Systematic evaluation of the gut microbiome of swamp eel (*Monopterus albus*) by 16S rRNA gene sequencing

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Background

Swamp eel (*Monopterus albus*) is a commercially important farmed species in China. T he dysbiosis and homeostasis of gut microbiota might be associated with swamp eel's diseases pathogenesis and food digestion. The contribution of gut microbiome in fish growth and health is being increasingly recognized. However, little was known about the microbial community in the intestine of swamp eel (*Monopterus albus*).

Methods

The intestinal microbiomes of the five distinct gut sections (midgut content and mucosa, hindgut content and mucosa, and stools) of swamp eel were compared by the Illumina MiSeq sequencing of the bacterial 16S rRNA gene sequencing and statistical analysis.

Results

The results showed that the number of observed OTUs decreased from proximal to distal of intestine. PCoA analysis revealed significant separations among samples from different gut sections. Nevertheless, there were 54 core OTUs shared by all gut sections and 36 out of 54 core OTUs significantly varied in the abundances in different gut sections. Furthermore, we discovered 66 section-specific enriched KEGG pathways. These section-specific enriched microbial taxa (e.g., *Bacillus*, *Lactobacillus*) and potential function capacities (e.g., amino acid metabolism, carbohydrate metabolism) might play vital roles in nutrients metabolism, immune modulation, and host-microbe interactions of swamp eel.

Conclusions

Our results showed that the microbial diversity, composition, and function capacity were varied substantially in different gut sections. The gut section-specific enriched core microbial taxa and function capacities may exert an important role in swamp eel's nutrients metabolism, immune modulation, and host-microbe interactions. This study should provide basic insights into the gut microbiome of swamp eel.

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Abstract

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55 microbiome of swamp eel.

56 **Key words: *Monopterus albus*; 16S rRNA sequencing; gut microbiome; function capacities**

57 **Introduction**

58 Swamp eel (*Monopterus albus*), taxonomically belonging to order Synbranchiformes, family
59 Synbranchidae, is an air-breathing teleost and widely distributed in swamps, streams, ponds, and
60 ricefields in areas of southern China, Japan, India and other Southeast Asian countries (FishBase,
61 http://fishbase.org/). Due to its great growth performance and rich nutrient content, swamp eel has
62 become a commercially important farmed species in China (Li et al., 2017), and in 2017 the
63 production of swamp eel reached 358,295 tons. Diseases and low feed efficiency are two major
64 factors restricting the development of swamp eel aquaculture. Probiotics are live microorganisms
65 that when administered in adequate amounts confer a health benefit on the host (De et al., 2014).
66 Many studies have revealed that the administration of probiotics could modulate gut microbial
67 balance, enhance immune status, reduce disease susceptibility and improve feed efficiency
68 (Caruffo et al., 2016; Hai, 2015; Newaj-Fyzul & Austin, 2015). It suggested that the dysbiosis and
69 homeostasis of gut microbiota might be associated with swamp eel's diseases pathogenesis and
70 food digestion.

71 Earlier researches on gut microbiota of freshwater and marine fishes had demonstrated that gut
72 microbiota played a crucial role in host nutrients metabolism, growth and health. Many cellulose-
73 decomposing bacteria were harbored in the intestine of grass carp (*Ctenopharyngodon idellus*),
74 such as *Anoxybacillus*, *Actinomyces*, and *Citrobacter* (Wu et al., 2012). Pompano (*Trachinotus*
75 *blochii*) showed high abundance of *Clostridia* which was associated with polysaccharides
76 degradation, when fed with commercial pellet (Rasheeda et al., 2017). The alpha diversity and the
77 dominant bacterial taxa significantly changed with the development of *Siniperca chuatsi* (Yan et
78 al., 2016). The interactions between threespine stickleback (*Gasterosteus aculeatus*) and gut
79 microbiota played a key role in the development of gut innate immunity (Small et al., 2017).
80 Moreover, gut microbial communities in the different gut sections exhibited distinct differences in
81 diversity and richness. The significantly higher alpha-diversity indices in the midgut (named as

foregut in Ye et al., 2014) than the hindgut in both Asian silver carp and gizzard shad. In salmon, microbial richness was higher in the digesta than in the mucosa (Gajardo et al., 2016), however, in the rabbitfish (*Siganus fuscescens*), the microbial richness significantly increased from content section to mucosal section (Nielsen et al., 2017). Since gut microbiome are complex and dynamic communities which have profound influences on fishes, it is important to systematically characterize the bacterial communities in different gut sections. However, to the best of our knowledge, there is few study about the gut microbiome of swamp eel.

Monopterus albus is a strict carnivore that prey on fishes, worms, crustaceans and other small aquatic animals in the wild (Liem, 1967). Under captive conditions, swamp eel are usually fed with surimi or mixed with commercial power feed. The gastrointestinal tract of swamp eel is a straight, uncoiled tube, passing directly to the anus, including the pharynx, esophagus, stomach, midgut (ileum) and hindgut (rectum). Bile enters the midgut by way of a short ductus choledochus (Liem, 1967).There is no external demarcation between mid- and hindgut, and histologically, the midgut and the hindgut are also similar that both of which contain four tunics: mucosa, submucosa, muscularis, and serosa. However, there are some microscopic di□erences. First, the mucosal folds of the midgut are in a reticular configuration, however, the mucosal folds of the hindgut are distinctly longitudinal and not as numerous as in the midgut. Second, in the midgut, the mucus secreting goblet cells are extremely numerous, while in the hindgut, the number of goblet cells is a food dependent feature, and starvation can cause a pronounced decrease in rectal goblet cells. Third, the serosa of the hindgut is much more prominent than that of the midgut. These make swamp eel likely to contain specific intestinal microbiota similar to those of carnivorous fish, such as the phylum *Cetobacterium, Clostridium, Fusobacteria*. Furthermore, the microscopic di□erences of gut may result in the different microbial structures in the midgut and hindgut.

The main objective of this study was to investigate gut microbial structures, compositions and function capacities of different gut sections of swamp eel using 16S rRNA gene sequencing. We wondered whether the different structural and functional characteristics of gut microbial community in different sections were correlated with swamp eel's nutrients metabolism, immune

109 modulation, and host-microbe interactions. This study would provide the first glimpse of gut
110 microbiome of swamp eel.

111 **Methods**

112 **Sample collection**

113 Swamp eels (40-45 g) were sampled from a commercial swamp eel farm in the Jiangxi Province,
114 China (28.4219 N, 116.4126 E), and acclimated in dechlorinated tap water at 25 ℃ in the 10 L
115 aquarium tanks. Then the swamp eel individuals were fed with minced fish once a day for 8 weeks
116 until dissection. The dechlorinated tap water was changed every day. All experimental swamp eels
117 were healthy and had not received any antibiotics, probiotics or prebiotics during the feeding
118 period. Fecal samples were collected immediately and separately before euthanasia. Fish were
119 anesthetized with tricaine methanesulfonate and the whole intestines were aseptically removed
120 from the abdominal cavity. The intestine was further dissected using sterile instruments to separate
121 the midgut (immediately after the stomach) and the hindgut (immediately before the anus) sections
122 according to Liem (Liem, 1967). The contents in each gut section were squeezed out and collected
123 separately. The proximal and distal of the intestine were then washed with sterile PBS three times
124 to remove remnants of the gut content. The gut mucosa was then scraped off with sterilized forcep
125 and transferred into a microcentrifuge tube. All Samples from different gut sections were
126 separately used for sequencing. All animal procedures were conducted according to the guidelines
127 for the care and use of experimental animals established by the Ministry of Agriculture of China
128 (No. SCXK YU2005-0001). Animal Care and Use Committee (ACUC) in Jiangxi Agricultural
129 University specially approved this study.

130 **DNA extraction and 16S rRNA gene sequencing**

131 Total DNA was extracted from gut content and gut mucosa of different individuals using the
132 PowerSoil® DNA Isolation Kit (Mo Bio, San Diego, CA, USA) according to the manufacturer's
133 instruction. Fecal DNA extraction was performed using the QIAamp Stool Mini Kit (QIAGEN,
134 Germany). The barcoded fusion forward primer 338F (5'-ACTCCTACGGGAGGCAGCA-3')
135 and the reverse primer 806R (5'-GGACTACHVGGGTWTCTAAT-3') were used to amplify the

V3-V4 hyper variable region of the 16S rRNA gene. The Barcoded V3-V4 amplicons were sequenced using the paired-end method on Illumina MiSeq 2×300 platform (Illumina, USA) following the standard protocols.

16S rRNA gene sequencing data analysis

To obtain the clean data, the barcodes and low quality sequences were filtrated using FASTX-Toolkit. FLASH software was used to merge high-quality paired-end reads into tags (Magoc & Salzberg, 2011). Operational Taxonomic Unit (OTU) picking was performed using the USEARCH pipeline with a 97% sequence identity (Edgar, 2010). We performed taxonomic assignments for the aligned sequences using the Ribosomal Database Project (RDP) classifier program with 80% confidence threshold (Wang et al., 2007). Microbial taxa abundance and diversity indices were generated using Quantitative Insights Into Microbial Ecology (QIIME) (Caporaso et al., 2010). Phylogenetic investigation of communities by reconstruction of unobserved states (PICRUSt) was used to predict the functional profile of the microbial community (Langille et al., 2013). We extracted the closed reference OTU table from quality control reads in QIIME against the Greengenes database. OTU normalization, gene family abundances prediction, and function categorization based on KEGG (Kyoto Encyclopedia of Genes and Genomes) pathway was performed by PICRUSt according to the default settings.

Statistical analysis

Microbial species richness was analyzed using the observed number of OTUs. Principal Coordinate Analysis (PCoA) of the beta diversity was performed based on the unweighted and weighted distance matrix. Permutational Multivariate Analysis of Variance (PERMANOVA) was performed to identify section specific enriched microbial taxa and functional capacities (Nielsen et al., 2017). The output results were visualized using ggplot2 and gplots in R package except the Venn diagrams which were drawn using the online tool (bioinformatics.psb.ugent.be/webtools/Venn/).

Results

Both data sets are accessible through NCBI's SRA, under study accession number SRP

163 [145040].

164 **Microbial diversities and compositions in different gut sections**

165 At first, 405, 642, 227, 372 and 171 OTUs were identified in the midgut content, midgut mucosa,
166 hindgut content, hindgut mucosa, and stools, respectively (Figure 1A). Then, we identified specific
167 and common OTUs in different sections via a Venn diagram (Figure 1B-D). 63 common OTUs
168 were detected among midgut content, hindgut content and stools. 315 OTUs were shared by both
169 the midgut mucosa and hindgut mucosa. Importantly, we found 54 common OTUs as a core
170 microbiota presented in all intestinal sections, while 53, 254, 5, 16, 37 specific OTUs were also
171 detected for midgut content, midgut mucosa, hindgut content, hindgut mucosa, and stools,
172 respectively. Moreover, PCoA analysis also revealed significant separations among samples from
173 different gut sections (Figure 2, Figure S2).

174 To further uncover characteristics of microbial compositions in different gut sections, we
175 analyzed the OTUs assigned for the phylum and genus level (Figure 3). At phylum level,
176 *Firmicutes*, *Fusobacteria*, *Proteobacteria*, *Bacteroidetes*, and *Actinobacteria* were the five most
177 dominant phyla. At genus level, *Cetobacterium*, *Ralstonia*, and *Rhodococcus* were the most
178 predominant genera. Interestingly, the abundances of these microbial taxa changed significantly
179 among different gut sections. For instances, *Firmicutes* occupied a large proportion of the gut
180 microbiota in both midgut and hindgut regardless of the locations sample obtained, but it only
181 occupied a small proportion of the gut microbiota in stools samples. *Fusobacteria* accounted for a
182 higher proportion of gut microbiota in the content section than in the mucosal section.
183 *Cetobacterium* was predominant in all samples, but a lower abundance in the midgut was observed
184 when compared to the hindgut and stools. In contrast, the abundance of *Rhodococcus* in midgut
185 was higher than that in hindgut and stools.

186 **Core microbial taxa enriched in different gut sections**

187 To identify which core microbial taxa showed different enrichment in specific gut section, we
188 analyzed the abundance of the 54 core OTUs across all sections. As shown in Figure 4, total 36
189 section-specific enriched OTUs were observed. In the midgut content, seven enriched OTUs were

annotated to *Enhydrobacter*, *Comamonadaceae*, *Caulobacteraceae*, *Microbacteriaceae*, *Peptostreptococcaceae*, *Bradyrhizobium*, and *Deinococcus*, respectively. Meanwhile, OTUs annotated to each of *Roseburia*, *S24-7*, *Bacillus*, *Acidobacteria*, *Paracoccus*, *Lactococcus*, and *Oxalobacteraceae* were enriched in the midgut mucosa. On the other hand, OTUs enriched in the hindgut content were annotated to *Cetobacterium somerae*, *Arthrobacter*, *Coprococcus*, *Bacteroidaceae*, *Ruminococcaceae*, *Epulopiscium*, and *Citrobacter*. OTUs annotated to *Clostridium*, *Pseudomonas*, *Rhodococcus*, *Ralstonia*, *Achromobacter*, *Streptococcus*, and *Lactobacillus* showed great abundance in the hindgut mucosa. Besides, 8 OTUs derived from *Chryseobacterium*, *Comamonas*, *Serratia*, *Acinetobacter johnsonii*, *Pedobacter*, *Plesiomonas shigelloides*, *Pseudoxanthomonas Mexicana*, and *Aeromonadaceae* increased in abundance in the stools samples.

Comparison of microbial potential capacities in different gut sections

To compare the potential functional capacity of microbial community in different gut sections, the relative abundances of KEGG pathways were predicted by PICRUSt. The results showed that 66 KEGG pathways exhibited significant differences in abundances across different gut sections (Figure 5). Among these, 26 pathways from the midgut samples, 28 pathways from the hindgut samples, and 12 pathways from stools samples. Notably, there were some characteristics of the distribution of differential pathways in specific gut section. For example, amino acid metabolism pathways such as lysine degradation, arginine and proline metabolism, and valine, leucine and isoleucine degradation were predominant in the midgut content. Cofactor and vitamins metabolism and signal transduction related pathways were overrepresented in the midgut mucosa. In the hindgut, carbohydrate and lipid metabolism pathways were prominent in the content, while bacterial replication, transcription, and translation related pathways were outstanding in the mucosa. In addition, we observed that microbial community was more capable of metabolizing secondary metabolites and xenobiotics in the stools.

Discussion

In this study, 16S rRNA sequencing analysis revealed the diversity, composition, and potential

functional capacity of microbial community across different gut sections in swamp eel. To our best knowledge, this is the first study systematically evaluating the gut microbiome of swamp eel (*Monopterus albus*).

At phylum level, *Firmicutes*, *Fusobacteria*, *Proteobacteria*, *Bacteroidetes*, and *Actinobacteria* were the five most dominant phyla. At genus level, *Cetobacterium*, *Ralstonia*, and *Rhodococcus* were the most predominant genera in the intestinal microbiota communities of swamp eel. Data analysis also showed that the most of the microbiome found in the intestine of swamp eel have been detected in other fish. This was in consistent with the results in Japanese eels (Huang et al., 2018). However, compared with Anguillid eel species, the intestinal microbial composition of swamp eel was markedly different. At the phylum level, *Proteobacteria*, *Fusobacteria* and *Bacteroidetes* were the dominant bacterial groups in European eels, and *Proteobacteria* is the most abundant phylum, accounting for 70.35±17.2% of the total number of reads (Huang et al., 2018). While in swamp eel, *Proteobacteria* accounted for only 12.88% of the total number of reads and were lower in hindgut mucosa and hindgut content, which accounted for 2.82% and 1.17% of the sequenced reads, respectively. The difference in genus is more obvious. Relative abundance of the top 5 genera in the intestinal mucosa of swamp eel were *Cetobacterium*, *Ralstonia*, and *Rhodococcus*, *Mycobacterium*, *Clostridium*. However, the top 5 bacterial genera in European eel intestine were *Aeromonas*, *Cetobacterium*, *Plesiomonas*, *Shewanella*, *Paludibacter*. Moreover, for the Giant-Mottled eels (*Anguilla. marmorata*) the dominant bacterial genera of were *Acinetobacter*, *Mycoplasma* and *Shewanella.* This difference may be related to the genetic characteristics of the species (Goodrich et al., 2014; Li et al., 2018). And also, the diet is one of the important factor that influences the community composition (Moschen et al., 2012; Piazzon et al., 2017).In this study, minced fish were used as feed, while the cultivated European eel were fed with commercial power feed and the Giant-Mottled eels were caught from the wild.

In this study, the number of observed OTUs decreased from the proximal to the distal section of intestine in swamp eel. This result was different from many vertebrate microbiome studies which revealed that the distal section of intestine had higher richness and diversity than the

proximal section. This difference may be associated with the specific physiological structure of swamp eel's intestine. Unlike omnivorous and herbivorous fish which usually have long and coiled intestines (Pereira et al., 2015; Santos et al., 2016), swamp eel is carnivorous which intestine is short and straight. And, generally, the mid-gut is thought to be the organ where the majority of digestion occurs (Egerton et al., 2015). Considering the crucial role of gut microbiota in host nutrients metabolism, we speculated that much more microbes inhabitation in the proximal section of swamp eel's intestine should benefit to host fast digestion and absorption of nutrients. Furthermore, much more OTUs presented in the mucosal section than the content section regardless of the locations that samples obtained. This result was consistent with gut microbiome studies in rabbitfish (*Siganus fuscescens*) and loach (Nielsen et al., 2017; Yang et al., 2017) and reinforced previous findings that the mucosal section might serve as a reservoir of diverse bacterial species (Lu et al., 2014). OTUs assigned for the phylum level and genus level further revealed some specific features of microbial compositions of swamp eel. Previous study demonstrated that the high abundance of *Firmicutes* was observed in the gut microbiome of omnivorous fishes and *Fusobacteria* was the predominant phylum in the gut microbiome of carnivorous fishes (Liu et al., 2016). Here, the microbial communities of swamp eel were dominated by, in order, *Firmicutes*, *Fusobacteria*, *Proteobacteria*, *Bacteroidetes*, and *Actinobacteria*. Furthermore, we found that *Firmicutes* was more predominant in the midgut and hindgut than the stools, while the abundance of *Fusobacteria* was higher in the content section than the mucosal section. The most dominant genus *Cetobacterium*, *Ralstonia* and *Rhodococcus* also varied in the abundances across different gut sections. These results suggested that using samples from single gut section to represent an overview of gut microbiota would likely fail to detect community variation responding to physiological variations of the gut (Durbán et al., 2011).

Although the gut microbiota showed distinct spatial heterogeneity, we still identified a core microbiota consisting of 54 common OTUs in all gut section. It was in line with previous fishes gut microbiome studies indicating that specific microbial taxa could form a stable core microbota in the intestine (Baldo et al., 2015; Rudi et al., 2018). Furthermore, we found that the enrichments

of these microbial taxa were associated with nutrients metabolism, immune modulation, and habitat adaptions. In the content section, most of the enriched microbial taxa were associated with nutrients metabolism. For example, dietary fiber degradation associated bacteria *Enhydrobacter* and *Comamonadaceae* (Premalatha et al., 2015; Sakurai et al., 2017), and amino acid metabolism associated bacteria *Caulobacteraceae* and *Microbacteriaceae* (Yin et al., 2017) were enriched in the midgut content. Gut microbial taxa equipped with multiple carbohydrate active enzymes such as *Bacteroidaceae*, *Ruminococcaceae*, *Coprococcus*, and *Citrobacter* (Luo et al., 2017; Tap et al., 2015; Wu et al., 2012) which involved in non-digestible dietary carbohydrates metabolism showed great abundance in the hindgut content. Notably, *Cetobacterium somerae* as a vitamin B-12 and antimicrobial metabolites producing species had a higher abundance in the hindgut content. It was similar to the results of many other fish gut microbiota studies (Bledsoe et al., 2016; Larsen et al., 2014).

Interestingly, stools samples as an end product of nutrients metabolism in the content section, several potential aquatic pathogenic bacteria were enriched, including *Serratia*, *Acinetobacter johnsonii*, *Plesiomonas shigelloides*, and *Aeromonadaceae* (González et al., 2000; Martins et al., 2013; Nadirah et al., 2012; Huang et al., 2018). *P. shigelloides* is a cause of diarrhea in human, usually isolated from the faeces (Khan et al., 2004) and has also been found from the gut of many fishes, such as tilapia (*Oreochromis niloticus*) (Nadirah et al., 2012), largemouth bass *Micropterus salmoides* (Larsen et al., 2014) and Japanese eel *Anguilla japonica* (Hsu et al., 2018). In grass carps, *P. shigelloides* has also been found to be associated with muscle erosive disease (Hu et al., 2014). *Acinetobacter johnsonii* rescently were regarded as opportunistic pathogens for farmed rainbow trout (Kozińska et al., 2014) and blunt snout bream *Megalobrama amblycephala* (Cao et al., 2017). However, they did not cause any infections or diseases in our feeding swamp eels. It indicated that these bacteria were native inhabitants of swamp eel's stools, and the intestine maybe has a certain ability to enrich harmful bacteria into feces and excrete them out of the body.

Meanwhile, many immune modulation associated bacteria were found inhabiting in the mucosal section. For instance, *S24-7* modulated mucosal immune homeostasis and *Roseburia* regulated

innate immunity (Liu et al., 2017; Patterson et al., 2017). Potential probiotics including *Bacillus*, *Acidobacteria*, and *Lactococcus* (Bernardeau et al., 2017; Lv et al., 2016; Wu et al., 2018) were predominant in the midgut mucosa and *Lactobacillus* were predominant in the hindgut mucosa. *Clostridium* and *Lactobacillus* involved in immune response, *Pseudomonas* and *Achromobacter* had strong antimicrobial activities, and *Rhodococcus* showed properties of probiotic (Nayak, 2010; Sharifuzzaman et al., 2017; Zothanpuia et al., 2016) were overrepresented in the hindgut mucosa. Besides, it was noteworthy that aerobic bacteria *Bradyrhizobium*, *Deinococcus*, *Arthrobacter*, and *Comamonas* preferred to thrive in the content section and anaerobes and obligate anaerobes such as *Paracoccus*, *Ralstonia* and *Streptococcus* were more prevalent in the mucosal section. This section-specific distributions might be related to its special respiration. *Monopterus albus* is an air-breathing teleost using the buccopharyngeal cavity for gas exchange (Damsgaard et al., 2014) and this is likely to cause a small amount of air into the intestine. Although there was no direct study on the oxygen concentration in the intestinal tract of *Monopterus albus*, early literature suggested that the intestine of *Monopterus albus* might have respiratory function (Petukat, 1965).

The potential functional capacities of microbial communities were distinctly different across different gut sections and these differential microbial functional capacities should be related to host physiological functions and host-microbes interactions. Amino acid metabolism pathways were more abundant in the midgut content, suggesting that gut microbiota in the midgut content may help swamp eel to digest dietary amino acids. Cofactors and vitamins metabolism and cellular signals processing pathways were enriched in the midgut mucosa. Since fishes lack the biosynthetic capacity for most vitamins, it is important that vitamins produced by gut microbiota will play a key role in host growth, intestinal mucosal immune and signaling molecules expression (Feng et al., 2016; Li et al., 2015). In the hindgut content, a high level of carbohydrate and lipid metabolism was identified. It was in line with previous studies that gut microbiome of fish hindgut had the ability of fermentation of non-digestible polysaccharides to short-chain fatty acids (SCFAs) (Geraylou et al., 2013; Mountfort et al., 2002). In the hindgut mucosa, microbial replications, transcriptions and translations related pathways were concentrated, which was in

consistent with previous studies that hindgut mucosa was an essential gut region where interactions between gut microbiota and host cells happened (Morgan et al., 2015; Sellers & Morton, 2014). Intriguingly, we observed that microbial xenobiotics and secondary metabolites metabolism pathways were more predominant in the stools samples. This result and the enriched microbial taxa in stools above indicated that stools may service as a "wastes dump" of swamp eel and microbial community.

Conclusions

In the present study, we comprehensively characterized the microbial communities in different gut sections of swamp eel. Our results showed that the microbial diversity, composition, and function capacity were varied substantially in longitudinal and radial parts of the intestine. The microbial diversity and composition across different gut sections could reflect the characteristics of swamp eel's intestine structures and feeding habit. The gut section-specific enriched core microbial taxa and function capacities may exert an important role in swamp eel's nutrients metabolism, immune modulation, and host-microbe interactions. Taken together, these results should provide a basis for further research on gut microbiome of swamp eel.

Conflict of interest

There is no conflict of interest related to this research.

Transparency document

The Transparency document associated with this article can be found in the attachment.

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Figure 1

The observed OTU numbers, unique and sharedOTUs in different gut compartments (n=4) .

(A) Bar plot shows the observed OTU numbers in midgut content (MC), midgut mucosa (MM), hindgut content (HC), hindgut mucosa (HM), and stools (S). (B) Venn diagram displays the number of shared and unique OTUs among midgut content (MC), hindgut content (HC), and stools (S). (C) Venn diagram displays the number of shared and unique OTUs between midgut mucosa (MM) and hindgut mucosa (HM). (D) Venn diagram displays the number of core OTUs shared by all gut compartments.

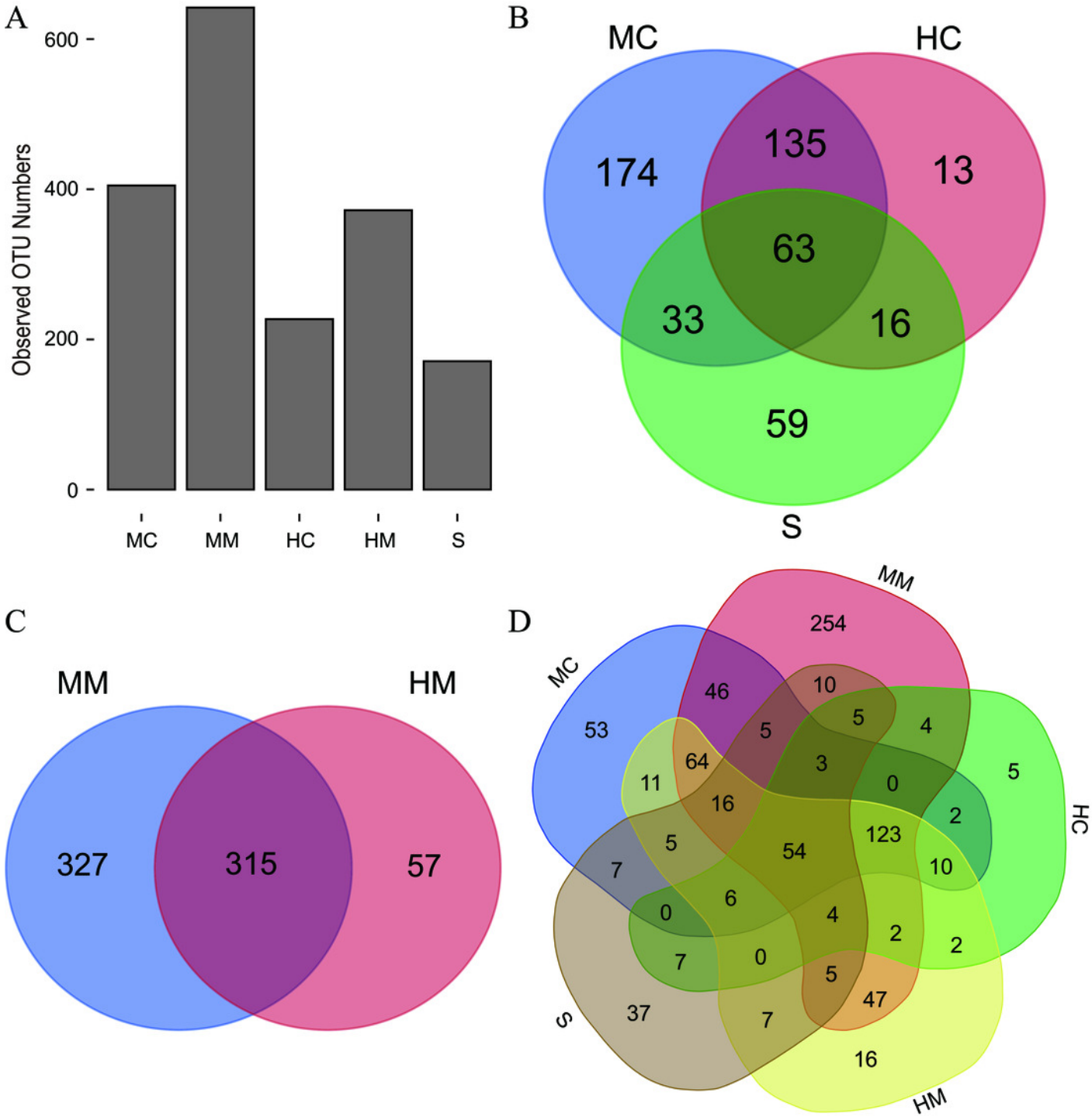
A
Observed OTU Numbers
600
400
200
0
MC
MM
HC
HM
S
B
MC
HC
S
174
135
13
63
33
16
59
C
MM
HM
327
315
57
D
MM
MC
HC
S
HM
254
46
10
53
5
5
4
64
3
5
11
0
16
2
123
5
54
10
7
6
0
4
2
2
7
0
5
47
37
7
16

Figure 2

Principal Coordinate Analysis (PCoA) ofmicrobial community in different gut compartments based on the UnweightedUniFrac distance matrix (n=4) .

The individual samples are color- and shape-coordinated according to the gut compartment.

MC : midgut content, MM: midgut compartment, HC: hindgut content , HM: hindgut mucosa,

S: stools

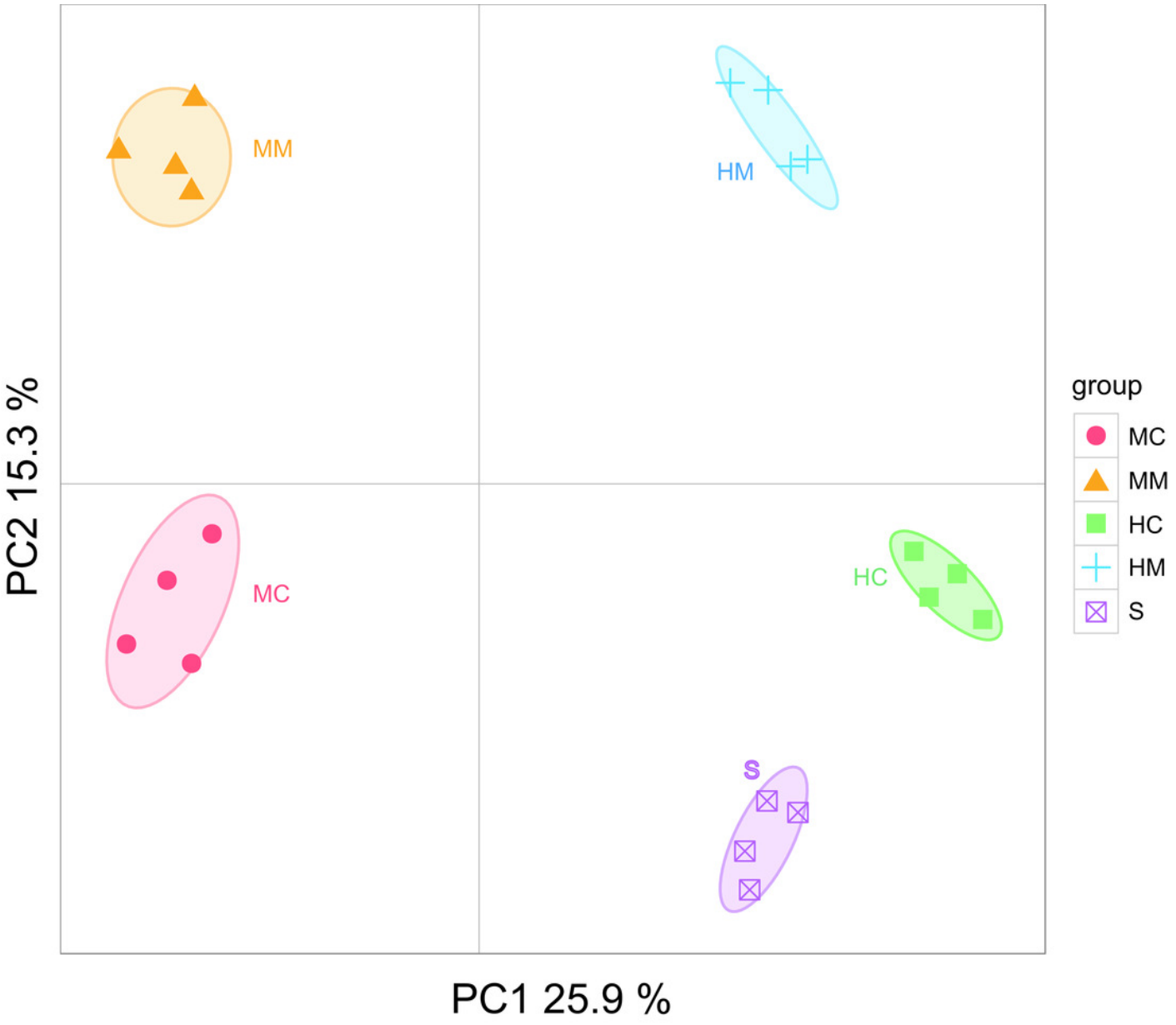

Figure 3

Gut microbial compositions at the phylumlevel and genus level (n=4) .

(A) Each bar represents average relative abundance of each phylum of gut microbiota in midgut content (MC), midgut mucosa (MM), hindgut content (HC), hindgut mucosa (HM), and stools (S). (B) Each bar represents average relative abundance of each genra of gut microbiota in midgut content (MC), midgut mucosa (MM), hindgut content (HC), hindgut mucosa (HM), and stools (S).

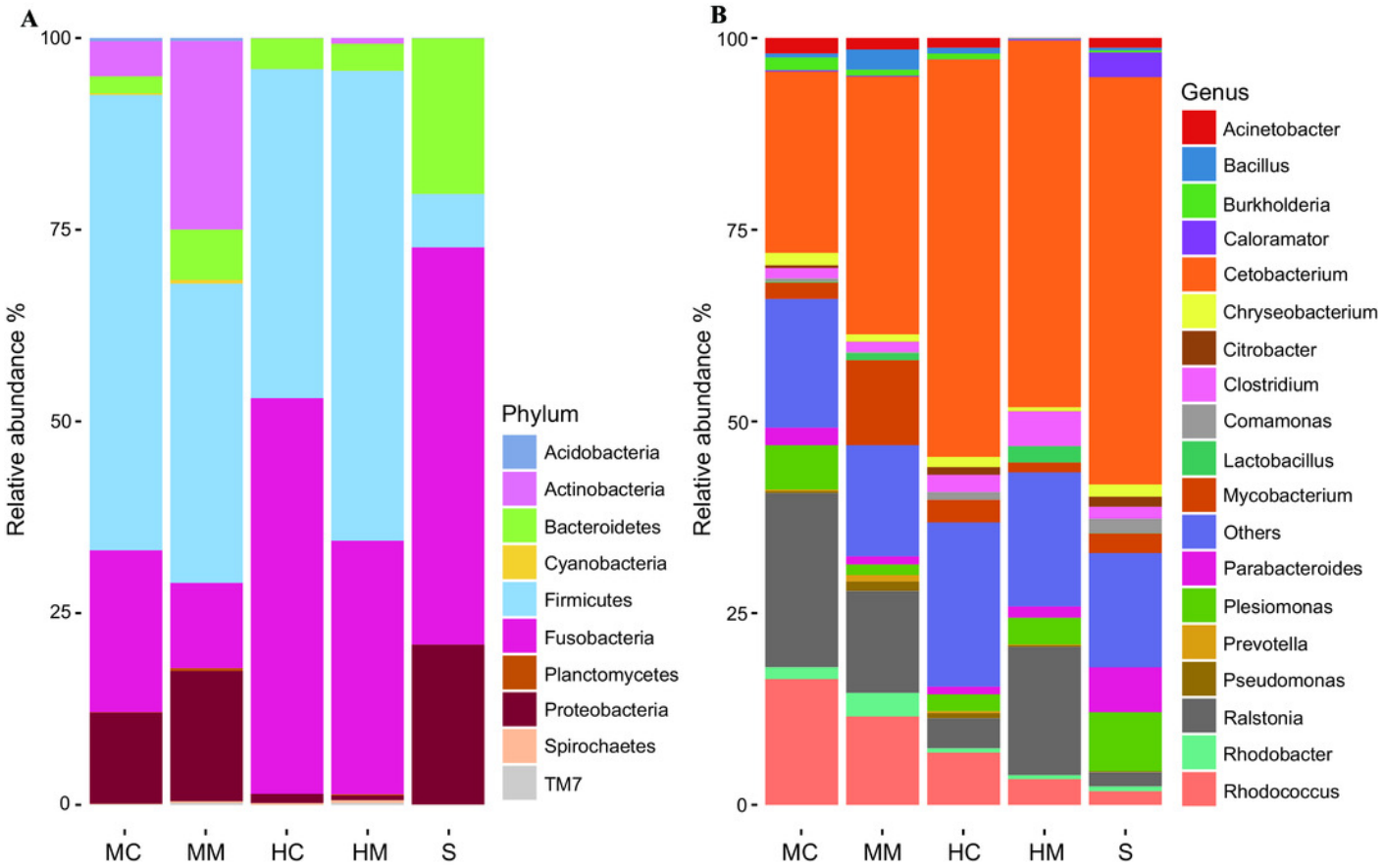

Figure 4

Gut compartment-specific enriched core OTUs (n=4) .

Heat map shows core OTUs significantly varied in abundances in different gut compartments (cell note on the heat map represents differentially abundant OTUs annotated to microbial taxa).

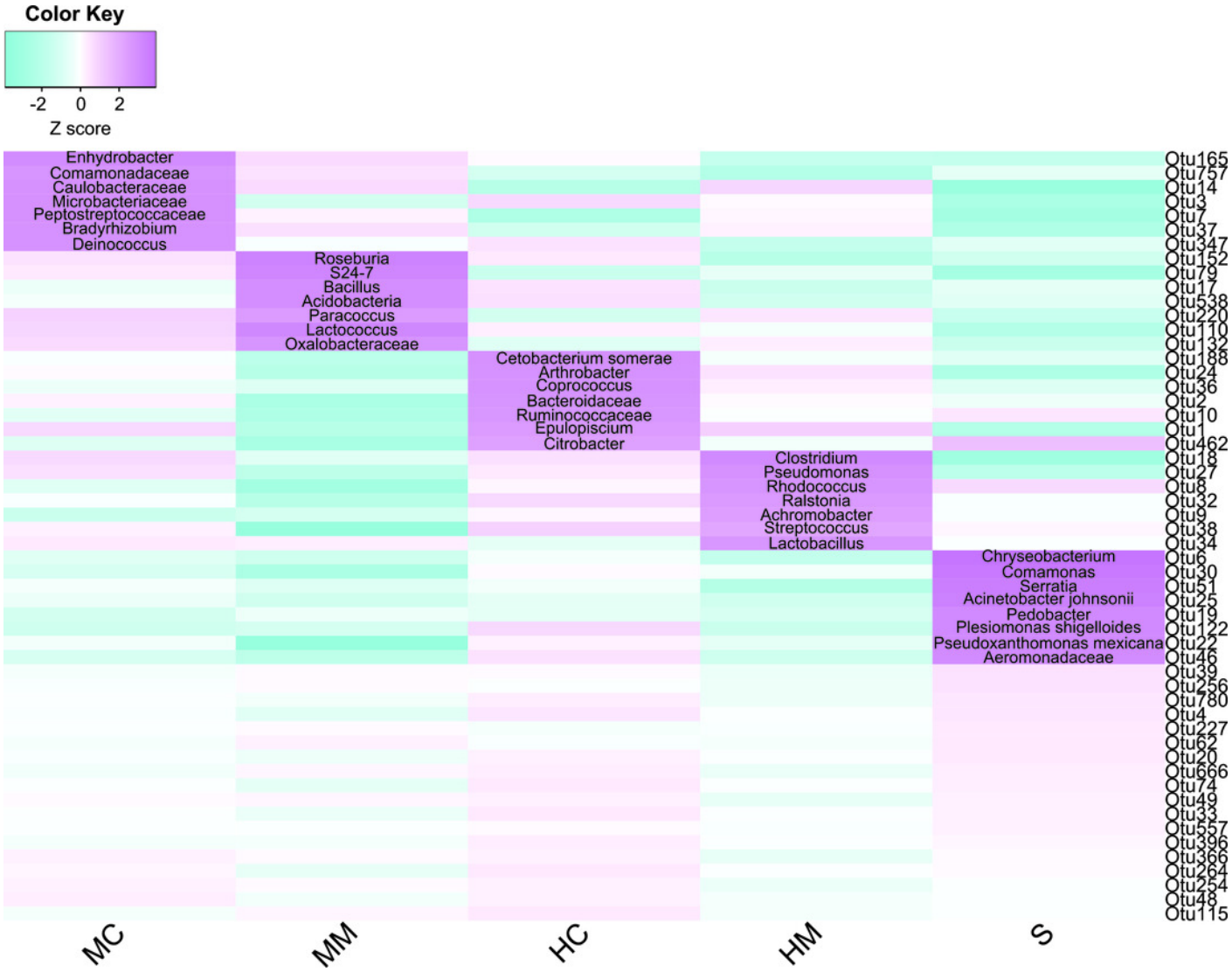

Figure 5

Comparison in the abundance of gutmicrobial potential function capacities in different gut compartments (n=4) .

Heat map shows the abundances of gut microbial KEGG pathways (level 3) significantly changed in different gut compartments (cell note on the heat map represents differentially abundant KEGG pathways at level 2).