Bacterial Atlas of Mouse Gut Microbiota

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Research

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Abstract

Background: Mouse model is one of of the most widely used animal models for exploring the roles of human gut microbiota, a complex system involving in human immunity and metabolism. However, the structure of mouse gut bacterial community has not been explored at a large scale. To address this concern, the diversity and composition of the gut bacteria of 600 mice was characterized in this study.

Results: The results showed that the bacteria belonging to 8 genera were found in the gut microbiota of all mouse individuals, indicating that the 8 bacteria were the core bacteria of mouse gut microbiota. The dominant genera of the mouse gut bacteria contained 15 bacterial genera. It was found that the bacteria in the gut microbiota were mainly involved in host's metabolisms via the collaborations between the gut bacteria. The further analysis demonstrated that the composition of mouse gut microbiota was similar to that of human gut microbiota.

Conclusion: Our study presented a bacterial atlas of mouse gut microbiota, providing a solid basis for investing the bacterial communities of mouse gut microbiota.

Background

The human gut microbiota plays key roles in human homeostasis, thus largely affecting human health [1]. The composition of gut microbiota develops dynamically in the first 2–3 years of life, which can affect risk factors related to adult health [2]. Increasing evidence has linked the human gut microbiota to diseases. The altered microbial communities are associated with obesity [3], gastrointestinal cancer [4], and type 2 diabetes [5]. To characterize human gut microbiota, murine models have been widely used, due to the extensive similarities in anatomy, physiology and genetics [6]. In mice, the dysbiosis of gut microbiota leads to severe diseases. Cognitive dysfunction is associated with the abnormal composition of the gut microbiota of mice [7]. The gut microbiota of mice influences the pathogenesis of malaria [8] and is identified as an important mediator of acute pancreatitis [9]. There are numerous lines of evidence suggesting that the gut microbiota of mice takes great effects on major depressive disorder [10] and type 1 diabetes [11]. In the gut microbiota of mice, *Bacteroides thetaiotaomicron* can affect the immune system because it is able to recapitulate the effects of the entire conventional microbiota and notably induces T_{reg} pathways [12]. *Chryseomonas*, *Veillonella* and *Streptococcus* may be the initial source of the atherosclerotic [13], while *Akkermansia muciniphila*, a mucin-degrading bacterium, elicits beneficial effects on metabolism and reduces atherosclerotic lesion formation [14]. As reported, type 2 diabetes is associated with a reduced abundance of butyrate producing bacteria and an increased abundance of *Lactobacillus sp* [15]. Although the gut microbiota of mice plays very important roles in health, the structure of gut microbiota of mice has not been extensively explored.

The colonization of the gut is influenced by many complex environmental factors, such as host genetics, age, diet, lifestyle, diseases and antibiotic use [16]. Based on microbial metagenome sequencing of some mice fed with low-fat or high-fat diets, the dominate genera in mice gut microbiota are *Faecalibacterium,*

Coprobacillus, Odoribacter, Anaerotruncus, Desulfovibrio, Enterococcus, Marvinbryantia, Pseudoflavonifractor, Coprococcus, Parabacteroides, Blautia, Eubacterium, Ruminococcus, Roseburia, Lactobacillus, Alistipes, Prevotella, Butyrivibrio, Clostridium and *Bacteroides* [17]. An increased ratio of the major phyla *Firmicutes* and *Bacteroidetes* (FIR/BAC ratio) and depletion of several bacterial species such as *Akkermansia mucinophilia* can promote the development of obesity in mice [18]. Generally the mouse gut microbiota can be divided into two enterotypes, including Ruminococcus enterotype and Bacteroides enterotype, a reproducible pattern of variation in the microbiota, according to their composition and community structure properties [19, 20]. At present, however, the diversity and abundance of the gut bacteria in healthy mice have not been characterized at a large scale.

To address this issue, the composition of the gut microbiota of 600 healthy mice was explored in the present investigation. The results revealed that at the genus level, the core bacteria of mouse gut microbiota contained 8 bacteria and the dominant bacteria consisted of 15 bacteria. There was a similarity between mouse and human gut bacterial communities.

Methods

Sample collection

A total of 300 ICR (Institute of Cancer Research) female and 300 ICR male mice (8 weeks old) purchased form Zhejiang Academy of Medical Sciences were raised in a sterilized condition for 3 days to stabilize the composition of gut microbiome. The mice were raised for another 4 days in the same sterilized condition. At day 3 and day 7, the body weight of mice was measured and the feces of each mouse were collected for later use.

Sequencing and sequence analysis of bacterial 16S rRNA

The bacterial genomic DNA was extracted directly from the fecal samples with FastDNA® SPIN Kit (MP Biomedicals, USA) according to the manufacturer's manuals. Subsequently the bacterial 16S rRNA gene was amplified by PCR using gene-specific primers (515F, 5'-GTGCCAGCMGCCGCGG-3'; 907R, 5'-CCGTCAA TTCMTTTRAGTTT-3') (M=A/C; R=A/G). The libraries of bacterial 16S rRNA gene were generated using NEB Next®Ultra™DNA Library Prep Kit for Illumina (NEB, USA) following the manufacturer's recommendation. The libraries were sequenced on an Illumina MiSeq platform.

Sequence analysis was performed by UPARSE software package using the UPARSE-OTU and UPARSE-OTUref algorithms. All primers, spacers, low-quality fragments and the sequences shorter than 50 bp were removed. The remaining sequences were further processed with the pre.cluster command and chimera.uchime command in Mothur. All sequences were denoised and screened for chimeric sequences. Then the sequences were assigned to the same operational taxonomic units (OTUs) by 97% sequence similarity. The uclust was used to annotate taxonomic information for each OTU. Mothur was used to analyze the community richness, community diversity and rarefaction curve.

Principal co-ordinates analysis

To compare two or more microbial communities, principal co-ordinates analysis (PCoA) was performed. The Bray-Curtis dissimilarity algorithm was applied for PCoA. The v egan of package R (version 3.4.4) (https://www.r-project.org/) were used.

KEGG (Kyoto encyclopedia of genes and genomes) pathway analysis

The bacterial 16S gene sequencing data were clustered with Greengenes database (http://greengenes.secondgenome.com) using closed reference OTU picking. The internal standardization was conducted to obtain the normalized species abundance. Subsequently, the abundance of three levels of KEGGorthology and pathway was obtained according to the relationship between the Greengenes database and the copy number of KEGGorthology.

Correlation analysis

Correlation analysis was performed using the OmicStudio tools at https://www.omicstudio.cn/tool/62. *Correlation plots were generated using the corrplot R package* (version 3.6.1).

Results

Bacterial communities in mice

To determine the composition of microbiota in the gut of mice, a total of 600 ICR (Institute of Cancer Research) mice (300 female and 300 male mice), one of the most commonly used laboratory mice, were subjected to the analysis of bacterial community (Fig 1A). At day 3 and day 10 after mouse raise, the bacterial 16S rRNA sequencing of mouse feces was performed. At day 10, the blood and intestinal tissues of mice were collected for examining physiological parameters (Fig 1A). The results showed that the bacteria of mouse gut were bacillus and cocci and vibrio (Fig 1B). The sequencing analysis of bacterial 16S rRNA gene of 1,200 mice yielded a total of 33,390,144 reads (Table S1). Based on these reads, 2478 operational taxonomic units (OTUs) were identified (GenBank accession no. PRJNA721276) (Table S1). All OTUs were defined by 97% similarity. The rarefaction curves of all samples approached plateaus (Fig 1C), indicating that the sequencing data represented the gut microbiome of mice.

In total, 29 phyla, 70 classes, 134 orders, 252 families, 624 genera and 828 species were classified (Fig 1D and Table S2). At the genus level, among 624 OTUs, only 19 OTUs could not be classified (3.04%), while the remaining 605 OTUs were matched to the known bacteria (96.96%) (Fig 1E and Table S3).

The dominant bacteria and core bacteria in the gut microbiota of mice

To determine the dominant bacteria and the core bacteria in the gut microbiota of mice, the composition of the gut microbiota of 600 ICR mice was characterized. The results of the principal coordinate analysis of mouse gut microbiota showed that there was no statistically significant difference of gut microbiota

composition at Day 3 and Day 10 (Fig 2A), indicating that the gut microbiota was stable and the data were reliable. However, the dots representing the gut microbiota of 600 mice were scattered in different locations (Fig 2A), showing the existence of individual differences of gut microbiota.

To determine the dominat baceria in the gut microbiota of mice, the bacteria of 600 mice were analyzed. The results revealed that at the phylum level, the dominant bacteria in the gut microbiota of mice mainly included *Firmicutes* (55.75%), *Bacteroidetes* (37.02%), *Proteobacteria* (4.05%), *Actinobacteria* (1.98%) and *Tenericutes* (1.09%), while the abundance of other bacteria was less than 1% (Fig 2B). The most dominant genus was *Bacteroidales S24-7 group_norank* (23.89%), followed by *Lactobacillus* (22.98%), *Faecalibaculum* (11.17%), *Alloprevotella* (5.44%), *Bacteroides* (4.31%), *Lachnospiraceae NK4A136 group* (4.17%), *Lachnospiraceae_ uncultured* (3.20%), *Escherichia-Shigella* (2.68%) and *Enterorhabdus* (1.73%), *Ruminococcaceae UCG-014* (1.37%), *Ruminiclostridium* (1.31%), *Alistipes* (1.30%), *Roseburia* (1.11%), *Mollicutes RF9_norank* (1.07%) and *Parabacteroides* (1.03%) (Fig 2B). At the species level, the dominant bacteria in the gut microbiota of mice were *Bacteroidales S24-7 group_uncultured bacterium* (23.89%), *Lactobacillus_ uncultured bacterium* (21.23%), *Faecalibaculum_uncultured bacterium* (11.17%), *Alloprevotella_uncultured bacterium* (5.44%), *Lachnospiraceae NK4A136 group_ uncultured bacterium* (3.71%), *Lachnospiraceae*_uncultured bacterium (3.16%), *Bacteroides_ uncultured bacterium* (3.10%), *Escherichia-Shigella_Unclassified* (2.68%), *Enterorhabdus_uncultured bacterium* (1.73%), *Lactobacillus_unclassified* (1.73%), *Ruminococcaceae UCG-014_uncultured bacterium* (1.34%), *Ruminiclostridium_uncultured bacterium* (1.31%), *Alistipes_uncultured bacterium* (1.25%) and *Roseburia_uncultured bacterium* (1.09%) (Fig 2B).

To reveal the core bacteria (the bacteria existing in all individuals) of the gut microbiota of mice, the gut microbiota of 600 mice were compared. The results showed that among the 624 known bacterial genera, 8 genera existed in all mouse individuals (Fig 2C), indicting that these bacteria were the core bacteria of mice. The 8 bacterial genera were *Bacteroidales S24-7 group_norank*, *Lactobacillus*, *Alloprevotella*, *Bacteroides*, *Lachnospiraceae NK4A136 group*, *Lachnospiraceae_ uncultured*, *Alistipes* and *Ruminiclostridium* 9, accouting for 23.91%, 22.99%, 5.44%, 4.31%, 4.17%, 3.39%, 1.30% and 0.89%, respectively. At the species level, the core microbiota contained 8 bacterial species, including *Alistipes_uncultured bacterium*, *Alloprevotella_uncultured bacterium*, *Bacteroidales S24-7 group_uncultured bacterium*, *Bacteroides_uncultured bacterium*, *Lachnospiraceae NK4A136 group_ uncultured bacterium*, *Lachnospiraceae_uncultured bacterium*, *Lactobacillus_ uncultured bacterium*and *Ruminiclostridium 9_ uncultured bacterium*, accounting for 1.25%, 5.44%, 23.91%, 2.97%, 3.61%, 3.34%, 21.22% and 0.89%, respectively (Fig 2D). However, the bacteria belonging to the 8 species could not be cultured.

Among the core bacteria, 7 out of 8 genera belonged to the dominate bacteria of mouse gut microbiota, including *Bacteroidales S24-7 group_norank* (23.91%), *Lactobacillus* (22.99%), *Alloprevotella* (5.44%), *Bacteroides* (4.31%), *Lachnospiraceae NK4A136 group* (4.17%), *Lachnospiraceae_uncultured* (3.39%) and *Alistipes* (1.30%). At the species level, 7 species of the core bacteria were the dominant bacteria, including *Bacteroidales S24-7 group_uncultured bacterium* (23.91%), *Lactobacillus_uncultured bacterium*

(21.22%), *Alloprevotella_uncultured bacterium* (5.44%), *Lachnospiraceae NK4A136 group_uncultured bacterium* (3.61%), *Lachnospiraceae_uncultured bacterium* (3.34%), *Bacteroides_uncultured bacterium* (2.97%) and *Alistipes_uncultured bacterium* (1.25%).

Collectively, these results revealed that the bacteria belonging to 8 genera were the core bacteria of the mouse gut microbiota. The dominant genera of the mouse gut bacteria contained 15 bacterial genera.

Bacterial composition in the gut microbiota of male and female mice

To compare the bacterial composition of male and female mice, the gut microbiota of mice were analyzed. The results showed that the female mice had a total of 1,041 OTUs, which could be classified into 27 phyla, 70 classes, 138 orders, 254 families, 626 genera and 841 species (Fig 3A). The male mice contained a total of 1038 OTUs, which were classified into 30 phyla, 75 classes, 141 orders, 256 families, 624 genera and 833 species (Fig 3A).

At the genus level, the dominant bacteria in the gut microbiota of male mice were *Bacteroidales S24-7 group_norank* (23.12%), *Lactobacillus* (22.85%), *Faecalibaculum* (12.81%), *Alloprevotella* (5.14%), *Bacteroides* (4.23%), *Lachnospiraceae NK4A136 group* (3.81%), *Lachnospiraceae_uncultured* (2.84%), *Escherichia-Shigella* (2.69%), *Enterorhabdus* (1.81%), *Citrobacter* (1.47%), *Ruminococcaceae UCG-014* (1.43%), *Alistipes* (1.19%), *Ruminiclostridium* (1.17%), *Erysipelotrichaceae_uncultured* (1.08%), *Mollicutes RF9_norank* (1.06%), *Roseburia* (1.02%), while the most dominant bacteria in the gut microbiota of female mice included *Bacteroidales S24-7 group_norank* (24.60%), *Lactobacillus* (23.11%), *Faecalibaculum* (9.62%), *Alloprevotella* (5.72%), *Bacteroides* (4.39%), *Lachnospiraceae NK4A136 group* (4.51%), *Lachnospiraceae_uncultured* (3.53%), *Escherichia-Shigella* (2.66%), *Enterorhabdus* (1.67%), *Ruminococcaceae UCG-014* (1.31%), *Alistipes* (1.40%), *Ruminiclostridium* (1.44%), *Mollicutes RF9_norank* (1.08%), *Roseburia* (1.20%) and *Parabacteroides* (1.05%) (Fig 3B). Among these bacteria, 2 genera (*Citrobacter* and *Erysipelotrichaceae_uncultured*) were dominant only in male mice, and *Parabacteroides* was dominant only in female mice. At the species level, the dominant bacteria in the gut microbiota of male mice contained *Bacteroidales S24-7 group_uncultured bacterium* (23.12%), *Lactobacillus_uncultured bacterium* (21.51%), *Faecalibaculum_uncultured bacterium* (12.81%), *Alloprevotella_uncultured bacterium (*5.14%), *Lachnospiraceae NK4A136 group_uncultured bacterium* (3.41%), *Bacteroides_uncultured bacterium* (2.86%), *Lachnospiraceae_uncultured bacterium* (2.81%), *Escherichia-Shigella_Unclassified* (2.69%), *Enterorhabdus_uncultured bacterium* (1.81%), *Citrobacter_Unclassified* (1.47%), *Ruminococcaceae UCG-014_uncultured bacterium* (1.40%), *Lactobacillus_Unclassified* (1.32%), *Ruminiclostridium_uncultured bacterium* (1.17%), *Alistipes_uncultured bacterium* (1.12%), *Bacteroides_uncultured organism* (1.09%), *Erysipelotrichaceae_uncultured bacterium* (1.08%) and *Roseburia_uncultured bacterium* (1.00%) (Fig 3B). The most dominate species in female mice was *Bacteroidales S24-7 group_uncultured bacterium* (24.60%), followed by *Lactobacillus_uncultured bacterium* (20.98%), *Faecalibaculum_ uncultured bacterium* (9.62%), *Alloprevotella_uncultured bacterium* (5.72%), *Lachnospiraceae NK4A136 group_uncultured bacterium* (3.99%), *Bacteroides_*

uncultured bacterium (3.32%), *Lachnospiraceae_uncultured bacterium* (3.48%), *Escherichia-Shigella_Unclassified* (2.66%), *Enterorhabdus_uncultured bacterium* (1.67%), *Ruminococcaceae UCG-014_uncultured bacterium* (1.28%), *Lactobacillus_ Unclassified* (2.12%), *Ruminiclostridium_uncultured bacterium* (1.44%), *Alistipes_ uncultured bacterium* (1.37%), *Roseburia_uncultured bacterium* (1.17%) (Fig 3B). Among these bacteria, 3 species (*Citrobacter_Unclassified, Bacteroides_uncultured organism* and *Erysipelotrichaceae_uncultured bacterium*) were dominant only in male mice.

At the genus level, the core bacteria of female mouse gut microbiota included *Bacteroidales* S24-7 group_norank, *Lactobacillus, Alloprevotella*, *Bacteroides*, *Lachnospiraceae NK4A136 group*, *Lachnospiraceae_uncultured*, *Alistipes*, *Ruminiclostridium* 9 and *Ruminococcaceae UCG-014* (Fig 3C). The core bacteria of male mice were *Bacteroidales* S24-7 group_ norank, *Lactobacillus, Alloprevotella*, *Bacteroides*, *Lachnospiraceae NK4A136 group*, *Lachnospiraceae_uncultured*, *Alistipes*, *Ruminiclostridium* 9, *Parabacteroides*, *Ruminococcaceae_uncultured* and *Lachnoclostridium* (Fig 3D). Among these core bacteria, the bacteria of 8 genera co-existed in male and female mice, including *Bacteroidales* S24-7 group_ norank, *Lactobacillus, Alloprevotella*, *Bacteroides*, *Lachnospiraceae NK4A136 group*, *Lachnospiraceae_uncultured*, *Alistipes* and *Ruminiclostridium* 9.

At the species level, the core bacteria of female mice contained *Alistipes_ uncultured bacterium*, *Alloprevotella_uncultured bacterium*, *Bacteroidales S24-7 group_uncultured bacterium*, *Bacteroides_uncultured bacterium*, *Lachnospiraceae NK4A136 group_uncultured bacterium*, *Lachnospiraceae_uncultured bacterium*, *Lactobacillus_uncultured bacterium*, *Ruminiclostridium 9_uncultured bacterium* and *Ruminococcaceae UCG-014_uncultured bacterium* (Fig 3E), while the core bacteria of male mice included *Alistipes_uncultured bacterium*, *Alloprevotella_uncultured bacterium*, *Bacteroidales S24-7 group_uncultured bacterium*, *Bacteroides_uncultured bacterium*, *Lachnospiraceae NK4A136 group_uncultured bacterium*, *Lachnospiraceae_uncultured bacterium*, *Lactobacillus_uncultured bacterium*, *Ruminiclostridium 9_uncultured bacterium*, *Bacteroides_ uncultured bacterium*, *Lachnoclostridium_uncultured bacterium*, *Parabacteroides_unclassified* and *Ruminococcaceae_uncultured bacterium* (Fig 3F). Except for *Ruminococcaceae UCG-014_uncultured bacterium* only in the core bacteria of female mice and *Bacteroides_uncultured organism*, *Lachnoclostridium_uncultured bacterium*, *Parabacteroides_unclassified* and *Ruminococcaceae_uncultured bacterium* in the core bacteria of male mice, the remaining bacteria of 8 species existed in the core microbiota of both male and female mice.

To determine wheather the core bacteria in the gut miccrobiota of male and female mice were dominate, the relative abundance of core bacteria was further analyzed. The results revealed that among the core bacteria, 8 out of 9 genera belonged to the dominate bacteria in the gut microbiota of female mice, including *Bacteroidales S24-7 group_norank* (24.62%), *Lactobacillus* (23.12%), *Alloprevotella* (5.72%), *Lachnospiraceae NK4A136 group* (4.52%), *Bacteroides* (4.39%), *Lachnospiraceae_uncultured* (3.73%), *Alistipes* (1.41%) and *Ruminococcaceae UCG-014* (1.34%). Among the core bacteria of male mice, 8 out of 11 genera were dominant, including *Bacteroidales S24-7 group_norank* (23.15%), *Lactobacillus*

(22.85%), *Alloprevotella* (5.14%), *Bacteroides* (4.24%), *Lachnospiraceae NK4A136 group* (3.81%), *Lachnospiraceae_uncultured* (3.02%), *Alistipes* (1.19%) and *Parabacteroides* (1.00%) (Fig3G).

Taken together, these findings revealed that the dominant bacteria in the gut microbiota of male and female mice contained of 16 and 15 genera of baceria, respectively. The core bacteria in the gut microbiota of male and female mice consisted of 11 and 9 genera, respectively.

Functional profiles of the bacteria in the gut microbiota of mice

To characterize the functions of the bacteria in the gut microbiota of mice, KEGG analysis was performed. The results exhibited that at level 1, the bacteria in gut microbiota of mice involved in metabolism (61.99%), none (11.51%), organismal systems (13.45%), genetic information processing (4.80%), environmental information processing (4.12%), human diseases (2.14%) and cellular processes (1.99%), indicating that the bacteria in the gut microbiota of mice mainly functioned in host's metabolism (Fig 4A).

At level 2, the gut bacteria of mice played important roles in global and overview maps (22.94%), energy metabolism (18.08%), endocrine system (12.85%), carbohydrate metabolism (8.08%), amino acid metabolism (3.89%), membrane transport (3.12%), metabolism of cofactors and vitamins (2.27%), translation (2.11%), replication and repair (1.79%), nucleotide metabolism (1.61%), lipid metabolism (1.16%), glycan biosynthesis and metabolism (1.09%), cellular community- prokaryotes (1.01%) and signal transduction (1.00%) (Fig 4A). At Level 3, the main functions of mouse gut bacteria included sulfur metabolism (15.81%), adipocytokine signaling pathway (12.52%), metabolic pathways (9.09%), biosynthesis of secondary metabolites (4.11%), biosynthesis of antibiotics (3.09%), microbial metabolism in diverse environments (2.22%), biosynthesis of amino acids (2.13%), carbon metabolism (1.47%) and ribosome (1.34%) (Fig 4A). These data showed that the main functions of the bacteria in the gut microbiota of mice were associated with host's metabolism.

To explore the functions of the dominant bacteria and the core bacteria in mouse gut microbiota, the dominant and the core bacteria were subjected to the KEGG analysis. The results showed that the dominant bacteria were mainly involved in host's metabolism, including carbohydrate metabolism, amino acid metabolism, energy metabolism, nucleotide metabolism, metabolism of cofactors and vitamins, lipid metabolism, glycan biosynthesis and metabolism, xenobiotics biodegradation and metabolism and metabolism of terpenoids and polyketides (Fig 2B). At the same time, the analysis indicated that the core bacteria in the mouse gut micrbiota mainly took part in host's metabolisms, including carbohydrate metabolism, amino acid metabolism, energy metabolism, nucleotide metabolism, metabolism of cofactors and vitamins, glycan biosynthesis and metabolism, lipid metabolism, metabolism of terpenoids and polyketides and xenobiotics biodegradation and metabolism (Fig 4C). These data revealed that the involvement of host's metabolisms was the major role of gut bacteria.

To reveal the relationship between the gut bacteria, the correlation analysis of the top 20 abundant bacterial genera of mouse gut microbiota was performed. The results indicated that *Citrobacter* was

positively correlated with *Erysipelotrichaceae_ uncultured*, *Ruminiclostridium* and *Ruminiclostridium 9*, while *Ruminiclostridium* was positively correlated with *Parabacteroides*, *Erysipelotrichaceae_uncultured* and *Ruminiclostridium 9*, showing the interactions between these gut bacteria (Fig 4D). There were also positive correlations between *Parabacteroides* and *Ruminococcaceae UCG-014*, as well as *Ruminiclostridium 9* and *Ruminococcaceae UCG-014* (Fig 4D). Negative correlation was found between *Escherichia-Shigella* and *Ruminococcaceae UCG-014*, *Escherichia-Shigella* and *Ruminiclostridium* (Fig 4D). In addition, *Alistipes* appeared to be negatively correlated with *Roseburia*, *Mollicutes RF9_norank*, *Erysipelotrichaceae_uncultured* and *Citrobacter* (Fig 4D). These interacted gut bacteria were associated with host's metabolism (Fig 4A, B and C).

Taken together, these findings presented that the bacteria in the gut microbiota mainly took part in host's metabolisms by the collaborations between the gut bacteria.

Similarity between human and mouse gut microbiota

To explore the similarity and difference between mouse and human gut microbiota, the dominant and core bacteria in human and mouse gut microbiota were compared. The sequencing data of human gut microbiota were obtained from the NCBI database (Fig S4), including 1,053 human fecal samples. At the genus level, the dominant bacteria in the human gut microbiota contained *Bacteroides* (19.73%), *Blautia* (8.81%), *Bifidobacterium* (7.56%), *Faecalibacterium* (5.85%), *Fusicatenibacter* (2.65%), *Anaerostipes* (2.00%), *Lachnoclostridium* (1.08%) and *Alistipes* (1.05%) (Fig 5A). Among these bacteria, *Alistipes* and *Bacteroides* were the dominant bacteria in the gut microbiota of mice while the remaining bacteria were the dominant bacterium unique in human gut microbiota. At the genus level, the human gut microbiota was partially similar to that of mouse gut microbiota.

At the species level, the dominant bacteria in the human gut microbiota included *Blautia_uncultured bacterium* (1.48%), *Bacteroides_Unclassified* (1.45%), *Bacteroides_uncultured bacterium* (1.43%), *Faecalibacterium_uncultured bacterium* (1.40%), *Streptococcus_Unclassified* (1.34%), *Lachnoclostridium_uncultured bacterium* (1.27%), *Fusicatenibacter_uncultured bacterium* (1.26%), *Roseburia_uncultured bacterium* (1.22%), *Butyricicoccus_uncultured bacterium* (1.21%), *Subdoligranulum_uncultured bacterium* (1.21%), *Lachnospiraceae_uncultured bacterium* (1.21%), *[Eubacterium] hallii group_uncultured bacterium* (1.21%), *Anaerostipes_uncultured bacterium* (1.20%), *Ruminococcaceae_uncultured bacterium* (1.18%), *Dorea_uncultured bacterium* (1.10%), *Intestinibacter_uncultured bacterium* (1.08%), *Ruminococcaceae UCG-013_uncultured bacterium* (1.08%), *Alistipes_uncultured bacterium* (1.06%), *Romboutsia_uncultured bacterium* (1.06%), *Ruminiclostridium 5_uncultured bacterium* (1.04%), *Lachnospiraceae NK4A136 group_uncultured bacterium* (1.04%), *Lachnoclostridium_uncultured organism* (1.03%), *[Eubacterium] ventriosum group_uncultured bacterium* (1.01%) and *Lachnospiraceae_Unclassified* (1.00%). Among these bacteria, *Bacteroides_uncultured bacterium*, *Roseburia_uncultured bacterium, Lachnospiraceae_uncultured bacterium, Alistipes_uncultured bacterium* and *Lachnospiraceae NK4A136 group_uncultured bacterium* were the dominant bacteria in the gut microbiota of mice while the remaining were the

dominant bacterium unique in human gut microbiota (Fig 5B). However, all of these bacteria are uncultured or not classified.

Based on the NCBI database (Fig S4), the human gut microbiota analysis showed that only two genera of bacteria were present in all human fecal samples. The bacteria were *Bacteroides* and *Blautia*, accounting for 19.73% and 8.81%, respectively. *Bacteroides* belonged to the core bacteria of mouse gut microbiota, while *Blautia* was unique in the human gut microbiota (Fig 5C). At the species level, there was no core species of bacteria in human gut microbiota.

Taken together, the findings indicated that the structure of mouse gut microbiota was similar to that of human gut microbiota.

Discussion

It is well known that the gut microbiota plays important roles in human health by affecting metabolisms. The gut microbiota participates in energy metabolism via inducing the expression of genes related to lipid and carbohydrate metabolism, whose dysbiosis can lead to obesity [21]. The structure of gut microbiota is always changed in patients with inflammatory bowel disease [22]. The gut microbiota is able to promote dysbiosis, barrier failure, colorectal cancer and inflammation [23]. Multiple neurological diseases, such as autism spectrum disorder, are related to gut microbiota, which regulates behaviors through production of neuroactive metabolites [24]. Many investigations demonstrate that the functions of gut microbiota depend on the structure of gut bacteria [25, 26]. The dysbiosis of gut microbiota can promote or boost susceptibility to metabolic disorders [26]. Therefore the gut microbiota has attracted more and more attentions. As mammalian models, rodents, especially mouse, are widely to explore the roles of gut microbiota [27]. For the better genetic and physiological similarities to humans, mouse model is most commonly employed. The mouse model has the ability to control environmental factors more easily in experiments to minimize changes in baseline gut microbiota between individuals. Although many investigations focus on mouse gut microbiota, the structure of gut microbiota of mouse has not been explored at a large scale [28]. Based on the analysis of mouse gut bacteria at a large scale, our findings revealed that the core bacteria of the mouse gut microbiota included 8 bacteria at the genus level, while the dominant genera of the mouse gut bacteria contained 15 bacteria. The structure of the gut microbiota of mice, including the core bacteria and the dominant bacteria, was similar to that of human being. Therefore, our study provided a solid basis for the investigations of gut microbiota.

Our findings revealed that two bacteria at the genus level, *Alistipes* and *Bacteroides*, were shared by mouse and human microbiota, suggesting the importance of *Alistipes* and *Bacteroides* in mammalian gut microbiota. *Alistipes*, one of the genus members of the *Bacteroidetes* phylum, is highly relevant to dysbiosis and metabolic diseases [29]. Various species of gut bacteria belonging to the genus *Alistipes* have been isolated from patients with appendicitis and abdominal and rectal abscess [30]. It is found that *Alistipes* is pathogenic in patients with colorectal cancer or depression [31, 32]. These data demonstrate that *Alistipes* in gut microbiota plays important positive roles in metabolic

diseases. *Bacteroides,* a dominant bacterial genus in gut microbiota of mouse and human being, can product sphingolipid, which is essential for the maintenance of the symbiotic relationship between gut microbiota and mammalian hosts [33]. Evidences indicate that *Bacteroides* is related to host- and diet-derived glycans and has tremendous capability to utilize complex recalcitrant glycans to sustaining gut microbial symbiosis [34]. These findings show that *Bacteroides* can confer health benefit to the host, therefore helping prevent or delay diseases. In this context, *Alistipes* could be a marker bacterium for metabolic diseases of human being as well as mammals and *Bacteroides* could be used as a bacterial indicator for human health.

Conclusions

The results revealed that at the genus level, the core bacteria of mouse gut microbiota contained 8 bacteria and the dominant bacteria consisted of 15 bacteria. There was a similarity between mouse and human gut bacterial communities. This study provided a solid basis for the investigations of gut microbiota.

Abbreviations

ICR: Institute of Cancer Research)

Operational taxonomic units: OTUs

Principal co-ordinates analysis: PCoA

KEGG: Kyoto encyclopedia of genes and genomes

Declarations

Ethics approval and consent to participate

All procedures treated with mice were in compliance with the Regulations for the Administration of Affairs Concerning Experimental Animals in China.

Consent for publication

Not applicable

Availability of data and materials

The datasets generated during and/or analysed during the current study are available at https://www.ncbi.nlm.nih.gov with accession number PRJNA721276, or was included in this published article (Table S4).

Competing interests

The authors declare that they have no competing interests

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Authors' contributions

MC acquired, analysed and interpreted the data and write this article. XZ designed the work and revised this paper. All authors read and approved the final manuscript.

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Figures

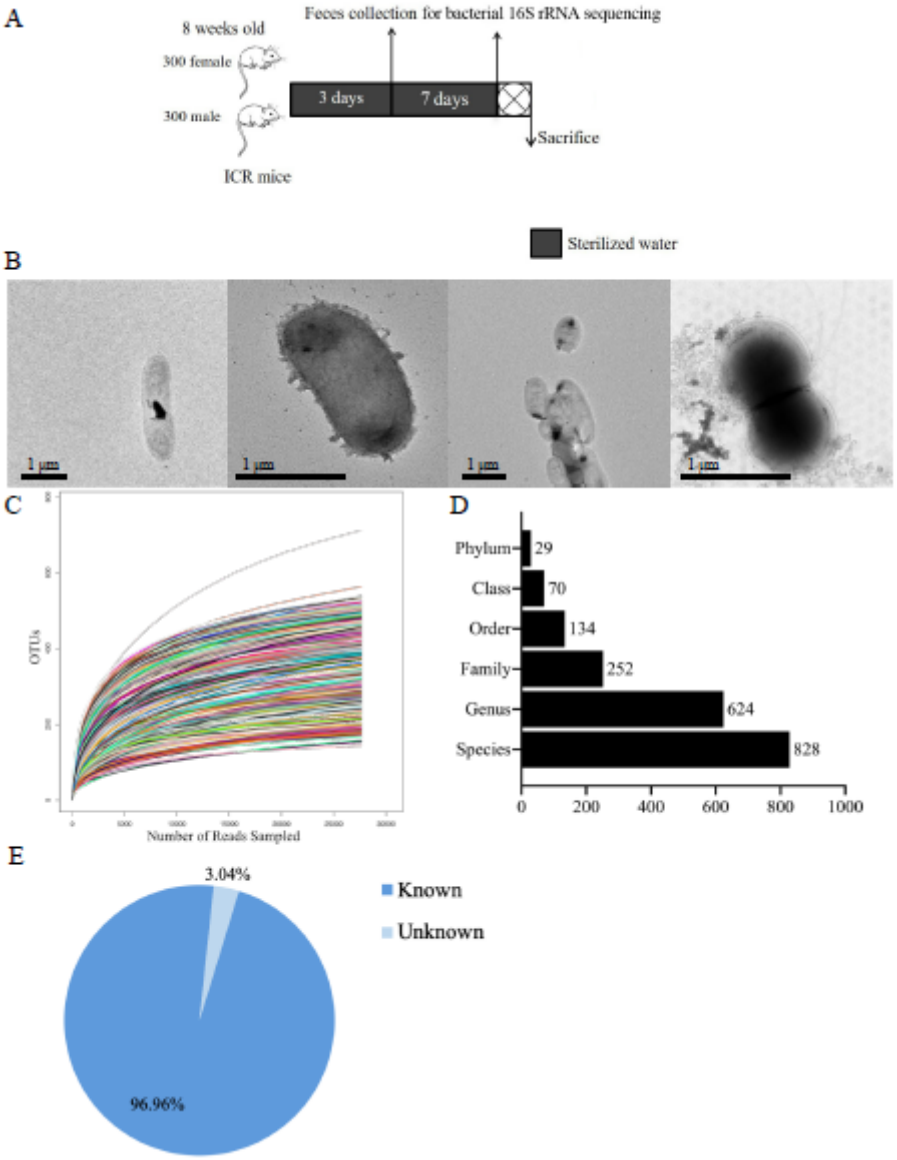

Figure 1

Bacterial communities in mice. (A) A flow diagram of the experiment. A total of 300 female and 300 male mice were fed sterilized water. Fecal samples were collected at day 3 and day 7 for bacteria 16S rRNA sequencing. At day 7, the blood and intestinal tissues of mice were subjected to the detection of physiological parameters. (B) Observation of microbes isolated from fecal samples of mice using transmission electron microscopy. The representative images were presented. Scale bar, 1μm. (C) Rarefaction curves of the bacterial 16S rRNA genes of the feces of 600 mice. (D) Numbers of OTUs homologous to the known bacteria in mouse gut microbiomta at each classification level. (E) Pie diagram of the known and unknown OTUs in all samples at the genus level.

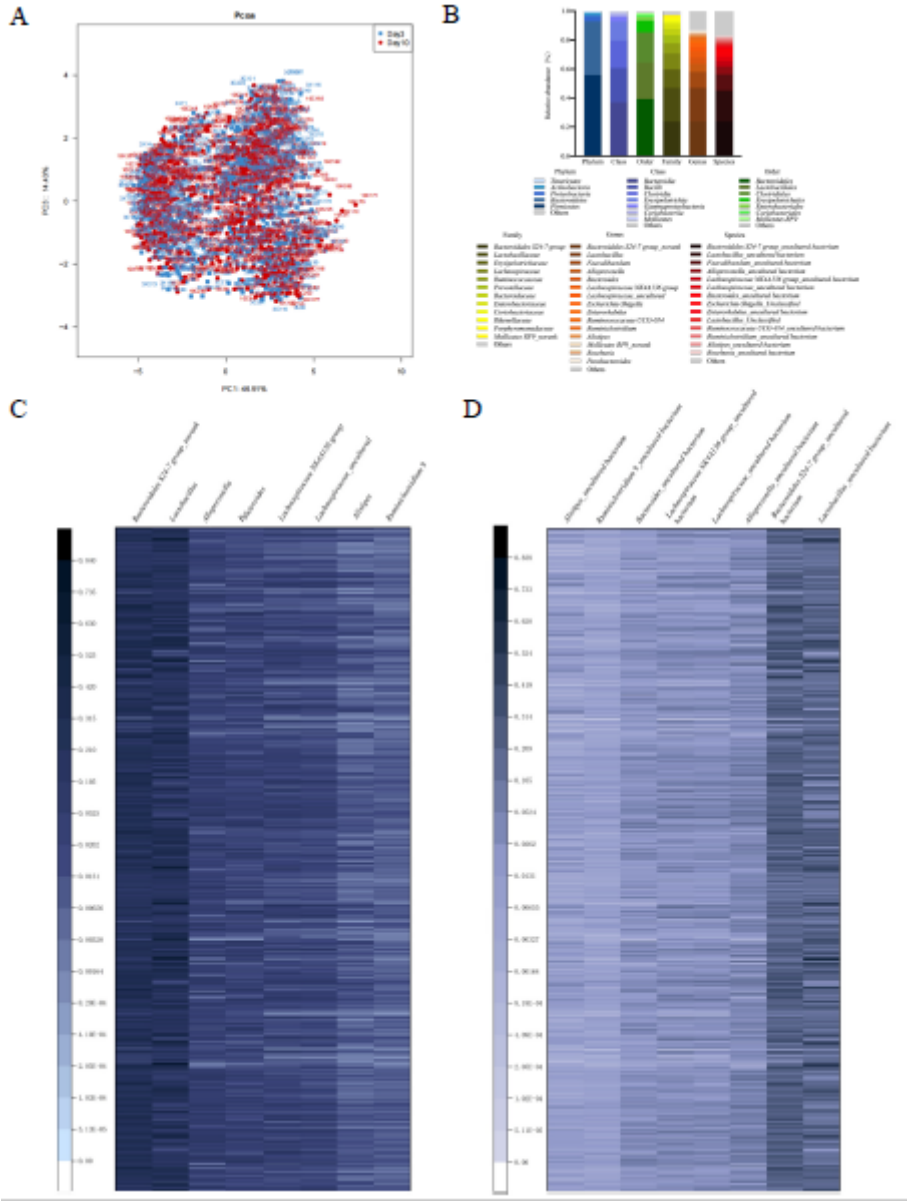

Figure 2

The dominant bacteria and core bacteria in the gut microbiota of mice. (A) Principal co-ordinates analysis (PCoA) of the bacterial communities of mouse fecal samples. Each dot represented the stucture of gut microbiota of mice at Day 3 (blue) or Day 10 (red). (B) Relative abundance of gut bacteria of mice. “Uncultured” represented the bacteria that could not be cultured. “Unclassified” indicated the sequences that could not be classified. “Norank” represented that there was no classification information or classification name. Bacteria with a relative abundance of less than 1% were classified as “Others”. (C) The core bacteria of mouse gut microbiota at the genus level. (D) The core bacteria of mouse gut microbiota at the species level. “Uncultured” represented the bacteria that could not be cultured.

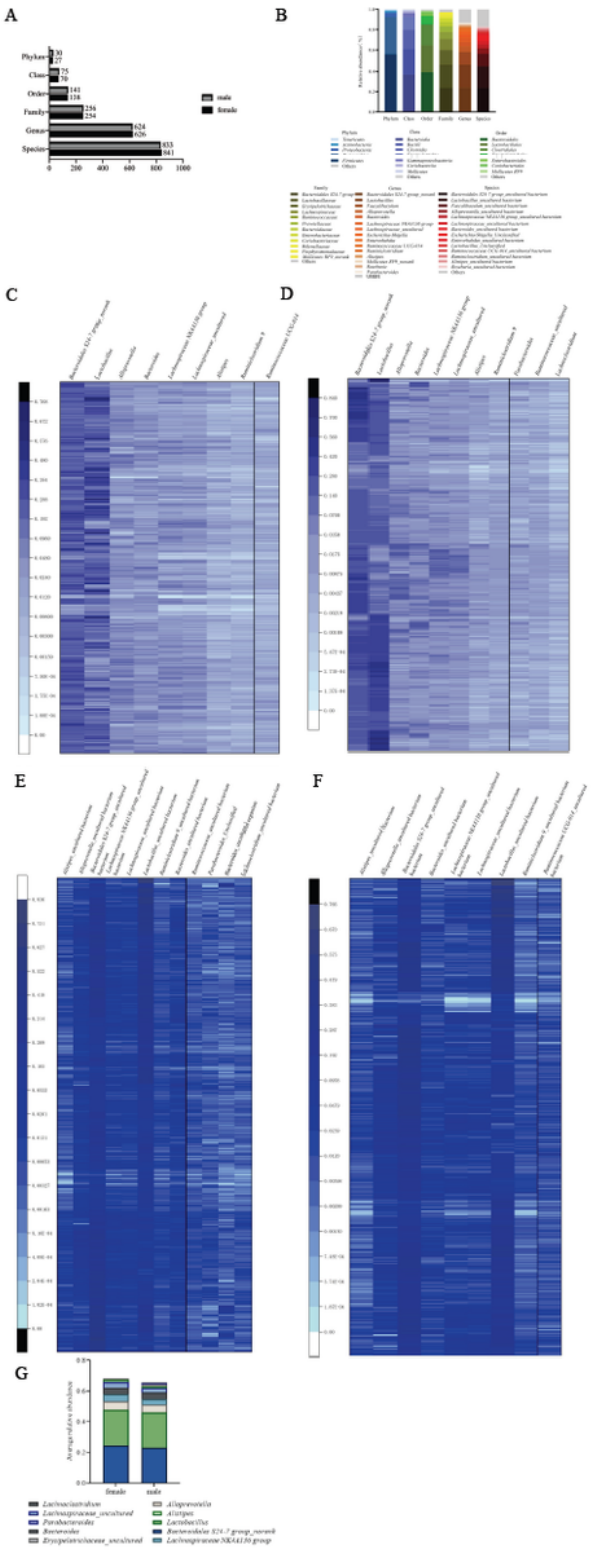

Figure 3

Bacterial composition in the gut microbiota of male and female mice. (A) Number of the bacteria in male and female mouse gut microbiota at each classification level. (B) Relative abundance of gut bacteria in male and female mice. “Uncultured” represented the bacteria that could not be cultured. “Unclassified” indicated the sequences that could not be classified. “Norank” represented that there was no classification information or classification name. Bacteria with a relative abundance of less than 1% were

classified as “Others”. (C) Core bacteria in the gut microbiota of female mice at the genus level. The left side of the black line represented the core bacterium shared by male and female mice, while the right side of the black line indicated the core bacteria different from male mice. (D) Core bacteria in the gut microbiota of male mice at the genus level. The left side of the black line indicated the core bacteria shared by male and female mice. (E) Core bacteria in the gut microbiota of female mice at the species level. The left side of the black line represented the core bacterium shared by male and female mice, while the right side of the black line indicated the core bacteria different from male mice. (F) Core bacteria in the gut microbiota of male mice at the species level. The left side of the black line represented the core bacteria shared by male and female mice. (G) Relative abundance of the core bacteria in the gut microbiota of female and male mice at the genus level. “Uncultured” indicated the bacteria that could not be cultured. “Unclassified” showed the sequences that could not be classified. “Norank” represented that there was no classification information or classification name. Only the bacteria with an relative abundance of more than 1% were listed.

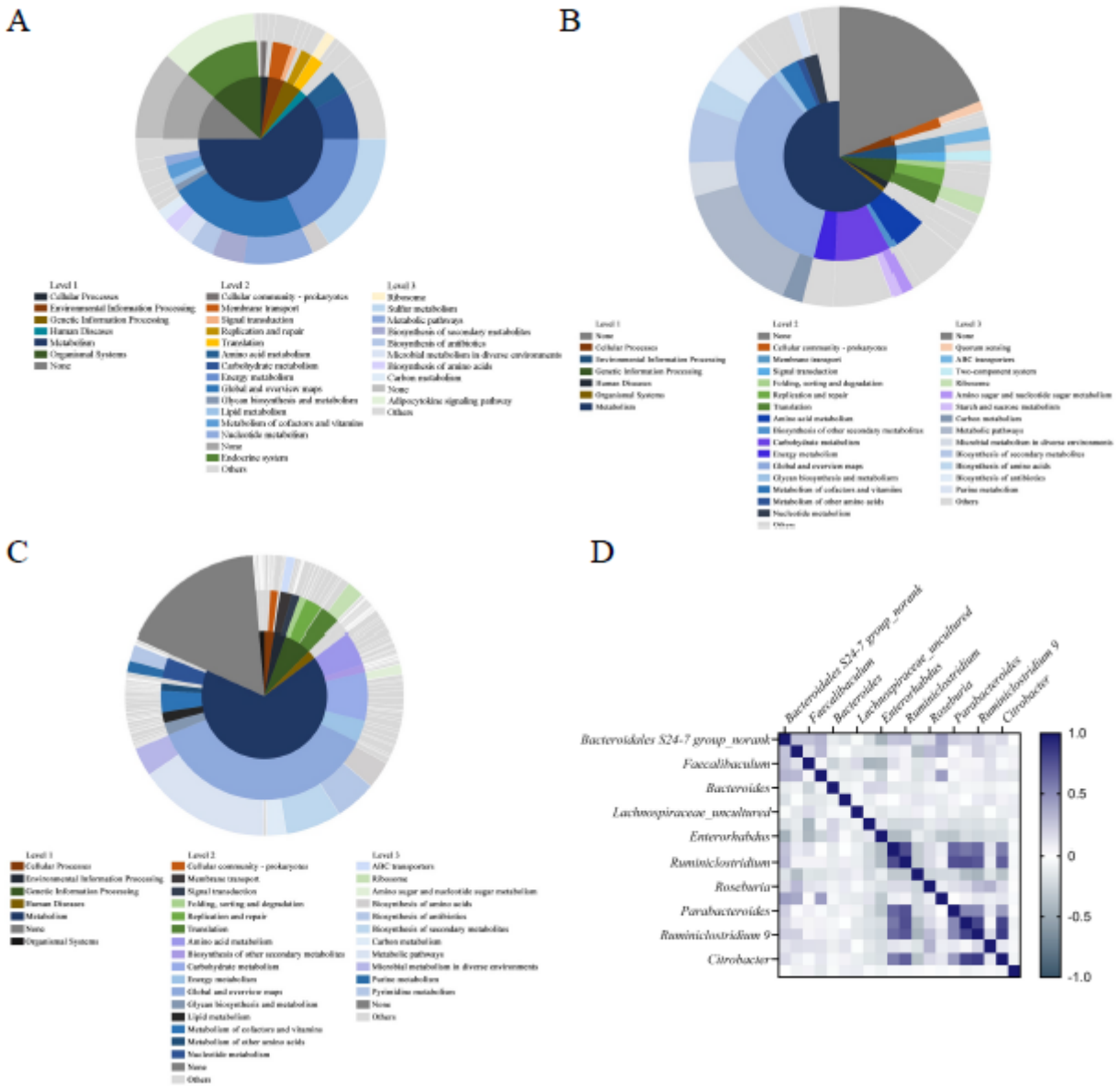

Figure 4

Functional profiles of the bacteria in the gut microbiota of mice. (A) The functional profiles of the bacteria in the gut microbiota of mice. The functions of the gut bacteria of mice were analyzed using KEGG. The KEGG pathways in level 1 (inner layer), level 2 (middle layer) and level 3 (outer layer) were indicated. Only pathways with a percentage more than 1% were shown and the remaining was labeled as “Others”. (B) The functions of the dominant bacteria in the gut microbiota of mice. The KEGG pathways involved by the gut bacteria were indicated. (C) The pathways involved by the core bacteria in the mouse gut microbiota. Based on the KEGG analysis, the pathways involved by the gut core bacteria were obtained. (D) Correlation analysis of the bacteria in mouse gut microbiota. The top 20 abundant bacteria were analyzed.

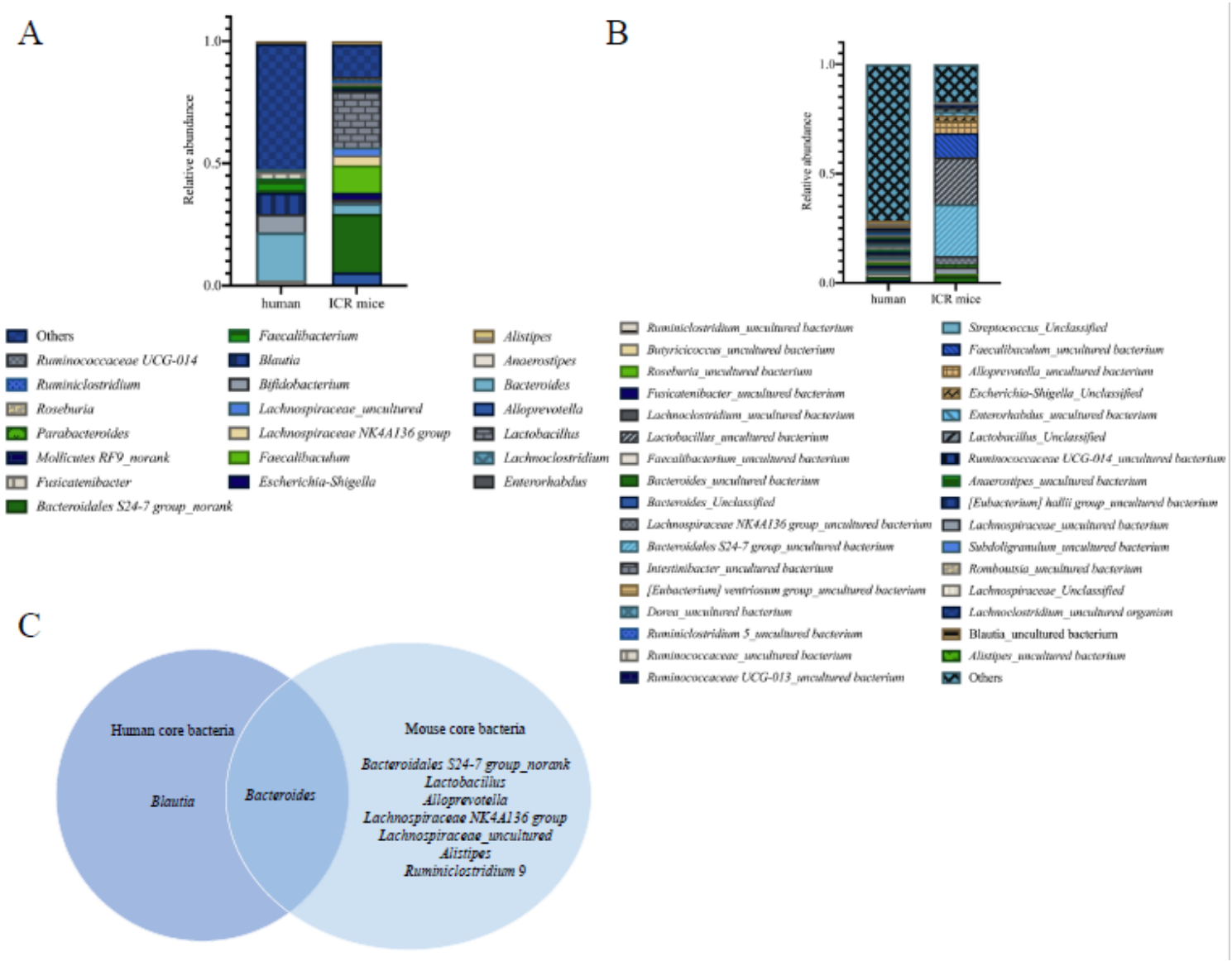

Figure 5

Similarity between human and mouse gut microbiota. (A) The dominant bacteria of mouse and human gut microbiota at the genus level. “uncultured” represented the bacteria that could not be cultured. “unclassified” indicated the bacteria that could not be classified. “norank” represented that there was no classification information or classification name. Bacteria with a relative abundance of less than 1% were

classified as “others”. (B) The dominant bacteria of mouse and human gut microbiota at the species level. (C) Comparison of the core bacteria in human and mouse gut microbiota.

Supplementary Files

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- TableS3.xls
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