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Original Article

Aging Gut Microbiota Markers, a Chinese Local Study

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SUMMARY

Background: Age-related physiological changes result in higher susceptibility to infections and geriatric emergency. Gut microbiota are accepted as an essential factor in the aging and shown geography-dependent.
Methods: Faecal samples of 81 volunteers (40 elderly aged 60-78 years, 41 young adult aged 21-38 years) living in Shenyang were collected and bacterial DNA was extracted for 16S rRNA gene PCR - denaturation gradient gel electrophoresis (DGGE) analysis. Maker genes related to aging were identified through gene sequencing and some were quantified by quantitative PCR (qPCR).
Results: The alternations in microbiota diversity and composition between young adult and elderly groups were demonstrated by 16S rRNA gene PCR - DGGE analysis, eight markers related to aging were found. Putative species corresponding to these markers were identified through gene sequencing, which might have potential association with the common digestive dysfunction and susceptibility to infections in elderly people. The qPCR methods of three species were established, two were testified to be significantly changed in the elderly.
Conclusion: The changed bacterial species in our study suggested the role of gut microbiota in Chinese aging and supported the location-dependent microbial alternation in aging. It provide a better understanding of microbial factors related to aging in Chinese guts, and provided suggestion for healthy aging.

1. Introduction

The gut microbiota plays a vital role in health maintenance of the host.[1] These important contributions of gut microbiota to host physiological and psychological functions can rationalize its significant and complicated involvement in host aging. Scientists all over the world have been trying to reveal the mysterious relationship between gut microbiota alternation and aging process.[2,3] Though independent studies have agreed that the old individuals were characterized by a lower microbiota diversity[4] and enriched pro-inflammatory bacteria,[5,6] more detailed age-related changes are very controversial with different results. A decrease in *Firmicutes* and increase in *Bacteroidetes* amount with age was observed in an Indian family study,[7] consistent with an Irish study where the core microbiota of the elderly were distinct from young adult with a striking increase in *Bacteroidetes* and *Clostridium* groups.[8] However, in a study on Italian elderly, *Bacteroidetes* proportion remained unchanged.[4] Furthermore, microbiota composition study performed in 4 European countries in Germany, France, Italy and Sweden reported that, *Eubacterium rectale-Clostridium coccoides* increased with age in the German population, but declined with age in Italian,[9] a similar decrease were observed in Dutch people.[10] In addition, it was found that *Faecalibacterim prausnitzii* decreased with age in the Swedish and Italian, but not in the French or German. *Atopobium* increased in German and Swedish elderly, but French and Italian elderly were not observed this change.[9] Even if the "study effect" or "method effect" cannot be excluded, these geography-dependent results in aging may be contributed to different human ethnics, lifestyle and eating habits, highlighting the importance of country specificity involved in age-related changes.

As for China, a recent research conducted in South China (Bama and Nanning) focused on centenarians and younger elderly reported that the amount of *Roseburia* and *Escherichia* were significantly higher in centenarians, whereas *Lactobacillus*, *Faecalibacterium*, *Parabacteroides*, *Coprococcus*, *Butyricimonas*, *Sutterella*, *Mitsuokella*, *Megamonas*, and *Akkermansia* was significantly decreased.[11] Another study carried out in a South China village (Gaotian) famous for its longevity revealed that the *Lactobacillus*, *Enterobacteriaceae*, *Enterococcus*, *Clostridium perfringens* and *Bacteroides* in Gaotian elderly villagers were significantly higher than control group.[12] A culture-based study in Jiamusi (located in Northeast China) indicated that there were more *Bifidobaterium* and *Lactobacillus* in younger students compared with elderly teachers.[13] The different results of these studies may be due to the different locations of participants and their living habits.

Considering the location-dependent changes (Table S1) and the role of gut microbiota with aging,[1,14] we took this opportunity by using denaturation gradient gel electrophoresis (DGGE) and quanti-

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tative PCR (qPCR) to look into elderly living in Shenyang, a typical city in Northeast China, seek potential microbial markers related to aging, and offer suggestions on healthy aging in Chinese population. Although high-throughput sequencing has been an developing technology in microbiological analysis, DGGE method also has potential to give information of gut microbiota due to its sensitivity and strain-specific typing possibility.

2. Materials and methods

2.1. Subject recruitment and sample collection

Total 81 healthy subjects entered this study, including 40 elderly (60 to 78 year, 24 female and 16 male) and 41 young adult (21 to 38 year, 13 female and 28 male). This study was followed the principles outlined in the Declaration of Helsinki, and approved by Ethics Committee at General Hospital of Shenyang Military (Shenyang, China). All donors, with normal physical index in the physical examination center and without past history including abdominal operation and serious disease, gave consent to be included in the study after receiving appropriate information, and were instructed on how to collect and store their stool samples and provided a convenient sample collection kit. All subjects had lived in Shenyang for at least 6 months before sampling, and who had recently used medication including antibiotics (in 3 months) or probiotic supplementation (in 1 month) were excluded from the study. Feces were stored at -80 °C and analyses were performed within 3 months. The details of demographics of two group of subjects (young adult and elderly) enrolled in this study were indicated in Table S2.

2.2. DNA extraction and PCR amplification

Total microbial DNA was extracted using QIAamp DNA Stool Mini Kit (Qiagen, Duesseldorf, Germany) following manufacture's protocol. Final DNA concentration was determined by NanoDrop 2000 Spectrophotometer (Thermo Scientific, the United States) and kept at -20 °C for storage. Bacterial 16S rRNA gene V3 region was amplified in an automated thermocycler (Mastercycler personal, Eppendorf, Hamburg, Germany) with the primers 341f plus GC clamp and 518r.

2.3. DGGE

DGGE was performed using the Dcode™ Universal Mutation Detection System (Bio-Rad Laboratories, Hercules, CA, USA) run at 60 °C, 120 V for 8 h, in 1 × TAE buffer (Tris-acetic acid - EDTA buffer, 0.02 mol/ l Tris, 0.5 mmol/l EDTA, 0.01 mol/l acetic acid, pH 8.2). Polyacrylamide gels (8% v/v) were prepared using acrylamide/bis acrylamide 37.5:1 in 1 × TAE with a denaturant linear gradient of 35–60% (a 100% denaturant solution contained 7 mol/l urea and 40% v/v formamide). A DGGE Mark II (Nippon Gene, Toyama, Japan) was used at a concentration of 100 ng per lane. Gels were stained with rapid silver staining method and visualized by Tannon GIS2010 Image system (Tanon, Shanghai, China).

2.4. Sequencing

DGGE gels were stained with SYBR Gold fluorescent dye and compared with the rapid silver stained. The bands stained with SYBR were excised from gel and socked overnight with ddH_2O, then was amplified with 341f plus GC clamp and 518r primers as described above and purified with a commercial gel extraction kit (Qiagen, Valencia, CA). The purified bands were ligated with pMD18-T vector (Takara, Dalian, China) and transformed into *E. coli* JM109 Cells (Takara, Dalian, China). Cloned genes were re-amplified with M13-F and M13-R primers, and selected for sequencing (Invitrogen, Shanghai, China). The sequences were analyzed with the BLAST program at the NCBI website (http://www.ncbi.nlm.nih.gov/blast).

2.5. Quantitative PCR

Quantitative PCR (qPCR) analysis was carried out in Applied Biosystems StepOnePlus™ Real-Time PCR System (Life Technologies, Singapore), using SYBR green master mix (Takara, Dalian, China). Primers of *Phascolarctobacterium faecium* (YH1), *Eubacterium biforme* DSM 3989 (YH3) and *Citrobacter freundii* (OH1) used for absolute quantification were listed in Table 1, which were designed according to 16S rRNA gene offered by NCBI Primer - BLAST (http://www.ncbi.nlm.nih.gov/tools/primer-blast/) and synthesized by Sangon Biotech (Shanghai, China). No appropriate qPCR primers were found for the other age-related bands. The amount of 16S rRNA gene copies of specific bacterial groups in the fecal samples was determined by comparing the Ct (threshold cycle) values of samples with those of the standard curves. The standards were prepared by a series of appropriate dilutions with 16S rRNA gene copies of recombinant plasmids of each bands.

2.6. Statistical analysis

The DGGE fingerprint profiles were analyzed with Gel Compare of Tannon GIS2010 Image System and transformed into data sets. Shannon diversity index (H') was calculated to measure the diversities of microbiota.[5] Differences in H' and the abundance of bands between young adult and elderly were determined using ANOVA with SPSS 20.0. Further, Pearson correlation was used to determine the correlation between DGGE fingerprint and aging with SPSS 20.0. Multivariate statistical analysis, including principal component analysis (PCA) and multidimensional scaling analysis (MDS),[15,16] were carried out using MVSP (Multi-Variate Statistical Package) 3.13 and SPSS 20.0 software. The qPCR data from StepOne Software v2.1 (Life

Table 1
Primers and bacterial genome copies in fecal samples of participants with qPCR.

Target organism	Primer	Sequence (5'-3')	PCR product (bp)	Annealing temperature (°C)	Young adult (log_{10}copies/ml, n = 41)	Elderly (log_{10}copies/ml, n = 40)
Phascolarctobacterium faecium	F	TCGACCCCTTCTGTGCCGGA	441	60	8.89 ± 1.87	8.73 ± 1.18
	R	TTGCTCTGCCTCGCGGCTTC				
Eubacterium biforme DSM 3989	F	CAGCATGTCGCGGTGAATAC	101	55	10.00 ± 1.69	8.65 ± 0.78**
	R	CTCCTCGAAAGGTTATGCCACC				
Citrobacter freundii	F	TCGGGCCTCTTGCCATCGGA	254	60	9.55 ± 1.93	11.42 ± 1.20**
	R	CCTCAGCACCTTCCTCCTCGCT				

* $p < 0.05$, ** $p < 0.01$. One-way ANOVA data were expressed as mean ± SD, elderly subjects compared with the young adult.

Technologies, China) were log-transformed and compared. The Independent-Samples T Test was used to determine the significant differences. All results were presented as mean ± SD and significance was accepted if $p < 0.05$.

3. Results

3.1. DGGE fingerprint in yound adult and elderly

All stools collected from yound adult and the elderly were subjected to PCR-DGGE, the quantitated results indicated that the number of bands of the elderly (20.1 ± 3.0) was significantly lower compared with the yound adult (21.9 ± 3.9, $p < 0.05$) (Fig. 1). Moreover, Shannon diversity index was measured to provide an indication for the bacterial distribution diversity. The diversity index of the elderly was 2.28 ± 0.33, which was significantly lower than that of the yound adult (2.49 ± 0.25, $p < 0.01$). These results indicated the differences of gut microbial structure between young adult and the elderly.

To further compare the structure of gut microbiota between the young adult and elderly, multivariate statistical analysis were performed on the DGGE results. After extraction of underlying components (eigenvector > 1) obtained from PCA, the highly diverse datasets were subjected to MDS. As shown in Fig. 2, most of the elderly samples aligned in the first, second and third quadrant except f1, f2, f3 and m14 which plotted in the fourth quadrant. The yound adult subjects mainly clustered in the fourth quadrant, the others scattered around the elderly.

3.2. Correlation analysis of the changes in DGGE fingerprint and age

To reveal age-related markers in gut microbiota community, correlation analysis between DGGE fingerprint and age was conducted. In detail, the changes in gut microbiota, as measured by abundance of the bands in the DGGE fingerprint, were correlated to the age of subjects with SPSS. As shown in Table 2, significant correlations were found for eight bands (YH1-YH4 and OH1-OH4) ($p < 0.05$). The YH1-YH4 bands were preponderant in young adult with abundance ranged from 2.26 to 2.58, while their abundances significantly decreased in elderly (0.09 to 1.07, $p < 0.05$). In particular, the abundance of YH1 decreased 25 folds in the elderly compared to young adult (0.09 ± 0.57 in the elderly and 2.26 ± 4.32 in young adult). On the other hand, significant increases of OH1-OH4 bands ($p < 0.01$) were observed in the elderly fingerprint (with abundance of 0.25-0.7 in the young adult and increased to 1.24-5.22 in the elderly). These results implied that some bacterial species were attenuated, while others were more prevalent in the elderly gut. Followed, these bands were sequenced and the similarity searches of GenBank database were performed using the BLAST in an attempt to identify known homologous species.

Prediction of bacteria based on the database showed that the bands of YH1-YH4 were closest to regions of *Phascolarctobacterium faecium* (YH1, 100% homology), *Eubacterium eligens* (YH2, 100% homology), *Eubacterium biforme DSM 3989* (YH3, 100% homology) and *Prevotella sp. DJF_RP53* (YH4, 100% homology) respectively (Table S3). *Phascolarctobacterium faecium*, *Eubacterium eligens* and *Eubacterium biforme DSM 3989* belong to *Firmicute*, while *Prevotella sp. DJF_RP53* is a member of *Bacteroidetes*. OH1 was 100% related to *Citrobacter freundii*, OH3 was 100% with regions of *Alistipes putredinis* and OH4 was 99% with *Bacteroides clarus YIT 12056*, however, OH2 was not hit in the databank. *Alistipes putredinis* and *Bacteroides clarus YIT 12056* are classified in *Bacteroidales* of *Bacteroidetes*, and *Citrobacter freundii* is fallen into *Proteobacteria* (Table S3). These results revealed that several species of *Firmicutes* (*Phascolarctobacterium faecium*, *Eubacterium eligens* and *Eubacterium biforme DSM 3989*) decreased with aging in Chinese elderly, meanwhile, some species of *Bacteroidales* altered, including two species (*Alistipes putredinis* and *Bacteroides clarus YIT 12056*) increased and one species (*Prevotella sp. DJF_RP53*) decreased in the aging process. Additionally, the amount of a species (*Citrobacter freundii*) of *Enterobacteriales* from *Proteobacteria* was also found to be enhanced in the elderly in our study.

3.3. Quantification of age-related gut microbial markers with qPCR

To confirm the markers found in DGGE, qPCR were conducted on *Phascolarctobacterium faecium*, *Citrobacter freundii* and

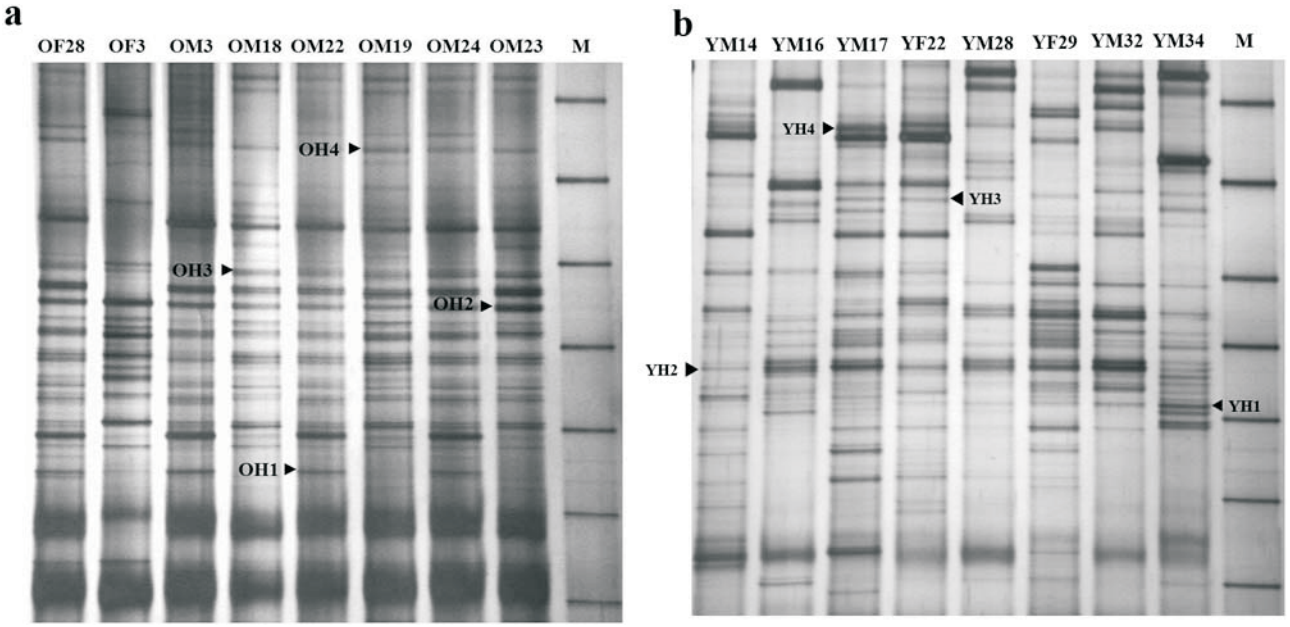

Fig. 1. Profiles of 16S rRNA gene PCR-DGGE fingerprint of the fecal microbiota from the elderly (A) and young adult (B) in Northeast China. OM, male elderly; OF, female elderly; YM, male young adult; YF, female young adult; M, reference marker (Nippon Gene DGGE Mark II).

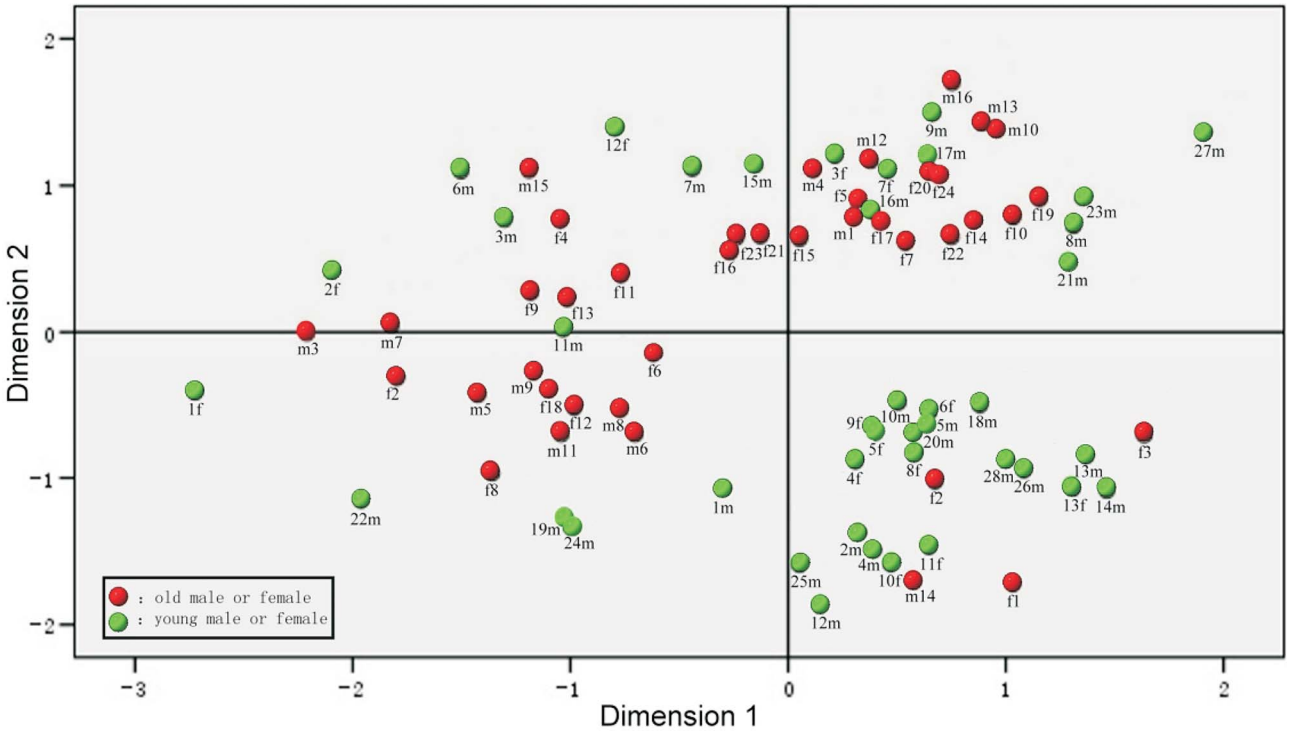

Fig. 2. Multidimensional scaling analysis (MDS) of 16S rRNA gene PCR-DGGE band patterns of the fecal microbiota from the elderly and young adult in Northeast China. Red: elderly subjects, green: young adult subjects.

Table 2
Age-related markers in 16S rRNA gene PCR-DGGE fingerprint of young adult and elderly in Shenyang and their related species.

DGGE bands		Pearson correlation coefficient	Abundance[a]		Related species (accession)	Identification
			Young adult (n = 41)	Elderly (n = 40)		
Bands decreased in elderly	YH1	0.33**	2.26 ± 4.32	0.09 ± 0.57**	*Phascolarctobacterium faecium* (NR_026111.1)	100%
	YH2	0.33**	2.32 ± 3.01	0.84 ± 1.87**	*Eubacterium eligens* (JN583454.1)	100%
	YH3	0.38**	2.58 ± 3.63	0.57 ± 1.62**	*Eubacterium biforme DSM 3989* (NR_044731.1)	100%
	YH4	0.28**	2.47 ± 3.31	1.07 ± 2.71*	*Prevotella sp. DJF_RP53* (EU728757.1)	100%
Bands increased in elderly	OH1	0.32**	0.25 ± 0.96	4.40 ± 8.53**	*Citrobacter freundii* (AB548827.1)	100%
	OH2	0.33**	0.06 ± 0.39	1.24 ± 2.33**	/	/
	OH3	0.43**	0.70 ± 1.56	5.22 ± 4.55**	*Alistipes putredinis* (AB554232.1)	100%
	OH4	0.37**	0.22 ± 1.05	3.29 ± 4.67**	*Bacteroides clarus YIT 12056* (AB547638.1)	99%

* $p < 0.05$, ** $p < 0.01$. [a] Independent-samples t test data were expressed as mean ± SD, the elderly subjects compared with the young adult.

Eubacterium biforme DSM 3989 which were filtered out appropriate primers. The qPCR results showed that more *Eubacterium biforme DSM 3989* ($p < 0.01$) were found in young adult faeces when compared with the elderly, and more *Citrobacter freundii* were found in elderly faeces compared to young adult ($p < 0.001$). These results were consistent with the results of DGGE. However, as showed in Table 1, although the amount of *Phascolarctobacterium faecium* was higher in young adult, the difference was not statistically significant compared with the elderly. This maybe explained by the different methods, new species maybe fit the primers detected with qPCR.

4. Discussion

Age-related physiological changes in the digestive tract, modification in lifestyle, and functions of immune system will affect microbiota composition, resulting in higher susceptibility to infections.[17] In this work, we attempted to investigate the gut microbiota changes in Shenyang to provide more understanding on age-related gut microbiota changes in Chinese population and their roles on aging. Our results proved that the elderly gut microbial community were differed from the young adult in Shenyang, which have a less diversity in the gut microbiota. 8 DGGE markers (YH1–YH4 and OH1–OH4) changed in the elderly gut microbiota were determined. *Eubacterium biforme DSM 3989* (YH3), *Prevotella sp. DJF_RP53* (YH4), *Alistipes putredinis* (OH3) and *Bacteroides clarus YIT 12056* (OH4) were first reported in Chinese gut (Table S4).

Eubacterium were listed as one of the top 10 predominant bacteria in human gut.[18] An early study on 20 healthy Japanese-Hawaiians with strict culture technique revealed that, the *Eubacterium* constituted 23% of total fecal microorganisms with the major species including *Eubacterium eligens* (YH2) and *Eubacterium biforme* (YH3),[19] their high frequencies in Japanese gut were later confirmed by molecular PCR methods.[20] *Eubacterium eligens* (YH2) was reported to can utilize pectin and polygalacturonic acid, it could ferment pectin with major fermentation products as formate and acetate, suggesting its contribution to pectin fermentation in the colon.[21] *Eubacterium biforme* (YH3) were observed to be dominant

in chimpanzees fed with high-fiber diets, implying a relationship between *Eubacterium biforme* and fiber digestion.[22] It was reported that *Eubacterium biforme* decreased in people suffering from long term diarrhea,[18] and exopolysaccharides were proved to be a selective enrichment of *Eubacterium biforme*.[23] *Prevotella* was dominant in people who consumed more carbohydrates especially fiber.[24] *Prevotella sp. DJF_RP53* (YH4) was detected in healthy young people of Gambia.[25] *Eubacterium eligens* (YH2), *Eubacterium biforme* (YH3) and *Prevotella sp. DJF_RP53* (YH4) were found to decreased in elderly faeces in our study, which might have potential role on fiber digestion disability or pectin utilization disorder, and resulting in digestive dysfunction in elderly people.

Citrobacter freundii (OH1) was 17 times increased in elderly faeces compared to young adult by DGGE analysis in our study, its increase in the elderly gut was further proved by qPCR. *Citrobacter freundii* was known to be an opportunistic pathogen accounting for approximately 29% of all opportunistic infections,[26] which did not cause diseases in healthy people, but affected patients with a weak immune system.[27] The increased *Citrobacter freundii* detected in the elderly might suggest the increased susceptibility to infections under a weakened immunity of the elderly. *Phascolarctobacterium faecium* (YH1) and *Alistipes putredinis* (OH3) were proved to be associated with cruciferous vegetable intake.[28] In our study, we found that *Phascolarctobacterium faecium* (YH1) decreased and *Alistipes putredinis* (OH3) increased in elderly. *Phascolarctobacterium faecium* was an asaccharolytic, succinate-utilizing bacterium isolated from Human Feces,[29] and *Alistipes putredinis* was described as a bile-resistant pigment-producing anaerobic from human sources.[30] The increased *Alistipes putredinis* in elderly gut may be involved in some diseases associated with pigment deposition, such as melanosis coli (a harmless pigmentation of the colorectum which occurred mostly in elderly people). However, this hypothesis need further researches.

Moreover, a decrease in *Firmicutes* and an increase in *Bacteroidetes* were as aging characteristics in many researches,[7,17] but opposite changes were found in German and Italian of Emilia Romagna.[31] In our study, *Phascolarctobacterium faecium* (YH1), *Eubacterium eligens* (YH2) and *Eubacterium biforme DSM 3989* (YH3) decreased in elderly were *Firmicutes*, *Alistipes putredinis* (OH3) and *Bacteroides clarus YIT 12056* (OH4) increased in elderly were *Bacteroidetes*.

In conclusion, our results suggested the age-related changes in gut microbiota of Chinese living in Shenyang. The changed bacterial markers detected in our study suggested the potential role of gut microbiota in aging process and supported the location-dependent microbial changes in aging. More investigations are necessary to explain further the relationship between gut microbiota and aging process.

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Supplement

Table S1
The details of demographics of two group of subjects (young adult and elderly) enrolled in this study.

		Elderly (n = 40)	Young adult (n = 41)
Age (yr): mean (range)		67.1 (60–78)	25.8 (21-38)
Gender (M/F)		16/24	28/13
Past history	Abdominal operation	-	-
	Chronic bronchitis	3	1
	Hypertension	2	-
Shenyang residence time		> 6 months	> 6 months
Medication intaken in 3 months		-	-
Probiotic supplementation in 1 month		-	-

Table S2
Age-related changes of gut microbiota in different regions.

Region	Methods	Experimental subjects	Age-related changes in gut microbiota	Reference
India	DGGE, qPCR and germiculture	Two healthy Indian families (one family: 8m, 26y, 56y; the other family: 14y, 42y, 62y)	With increasing age: *Firmicutes* decreased, *Bacteroidetes* increased	7
Ireland	Pyrosequencing	Elderly (> 65y) and younger control subjects (28–46y)	Elderly: *Bacteroides* increased	8
Emilia Romagna, Italy	Human intestinal tract chip (HITChip) and qPCR	Young adults (average 30y), elderly (average 70y) and centenarians (average 100y)	Centenarians: microbiota diversity, *Faecalibacterium prauznitzii* and *bifidobacteria* decreased, *Bacilli* and *Eubacterium limosum* increased	4
Milan, Italy	Enumeration of incubated bacteria	Young adults (24–57y) and centenarians (100–104y)	Centenarians: *Enterobacteriaceae*, *Bifidobacteria*, and *Bacteroides* decreased, *Clostridia sensu stricto* increased	2
France Germany Italy Sweden	Fluorescence in situ hybridization (FISH) coupled with flow cytometry	Young adults (20–50y, mean 35y) and elderly (> 60y, mean 75y)	Elderly: *Enterobacteria* and *Lactobacillus-Enterococcus* incresed Elderly: *Eubacterium rectale-Clostridium coccoides*, *Atopobium*, *enterobacteria*, *Lactobacillus-Enterococcus* increased Elderly: *Eubacterium rectale-Clostridium coccoides* and *Streptococcus-Lactococcus* decreased, enterobacteria increased Elderly: *Faecalibacterim prausnitzii* decreased, *Atopobium* and *enterobacteria* increased	9
Bama and Nanning, China	High-throughput sequencing	Younger elderly (85–99y) and centenarians (100–108y)	Centenarians: *Roseburia* and *Escherichia* increased, *Lactobacillus*, *Faecalibacterium*, *Parabacteroides*, *Coprococcus*, *Butyricimonas*, *Sutterella*, *Mitsuokella*, *Megamonas* and *Akkermansia* decreased	11
Gaotian village, Liuyang, China	High-throughput sequencing and qPCR	Gaotian villagers (> 60y) and control group (average 50y)	Gaotian villagers: Microbiota diversity, *Lactobacillus*, *Enterobacteriaceae*, *Enterococcus*, *Clostridium perfringens* and *Bacteroides* increased	12
Jimusi, China	Enumeration of incubated bacteria	Young students (19–22y) and elderly teachers (56–60y)	Elderly teachers: *Bifidobaterium* and *Lactobacillus* decreased	13

Table S3
Top four Blast results of YH1-YH4 and OH1-OH4 in Genbank.

Characteristic bands	Description (accession)	Max ident	E value	Max score	Query coverage	Gaps
YH1	*Phascolarctobacterium faecium* (NR_026111.1)	100%	1e-97	352	100%	0%
YH2	*Eubacterium eligens* (JN583454.1)	100%	4e-80	306	100%	0%
	Lachnospira pectinoschiza (L14675.1)	99%	6e-78	298	100%	0%
	Lactobacillus rogosae (GU269544.1)	99%	2e-77	297	100%	0%
YH3	*Eubacterium biforme DSM 3989* (NR_044731.1)	100%	4e-97	351	100%	0%
YH4	*Prevotella sp. DJF_RP53* (EU728757.1)	100%	5e-91	342	100%	0%
OH1	*Citrobacter freundii* (AB548827.1)	100%	1e-93	351	100%	0%
	Citrobacter sp. FCC134 (JF772099.1)	99%	5e-92	351	100%	0%
	Citrobacter sp. LSRC194 (AB675738.1)	99%	5e-92	351	100%	0%
	Citrobacter sp. LRC99 (JF772065.1)	99%	5e-92	351	100%	0%
OH2	*C.cellulolyticum* (X71847.1)	93%	2e-65	257	100%	0%
	Clostridium sp. YIT 12069 (AB491207.1)	93%	6e-65	255	100%	2%
	Clostridium josui (AB675738.1)	92%	7e-64	251	100%	0%
OH3	*Alistipes putredinis* (AB554232.1)	100%	6e-91	342	100%	0%
	Alistipes sp. NML05A004 (EU189022.1)	98%	1e-86	327	100%	0%
	Alistipes massiliensis (AY547271.1)	98%	1e-86	327	100%	0%
	Alistipes putredinis (NR_025909.1)	98%	1e-86	327	100%	0%
OH4	*Bacteroides clarus YIT 12056* (AB547638.1)	99%	2e-89	333	100%	0%
	Bacteroides finegoldii DSM 17565 (NR_041313.1)	98%	5e-89	324	100%	0%
	Bacteroides uniformis strain JCM 5828 (NR_040866.1)	98%	5e-89	324	100%	0%
	Bacteroides intestinalis DSM 17393 (NR_041307.1)	97%	2e-87	318	100%	0%

Table S4
The age-related species in this study.

Bacterial species	Ever reported in Chinese before	Reported function
Phascolarctobacterium faecium (YH1)	Yes[S1]	- Associated with cruciferous vegetable consumption[29]
Eubacterium eligens (YH2)	Yes[S2]	- Utilizes pectin or polygalacturonic acid[22]
Eubacterium biforme DSM 3989 (YH3)	No	No reported function for the strain As for *Eubacterium biforme*: - Lost in people with long term diarrhea[19] - The dominant species of feces in chimpanzees on high-fiber diets[23] - Selectively enriched by EPS[24]
Prevotella sp. DJF_RP53 (YH4)	No	No reported function for the strain As for *Prevotella*: - Dominant in people consuming more carbohydrates especially fiber[25] - Cause infections such as abscesses, bacteraemia, bite infections, wound infection, periodontitis and genital tract infections[S3]
Citrobacter freundii (OH1)	Yes[S4]	- Can metabolize glycerol, lactose or citrate as a carbon source - An opportunistic pathogen which can cause infections of respiratory tract, urinary tract, and blood, hepatic, biliary and pancreatic diseases.[27,28] Other reported cases included neonatal meningitis,[S5] necrotizing fasciitis,[S6] peritonitis and tunnel infection.[S7] - Can convert nitrate or the ammonium ion to nitrite in the environment[S8] - Genome contain a plasmid encodes L-methionine γ-lyase (MGL)[S9] - Produce the enzymes which play important roles in the Biotech industry such as phosphatase
Alistipes putredinis (OH3)	No	- Associated with cruciferous vegetable consumption[29] - Produce pigment[22]
Bacteroides clarus YIT 12056 (OH4)	No	No reported function for the strain or the species *Bacteroides clarus*

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