The Regional Diversity of Gut Microbiome Along the GI Tract

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Abstract

**Background:** The proliferation and survival of microbial organisms including intestinal microbes are determined by their surrounding environments. Contrary to popular myth, the nutritional and chemical compositions, water contents, O2 contents, temperatures, and pH in the gastrointestinal (GI) tract of human are very different in a location-specific manner, implying heterogeneity of the microbial composition in a location specific manner.

**Results:** We first investigated the environmental conditions at 6 different locations along the along the GI tract and feces of ten weeks’ old male SPF C57BL/6 mice. As previously known, the pH and water contents of the GI contents at the different location of the GI tract were very different from each other in a location-specific manner, and none of which were not even similar to those of feces. After confirming the heterogeneous nature of the GI contents in specific location and feces, we thoroughly analyzed the composition of microbiome of the GI contents and feces. Metagenome sequencing on the GI contents and feces showed presence of 13 different phyla. The abundance of Firmicutes gradually decreased from the stomach to feces while the abundance of Bacteroidetes gradually increased. The taxonomic α-diversities measured by ACE richness, Shannon diversity and Fisher’s alpha all indicated that the diversities of gut microbiota at colon and cecum were much higher than that of feces. The diversities of microbiome compositions were lowest in jejunum and ilium while highest in cecum and colon. Interestingly, the diversities of fecal microbiome were lower than those of cecum and colon. Beta diversity analyses by NMDS plots, PCA, and un-supervised hierarchical clustering all showed that the microbiome compositions were very diverse in location-specific manner. Direct comparison of the fecal microbiome with the microbiome of the whole GI tracts by α-and β-diversities showed that fecal microbiome did not represent the microbiome of the whole GI tract

**Conclusion:** The fecal microbiome is different from the whole microbiome of the GI tract, contrary to the baseline assumption in the large majority of contemporary microbiome research work.

Background

The human gut microbiome is consisted of 100 trillion microbes in the intestine whose collective genome contains at least 100 times more genes than our own genome [1–3]. The results of the interactions between gut microbiome and its host are various; negligible, negative or positive [4]. Despite negative consequences in some cases, the presence of gut microbiome is essential to our health and well-being in most cases [4]. It is now clear that the gut microbiome contributes significantly to the traits of humans as much as our genes, especially in the case of atherosclerosis, hypertension, obesity, diabetes, metabolic syndrome, inflammatory bowel disease (IBD), gastrointestinal tract malignancies, hepatic encephalopathy, allergies, behavior, intelligence, autism, neurological diseases, and psychological diseases [4, 5]. Alteration of the composition of the gut microbiome even affects the behavior, intelligence, mood, autism, psychology, and migraines of its host through the gut-brain axis [6]. It is now clear that the relationship between gut microbiome and humans is not merely commensal but rather a
mutualistic relationship [6–9]. Thus, recent advances in the field of gut microbiome are not only elucidating our understanding of human biology but also present a new paradigm of opportunities for medical and food applications.

The gut microbiome comprises all intestinal microorganisms residing along the gastrointestinal (GI) tract which include commensal, symbiotic and pathogenic microorganisms. Almost all of the current researches on gut microbiome strictly rely on metagenome sequencing analyses of microbiomes isolated from fecal samples under the baseline assumption that fecal microbiome represents whole gut microbiome or at least similar [10–12]. The GI tract is a hollow organ system but divided into sections which digests food, extracts and absorbs nutrients, and discharges waste materials in a location-specific manner. The environmental conditions such as pHs, water contents, chemical profiles, O$_2$ contents, etc. in the GI tract are constantly changed location by location as the specific components of foods are mechanically and enzymatically broken down into substances for absorption into the bloodstream [13].

The growth of microbial organisms is ultimately determined by environmental factors such as chemical components, water contents, O$_2$ contents, temperatures, and pH [14–16]. Intestinal microbial organisms are not an exception. The nutritional and chemical compositions, water contents, O$_2$ contents, temperatures, and pH in the gastrointestinal (GI) tract of human are very different in a location-specific manner [17–21], which implies that the compositions of gut microbiome in the GI tract could be also differ in a location specific manner. Since none of these environmental factors in feces are represented in any part of the GI tract even in large intestine, it would be reasonable to question whether fecal microbiome does represent the microbiome of the gastrointestinal tract or not.

Considering these variances in the GI tract, we investigated variations of gut microbiome at different locations of the GI tract, and compared the compositions of microbiome in the GI tract with the fecal microbiome by using thorough statistical methods. This work showed that the compositions of gut microbiome were constantly changing at a location specific manner reflecting its environmental difference.

**Results**

**The environmental conditions in the GI tract varied in a location-specific manner**

Realization of the variable nature of environmental factors in the GI tract prompted us to investigate the possibility of the location-specific environmental variations in the GI tract by using male SPF C57BL/6 mice. The whole GI tracts of mice of ten weeks’ old were divided into six parts (Figure S1), and the GI contents from each location as well as feces were collected and analyzed. As expected, the pHs and water contents of the GI contents were very different from each other in a location-specific manner along the GI tract and those of feces was not similar to the GI contents at any location (Table S1, S2), indicating heterogeneous environments along the GI tract. These results clearly showed that the environmental
conditions in the GI tract vary reflecting the local function in the GI tract. The environmental condition of feces was not similar to those of any part of the GI contents, nor the overall GI content.

**Metagenome sequencing unveiled the location specific diversity of gut microbiome in the GI tract**

We next investigated the diversity of gut microbiome at different locations within a same mouse by metagenome analyses. The V3-V4 sites of the 16Sr rRNA genes of the isolated genomic DNAs of the gut microbiome of the GI contents were sequenced using the MiSeq™ platform (Illumina). The sequence reads containing incorrect primer, barcode sequences, sequences with more than one ambiguous base, low quality sequences or chimeras were 2.2%, and these sequence reads were removed. The filtered 16S rRNA sequences were used to identify individual microbes by matching the 16S rRNA sequences with the SILVA reference (region V3-V4) database (https://www.arb-silva.de/). All of the identified 16S rRNA sequences were able to be classified into 13 different phyla; Bacteroidetes (51.5%), Firmicutes (35.88%), Proteobacteria (8.29%), Epsilonbacteraeota (1.26%), Cyanobacteria (9.4%), Actinobacteria (63%), Patescibacteria (5%), Deferribacteres (17%), Tenericutes (62%), Verrucomicrobia (0.8%), Planctomycetes (0.4%), Fusobacteria (0.3%), and Gemmatimonadetes (0.1%) (Figure 1A). Interestingly, the abundance of the two most abundant groups of microbes was reversed from stomach to feces along with the GI tract (Fig. 1B, 1C), suggesting that microbial flora change reflecting the environmental change in the GI tract. The abundance of Firmicutes gradually decreased from the stomach to feces while the abundance of Bacteroidetes gradually increased (Fig. 1B, 1C).

*Alpha-diversity analysis showed that microbiomes in the different locations of the GI tract completely differed from each other*

The gross microbiome analysis at the phylum level along the GI tract indicated that the microbiome was ever changing along the GI tract reflecting their various environments and the microbiome of the GI tract was very different from fecal microbiome (Fig. 1A, 1B, Figure S2 ~ S4). The discrepancy of microbiome compositions depending on locations became more evident at lower taxonomic levels (Figure S5). Surprisingly the microbiome in the upper GI tracts and small intestines completely differed from those the lower GI tracts within a mouse and the degree of differences gradually decreased from stomach to feces (Figure S5, Table S3 ~ S7). It should be noted that the microbiome differences of large intestines among different mice were significantly decreased, demonstrating quite similar microbiome compositions of large intestine and feces among different mice. The microbiome analysis at the class level demonstrated that Bacteroidia was unanimously abundant along the GI tracts while most abundance were observed with Bacilli and Clostridia in the stomach, with Bacilli and Erysipelotrichia in the small intestine, and with Clostridia in the large intestine and feces (Table S4). Likewise, GI tract was unanimously abundant with the order of Bacteroidales followed by Lactobacillales and Clostridiales in the stomach, Lactobacillales and Erysipelotrichales in the small intestine, and Clostridiales in the large intestine and feces, respectively (Table S5). At the family levels, Muribaculaceae was unanimously abundant followed by Lactobacillaceae and Lachnospiraceae in the stomach, Lactobacillales in the small intestine, and
Lachnospiraceae and Ruminococcaceae in the large intestine and feces, respectively (Table S6). At the genus levels, there were clearly distinguished pattern along the GI locations despite of the presence of unidentified groups (Table S7). Helicobacter was in the stomach as well as large intestine but not in small intestine. Lactococcus, Dubosiella, Parasutterella and Turicibacte were specifically observed in the small intestine while Helicobacter, Bacteroides, Alloprevotella, Odoribacter, and Alistipe in large intestine and feces (Table S7).

Our initial comparison of the microbiome compositions at locations along the GI tracts was followed by a thorough diversity analysis of the microbiome. The taxonomic α-diversities measured by ACE richness, Shannon diversity and Fisher’s alpha all indicated that the diversities of gut microbiota at colon and cecum were much higher than that of feces (Fig. 2, Table S8). The diversities of microbiome compositions were lowest in jejunum and ilium while highest in cecum and colon. It should be noted that the diversities of fecal microbiome were lower than those of cecum and colon. Clearly, the α-diversity analyses indicated that the fecal microbiome did not represent the microbiome in the GI tract of its host, contrary to the general baseline assumption.

**Beta-diversity analysis confirmed that microbiomes in different locations of the GI tract completely differed from each other**

Discrepancy of the composition of gut microbiome along the GI tract became more evident with analysis of the ratio between location and local species (Fig. 3,4, Figure S6). To compare the diversities of the microbiomes at different locations, β analysis method was applied. The NMDS plots based on Bray-Curtis distances showed that the microbiome compositions were very diverse in location-specific manners in all of tested three mice and that, more significantly, fecal microbiome did not represent the microbiome of the GI tracts (R² = .49, P = .003 ADONIS) (Fig. 3A). We transformed the OTUs of each microbiome into principal components using an unweighted UniFrac metric for Principal coordinates analysis (PCoA). Eigenvalues of each microbiome in different locations of the GI tracts were very different from each other (Fig. 3B, 3C). PCoA confirmed again that the fecal microbiome communities in all of the tested three mice did not represent any part of the microbiome communities in the guts. The correlation analysis of OTU values with respect to the locations of the GI tract by drawing a heat map of the top ranked OTUs defined at the bray curtis distance level revealed that feces had a distinguished microbial profiles compared with any locations of the GI tracts (Fig. 3D). Un-supervised hierarchical clustering clearly partitioned the samples into two distinguished groups, and this pattern was observed repeatedly over a wide range of phylogenetic levels (Figure S6).

**Alpha-diversity analysis showed that fecal microbiome did not represent the microbiome of the whole GI tract**

Location-specific analysis on microbiome clearly indicated that microbiome in the GI tract varied on its location under varied physical and chemical environments, and that fecal microbiome might not represent the real microbiome in the GI tract (Fig. 1 ~ 4, Figure S2 ~ S6). To investigate that possibility, we
directly compared fecal microbiome compositions with the microbiome composition of the whole GI tracts in each mouse. As expected, the gross microbiome analyses revealed that the microbiome composition of the GI tracts were clearly different from fecal microbiome composition (Fig. 5A, B, Figure S7A, S7B). The microbiome discrepancy between feces and the GI tract became more evident at lower taxonomic levels (Fig. 5B, Figure S7C). The most abundant microbial families in the GI tracts were Muribaculaceae, Lactobacillaceae, Lachnospiraceae Ruminococcaceae, and Erysipelotrichaceae in the decreasing order while Muribaculaceae, Ruminococcaceae, Lachnospiraceae, and Prevotellaceae were in fecal microbiomes (Fig. 5B). At the genus level, Lactobacillus, Lactococcus, Dubosiella and Turicibacter were highly represented in the GI tract but not in feces (Figure S7D and Table S7).

After noting the microbiome discrepancy between feces and the GI tract by direct comparison, thorough taxonomic α-diversity analyses were performed. The taxonomic α-diversities measured by ACE richness, Shannon diversity and Fisher’s alpha all indicated that the diversities of the microbiome of the GI tracts were much higher than fecal microbiome (Fig. 5C). Also, fecal microbiome did not represent the microbiome of the GI tracts in all of the three mice. Shannon diversity (p < .05), ACE richness (p < .01) and Fisher’s alpha (p < .01) concluded that the microbiome of the GI tract is statistically different from fecal microbiome and fecal microbiome does not represent the microbiome of GI tract.

*Beta-diversity analysis confirmed that fecal microbiome did not represent the microbiome of the whole GI tract*

Comparative analysis on the microbiome of feces and the GI tracts by β diversity analyses (community structure: R² = .1, p < .05 ADONIS) further solidified that fecal microbiome did not represent the microbiome in the GI tract of its host. Both NMDS and RDA plots showed that fecal microbiome were distinctly different from the microbiome of the GI tracts in all of the tested mice (Fig. 6A, 6B). Interestingly, the microbial community of fecal microbiome was closer to each other in individual mice than to that of the GI microbiome within the same mice. The distinct difference of microbiome compositions between feces and the GI tract within a mouse became more evident with a correlation analysis of total OTUs with respect to feces and the GI tract. The heat map of the all OTUs defined at the Bray-Curtis distance level revealed that the fecal microbiome was completely different from that of the GI tracts as demonstrated in a distinguished pattern of microbial profile among feces and also among the GI tracts in all tested mice rather than between the microbiome compositions of feces and the GI tract within same mice (Fig. 6C).

**Discussion**

The gut is the place where food is broken-down and metabolized, nutrients are absorbed, water and minerals absorbed, waste metabolites are dumped from body, and pH and oxygen levels fluctuates [22]. These activities in gut are precisely regulated and thereby location-specific, which means that the environment condition inside gut is different location by location [23–27]. Accordingly, the chemical and physical composition of feces differ from the GI contents in large intestine [28]. In fact, this work showed that the pH and water content of feces even differed from those of the GI contents in large intestine [29].
The pH differences noted here are well matched with previous reports that the intraluminal pH at different locations ranged from 1.0 ~ 2.5 (stomach) to 6.6 ± 0.5 (large intestine) while the pH of feces was 7.5 ± .4[19]. Considering these differences, it would not be surprising to note that the fecal microbiome cannot represent the gut microbiome of its host because the growth and propagation of microbial organisms, including the intestinal microbes of mammals, depend on their surrounding environments.

Although the fact that fecal microbiome differ from gut microbiome has not been recognized, there has been growing concerns regarding using feces as a proxy to study the gut microbiome. Yan et al. found a certain degree of discrepancy between the fecal microbiome and the gut microbiome in chicken [30]. It has been reported that stool sampling affects the heterogeneity and inconsistency of fecal microbiome [29, 31, 32]. This works showed that even the microbiome in the GI content of the large intestine is different from the fecal microbiome (Fig. 1 ~ Fig. 4). Since stool can be excreted at various state from the large intestine, the heterogeneity and inconsistency of fecal microbiome could be expected. Therefore, this work seems to well explain the previous works which accounted for the heterogeneity and inconsistency of fecal microbiome as stool inconsistency[29, 31, 32].

Although validity of fecal microbiome as a proxy of gut microbiome have been questioned previously, the question never been seriously investigated. Rather, fecal samples have been customarily used for investigation of gut microbiome after neglecting the fact that even stool sampling generates heterogeneity and inconsistency in fecal microbiome. To compare the gut microbiome and fecal microbiome in a same condition, we used genetically homogenous sibling male mice grown in a co-housed condition to ensure that the experimental condition is identical for each mouse. Thorough statistical analyses showed that microbiome in the GI tract is consistently changing reflecting environmental conditions at the location of the GI tract and thereby fecal microbiome is different from the whole gut microbiome of the GI tract.

While numerous recent researches successfully showed that gut microbiome plays determinant roles in various phenotypes and diseases of its host [10, 33–35], those researches are largely associative in nature and may fail to pinpoint the causative intestinal microbes for the phenotypes or diseases along with difficulties in consistency and reproduction by other researchers [36–38].

Conclusions

This work suggests that fecal microbiome could be merely the result of gut microbiome dysbiosis while a gut microbiome dysbiosis might be responsible for phenotypes and diseases of host. Rather than fecal analysis, it would be reasonable to investigate gut microbiome among individual hosts with reliable tools such as detecting a blood signature of gut microbiome or developing an endoscopic method.

Methods

Animals and sample collection
All animal care and use protocols were performed strictly in accordance with the ethical guidelines of the Ethics Committee of the Chonbuk National University Laboratory Animal Center (Permit Number: CBU 2012-0040) in accordance with the 'Guide for the Care and Use of Laboratory Animals.

Six-week-old male C57BL/6 mice (Joongang Experimental Animal Co., Seoul, Korea) were purchased and acclimatized for 4 weeks. During the experimental period, the mice were housed in an animal room under controlled environmental conditions at a temperature of 22 ± 2 °C, relative humidity of 50 ± 5%, and a 12-h light/dark cycle, with food and water readily available. Three male SPF C57BL/6 mice aged ten weeks were used in this study. All mice were transferred to freshly sterilized cages, and the feces were collected within two hours from the cages. All mice were sacrificed by cervical dislocation. After sacrificing the mice, the whole GI tracts were segmented immediately into stomach, duodenum, jejunum, ileum, cecum, and colon according to the anatomical feature. The segments were subsequently opened along their cephalocaudal axis using a sterile scissor, and the GI contents in each segment were thoroughly harvested by collecting and followed by swapping with spatula. Each sample, except for pH measurement, was weighed and immediately frozen in liquid nitrogen and were stored at -80°C until DNA extraction.

**Determination of the water contents of the GI contents**

The water contents of each GI contents were determined by subtracting dry weights from the wet weights. The wet weights of the GI contents were measured before lyophilization and the dry masses were measured after lyophilization.

**pH determination of the GI contents**

Approximately .1 g of each GI content was transferred into Eppendorf tube containing 0.9 ml ddH₂O. After thorough mixing followed by standing 1 hour at room temperature, pH of each sample was measured, using a pre-calibrated Orion Star™ A210 series benchtop pH meter (Fisher Scientific). pH was measured three times and averaged.

**Microbiome DNA preparation**

Total genomic DNA from each sample was extracted using the phenol-chloroform isoamyl alcohol extraction protocol, as described previously [39]. Briefly, lysis buffer (200 mM NaCl, 200 mM Tris-HCl (pH 8.0), 20 mM EDTA) suspended samples were processed by bead beating, and the genomic DNA recovered from aqueous phase by phenol:chloroform:isoamylalcohol. DNA precipitated with the addition of 3M sodium acetate followed by isopropanol. After rinsing with 70% ethanol and drying, the DNA pellet was dissolved in TE buffer (10 mM Tris-HCl pH 8.0, 1 mM EDTA). DNA was quantified using a BioSpec-nano spectrophotometer (Shimadzu Biotech).

**Bacterial 16 s rRNA genes Sequencing**

The sequencing samples are prepared according to the Illumina 16S Metagenomic Sequencing Library protocols. The 16S rRNA genes were amplified using 16S V3-V4 primers (16S Amplicon PCR Forward
Primer: 5’ TCGTCGGCAGCGTCAGATGTATAAGAGACCA GCCTACGGGNGGCWGCAG; 16S Amplicon PCR Reverse Primer: 5’ GTCTCGTGGGCTCGAGATGTATAAGAGACAGGACTACHVGGGTATCTAATCC).

Input gDNA was amplified with 16S V3-V4 primers, and a subsequent limited cycle amplification step was performed to add multiplexing indices and Illumina sequencing adapters[40]. The final products were normalized and pooled using the PicoGreen, and the size of libraries were verified using the TapeStation DNA screen tape D1000 (Agilent). And sequencing (2 × 300) was done using the MiSeq™ platform (Illumina) according to the standard protocol.

### Sequencing data analysis

To improve genome assembly, the paired-end reads from NGS (Next Generation Sequencing) were merged using FLASH (Fast Length Adjustment of Short reads) [41]. The amplicon error was modelled from merged fastq using DaDa2 and filtered out noise sequence, corrected errors in marginal sequences, removed chimeric sequences, removed singleton, and then dereplicated those sequences [42]. The Q2-Feature classifier is a Naive Bayes classifier trained based on SILVA reference (region V3-V4) database (https://www.arb-silva.de/) to classify the dataset used in the experiment [43]. The q2-diversity used in the diversity calculation and statistical tests [44].

### Data preprocessing

The metagenomics data, OTU and taxonomic classification tables were imported into phyloseq (1.28.0) package in R version 3.6.1 [45]. The OTUs that are not present in at least one sample were considered as OUTs generated by sequencing errors, thereby removed for further analysis. The OUT data was converted to metagenomeseq object and normalized by cumulative-sum-scaling (CSS), which was specially built for metagenome data in the bioConductor package metagenomeSeq (1.16.0.) [46]. Normalized data was imported as phyloseq class object in R for further analysis and visualization.

### Alpha diversity and abundance evaluation of microbiome

Alpha diversity (ACE richness, Simpson diversity, and Fisher’s alpha) metrics were calculated based on CSS normalized values without filtering in phyloseq package [47]. To detect differences in richness and alpha diversity between groups, we used Kruskal-Wallis rank sum test., and filtered data was converted into relative abundance. Further, unclassified phyla were removed from total samples. Any taxa with a total of less than 0.5% were collapsed into “other” and each taxonomy level was glmmed before plotting.

### Beta diversity and abundance evaluation of microbiome

Beta diversity metrics were computed and visualized using log transformed, normalized OTU data in phyloseq package including Bray-Curtis dissimilarity [48]. To detect statistical differences in beta diversity metrics between groups, we used permutational multivariate analysis of variance (PERMANOVA) in the vegan package in R. ADONIS was used with 999 permutations in the vegan package in R to quantify the effect size of variables explaining Bray-Curtis distance [49]. Unweighted PCoA was calculated and
visualized by QIIME2, however; NMDS, RDA, MDS, CCA, and DCA were plotted in the phyloseq package in R.

**Construction of heatmap and phylogenetic tree**

A heatmap and cluster analysis were generated using the relative abundances of genera from both all OTU values or core abundant OTU values in Heatplus (2.30.0.) package from bioconductor and vegan package in R. Average linkage hierarchical clustering and Bray-Curtis distance metric were used for cluster analysis and heatmap generation respectively [50]. *Unsupervised* prevalence filtering was done with threshold 5% of total samples to collect mostly abundant taxonomy for heatmap generation.

Phylogenetic trees for each sampling site were constructed from row sequences without any filtering to show direct visualization of sample richness with relation to taxanomy classification. Taxonomies that could not be classified down to the species level were reclassified based on NCBI accession number using taxonomizr (0.5.3) package in R [51]. 16 s rRNA sequences from each sampling site were aligned in ClustalW [52] with default parameter, and resulted alignments were used to construct the Maximum-likelihood phylogenetic trees in MEGAX [53] with 500 bootstraps replicates. All phylogenetic trees were visualized in iTOL [54].

**Statistical analysis**

All statistical analyses are reported as the mean ± SEM, and the differences in relative abundance of bacterial populations among feces to GI parts were analysed using the Mann-Whitney sum rank tests in R software. Significance was declared at *P* < 0.05. All graphs were prepared with R software.

**Abbreviations**

CCA  
*canonical correspondence analysis*; CSS: cumulative-sum-scaling; DCA: *detrended correspondence analysis*; FLASH: fast length adjustment of short reads; gDNA: genomic deoxyribonucleic Acid; GI: gastrointestinal; IBD: inflammatory bowel disease; MDS: multidimensional scaling; NGS: next generation sequencing; NMDS: non-metric multidimensional scaling; OUT: operational taxonomic unit; PCoA: principal coordinates analysis; PCR: Polymerase chain reaction; RDA: redundancy analysis; rRNA: *ribosomal* ribonucleic acid, 16SrRNA: 16S ribosomal ribonucleic acid.

**Declarations**

- Ethics approval and consent to participate:

This study was approved by the ethical guidelines of the Ethics Committee of the Chonbuk National University Laboratory Animal Center.

- Consent for publication: Not applicable.
Availability of data and materials:
The datasets supporting the conclusions of this article are included within the article and its additional files.

Competing interests: The authors report no conflict of interest.

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Authors’ contributions:
Author Contributions are as fellows. S.T.H. and S.L. designed the project and experiments; E.L., H.J.C., S. L. L. and S.T.H. analyzed results; E.L., J.H. and H.J.C. performed the experimental works; E.L., W.H.T., S.T.H. and S.L. wrote the manuscript. All authors read and approved the final manuscript.

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Figures
Figure 1

Comparison of microbial diversity at the different locations of the GI tract in a same mouse. A. Relative abundance of phyla occupying in the GI tract and feces. B. Comparison of relative abundances of the three main bacterial phyla found in every sampling site: Bacteroidetes, Firmicutes, Proteobacteria represented as relative abundances on the Y-axis. C. The relative abundance of Bacteroidetes and Firmicutes along the GI tract.
Figure 2

Comparison of microbial diversity at the different locations of the GI tract in a same mouse by α-diversity analysis. Species richness and diversity measured by ACE richness, Shannon diversity and Fisher’s alpha, Evenness, Inverse Simpson, Simpson at the different locations of the GI tract.
Figure 3

Comparison of microbial diversity at the different locations of the GI tract in a same mouse by β-diversity analysis. A. Non-metric multidimensional scaling (NMDS) plots showing the difference of microbiome in different locations of the GI tract at OTU level based on Bray-Curtis distances. B. Three-dimensional unweighted PCoA based on the unweighted Unifrac metric of microbiome among all samples. C. Principal coordinate analysis (PCoA) based on the unweighted Unifrac metric of microbiome among all
samples. The percentage of variation explained by PC1 and PC2 are indicated in the axis. D. Heatmap of the microbial composition and relative abundance of all samples based on the Bray–Curtis distance matrix calculated from relative OTU abundances at genus level.

**Figure 4**

Ordination plots based on the Bray-Curtis distances in the microbial communities of the GI tracts. 2D stress values were 0.03, 0.29 and 0.086 for mouse 1, mouse 2 and mouse 3 respectively. A. Redundancy
analysis (RDA), B. Detrended correspondence analysis (DCA), C. Multidimensional scaling (MDS) and D. Correspondence analysis (CA).

**Figure 5**

Comparative analysis on microbiome diversity in the GI tract and feces by α-diversity analyses. A. Maximum-likelihood phylogenetic tree comprising all of the taxa in the GI content and feces respectively. Clades are labelled according to family and the corresponding phylum is depicted in the first outer layer.
B. Relative abundance of family level occupying in feces or the GI tract. C. Species richness and diversity measured by the indices of ACE richness, Shannon diversity and Fisher's alpha.

Figure 6

Comparative analysis of microbiome diversity in the GI tract and feces by β-diversity analysis. A. The NMDS plot showing the difference of microbiome between feces and the GI tract at OTU level based on Bray-Curtis distances. The 2D stress was 0.109. B. The RDA plot showing the difference of microbiome...
between feces and the GI tract at OTU level based on Bray-Curtis distances. C. Heatmap of the microbial composition and relative abundance of all samples based on the Bray–Curtis distance matrix calculated from relative OTU abundances at genus level.

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