The gut microbiota of healthy aged Chinese is similar to the healthy young

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Abstract

We collected and examined the gut microbiota of a cross-sectional cohort of more than 1000 very healthy Chinese individuals who spanned the ages from 3 to over 100. The analysis of 16S rRNA gene sequencing results used a compositional data analysis paradigm coupled with measures of effect size, where ordination, differential abundance and correlation can be explored and analyzed in a unified and reproducible framework.

The Cohort and Consent

Volunteers filled out a self-reporting health information questionnaire and the age and detailed geographic locations of all persons was collected. These were divided into eight groups by age and are referred to by their group: kindergarten students aged 3-6 (kin); primary school student age 6-12 (pup); middle school students aged 12-18 (ms); college students aged 18-30 (you); soldier and police recruits aged 18-25 (ys); middle aged people aged 30-60 (mage); elderly people aged 60 (eld); and centenarians aged at least 90 (cent).

Inclusion criteria were: nonsmoker, teetotaler, mood was stable (self-assessed/reported), absence of any diseases, no prescription medication and antibiotics for the past 3 months, no personal and family disease history (such as cardiovascular, gastrointestinal, metabolic, neurological/mental and respiratory diseases as well as cancers), and parents and grandparents are all alive or passed away after 80 years of age. This last criterion was not applied to subjects recruited older than 31 years of age. These stringent criteria excluded between 97% - 99% of potential volunteers depending on age. The volunteers in the ys category were chosen with all the above-listed criteria and the following two additions: firstly, they had passed the standard military entrance medical examination, and secondly, their grandparents lived to be at least 85 years.

Description of informed consent, and ability to use data for publication.

Before sampling, all volunteers were informed about the purpose of this study, and signed an informed consent, which included provision of data acquired by examination of the samples they provided. The study was approved by the University of Jiangsu Affiliated Hospital Ethics Committee for Biomedical Research (Zhenjiang City, Jiangsu Province, China).

Methods

The V4 region of the 16S rRNA gene was amplified using the Earth Microbiome primer sets with custom barcodes, and paired-end sequenced on an illumina MiSeq. Reads were demultiplexed, overlapped and assigned to genera as described [6]. Analyses were conducted in - as close to possible - a CoDa framework [4]. Ordination was was done using compositional biplots [1], differential abundance was performed using ALDEx2 [3], and compositional association was determined using a symmetrical version of the ϕ metric [5] (the denominator was the sum of the variances, rather than the single variance). Differential abundance and association were calculated as the expected value of the posterior distribution of the data as described [2]. This robustly estimates the likelihood of a 0 count in these sparse, high-dimensional, count compositional data.

Results

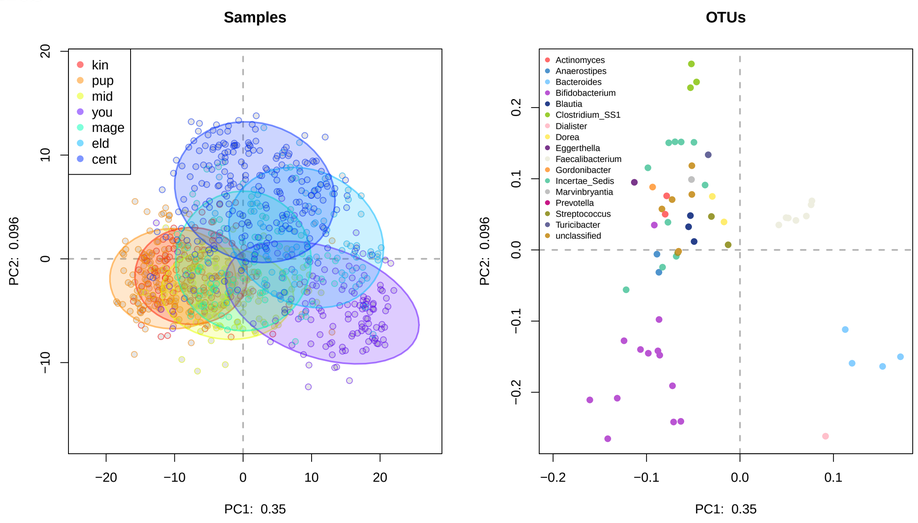

Figure 1: PCA plot including taxa that are significantly different between any pair of groups (ALDEx2 effect ≥ 1), or that are compositionally associated ($\phi \leq 0.23$). These two plots show the first two principle components of the centre log-ratio transformed data for the dataset comprising samples from people aged 3 to 100. The left panel shows a plot where each point is a sample. Samples are coloured by their grouping, and the data ellipses encompass 75% of the points in a group. This demonstrates that groups separate broadly by age cohort. The right panel shows the contribution of the selected OTUs to the separation of the samples. The ability to directly interpret these plots is limited by the small proportion of variance explained. OTUs are coloured by their assigned genus.

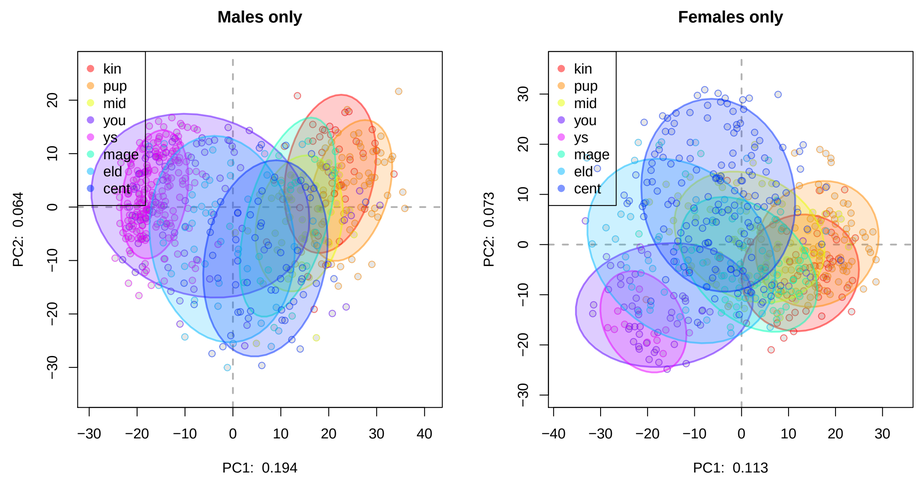

Figure 2: Male and female participants ordinated similarly, but the female ordination was substantially less variable than the male ordination.

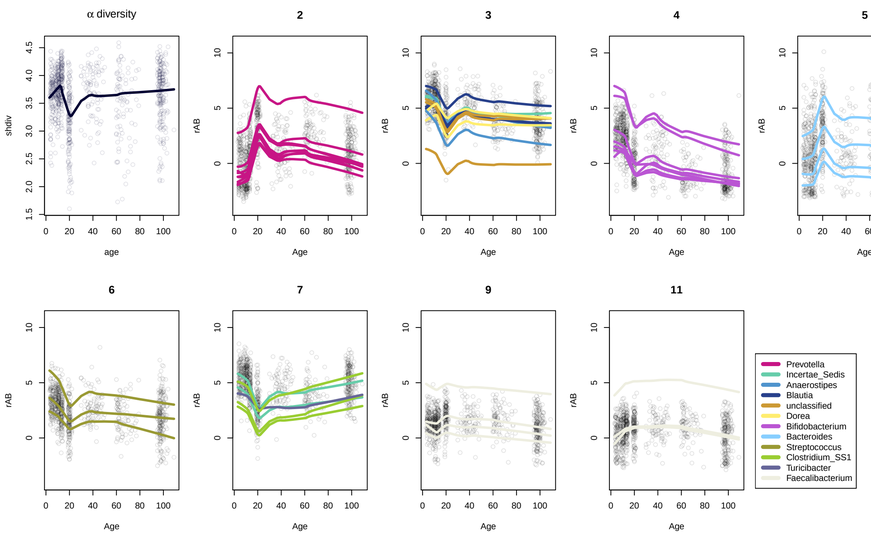

Figure 3: Exploration of compositionally associated taxa using the expected value of a symmetrical version of the ϕ metric with a cutoff of 0.23. The loess lines of best fit are shown for each line is a taxon in the group. The actual values for are shown in the background in light circles to illustrate that while the mean fit is very close for a given pair of taxa, the dispersion is very large.

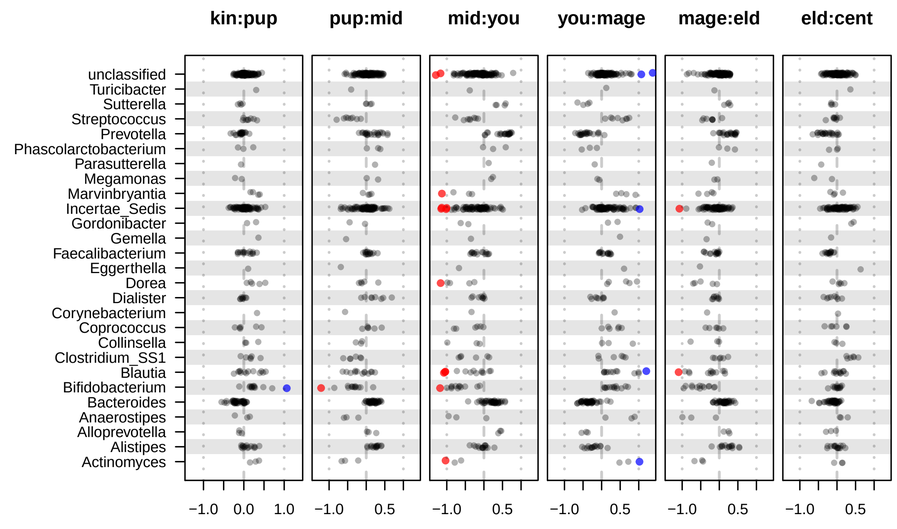

Figure 4: Differential abundance of major taxa for successive pairwise comparisons. All possible comparisons of cohorts revealed 102 OTUs that were reproducibly different between at least one pair of cohorts. These were grouped into 26 genera, and one set of unknown genera. The plots show all OTUs in these 26 genera for four typical pairwise comparisons: kindergarten vs youth, elderly vs kindergarten, middle age vs youth and centenarians vs middle age. Each plot shows a point for each OTU grouped by genus with the mean log2 difference between the two groups on the x axis. Points are coloured as red or blue if they have an effect size ≥ 1 as determined by ALDEx2 for the comparison.

Conclusions

A compositional paradigm is entirely appropriate for, and can be easily adapted to, the analysis of very large microbiome cohorts. The results obtained by exploratory ordination, differential abundance and compositional association are concordant.

Our analysis showed several surprising results compared to other cohorts. First, the overall microbiota composition of the healthy aged group was similar to that of people decades younger. Second, the major changes in the gut microbiota profiles were found to occur before age 20. Third, the gut microbiota of the age 30 cohort was observed nearly unchanged for all cohorts of greater age. Fourth, the gut microbiota of males appeared to be more variable than that of females.

Taken together, the findings suggest that the microbiota of the healthy aged in this cross-sectional study differ little from the healthy young, although the minor variations that do exist depend upon the comparison cohort.

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Acknowledgements

*Tiyani Health Sciences Consortium Team: Gaorui Bian, Gregory B. Gloor, Aihua Gong, Changsheng Jia, Wei Zhang, Jun Hu, Hong Zhang, Yumei Zhang, Zhenqing Zhou, Jiangao Zhang, Jeremy P. Burton, Gregor Reid, Yongliang Xiao, Qiang Zeng, Kaiping Yang, Jiangang Li1

Collection, sequencing and initial analysis was supported by the following grants. JL - Zhenjiang Tianyi Biotech Co. Ltd., Human Microbiome Project Grant TY2015001: KY - International Science and Technology Cooperation Program, Zhenjiang GJ2015005 and Jinshan Excellence Projects, Zhenjiang ZJX2016170: WZ - Social Development of Science and Technology Support Projects, Zhenjiang SH2013036 and Science and Technology Assistance Projects, Xinjiang 2014AB045. Development of the compositional data analysis approach was supported by an NSERC Discovery grant RGPIN-03878-2015 to GBG.