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Molecular characterization and structure of intestinal micro flora for postmortem interval estimation in SD rats

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Abstract

Background The human rectum flora consists of a huge variety of bacteria and the association between individuals and their rectum bacterial community begins presently after birth and continues the whole lifetime. Once the body dies, the inherent microbes begin to break down from the inside and play a key role thereafter. Results The aim of this study was to investigate the probable shift of the rectum flora at different time intervals up to 15 days after death and to characterize the contribution for of this shift to estimate the time of death. The rectum of rats was wiped with a sterile cotton swab and the samples were proceeded for DNA extraction, PCR amplification of the 16S rRNA gene with the V3+V4 variable regions, and high throughput sequencing carried out on IonS5TMXL platform. The results were analyzed for intra-group and inter-group diversity, similarity and difference at different time points. At phylum level, Proteobacteria and Firmicutes showed major shifts, checked at 11 different intervals and emerged in the most of postmortem intervals. At the genus level, Enterococcus appeared in all groups except alive samples, Lactobacillus and Proteus appeared in most time points, and the latter showed an increasing trend after 3 days postmortem samples. At the species level, Enterococcus_faecalis and Proteus_mirabilis existed in most postmortem intervals, and the former had a downward trend after day 5 postmortem, while the latter had an upward trend. Corynebacterium_amycolatum , Entero_isolate_group_2 , Bacteroides_uniformis , Enterococcus_faecalis , Streptococcus_gallolyticus_subsp_macedonics , Clostridium_sporogenes were more abundant in 0-hour, day 1, 3, 5, 7, 13 postmortem intervals, respectively, while Proteus_mirabilis and Vagococcus_lutrae were abundant in day 15 postmortem. In addition, functional capacity analysis of Membrane_Transport, Amino_Acid_Metabolism, Nucleotide_Metabolism and Energy_Metabolism showed significant differences between alive and almost all other time points after death ($P < 0.05$). Conclusions All in all, bacteria at different levels (phylum, genera, species) showed different characteristic during the process of decomposition and possessed entirely different relative abundance and the structure of bacterial community in each time point shifted obviously, which suggested that the specific bacteria might imply the specific postmortem interval during decomposition.

Background

The time of death refers to the time passed from death to forensic autopsy. Post-mortem interval (PMI) plays an immense role in the detection of abnormal death cases. There are some methods for PMI, including pure physical methods, combined physical and chemical methods, cadaver self-degradation and corruption [1]. The above methods can offer estimation of PMI but disadvantaged by high capriciousness and the capriciousness is from days to months. Recently, using dead microorganisms to estimate post-mortem interval is getting more concern. [2]. However, utilizing microorganisms to infer the PMI and the death microbiome is still existing large space in research.

The microbiota inhabit mostly in the intestinal tract, specifically anaerobic, where they can obtain abundant nutrition and serves as a favorable site for intestinal microbial occupation [3]. The host microorganisms are affected by many factors, such as environment they are exposed to, whether the hosts are sick or not, and the progression of the disease and nutritional status of the patients are involved [4]. Microorganisms also play an important role in the degradation of corpses [5]. Although the microorganisms in the dead body have not received such attention like bacteria related with diseases, more and more researchers have involved in it. Pechal *et al.* put pigs in an environment without human intervention, and established a data model to explain the metabolites of 95% bacteria in the process of cadaveric degradation, therefore the problem that microorganism is influenced by environment was reviewed [6]. Carter *et al.* found that seasonal shifts had significant effects on the microorganism of pigs after death [7]. The post-mortem microorganisms can be used to estimate the time of death within three days after death [2].

In this study, 88 intestinal flora samples of 11 time points from 8 SD rats were sequenced at IonS5TMXL platform to find the characteristic flora and the main metabolic functions at each time point, in order to explore the time points with the characteristic flora and corresponding metabolic function.

Methods

2.1 Ethic statement

This study was approved by Institutional Animal Use and Care Committee of Xi'an Jiaotong University, Shaanxi, China (Ethics Approval Number: 2017-388).

2.2 Laboratory animals with their basic information

8 male healthy SD (Sprague Dawley) rats were purchased from the Experimental Animal Center of Xi'an Jiaotong University with an average of body weight about 200-220 g/rat and numbered C-J. The rats were feed with normal food in laboratory cages (0.46×0.30×0.16 m) for two weeks and then sacrificed with cervical dislocation. The dead rats were placed in 11 cages (0.46×0.30×0.16 m) which communicated with the outside environment but excluded insects. The experimental samples were collected from same batch of alive (one time point) and dead (10 time points) rats.

2.3 The ways of sample collection and time points selection

Fecal samples were collected before death (alive). The rats' postmortem putrefaction happened in Xi'an City, Shaanxi Province, China (34°15'39.9"°N, 108°56'33.32"°E) in December. Samples of rectum flora from dead rats were rubbed with sterile cotton swabs dipped in sterile saline for one minute before and after execution for taking more rectum bacterial sample. The time points of sample collection were as follow: before death, 0 hour, 8 hours, 16 hours, day 1, 3, 5, 7, 9, 13, 15 after death (time points of alive, h0, h8, h16, D1,3, 5, 7, 9, 13, 15). Swabs were placed in 1.5 mL sterile EP tube and then stored at - 80℃ for further using. The sampling time was from December 6 to 21, 2017. The average sampling temperature was 20.63±0.93℃ and the humidity was 15.37±2.79%.

2.4 DNA extraction

The total bacterial genomic DNA was extracted from feces and swabs by QIAamp DNA Stool Mini kit (Qiagen, Germany) and QIAamp DNA Mini kit (Qiagen, Germany) and the specific procedures were according to the corresponding manufacturer's recommendations. The DNA concentration was determined by NanoDrop2000 (Thermo Science, Waltham, MA, USA) and stored in a refrigerator at - 80°C for further using.

2.5 High throughput sequencing

2.5.1 Operation flow

Based on Thermofisher's IonS5TMXL sequencing platform, single-end sequencing (SE600) was used for high throughput sequencing. The total DNA of microorganisms was amplified with 16SrRNA V3+V4 universal primers 341F (5'-CCTAYGGRBGCASCAG-3') and 806R (5'-GGACTACNNGGTATCTAAT-3'), the amplified products were recovered purified and quantified, and the corresponding mixing ratio of each sample was adjusted according to the quantitative results. Then the library was prepared and sequenced on the machine.

2.5.2 Sequencing data processing

Cutadapt V1.9.1 [8] was used to process the reads and get the original data. Clean reads were obtained by comparing UCHIME Algorithms with species annotation database.

2.5.3 OTU clustering and species annotation

Uparse v7.0.1001[9] was used to cluster Clean reads with 97% consistency to form Operational taxonomic unit (OTUs). The OTUs with the highest frequency were selected as representative sequences. Mothur method was used to analyze species annotations with SSUrRNA [10] database of SILVA132 [11]. MUSCLE (Version 3.8.31) [12] software was used for rapid multi-sequence alignment to obtain phylogenetic relationships of all OTUs sequences.

2.5.4 Sample complexity analysis

Alpha diversity (Chao1, ACE, Shannon index, Simpson) was calculated by QIIME (1.9.1) [13] software and Rarefaction Curve, species accumulation curves were drawn by R software (Version 2.15.3). Alpha diversity among groups was compared, and the differences were tested by non-parametric test of Friedman test for Dunn's multiple comparison test and results were considered significant when $P < 0.05$.

2.5.5 Diversity comparison analysis

Unifrac distance was calculated by QIIME (1.9.1) software, and hierarchical clustering of samples was constructed by UPGMA (Unweighted Pair-group Method with Arithmetic Mean). The NMDS diagram was drawn by vegan package of R software. The Beta diversity index was analyzed by R software, and the parameters and non-parameters were tested respectively. Linear discriminant analysis effect size (LEfSe) [14] was used to recognize microbial taxa and predicted functional genes (PICRUSt) that were abundant in gut of succession time points, based on a LDA score >2.0. and $P < 0.05$ uses. Anosim analysis uses Adonis function of R vegan package, and species analysis with significant differences between groups uses R software.

2.5.6 PICRUSt

PICRUST(Phylogenetic Investigation of Communities by Reconstruction of Unobserved States) [8] constructed bacterial function based on the Kyoto Encyclopedia of Genes and Genomes (KEGG) [15] database and abundance of metabolic pathways, and Boot strap Mann-Whitney U test was also applied for detecting gene pathways or OTUs with significantly diverse abundance among groups.

2.6 Statistical analysis

Altogether statistical analysis was evaluated by GraphPad Prism (v 5.01) or R (v2.11.1) packages and testified with Friedman test for Dunn's multiple comparison test and P value of<0.05 was measured and considered significant difference.

Results

3.1 The relative abundance of gut flora in different groups

A total of 7,029,815 raw data and 6,674,323 clean data were obtained by high throughput sequencing, with an effective rate of 94.97% (the ratio of Clean Data to Raw Data). A total of 22,625 OTUs were based on 97% similarity allocated, with an average of 257 OTUs per sample. The total usable sequences were classified into 33 phyla, 49 classes, 108 orders, 203 families, 465 genera and 306 species. Species accumulation boxplot and rarefaction curves of all samples became smooth with the quantity of sequences enlarged, demonstrating that this sequencing profundity could mirror the whole bacterial species richness (Figure. 1). A Venn diagram was applied for comparing the similarities and variances among the communities in the different groups. The eleven groups showed communities of 36 OTUs in shared, with the unique OTUs composed of 84.94%, 90.00%, 74.83%, 87.84%, 21.74%, 14.29%, 5.26%, 10.00%, 16.28%, 23.4%, 26.53%in time points of alive, 0 hour, 8 and 16 hours, day 1, 3, 5, 7, 9, 13, 15, respectively (Figure. 2). Ternary plots displayed by Ternary plot order of VCD package in R software. Figure. 3A indicated that the abundance of *Corynebacterium_amycolatum* was the highest and the most high-load in h0 among the three time points, and that of *Baterium_mpn_isolate_group_2* and *Falsiporphyromonas_endometrii* were higher in alive, 8 hours after death, respectively. Figure. 3B showed that the richness *of Enterococcus_faecalis* was increased in 16 hours and day 1, 3 post-mortem, followed by *Proteus_mirabilis*, which was higher in day 3 post-mortem as compared to the other two time points; Figure. 3C showed that the relative abundance of *Enterococcus_faecalis* was increased in day 5 post-mortem, and possessed almost same abundance in day 7, 9 post-mortem; Figure. 3D showed *Proteus_mirabilis* was the highest

one among the three time points of day 15 after death and followed by *Vagococcus_lutrae*, and *Enterococcus_faecalis* was much higher in day 9, 13 post-mortem than day 15 after death.

3.2 Microbial analysis at different levels

The microbial community structure succession was identified during decomposition, all of the 16S rRNA sequences were classified at phylum, genus and species level. The notable tendencies and fluctuations exhibited in the relative richness of the diverse bacterial taxa in the rectum of the rat cadavers through the decaying process (Figure. 4A, B, C; Figure. 5A, B, C; Table 1). Figure. 4A, B, C showed variations of bacteria with their proportions at different levels and Figure. 5A, B, C showed the relative abundance of 10 topmost bacteria in the study samples.

At the phyla level, *Firmicutes*, *Proteobacteria*, *Bacteroidetes* and *Actinobacteria* existed in wholly time points. *Bacteroidetes* (54.57%), *Firmicutes* (45.83%) and *Proteobacteria* were the dominant phylum in alive samples, 0-hour post-mortem and other time points, respectively. The *Bacteroidetes* began to show a downward trend and even disappeared, rising to 5.52% in 15 days post-mortem samples. The relative abundance of *Proteobacteria* in alive samples was much lower than 8-hours, day 1, 9 post-mortem, while *Bacteroidetes* was greatly higher in alive samples than 8-hours, day 3, 5, 7, 9, 13, 15, and *Actinobacteria* was significantly higher in 0-hour, day 5, 7, 9, 13, 15 post-mortem ($P<0.05$).

At the genus level, *Lactobacillus*, *Enterococcus* were appeared as the dominant genera before day 1 post-mortem and day 3-13, respectively, and *Helicobacter* disappeared on 7, 9, 15 post-mortem intervals and *Proteus* was the most abundant in day 15 post-mortem. The relative abundance of rectum flora in alive samples was greatly lower than in day 3, 5, 7, 9, 13 post-mortem intervals, nevertheless, *Helicobacter* was much higher in these days ($P<0.05$). The proportion of *Proteus* and *Lactobacillus* showed significantly lower in alive and 0-hour than in day 13 post-mortem samples, while *vagococcus* displayed opposite result ($P<0.05$).

At the species level, the 10 topmost species existed in 8 hours post-mortem, but *Clostridium_sporogenes* and *Falsiporphyromonas_endometrii* disappeared before day 1 post-mortem and after day 3 post-mortem, respectively. *Enterococcus_faecalis* and *Proteus_mirabilis* appeared at the whole decomposition process of 15 days after death, the former one showed a downward trend from the day 5 post-mortem, while the latter one showed an upward trend. *Bacterium_mpn-isolate_group_2* disappeared (day 5-13 post-mortem) or decreased after alive. The relative abundance of *Enterococcus_faecalis* was much lower in alive samples than in day 5, 7, 9, 13, 13, 15 after death, while *Bacterium_mpn-isolate_group_2* was distinctly higher in these days ($P<0.05$). The relative abundance of *Corynebacterium_amycolatum* and *Lactobacillus_intestinalis* were greatly higher in 0-hour, day 7, 9, 13, 15 post-mortem intervals, and *Lactobacillus_reuteri* was higher in alive, 0-hour, 16-hours and day 1 than in day 13 post-mortem.

3.3 Characterization of bacterial diversity and community structure

The whole rectum flora community was evaluated by diversity and richness as calculated at the level of 97% similarity. Alpha diversity indexes of the observed_species, abundance based coverage estimators (ACE) and chao1 values for the rectal bacteria in the alive samples were significantly higher than those in day 5, 7, 9, 13, 15 post-mortem, suggesting that richness and diversity of the rectum flora decline a lot after day 5 post-mortem (Table 2). All of the Alpha diversity indexes were showed in Table 2 that there were having significant differences in overall rectum bacterial community structure in the eleven post-mortem intervals.

The similarity of the gut flora communities of rats in eleven groups were measured by beta diversity metrics such as Non-metric dimensional scaling (NMDS) and Beta diversity heatmap to define the differences of each individual animal gut microbial structure at different post-mortem interval. As displayed in Figure. 6A, the difference coefficients among other groups were almost greater than 0.5, telling that bacteria community in different groups had great diversity. All of the samples were gathered into 11 prime clusters. According to Non-Metric Multi-Dimensional Scaling (NMDS, stress=0.152), the bacterial communities of the gut samples were separated into three clusters between late and early PMI (Figure. 6B). Conspicuously, 8 hours post-mortem could be significantly separated from other groups, but samples of 8 hours post-mortem were clustered, indicating that the gut flora at 8 hours after death differed with other two clusters obviously.

LDA Effect Size (LEfSe) is a biomarker detection and descriptive device for high-dimensional statistics. The LEfSe analysis was performed for comparing the projected bacterial community among 11 time points at different levels (Figure. 6C). The results suggested that the provision of related taxa significantly diverse among overall groups. The LDA scores showed that the relative abundances of *Corynebacterium_amycolatum*, *Entero_isolate_group_2*, *Bacteroides_uniformis*, *Enterococcus_faecalis*, *Streptococcus_gallolyticus_subsp_macedonics* and *Clostridium_sporogenes* were more abundant in 0-h, day 1, 3, 5, 7, 13 post-mortem intervals, respectively, while *Proteus_mirabilis* and*s_Vagococcus_lutrae* were in day 15 post-mortem.

3.4 PICRUSt

The shifts in the probable functions of the gut flora of rats before and after death were inspected by predicting the 16S rRNA genes using PICRUSt (Figure. 7A, B). The top four different pathways at Kyoto Encyclopedia of Genes and Genomes (KEGG) at level 1 were Metabolism, Genetic_Information_Processing, Environmental_Information_Processing and Cellular_Processes and at level 2 were Membrane_Transport, Carbohydrate_Metabolism, Amino_Acid_Metabolism and Replication_and_Repair. The functional pathway related with Environmental_Information_Processing such as Membrane_Transport as the dominant one in the whole time points, then the followed pathways associated with Genetic_Information_ Processing and Metabolism, including Carbohydrate_Metabolism, Amino_Acid_Metabolism and Replication_and_Repair, respectively, possessing large number associated genes in all samples (Figure. S1). The pathways of Environmental_Information_Processing and Organismal_ Systems were significantly higher in rectum bacterial community day 5, 7, 9, 13, 15 after death as compared to alive and Membrane_Transport associated with the first mentioned pathway displaying identical result (Table S1, S2). Amino_Acid_Metabolism, Energy_Metabolism and Metabolism_of_Cofactors_and_Vitamins exhibited significant difference between alive and day 5, 7, 9 post-mortem intervals, while Energy_Metabolism showed notable differences between alive and after day 5 post-mortem (Table S1, S2) ($P<0.05$).

Discussion

The microbiome in alive body is complicated and multiple significant differences had been observed between and within individuals [16]. Microorganism exist inside and outside throughout the dead body and happened distinctly and temporally shift during decay [17]. The results of this study displayed that the main phyla in intestinal samples before rats death were *Firmicutes* and *Bacteroidetes*, which was similar to the NIH Human Microbiome Project (HMP) [18] (Figure. 5A). We had also explored the composition and structural features of gut microbiota at species level. The results showed that *bacterium_mpn-isolate_group_2* and *Lactobacillus_reuteri*as were the dominant species in alive samples. The forte of using a rat model is that huge quantity samples provided convenience to evaluate the intra-individual microbial distinction during decomposition and the changes of microbiota existed in alive people and other mammals had known [19].

The bacterial communities in the decomposing rats had shifted distinctly in category and relative abundance comparing with alive rats. Indexes of evaluating bacterial community richness such as observed_species, shannon, chao1, ACE, goods_coverage significantly reduced in several post-mortem intervals as compared to pre-mortem, while Simpson index and PD_whole_tree showed no significant difference which was incoordinate with previous study [20], suggesting that different environmental factors can influence the gut bacterial community richness after death.

This study indicated a notable variation in gut bacterial diversity and relative abundance during the decomposition course of 15 days at phyla, genera and species level. Despite the dominant bacteria community diversity at phyla level had no obviously fluctuation, the relative abundance of *Bacteroidetes* notably deduced after death time, which was similar with preceding analysis [21]. As previously reported [22], high level of *Vagococcus* might account for the larvae of blow fly, indicating that the carrion insects might participate in the decomposition process (the eggs of larvae might be from the rats themselves for *Vagococcus* in the alive samples abundance was 0.001%). Changes in phyla *Firmicutes* and *Proteobacteria* were destined in opposition direction of the relative abundance over time which was compatible with previous work [23]. At the genus level, *Bacteroides* was the most obvious variation at the early death time, while *Enterocuccos* and *proteus* were at the late time. *Lactobacillus* as Gram-positive, facultative anaerobic bacteria was capable of converting sugars to lactic acid and also participated in tryptophan metabolism producing smell indole-3-aldehyde (I3A) [24]. Our consequences maintained the concept that the above four mentioned genera might act as imperative donors to the process of decomposition. The bacterial community at species level was scarcely reported, therefore we investigated to this sort of microorganism. Our study analysis showed an obvious shift of *Corynebacterium_amycolatum* at early postmortem, while *Enterococcus_faecalis*, *Proteus_mirabilis*, *Clostridium_sporogenes* and *Vagococcus_lutrae* at late post-mortem. Interestingly present findings of *Proteus_mirabilis* and *Vagococcus_lutrae* displayed parallel change almost though percentage was different. According to previous study, *Proteus_mirabilis* was able to attract blow fly who was the reason of high percentage of *Vagococcus_lutrae* [25]. The above two species of bacteria after day 15 post-mortem had a downside, while *Enterococcus_faecalis* and *Clostridium_sporogenes* was trending upward. The *Clostridium_sporogenes* mainly occurring before day 1 post-mortem as Gram-positive, obligate anaerobic species possessed the capacity of decomposing carbohydrates and peptones to organic acids and alcohols and also taken part in tryptophan metabolism producing smell 3-Indolepropionic acid (IPA) [26], suggesting that the spilled odors in four time points (0 hour, 8 hours, 16 hours and day 1 post-mortem) were primarily produced by the *Clostridium_sporogenes*. The Gram-negative, facultatively anaerobic bacteria of *Proteus_*

mirabilis appeared in day 13, 15 post-mortem intervals could also produce hydrogen sulfide gas, indicating that the smell in last two time points of this study was associated with *Proteus*

_mirabilis. However, further studies are required to observe the role of the mentioned species for PMI after day 15 post-mortem.

The results of this study provide qualitative and quantitative appearance with the post-mortem rats' rectum microbiome over first 15 days. LEfSe results suggested that seven categories were identified as seven PMI indicator. Thereinto, *Bacteroides_uniformis* belongs to *Bacteroides spp.* can be regarded as PMI indicator of day 3 post-mortem. It had been reported that [24] *Bacteroides spp.* could be used as quantitative indicator of PMI. *Enterococcus_faecalis*, *Streptococcus_gallolyticus_subsp_macedonics*, *Clostridium_sporogenes* were included in *Enterococcus*, *Streptococcus* and *Clostridium spp.* can also be used as PMI indicator of day 5, 7, 13 post-mortem, which have been reported as the most abundant species during decomposition [27].

The PICRUSt analysis was applied to deduce functional abilities of different bacteria taxa. The results of PICRUSt suggested that the microbial function shifted significantly between pre- and several post-mortem intervals (Table S1, S2). *Bacteroides*, *Lactobacillus*, and *Streptococcus* were recognized bacterial species for their involvement in proteolysis. These four bacteria participated in *Amino_Acid_Metabolism* and *Carbohydrate_Metabolism* as the main force in different time points, transforming proteins into smell gases such as H_2S, methane, ammonia, sulfur dioxide and organic acids (e.g. propionic acid, lactic acid). The above processes were resulted by a reduction in the obtain ability of oxygen [28]. The gases leads to abdominal cavity rupture and then resulted in the shift of bacteria for the cavity becoming aerobic [19].

Although the results offered a detailed considerate of bacteria within a decomposing cadaver system and recommend that microbial community data can be evolved into a legal medicine means for estimating post-mortem interval (PMI), however, an additional work is essential to better comprehend this perception

Conclusions

Taken together, the bacteria community emerged distinct shift during 15 days of decay, such as *Proteobacteria* and *Firmicutes* showed opposite relationship, *Proteus* showed an increasing tendency after day 3 post-mortem. These findings offer the foundation to analysis the bacterial community in the specific time points after death, particularly, the species level analysis can be considered. *Corynebacterium_amycolatum* and *Bacteroides_uniformis* in 0- hour and day 1 post-mortem, respectively, emerged as the characteristic bacteria at specific time points as described above. The bacteria at species level with high relative abundance and significant difference at the specific post-mortem interval, might be the indicators of PMI.

Abbreviations

SD: Sprague Dawley; Postmortem interval: PMI.

Declarations

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Availability of data and materials

The datasets used and/or analyzed during the current study are available from the

corresponding author on reasonable request.

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Contributions

HL: Conceptualization, Data curation, Formal analysis, Methodology, Project administration, Software, Writing – original draft. **LY, RNL, SRZ, EY, SU, NM, DW, YS, CHA, HLL:** Writing – review & editing. **ZYW and JRX:** Funding acquisition, Writing – review & editing. All authors read and approved the final manuscript.

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Ethics approval and consent to participate

All of the animal handling and treatment were approved by the institutional ethics committee of the Institutional Animal Use and Care Committee of Xi'an Jiaotong University according to paragraph 7 (Ethics Approval Number: 2017-388).

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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Tables

Table 1 Significant difference in Abundance of OTUs at phylum, genera and species levels in pre- and post-mortem.

Bacteria	Mean relative abundance									
	alive	0 hour	8 hours	16 hours	Day1	Day3	Day5	Day7	Day9	Day13
Phylum										
Proteobacteria	0.018967^{a}	0.238017^{c}	0.685157^{b}	0.515944	0.783757bd	0.603994	0.477884	0.509146	0.655225b	0.445179
Bacteroidetes	0.575744^{a}	0.055408	0.046913^{b}	0.039948	0.009058	0.090197^{b}	0.002348^{b}	0.003179^{b}	0.000968^{b}	0.001218^{b}
Actinobacteria	0.00136	0.176496^{c}	0.008813	0.011762	0.002587	0.00605	0.000186^{d}	0.000137^{d}	0.000298^{d}	0.001174^{d}
Genera										
Enterococcus	0.000381^{a}	0.127666	0.013376^{e}	0.016927^{g}	0.028338^{i}	0.207753^{b}	0.491857^{bfhj}	0.382742^{b}	0.332367^{b}	0.374643^{b}
Proteus	0.000049^{a}	0.00023^{c}	0.000577	0.000455^{g}	0.000597^{i}	0.104454^{bj}	0.044976^{bj}	0.046492	0.084841	0.216454^{bj}
Lactobacillus	0.022459^{a}	0.189569^{c}	0.174197^{e}	0.336959^{g}	0.162977^{i}	0.015514	0.003732	0.045177	0.001712^{h}	0.000176^{bdfhj}
Vagococcus	0.00001^{a}	0.00047^{c}	0.003551	0.004299	0.003737	0.03471^{b}	0.005624^{b}	0.014966	0.00558	0.045661^{bd}
Helicobacter	0.001624^{a}	0.041934^{c}	0.000572	0.00201	0.000059	0^{bd}	0^{bd}	0^{bd}	0^{bd}	0.000005^{bd}
Species										
Enterococcus_faecalis	0.000059^{a}	0.008583^{c}	0.001355^{e}	0.006862	0.01947^{i}	0.115879^{b}	0.39001^{bdf}	0.241289^{bf}	0.230305^{b}	0.22268^{bfj}
Proteus_mirabilis	0.000034^{a}	0.000127^{c}	0.000372^{e}	0.000372	0.00045^{i}	0.079285^{bdf}	0.026675^{bf}	0.036319	0.071259	0.180374^{bfj}
Clostridium_sporogenes	0	0	0.000352^{e}	0^{g}	0^{i}	0.000024	0.000044	0.00021	0.000386	0.104938^{fhj}
Corynebacterium_amycolatum	0.000044	0.000665^{c}	0.001585	0.001047^{g}	0.000181	0.008432^{d}	0.014164^{d}	0.043347^{df}	0.001477^{df}	0.00114^{df}
Lactobacillus_intestinalis	0.00045	0.012618^{c}	0.0206	0.027188^{g}	0.019549	0.000298	0.000034^{dh}	0.000088^{dh}	0.000015^{dh}	0^{dh}
bacterium_mpn-isolate_group_2	0.025628^{a}	0.000044	0.000034	0.000015	0.000015	0.000005^{b}	0^{b}	0^{b}	0^{b}	0^{b}
Lactobacillus_reuteri	0.006877^{a}	0.014628^{c}	0.00941	0.018869^{g}	0.017822^{i}	0.006383	0.000191	0.002005	0.000127^{h}	0.000015^{bdhj}

The significant findings were testified with Friedman test for Dunn's multiple comparison test and $P<0.05$ by GraphPad Prism. “a, c, e, g, i” represent alive, 0 hour, 8 hours, 16 hours and day 1 pre- and post-mortem comparing with other time points, and “b, d, f, h, j” means having significant difference ($P<0.05$).

Table 2. Alpha diversity index of high-throughput analysis of intestinal microbial richness and diversity

Time points	Observed_species	Shannon	Simpson	Chao1	ACE	Goods_coverage	PD_whole_tree
alive	574^{a}	6.284^{a}	0.957^{a}	681.781^{a}	691.263^{a}	0.995^{a}	47.217^{a}
0 hour	410^{c}	4.845	0.842	472.923^{c}	474.917^{c}	0.997	45.098^{c}
8 hours	191^{e}	2.77^{b}	0.681^{b}	256.809^{e}	276.089^{e}	0.998	29.156^{e}
16 hours	321	3.67	0.772	393.452	414.277	0.997	54.33
day 1	159	2.414^{b}	0.665^{b}	238.27	269.216	0.997	23.572
day 3	167	3.316	0.781	210.444	214.123	0.998	20.155
day 5	61^{bdf}	2.437	0.658^{b}	80.908^{bd}	92.725^{bdf}	0.999^{b}	14.241^{bd}
day 7	64^{bd}	2.633^{b}	0.739	78.199^{bdf}	90.613^{bdf}	0.999^{b}	10.862^{bd}
day 9	81^{bd}	2.629^{b}	0.705	107.505^{bd}	114.157^{bd}	0.999^{b}	13.614^{bd}
day 13	102^{b}	2.388^{b}	0.656	148.395^{b}	163.889^{b}	0.998^{b}	17.613
day 15	103^{bd}	2.60^{b}	0.697^{b}	134.112^{bd}	139.958^{bd}	0.999^{b}	11.867^{bdf}
*P**	<0.0001	<0.0001	0.0011	<0.0001	<0.0001	<0.0001	<0.0001

The values signified in the table are the mean values of each group, significant findings were testified with Friedman test for Dunn's multiple comparison test and $P<0.05$ by GraphPad Prism. “a, c, e” represents alive, 0 hour, 16 hours pre-and post-mortem comparing with other time points, and “b, d, f” means having significant difference.

Figures

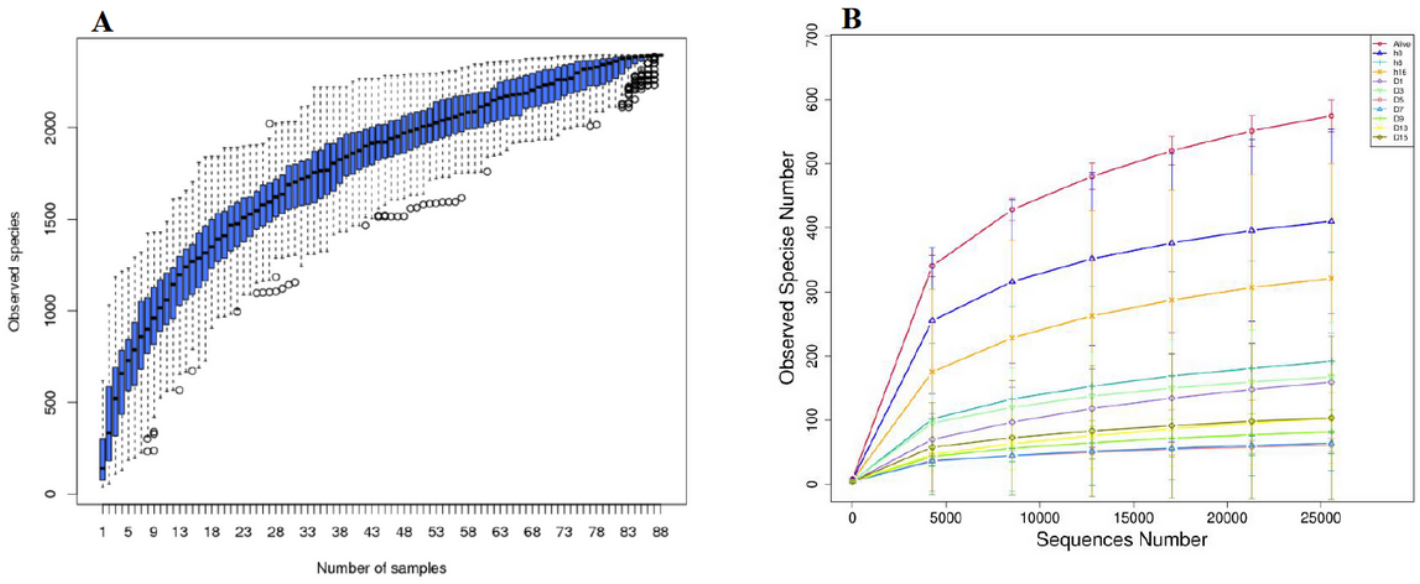

Figure 1

Species accumulation boxplot, evaluating whether sample size was sufficient or not and determining species richness (A). Rarefaction analysis of the microbiota of the rat rectum (B).

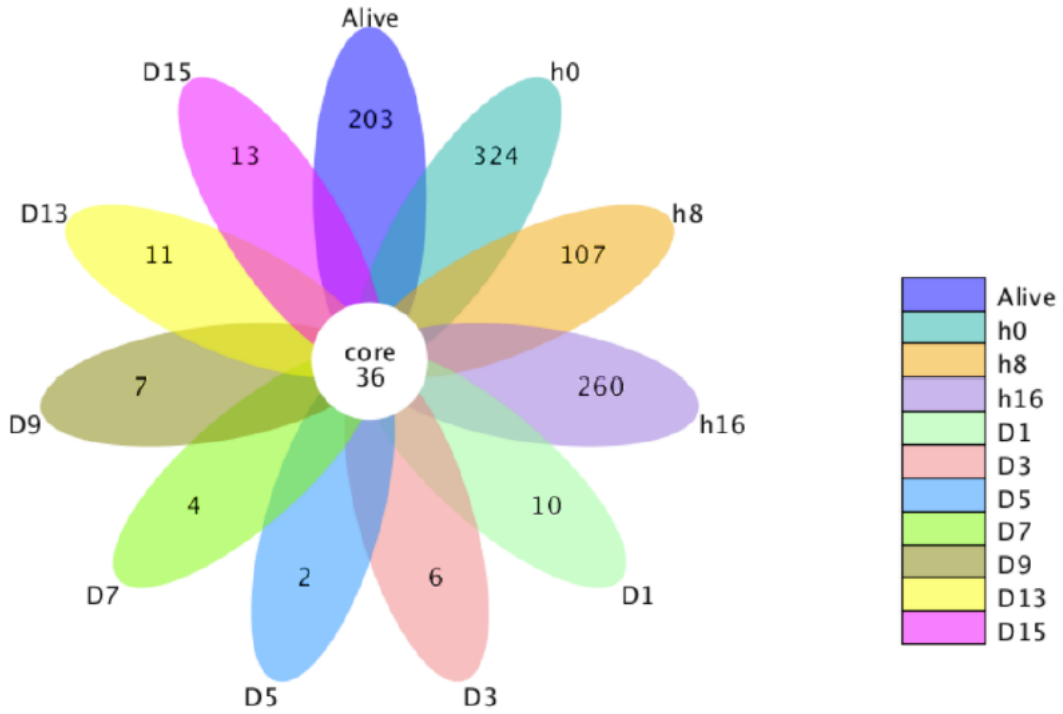

Figure 2

Species accumulation boxplot, evaluating whether sample size was sufficient or not and determining species richness (A). Rarefaction analysis of the microbiota of the rat rectum (B).

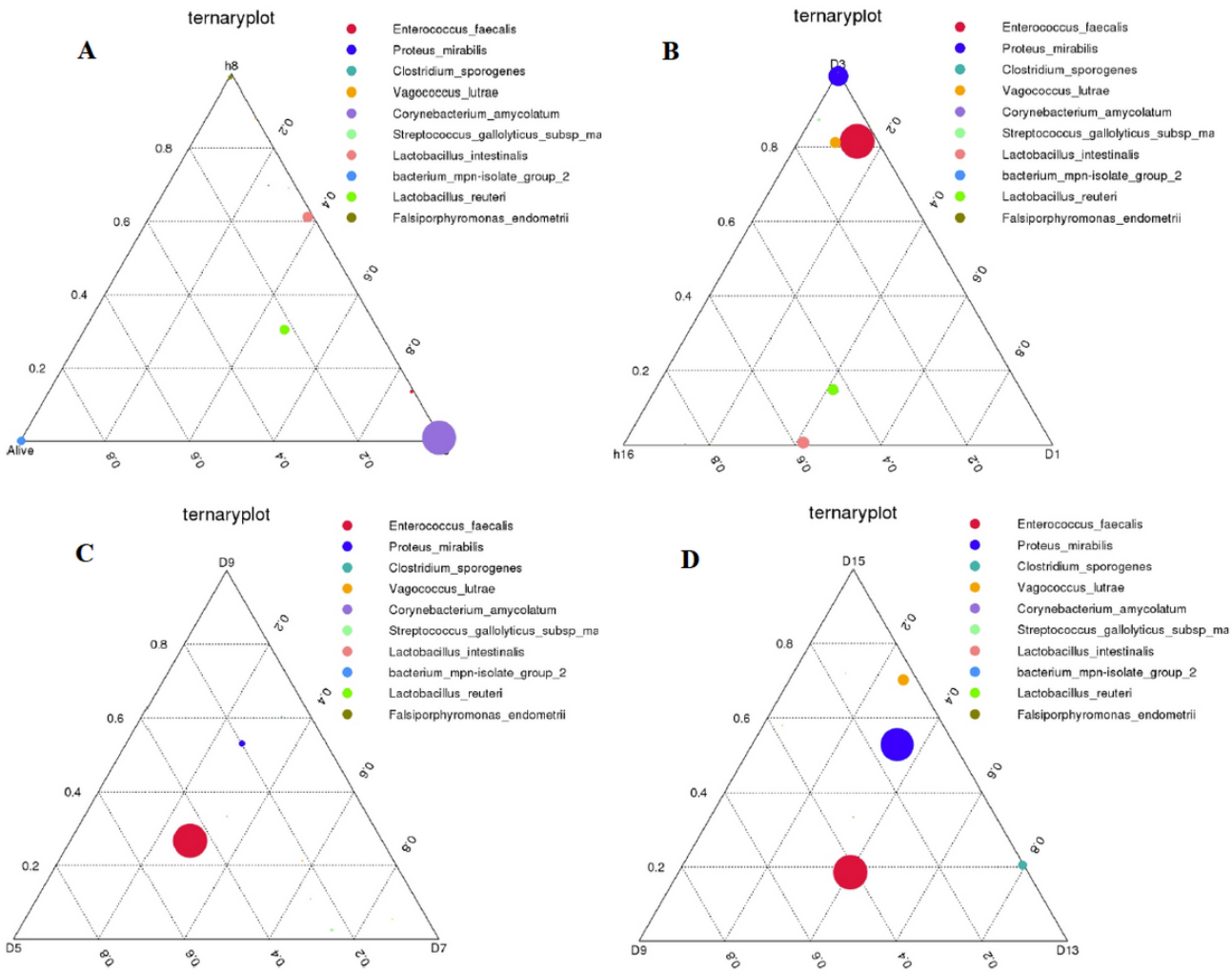

Figure 3

Ternary plots displaying the distribution of microbial based on OTUs. Each circle represents an individual OTU while its size suggests quantity of reads related. The position of each OTU is determined by its quantity of sampling time point to the total bacteria count (n = 3).

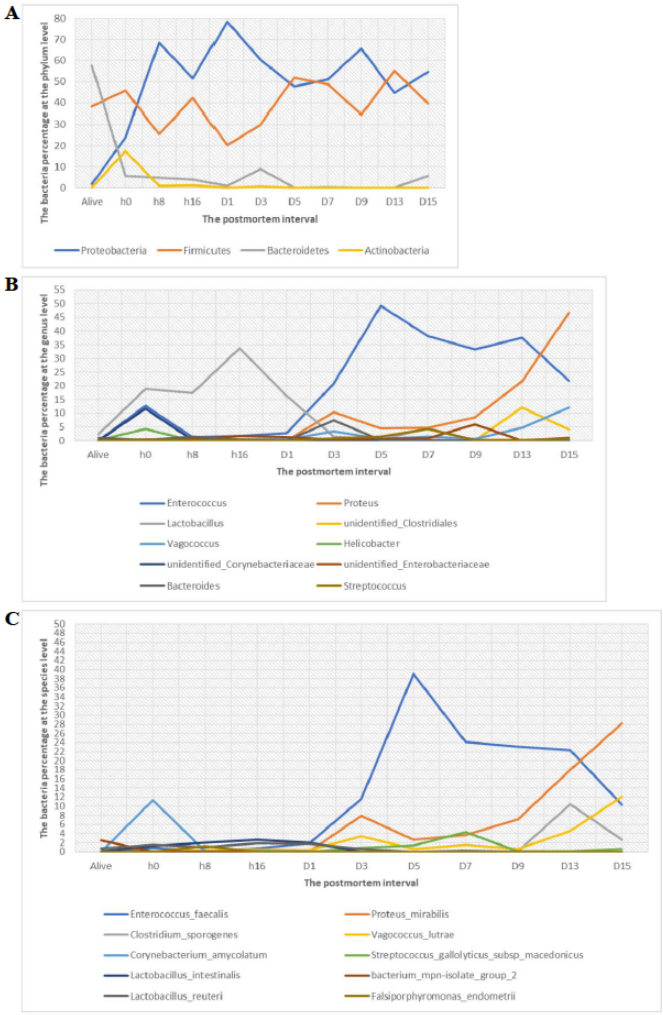

Figure 4

Line charts exhibit obvious shift of microorganism percentages at phylum (A), genus (B), species level (C).

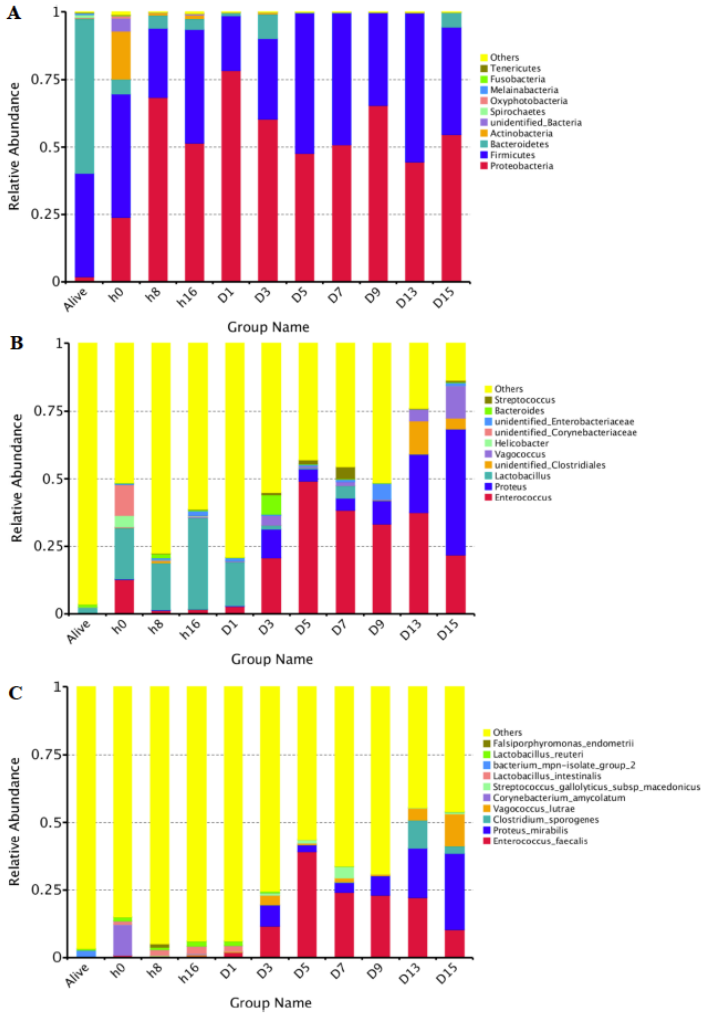

Figure 5

Taxonomic profiles of the rectum bacteria in eleven time points. Values represent the relative abundance of the top 10 phylum (A), the top 10 genus (B) and top 10 species (C) present in eleven time points.

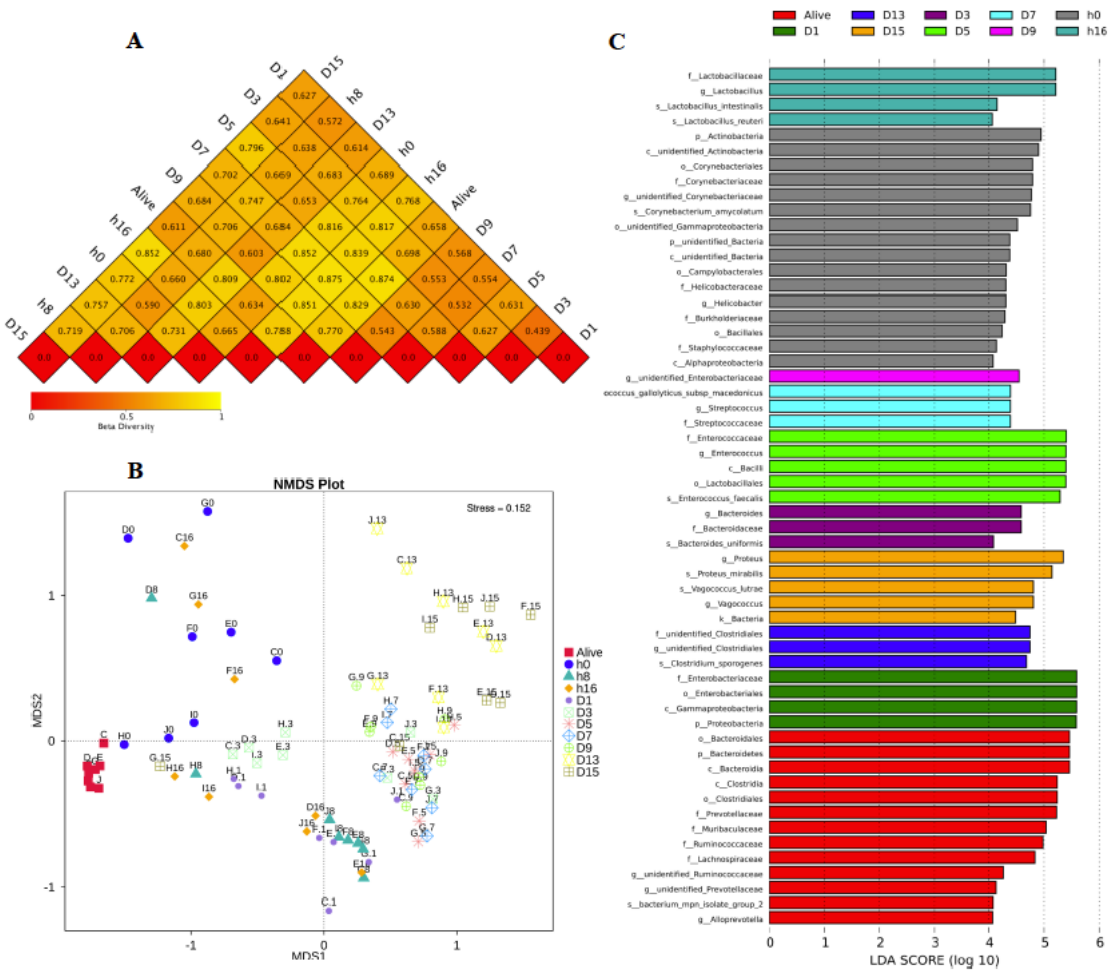

Figure 6

Beta diversity of bacterial populations in eleven time points reflects inter-group differences. Heatmap was drawn by the Unweighted Unifrac distance (A). The microbial diversity in a certain time point increase follow with the size of value. NMDS (non-metric multi-dimensional scaling) coordination plot among the whole samples (B). Stress value less than 2 indicates that NMDS can accurately reflect the difference between samples. Significant taxa obtained in the sampling time points using LEfSe analysis. Linear discriminant analysis (LDA) plots of bacteria at different levels as results of all time points (C).

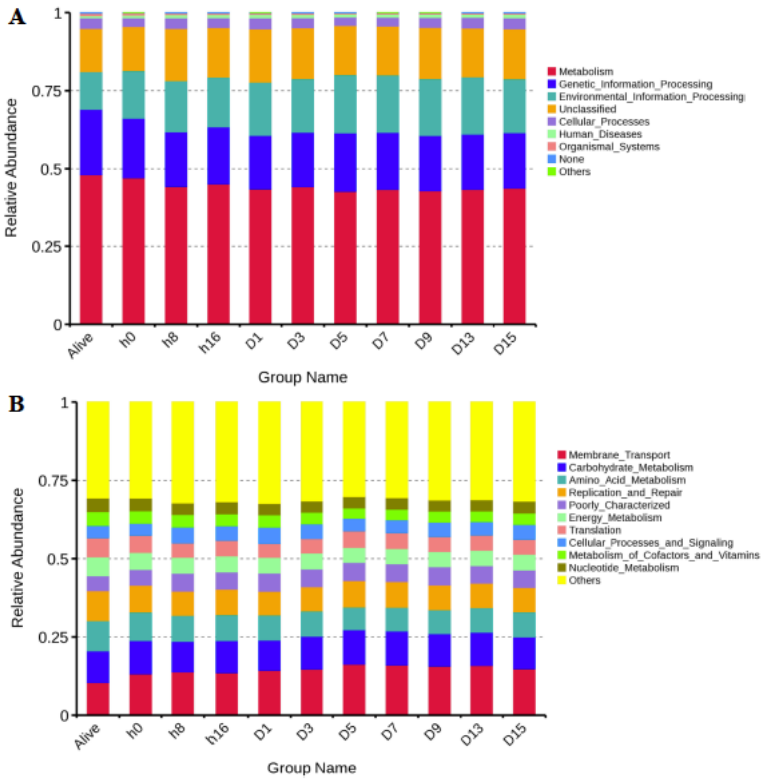

Figure 7

Function profiles of the rectum bacteria in all postmortem intervals. Values represent the relative abundance of the top 10 function at level 1 (A) and 2 (B) present in each time points.

Supplementary Files

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- Tables.pdf
- Figure.pdf
- Additionalfile.docx