USA) has been criticized for its relatively high sequencing error rate; however, the sequencing fidelity can be greatly improved by applying the
circular consensus sequencing (CCS) strategy together with statistical correction via the software pbccs (https://github.com/PacificBiosciences/unanimity) (Anslan et al. 2017). One way to improve the detection of relative minor taxa in the metagenomic samples would be to significantly increase the sequencing depth and coverage; however, this would largely increase the sequencing cost. Another way that may improve the detection sensitivity of target microbes would be to use taxa-specific primers instead of common universal primers. This study thus opted for using two pairs of target-specific primers that were previously designed by our laboratory to amplify LAB and Bifidobacterium 16S rRNA genes from human fecal samples (Hou et al. 2018; Sakandar et al. 2020). The application of these two pairs of primers in combination with the PacBio sequencing technology would be able to generate species-level LAB and bifidobacterial microbiota profiles. This will help us to study potentially beneficial bacteria LAB and Bifidobacterium with low abundance in the gut of longevity people.

Our overall objective was to improve the understanding of the taxonomic composition of LAB and bifidobacteria in the intestinal tract of elderly of different age ranges. The generated data could serve as reference information in the intestinal tract of elderly of different age ranges. The generated data could serve as reference information in the intestinal tract of elderly of different age ranges. of the taxonomic composition of LAB and bifidobacteria in the intestinal tract of elderly of different age ranges. The generated data could serve as reference information in the intestinal tract of elderly of different age ranges.

### Materials and methods

#### Subjects and sample collection

This observational study mainly compared the gut LAB and Bifidobacterium of subjects of different age groups. Fifteen longevous elderly (aged ≥ 90; mean ± SD = 91.06 ± 1.28; “L” group), 46 elderly (aged 75–87; mean ± SD = 82.06 ± 3.82; “E” group), 12 young elderly (aged 65–74; mean ± SD = 69.08 ± 3.23; “YE” group), and 32 young adults (aged 20–28; mean ± SD = 23.06 ± 2.12; “Y” group) were recruited from nine Chinese cities across eight different provinces or provincial-level municipalities (Supplemental Table S1). All recruited elderly subjects were living in the county town independently or with their children. Every participant had to fill out a written consent form and a questionnaire for recording specific information, including age, gender, previous medical history, antibiotics and probiotics use, and main dietary characteristics.

The inclusion criteria were (i) no antibiotics or probiotics use and no significant change in dietary structure 3 months before starting the study; (ii) no chronic diseases, such as uncontrolled hypertension or diabetes, malignant neoplasia, and gastrointestinal diseases; and (iii) participants did not know each other and there was no direct relationship between them. Subjects were required to self-collect fecal samples into sterile stool specimen collection tubes provided in advance and return the samples to members of the research team. Samples were then frozen immediately in liquid nitrogen and transported to the laboratory within 12 h. After arriving in the laboratory, all samples were kept at –80 °C until further analysis.

### Extraction of metagenomic DNA, amplification of target fragments, and SMRT sequencing

According to the manufacturer’s instructions, DNA was extracted from all samples using the QIAamp Fast DNA Stool Mini Kit (Qiagen GmbH, Hilden, Germany), and the quality of the extracted DNA was examined by 1% agarose gel electrophoresis and a NanoDrop spectrophotometer (Thermo Fisher Scientific, Waltham, USA). All extracted DNA samples were temporarily stored at –20 °C. A reagent blank control consisted of all reagents used during sample handling but contained no sample was processed in parallel to detect DNA contamination.

The purified metagenomic DNA was used as templates to amplify the gene fragments from all samples by PCR using specific primer pairs targeting LAB [forward (5′-GCTCAG GAYGAACGCGYG-3′) and reverse (5′-CACCGCTACACA TGRADTTC-3′)] (Hou et al. 2018) and Bifidobacterium [forward (5′-GTAAGGTGGAAGCGCTGTGCAATAA-3′) and reverse (5′-GAAAGAGAAGGCCACACTAGTAA-3′)] (Sakandar et al. 2020). A 16-nt barcode was added to each end of the primer to distinguish between different samples in the same library. The specific amplification conditions were as follows: 95 °C for 1.5 min, 95 °C for 30 s, 60 °C for 40 s, 72 °C for 1 min, 28 cycles, and then 72 °C for 7 min. The amplification reaction was performed in an Applied Biosystems PCR (Agilent Technologies, Santa Clara, USA) instrument, and the PCR reaction products were checked by 2% agarose gel electrophoresis and stored in a –20 °C refrigerator for later use. Amplicons (2 μg) were used to construct DNA libraries with the Pacific Biosciences Template Prep Kit 2.0 (Pacific Biosciences Inc., CA, USA) following the manufacturer’s recommendations, and the DNA libraries were sequenced using P6/C4 chemistry (PacBio RS II instrument) in the circular consensus sequencing mode.

### Analysis of high-quality sequences

Quality control of raw data was processed by the RS_ReadsOfInsert1 protocol (available in the SMRT Portal version 2.7) (Hou et al. 2015). The minimum cycle sequencing number, minimum prediction accuracy, minimum insertion read length, and maximum insertion read length were 5, 90, 650, and 850, respectively, for LAB sequences. Slightly different restrictive filter criteria were applied to the Bifidobacterium...
sequences due to the shorter anticipated amplicon length. The minimum cycle sequencing number and minimum prediction accuracy remained 5 and 90, while the minimum and maximum insertion read length for *Bifidobacterium* sequences were set to 600 and 800, respectively. Then all sequences were classified into different samples according to the primer barcodes. High-quality sequences were extracted through the Quantitative Insight into Microbial Ecology (QIIME) software package (version 1.7) (Caporaso et al. 2010a, b) after barcode removal.

The main steps in LAB and *Bifidobacterium* sequence analysis are as follows: (i) PyNAST (Caporaso et al. 2010a, b) was used to calibrate and align the sequences, and UCLUST (Edgar 2010) was used to select the most abundant sequence in each cluster as the representative sequence by clustering at 100% sequence identity. Then the representative sequences were classified into operational taxonomic units (OTUs) at a 97% threshold. (ii) The Chimeraslayer (Haas et al. 2011) tool was used to detect and remove any sequence containing chimeric OTUs. Each representative OTU was compared with the Ribosomal Database Project (RDP-II, Release 11.5) (Cole et al. 2007), Silva (version 128) (Quast et al. 2013), and Greengenes (version 13.8) (DeSantis et al. 2006) databases at an identity threshold of 80%, and the results were integrated using internal scripts. (iii) A de novo taxonomic tree was constructed from the representative OTU set using FastTree (Price et al. 2009) for calculating alpha-and beta-diversity. A similar process was employed for analyzing the *Bifidobacterium* sequences except for the final step of taxonomic annotation. One major difference between the two datasets was that the rpsK gene was the target gene fragment for *Bifidobacterium*. Thus, the annotation of *Bifidobacterium* was based on a reference database constructed from known rpsK gene sequences of *Bifidobacterium* species extracted from public databases (Sakandar et al. 2020).

**Statistical analyses**

All statistical analyses were performed using R software (version 4.0.2) (Chambers 2008). Unless otherwise stated, data are expressed as mean ± SD. To evaluate the structural difference between microbiota of different groups of samples, principal coordinate analysis (PCoA) was performed by vegan and visualized using ggpur. Wilcoxon and Kruskal–Wallis tests were used to evaluate microbial differences between two or multiple groups with a cut-off confidence level of 95%, and the corr.test function in the “psych” package was used for Spearman correlation analysis. Analysis of similarity (ANOSIM; 999 permutations) was applied to detect significant differences between groups. All graphical presentations were generated under the R environment.

**Results**

**Dataset characteristics and alpha diversity of the gut LAB and Bifidobacterium microbiota**

A total of 105 volunteers were recruited from nine regions in China (Fig. 1a; Supplemental Table S1), and 343,580 high-quality sequences (252,930 *Bifidobacterium* and 90,650 LAB sequences) were generated from all samples. The values of Shannon diversity index of *Bifidobacterium* of most groups were similar except that the young elderly group showed a significantly higher value than the longevous elderly (*P* < 0.05). The young adult group showed a significant lower number of observed *Bifidobacterium* OTUs than the young elderly and elderly groups (*P* < 0.01 in both cases), while no significant difference was observed between the young adult and longevous elderly groups (Fig. 1b). There was no significant difference in the Shannon diversity and number of observed species of LAB among the four groups (Fig. 1c). Moreover, as the age increased, the Shannon diversity and the number of observed species of the *Bifidobacterium* subpopulation decreased, though the correlation was non-significant (*P* < 0.06), and such trends were not observed in the LAB microbiota (Fig. 1d, 1e).

**Beta diversity of the gut LAB and Bifidobacterium microbiota**

To visualize the structural difference in the gut *Bifidobacterium* and LAB communities between different age groups, PCoA analyses (Bray–Curtis distance) were performed. The PCoA plot of the gut *Bifidobacterium* exhibited no distinct clustering pattern (Fig. 2a), except that most of the symbols representing the longevous elderly group were distributed to the right side of the PCoA score plot though there was some extent of overlapping with symbols representing the other groups. Nevertheless, such result suggested that the gut bifidobacterial microbiota structure of the longevous elderly group was likely more different from that of the other age groups. Further analysis by ANOSIM confirmed that the bifidobacterial microbiota structure of the longevous elderly group was significantly different from the other groups (*R* = 0.066 to 0.114; *P* = 0.001 to 0.015; Fig. 2b). The results of ANOSIM also revealed a significant *P* value between the young adult and young elderly groups though with a small *R* value (*R* = 0.05, *P* = 0.003), suggesting only a weak difference; and no significant difference between the young elderly and elderly groups (*R* = 0.02, *P* = 0.23; Fig. 2b). In contrast, symbols representing the gut LAB microbiota of
the young adult group clustered distinctly from the three elderly groups, and the cluster shifted in a different direction from that of the elderly, suggesting that the young adults had an apparently different gut LAB subpopulation from the older subjects (Fig. 2c). Significant differences in the gut LAB microbiota between the young adults and the elderly groups were confirmed by ANOSIM ($R^2 = 0.095$ to $0.11$; $P < 0.001$, young adult group versus all three elderly groups), while no significant difference was found among the three elderly groups (Fig. 2d).

**Composition of gut bifidobacterial and LAB microbiota of four age groups**

A total of 30 species of bifidobacteria were detected in all samples. Several dominant species with high abundance in the overall bifidobacterial microbiota were *Bifidobacterium breve* (29.93%), *Bifidobacterium catenulatum* (24.74%), *Bifidobacterium adolescentis* (20.74%), *Bifidobacterium dentium* (12.97%), and *Bifidobacterium bifidum* (5.01%). It is interesting to note that comparatively more *B. breve* sequences (67.59%) were detected in the longevous elderly group, while the young adult group had comparatively more *B. adolescentis* (42.30%). Moreover, the relative abundance of *B. adolescentis* showed a decreasing trend with aging (42.30% in young adult; 18.54%, 11.23%, and 5.71% in young elderly, elderly, and longevous elderly groups, respectively; Supplemental Fig. S1a).

A total of 118 species of LAB were detected in all samples. Several dominant species with high abundance in the overall LAB microbiota were *Streptococcus sanguinis* (17.92%), *Vagococcus fessus* (13.78%), *Lactobacillus saerimneri* (12.80%), *Lactobacillus murinus* (6.33%), and *Streptococcus parauberis* (4.86%) (Supplemental Fig. S1b). The three elderly groups were characterized by having a high relative abundance of *V. fessus* (17.04%) and *S. sanguinis* (17.02%), while *L. saerimneri* (38.71%) and *S. sanguinis* (19.97%) were the dominant species in the young adult

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**Fig. 1** Map of sample collection and alpha diversity of gut lactic acid bacteria (LAB) and *Bifidobacterium* of different age groups. One hundred and five volunteers were recruited from nine cities in China. The legend at the bottom right shows the sample size in each region (a). Shannon diversity index and average number of observed species of the subpopulations of gut LAB (b) and *Bifidobacterium* (c) of the longevous (L), elderly (E), young elderly (YE), and young (Y) groups. Correlation between subjects’ age with Shannon diversity index and number of observed species of the subpopulations of gut LAB (d) and *Bifidobacterium* (e). * $P < 0.05$; ** $P < 0.01$.
The different profiles of dominant species of the gut LAB microbiota of young adults and the elderly groups were consistent with the distinction in LAB microbiota structure between young and elderly subjects observed in PCoA analysis, suggesting that these dominant species were the main contributors responsible for the structural difference in the gut LAB microbiota.

To identify age-specific differential abundant species, the relative abundances of Bifidobacterium and LAB species detected in the four age groups were compared (Fig. 3; Supplemental Table S2). There were 3 species of Bifidobacterium and 14 species of LAB with significant differences in relative abundance between the longevous elderly group and the other three elderly groups, and 2 species of Bifidobacterium and 1 species of LAB with significant difference between the young adult group and the elderly group (P < 0.05; Fig. 3). On the other hand, significantly more B. adolescentis and B. bifidum were detected in the young adult group than the other elderly group (P < 0.01; Fig. 3). For the gut LAB microbiota, the differential abundant species between the young adult group and the three elderly groups were L. murinus, L. saerimneri, Lactobacillus sanfranciscensis, V. fessus, and Lactobacillus brevis (P < 0.05; Fig. 3). It is worth noting that significantly more L. saerimneri sequences were detected in the young adult group than the three elderly groups (P < 0.05; Fig. 3).

**Core, common, and unique OTUs of Bifidobacterium and LAB in the four age groups**

The study further investigated the distribution of common and unique OTUs of four different age groups. A total of 1006 Bifidobacterium OTUs were identified in the complete dataset, nine of which could not be identified to the species level. Most of the OTUs belonged to B. breve (51.5%), B. catenulatum (35.39%), and B. adolescentis (10.54%) (Fig. 4a). A total of 113 LAB OTUs were identified, 14 of which could not be identified to the species level. Most OTUs belonged to Streptococcus salivarius (20.35%),...
Lactobacillus delbrueckii (7.96%), Streptococcus parasanguinis, and Lactobacillus mucosae (5.31%) (Fig. 4b). It was observed that the elderly group contained more unique Bifidobacterium (13,451) and LAB (3,003) OTUs, while the longevous elderly had the least unique OTUs (Bifidobacterium 371 and LAB: 63 unique OTUs, respectively).

The concept of “core microbiota” is used to identify and describe key microorganisms that are stable and permanent in a microbial community (Astudillo-Garcia et al. 2017). Here, the core OTUs is defined as OTUs that existed in at least 50% of the samples of each group (Wu et al. 2019). The longevous elderly group had a higher richness in Bifidobacterium OTUs compared with the other three groups, containing 158 unique core OTUs, all of which belonged to the species B. breve except one B. catenulatum OTU. The longevous elderly group and the young adult group shared 15 core OTUs, which were identified as B. breve and B. bifidum (Fig. 4c). The three elderly groups shared 24 core LAB OTUs, only three of which could not be identified to the species level. The dominant core species included S. salivarius (20.83%), L. mucosae, and L. delbrueckii (12.5%). No common core LAB OTU was found between the three elderly groups and the young adult group (Fig. 4d). Contrasting to the other three groups, no unique core Bifidobacterium and LAB OTUs were identified in the elderly group. In conclusion, for bifidobacterium, the longevous elderly group had the lowest number of unique OTUs, but the largest number of unique core OTUs. While in the elderly group, the opposite was true.

**Correlation networks between gut Bifidobacterium and LAB**

To infer potential interactions among the gut Bifidobacterium and LAB communities, co-occurrence networks of bacterial species were constructed for each age group based on taxonomic distribution (only included major species with over 1% average relative abundance; Fig. 5). Generally, the co-occurrence networks of the longevous elderly group exhibited greater complexity than the other groups (number of significant correlations: longevous elderly: 73; elderly: 18, young elderly: 61, and young adult: 30). Almost all identified associations were positive correlations. Three negative correlations were found in the longevous elderly groups. The dominant species B. breve showed strong negative correlation with Enterococcus faecalis (r = −0.73; P = 0.001) and B. catenulatum (r = −0.78; P = 0.001), and the two species, B. longum and B. catenulatum, also correlated negatively (r = −0.73; P = 0.001). For the young adult group, two negative correlations were identified, which was between B. adolescentis and B. catenulatum (r = −0.65; P < 0.001); L. saerimneri and S. sanguinis (r = −0.66; P < 0.001; Fig. 5).
Discussion

The human gut represents a highly complex ecosystem densely colonized by countless microorganisms, and the ecosystem has now shown potential to be a promising target for improving the health of the elderly and extending healthy lifespan (Biagi et al. 2013; Turroni et al. 2014). Bifidobacterium and LAB are human gut inhabitants, and many commercially developed probiotic products contain these bacteria, as they might have an important impact on human health maintenance. It was reported that some of these bacteria were deprived in the elderly (Claesson et al. 2012; Hopkins and MacFarlane 2002), which might hinder healthy aging. So, it would be of interest to characterize the gut bifidobacterial and LAB microbiota in older adults. However, due to their low abundances in the intestine, conventional metagenomic analysis might easily underestimate the amount and diversity of these taxa. It would thus be necessary to apply new methods to accurately detect and profile these microbial subpopulations in human fecal samples. To improve the understanding of how the gut microbiota was related to longevity, this study conducted a cross-sectional survey to generate accurate taxonomic profile of the gut LAB and bifidobacteria in elderly of several age ranges, and the gut microbiota of the elderly was compared with that of young adults. This study was unique and novel in applying the PacBio SMRT sequencing technology to sequence amplicons generated by bifidobacteria-and LAB-specific primers, targetting to the gut LAB and bifidobacteria microbiota.

The alpha diversity reflects the diversity and richness of microbial communities. Our research found that the diversity and number of gut Bifidobacterium decreased as the elderly subjects’ age increased. In contrast, although the gut LAB subpopulation of young adults and elderly groups differed significantly, no significant correlation was found between the gut LAB diversity and richness with age. Our results suggested that the gut Bifidobacterium microbiota was more affected by age than the LAB subpopulation. Contradictory results have been reported on the gut microbial diversity and composition in elderly. For example, some studies...
observed that longevous elderly had a greater gut microbiome diversity than the young adults, and it was proposed that having a higher diversity of gut microbiota might be health-promoting in long-lived elderly (Kong et al. 2016, 2019). Another study found that the gut microbial diversity of centenarians and young people was lower than that of the elderly (Wu et al. 2020). Our study observed that the diversity of Bifidobacterium in longevous elderly was significantly lower than that of the elderly group, and there was indeed a decreasing trend in the diversity of gut bifidobacterial microbiota with age, which was in line with the results of Hopkins and MacFarlane (2002). Collectively, these conflicting findings suggested that the alpha diversity only measured the outcome of ecological processes but not...

Fig. 5 Co-occurrence network analysis of gut lactic acid bacteria and Bifidobacterium of different age groups. The four independent networks correspond to the longevous (L), elderly (E), young elderly (YE), and young (Y) groups, respectively, and significant correlations with Spearman’s correlation coefficient > 0.6 or < − 0.6 are shown.

Each network node represents a bacterial species, node size represents the relative abundance of the species, and the line represents significant correlation between two species. Positive and negative correlations are shown in gray and red, respectively.
the ecological process itself. Shade pointed out that a high diversity is not necessarily “better” or “healthy”, but “a starting point for further inquiry of ecological mechanisms rather than an “answer” to community outcomes” (Shade 2017). Therefore, it might be more meaningful to focus on analyzing age-dependent distribution of specific gut probiotic species/strains and their role in longevity.

Although the large cohort study of Bian et al. (2017) showed that the overall microbiota composition of the healthy aged group was similar to that of people decades younger, the gut microbiota differed little between individuals from the ages of 30 to >100. Wu et al. (2020) found that the gut bacterial communities in centenarians clustered separately from those of the young and elderly subjects. Our results found that the composition of the gut bifidobacterial microbiota of the longevity elderly group was different from the other age groups. The major age differential species were B. breve, B. longum, and B. adolescentis; especially, a higher relative abundance of B. breve and B. longum was detected in the gut of the longevity elderly group. Wang et al. (2015) reported similar results that comparing with young elderly and young adults significantly more gut B. longum was found in centenarians in Guangxi, China, and the abundance of B. adolescentis decreased significantly with age. Kato et al. (2017), however, reported that B. longum was widely detected in healthy Japanese gut of various ages, and B. breve was more abundant in young people. The disagreement could be related to the different ethnicities of recruited subjects and differences in other environmental factors, such as living habits, diet, and geographic region (He et al. 2018).

The B. breve species was found to effectively reduce intestinal inflammation and might prevent and improve mental diseases, such as Alzheimer’s disease (Lecceese et al. 2020). A clinical trial found that providing B. breve-containing probiotics with moderate resistance training to healthy elderly improved subjects’ mental condition, body weight, and bowel movement frequency (Inoue et al. 2018), supporting that ingesting B. breve was beneficial in promoting healthy aging. Our results found that, apart from being a dominant species, many core and unique OTUs of the longevity elderly group belonged to B. breve. This species showed a strong negative correlation with E. faecalis, suggesting a possible inhibitory effect of B. breve against potentially pathogenic gut bacteria. Such result also supported that B. breve was a beneficial species that helped promote longevity. There is a growing awareness concerning the benefits of the prophylactic and personalized use of probiotics in maintaining health and improving quality of life of the elderly population (Tsai et al. 2021); thus, it would be worth further investigating the beneficial effect of B. breve on promoting healthy aging on a personalized level. Our study also found that the longevity elderly group had the highest number of unique core OTUs. It has been reported that the gut microbiome would become more unique as a person reached middle age (from 40 to 50 years old), but similar changes were not observed in older people who were in poorer health (Wilmsanski et al. 2021).

The young adults and elderly subjects had significantly different profiles of gut LAB microbiota. Some age-associated species were identified. For example, L. murinus, L. sanfranciscensis, V. fessus, Lactobacillus curvatus, and Lactobacillus helveticus were significantly more prevalent in all three elderly groups. In contrast, the species L. saerimneri, Lactobacillus ruminis, Ruminococcus flavefaciens, Weissella confusa, and Weissella ghanensis were more prevalent in young adults. A previous report found that L. saerimneri had potent tumor necrosis factor (TNF)-inhibitory activity (70% inhibition), while L. ruminis exhibited immunostimulatory properties by activating TNF production in THP-1 monocytes. Thus, both human gut-inhabiting species could reduce inflammation and organ damage (Taweechotipatr et al. 2009). Our results showed a significantly negative correlation between L. saerimneri and S. sanguinis, suggesting a potential inhibitory effect of L. saerimneri on S. sanguinis, which is an oral pathogenic anaerobe associated with dental caries and periodontitis (Wuersching et al. 2021). Thus, aging might be associated with the decrease or even absence of beneficial species, such as L. saerimneri and L. ruminis, in the elderly gut.

Correlation analyses of the current dataset revealed that there were close interactions among the gut bifidobacterial and LAB subpopulations and that the complexity of microbiota networking varied with age, with the highest complexity of interactions among these species in the longevity elderly group. In our study, B. catenulatum was the dominant species in the four age groups, which was negative association between some dominant species (B. breve and B. longum). The results of a previous study were not consistent with our observation in that B. catenulatum is not detected in Italian centenarians (Drago et al. 2012). A study of the intestinal microbiota of adult patients with hepatitis B virus-induced chronic liver disease showed that the prevalence of B. catenulatum significantly decreased compared with healthy subjects (Xu et al. 2012), while a study in allergic diseases in infants at 1 month of age had a higher prevalence of the B. catenulatum (Suzuki et al. 2007). Thus, this variation indicated that B. catenulatum may not have the same beneficial effect in different populations, and the next study needs to further clarify its role in the gut of longevity elderly people. Although a “healthier” gut microbiota might facilitate healthy aging, the digestive system of the elderly inevitably suffers from a series of aging and degeneration from structure to function, such as decrease in chewing ability of the mouth, smaller esophageal contraction, and lower digestion and utilization rate of...
food (Soenen et al. 2016); aging-associated degeneration in physiological function might relate to systemic shift or variation in individual functional microbes in the elderly gut. For instance, our dataset showed that B. dentium was widely distributed among the elderly, and a previous study sequenced the genome of B. dentium and revealed that it was well adapted to the human oral cavity in subjects over 60 years old (Ventura et al. 2009). Moreover, normally, it would be hard for certain oral bacteria, e.g., Streptococcus oralis, to reach the gut due to gastrointestinal tract barriers (Odamaki et al. 2016), but our results showed that some of these bacteria were enriched in the elderly gut microbiota. These data might imply that the decline in gastrointestinal tract functionality in the elderly could lead to significant changes in the distribution of some oral microbes (e.g., B. dentium and S. oralis), leading to enrichment in the gut.

As the current study was a cross-sectional survey, it is not easy to know whether the health-associated features of the elderly or longevous elderly gut microbiota were already present at a younger age, and/or if they were somewhat related to subjects’ past lifestyle. Moreover, even though it would be interesting to perform long-term tracking of changes in the human gut microbiota to confirm if certain taxa would be lost or reacquired at certain life stages of longevous individuals, however, it would be very difficult to perform longitudinal studies of such nature, as it would be extremely hard if not impossible to predict human longevity.

In summary, our study found interesting age-dependent features of the species-level gut bifidobacterial and LAB microbiota. The study found that the intestines of long-lived elderly have unique characteristics, and identified the potential probiotic species, B. breve and B. longum, which were enriched in it. Although it is still early to draw any causal relationships between healthy aging with potentially beneficial gut microbial subpopulations, these age-related species might serve as microbial biomarkers of aging and/or longevity.

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Author contribution ZHS, TM, and CQY conceived and designed the experiments; XS, HJ, ZG, QXZ, LYK, and HPZ contributed sample collection, reagents preparation, and data analysis. All authors were involved in the manuscript preparation.

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Data availability The raw sequencing dataset and analysis codes generated in this study have been deposited to the NCBI SRA database (BioProject: PRJNA702904).

Code availability The code related to the research was stored under https://github.com/TengMa-Cleap/Longevity-project.

Declarations

Ethics approval The study protocol was reviewed and approved by the Ethical Committee of the Inner Mongolia Agricultural University (Hohhot, China).

Consent to participate Not applicable.

Consent for publication Not applicable.

Conflict of interest The authors declare no competing interests.

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