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# Effect of dietary interventions on the intestinal microbiota of Mongolian hosts

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**Abstract** The gut microbiota of Mongolian hosts has distinctive characteristics due to their meat- and dairy-oriented daily diets and unique genotype. The aim of the present study was to investigate the effect of switching from the typical high protein and fat Mongolian diets to carbohydrate-rich meals composed principally of wheat, rice and naked oats on the host gut microbiota within 3 weeks. Our study took the advantage of the long sequence reads produced by the PacBio single molecule real-time sequencing technology to enable the profiling of subjects' gut microbiota communities along the diet intervention to the species precision. We found that the bacterial richness and diversity decreased apparently along the diet intervention. During the diet intervention, the gut microbiota composition displayed no significant difference at phylum level (with major phyla of Firmicutes, Bacteroidetes, Tenericutes and Proteobacteria). The relative abundances of some genera such as *Bacteroidetes*, *Faecalibacterium*, *Roseburia*, *Alistipes*, *Streptococcus*, and *Oscillospira* were significantly altered after the diet switching started. Notably, significant changes were also observed in the proportions of the species *Bacteroides dorei*, *Bacteroides fragilis*, *Bacteroides thetaiotaomicron*, *Ruminococcus albus*, *Ruminococcus faecis*, *Roseburia faecis* and *Eubacterium ventriosum*. These results have demonstrated that diet and host gut microbiota is closely linked.

**Keywords** Gut microbiota · Diet intervention · Mongolian · PacBio single molecule real-time sequencing technology (SMRT sequencing)

## 1 Introduction

The gut microbiota plays a significant role in overall human immunology, nutrition and pathological processes [1]. The composition of human gut microbiota is related to the host genetics [2] and diet [3]. The host genotype contributes to the human intestinal microbial diversity by long-term co-evolution via a symbiotic host-microbe interaction [4]. Yet, the intestinal microbiota can rapidly respond to changes in dietary intake [5], and modulate the efficacy of energy harvest from food [6].

Mongolia is situated in East Asia and bordered by China to the south and Russia to the north. Traditionally, Mongolian people adopt a typical nomadic life style. In the thirteenth century, the country straddled across Europe and Asia, which promoted the East–West cultural exchanges and intermediation [7]. As an ancient tribe, the Mongolians have retained distinct genotype [8]. The host genetics strongly influences the host-microbe interaction and may contribute to select for specific spectra of human gut commensal microbiota [2]. De Filippo reported significant differences between the intestinal microbiota of children from Europe and rural Africa [3]. The signatures of Korean gut microbiota were distinct from the American, Chinese and Japanese ones, possibly because of the unique characteristics of each country such as host genetics and diet

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styles [9]. Yatsunenko also demonstrated the fact that microbial flora differed between different human populations [10].

The daily Mongolian diet is mainly composed of meats and fermented dairy products, contributing to a high animal protein, fat content and probiotics or prebiotics. Moreover, their typical diets contain a lot more fried food than vegetables and fruits, and a low proportion of coarse food grain such as oat and millet. Most Mongolians frequently consume liquors against the cold weather [7]. Mongolian unique genotype in combination with their typical diet probably contributed together to shape their gut microbiota. Our previous study showed that diet affect the microbiota composition of Mongolians living in different circumstances [11], and found that the Mongolian core gut microbiota which was mainly composed of Firmicutes [12] changed with season varied.

The advent of high-throughput sequencing technology has led to the development of culture independent metagenomic approaches that facilitate the characterization of intestinal microbiota [13, 14]. The Pacific Biosciences (PacBio) single molecule, real-time sequencing technology (SMRT) based on DNA polymerization is a promising third generation high-throughput technique that produces longer reads than the second generation sequencing technology [15]. Many studies have taken the advantage of the longer reads of SMRT sequencing to profile environmental microbial communities based on the full-length 16S rRNA gene to achieve a high taxonomic resolution of up to species level [16–18].

In this study, we investigated the effect of diet switching of 26 Mongolian subjects (from typical Mongolian to low protein and fat meals composed principally of wheat, rice and naked oats) on the host gut microbiota using the PacBio SMRT platform.

## 2 Materials and methods

### 2.1 Ethics statement

Written and informed consent were obtained from all volunteers. The study protocol was approved by the Ethical Committee of the Inner Mongolia Agricultural University (Hohhot, China).

### 2.2 Participant recruitment and study design

Twenty-six healthy adults (22–35 years old) were recruited from Mongolia to participate in the controlled feeding experiment, who arrived in China 1 d before the experiment. All subjects did not suffer from any gastrointestinal disorder and did not take any antibiotics, and ate the fermented dairy products from the 3 months before the experiment started and until the end of the experiment. The bowel movement frequency of each subject was reported to be 1–2 d throughout the experiment. We collected the diet information of each participant using a food frequency questionnaire; and the detailed information of subjects and the average Mongolian diet composition during the 7 d prior to the experiment are listed in Tables S1–4.

Three types of Chinese foods were prepared for the participants along the experimentation period. All the Mongolian participants consumed identical meals for lunch and dinner of three continuous weeks without unifying their breakfast from Mongolia which include pastry, milky tea and dairy products. The meals comprised a relatively low protein and fat makeup compared to the typical Mongolian diet. Mainly, the content of staple food in the diet was changed every week. For the first week, the staple food was wheat-based, in combination with vegetables and red meat. For the second week, rice was the staple food, accompanied with vegetables and a reduced amount of beef compared to the first week. For the last week, meals were rich in dietary fiber, i.e. oat-based noodle together with vegetables and only a small amount of red meat (Table S4).

### 2.3 Fecal sample collection and DNA extraction

The first fecal samples were collected on the first day the subjects arrived in China before the diet intervention experiment started. Stool samples were collected every Sunday morning over 3 weeks from each individual before breakfast was eaten. The collected fecal samples were preserved at −80 °C until genomic DNA extraction of fecal microbiota was performed using a QIAGEN DNA Stool Mini-Kit (QIAGEN, Hilden, Germany) following the manufacturer's instructions [19]. The quality of the extracted genomic DNA was checked by agarose gel electrophoresis and spectrophotometric analysis (optical density at 260 nm/280 nm ratio). All extracted DNA were stored at −20 °C until further experiment.

### 2.4 Full-length 16S rRNA gene amplification

Bacterial 16S rRNA gene sequences were amplified from all genomic DNA samples by PCR for SMRT barcode sequencing using the forward 27F (5′-AGAGTTTGATCMTGGCTCAG-3′) and reverse 1492R (5′-ACCTTGTTACGACTT-3′) primers [18]. A set of 16-base barcodes for every DNA sample was added to the forward and reverse PCR primers. The PCR amplifications were performed as described previously [20]. The PCR program was as follows: 95 °C for 4 min; 30 cycles of 95 °C for 30 s, 58 °C for 30 s, and 72 °C for 30 s with a final extension of 72 °C for 5 min.