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Differences of Mice Gut Microbiota between SPF and Clean Environment

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Abstract

Objective: Compare the difference of intestinal microbiota between Specific Pathogen Free (SPF) mice and clean environment mice.

Methods: Twelve mice were divided into two groups. One group is Specific Pathogen Free (SPF) mice, another is clean environment mice. The stool samples were collected for nine and eighteen days both in SPF and clean environment, and the total DNA was extracted. PCR-DGGE technology were used to obtain microbial flora fingerprint, Quantity one software were used to build phylogenetic tree, the similarity and diversity of the map can be used for analysis, differential bands were selected for sequencing.

Results: Under the conditions of SPF, the intestinal microbiota of mice were significantly different ($p<0.05$) from that of the clean grade, and the Enterobacteria were specific in SPF mice. With the passage of time, *Lactobacillus* were decreased in SPF mice.

Conclusion: Differences exist in intestinal microbiota of mice under different feeding conditions.

Keywords: SPF Mice; Clean Environment Mice; Intestinal Microbiota; Diversity

Introduction

Different colonies begin to colonize the intestine before and after the mammal are born, and the intestinal flora changes with the living environment and lifestyle of the host. Under the condition of bacteria, the number of intestinal flora in mice increased in number and species. Different intestinal colonies had different effects on the construction of mouse immune system. Studies have shown that when humans and other mammals are born, colonization of the flora in the gut plays an important role in the construction of the immune system [1,2]. The purpose of this study is to compare the differences between the SPF environment and the intestinal flora of the mice in a clean environment, so as to provide a theoretical basis for researchers to select the corresponding level of mice according to different experimental purposes in the future, and improve the reliability of the experiment.

Materials and Methods

Subjects

Ten experimental animals 18 ~ 22 g Male C57BL/6j mice were purchased from Experimental Animal Center of Dalian Medical University. Animals were divided into two groups randomly. Each group is six mice. One group is specific pathogen free (SPF) mice, another is clean environment mice.

Methods

Extraction of fecal DNA

Weigh 200 mg of fecal samples, extract the total DNA of all samples according to FOREGENE's DNA extraction kit instructions, and extract the extracted DNA samples in a -20°C refrigerator.

PCR amplification

PCR reaction system Composition in 50 μL:

10*EXPCR Buffer (including Mg^{2+})	5 μL
dNTPs mixture	8 μL
1% BSA	5 μL
20 μM upstream primer	1 μL
20 μM downstream primer	1 μL
ExTaq DNA Polymerase (5 U/μL)	0.5 μL
DNA template	3 μL
ddH_2O	26.5 μL

In the reaction system: upstream primer: GC-341F (5'-GC clamp-CCT ACG GGA GGC AGC AG-3'), downstream primer: 518R (5'-ATT ACC GCG GCT GG-3'), in which the upstream primer 5'-end GC-clamp is a 40 bp "GC clamp" whose sequence is: CGC CCG GGG CGC GCC CCG GGC GGG GCG GGG GCACGG GGG G.

PCR reaction conditions:

95°C pre-denaturation	5 min	
94°C deformation	30 s	30 Cycles
54°C annealing	30 s	
72°C extension	30 s	
72°C fully extended	7 min	

PCR reaction conditions are given above.

Denaturing gradient gel electrophoresis (DEEG)

The product after 20 μL PCR was mixed with 5 μL of 6*loading buffer, and then analyzed by DGGE electrophoresis in a constant temperature water bath at 60°C. The concentration gradient of the denatured gel was selected to be 30%-60%. According to the preliminary experiment, the concentration was the optimal concentration, the loading amount was 25 μL, and the electrophoresis was carried out for 5 min under high voltage. After the sample entered the separation gel, the current was changed to 14A, and the electrophoresis was carried out for 6 h, where in the running buffer is 1*TAE Buffer. After the end of electrophoresis, EB staining was performed for 1 h, gel imaging photo analysis and gel recovery.

Analyze the DGGE

Cut the specific strip into the EP tube after high pressure. Wash it repeatedly with deionized water after high pressure for 3 times, absorb the supernatant as much as possible, and finally add 50 μL to the EP tube after high pressure. Deionized water after high pressure was stored in a refrigerator at -20°C overnight. Then, it was centrifuged at 90°C for 10 min, centrifuged at 10000*g for 5 min, and 3 μL of the supernatant was taken as a template for PCR amplification (the upstream primer used for this amplification was a GC-free upstream primer). The amplified PCR product samples were sent to the company for sequencing, and the sequencing results were subjected to blast analysis in the NCBI database. Cluster analysis of DGGE maps using Quantity One software.

ELISA analysis

Pro-inflammatory cytokine interleukin-1-beta (IL-1β) measurement with ELISA in serum. The ELISA kits were purchased from Shanghai Lengton Bioscience Co, Ltd., Shanghai, China.

Results

PCR amplification results

The total DNA of SPF and clean mouse feces was amplified by PCR, and the amplified product was detected by 1% agarose gel electrophoresis. The results showed that the PCR fragments were both 200 bp and there were no non-specific amplified fragments (Figure 1).

DGGE Fingerprint of SPF and Clean Grade Mice

The distribution of intestinal flora in SPF mice and clean mice is shown in Figure 2. In the DGGE map, the bands at each position represent different types of bacteria, and the brightness of the bands reflects the individual bacteria. The relative amount of the group. Therefore, the distribution of both the intestinal flora and the clean-grade mice of SPF mice can be reflected by the DGGE map: the dominant flora between the two groups produced a large difference.

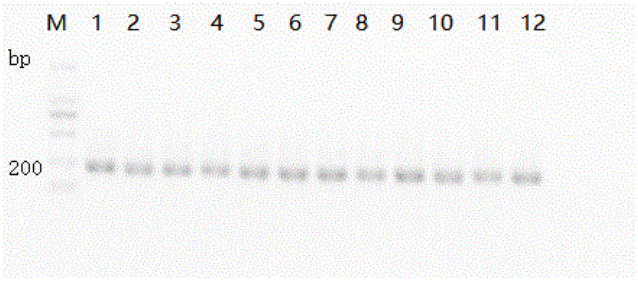

Figure 1: Gel electrophoresis pattern of total DNA amplification product of mouse feces (M: DNA Marker). 1-3: 9-day clean grade mouse group; 4-8: 18-day clean grade mouse group; 8-12: SPF grade mouse group.

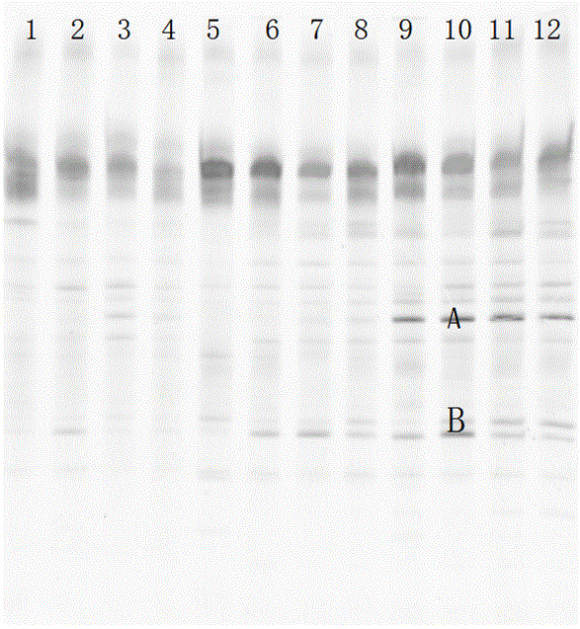

Figure 2: DGGE fingerprint of intestine flora of clean grade and SPF mice. 1-3: 9 days clean grade mouse intestinal flora; 4-8: 18 days clean grade mouse intestinal flora; 8-12: SPF grade mouse intestinal flora.

Gelation sequencing results

By comparing and analyzing the DGGE fingerprints, the bands with significant differences were cut and sequenced, and the measured sequences were blasted by NCBI database to obtain the names of the flora, as shown in Table 1.

Strip number	Bacteria name	species	Similarity	Login ID
A	*Lactobacillus intestinalis*	*Lactobacillus*	99%	NZ AZGN01000031.1
B	*Lactobacillus animalis*	*Lactobacillus*	99%	NZ AYYW01000015.1

Table 1: DGGE gel differential bands after sequencing, NCBI database bacterial group comparison.

Clustering and similarity analysis of intestinal flora in SPF mice and clean mice

The DGGE map was analyzed by the software Quantity One, and each lane was clustered by UPGMA to obtain a dendrogram. The clean-grade mouse intestinal flora and the SPF-grade mouse intestinal flora were classified into two clusters respectively. There was a significant difference in the intestinal flora structure between the SPF-grade mice and the clean-grade mice (P=0.0328, p<0.05), and the differences between the groups were relatively large, but the differences among the individuals in the group were relatively small (Figure 3).

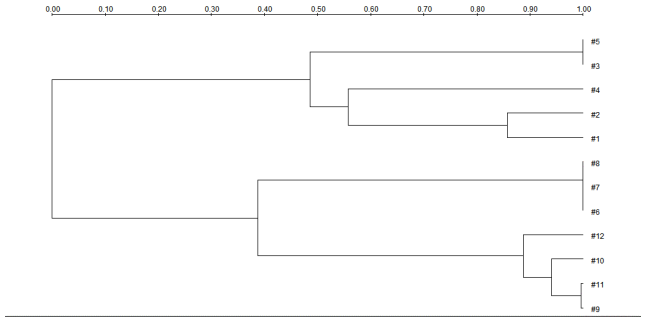

Figure 3: UPGMA cluster analysis chart. (Note: #1-#3: 9 days clean grade mouse intestinal flora; #4-#8: 18 days clean grade mouse intestinal flora; #9-#12: SPF grade mouse intestinal bacteria group)

Pro-inflammatory cytokine level

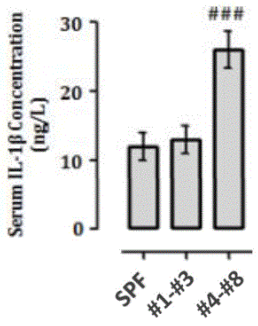

Figure 4: Pro-inflammatory cytokine level. (Note: #1-#3: 9 days clean grade mouse intestinal flora; #4-#8: 18 days clean grade mouse intestinal flora; #9-#12: SPF grade mouse intestinal bacteria group)

We measured serum interleukin-1-beta (IL-1β) cytokine levels in clean grade environments and SPF grade mice. Studies have shown that SPF-level mice have the lowest levels of IL-1β ($p<0.01$), and the more IL-1β content is agreed with the number of days of growth in a clean-grade environment (Figure 4).

Discussion

Analysis of the results of the study

The intestinal flora of mice in the SPF environment and clean environment, the composition of the intestinal flora is significantly different, of which lactobacilli are more specific strains of SPF mice. It proves that the cleanliness of the environment has an effect on the colonization of the mouse flora, and by comparing the differences in the intestinal flora of the mouse in the SPF environment and the clean environment, the changes in the microbial environment experienced by the human body caused by the modern living environment can be simulated. Further improve the accuracy and reliability of the experiment.

Gut microbes are a positive and useful health substance that primarily affects the normal structure and function of the mucosal immune system. The structure and composition of the gut flora not only reflects the choice between the microorganism and the host, but also promotes the stability of its interaction and function. Intestinal development in the early fetus is incomplete, and the intestinal flora of the fetus is affected by the mode of delivery, diet, hygiene, and medication [3] and colonization begins immediately after birth. Although infants and formula-fed infants differ in their intestinal microbial composition and incidence of infection [4], Enterobacteria and mycobacteria are representative of early colonization. Such bacteria can regulate gene expression in the host and create a suitable environment for themselves to prevent the growth of other invading bacteria [5]. Although adult intestinal flora changes with lifestyle, eating habits and age [6]. In many studies it has been shown that host genotypes have a greater impact on individual gut microbiota than environmental factors [7].

The natural defense barrier formed by the gut flora has multiple protective effects on intestinal epithelial cells, stabilizes the structure and promotes metabolism. The physiological effects of the gut flora in the gut can already be shown by comparison of sterile animals and animals with colonized flora. Sterile animals are more susceptible to infection and reduce their vasoactive, digestive enzyme activity, muscle wall thickness, cytokine production, and serum immunoglobulin levels. However, the use of microorganisms to reconstitute the intestinal flora of sterile mice can restore their mucosal immune system [8]. Studies have shown that the combination of sterile mice with a single type of *Bacteroides* the *taiotaomicron* affects nutrient intake, metabolism, angiogenesis, mucosal barrier function and synthesis of the intestinal nervous system [9]. Therefore, the

composition of the colonization flora may affect the individual's immunity.

Significance of the results of this experimental study

Clinically, some patients suffering from trauma, advanced tumors, and immunocompromised disorders usually develop intestinal flora disorders. In the past, people used some physical and chemical methods to kill tumor cells. However, the drawbacks of these methods are that they are non-specific to the destruction of immune function, and it is difficult to ensure that the immune function is impaired without damaging the normal functions of other systems in the body, making it difficult to define immune function and intestinal tract. Is there an exact relationship between the flora .By understanding the differences between the intestinal flora in SPF and clean environment, the interference of the bacteria on the experimental results can be avoided, and the appropriate animal model can be selected to simulate the human body, thus providing the theory for the researchers to select the corresponding level of mice according to different experimental purposes in the future. Based on, and improve the reliability of the experiment.

Innovativeness of this experimental study

There are few reports on the differences between SPF-grade and clean-grade mice. The domestic literature only compares the diversity of lower respiratory flora between SPF and normal mice [10]. But there is no analytical comparison of gut flora. This experiment shows the difference between SPF-grade and clean-grade mice by comparing the differences in intestinal flora, filling in the gaps in the domestic literature comparing the differences in intestinal flora between SPF mice and clean-grade mice. .

Insufficiency and assumptions of this experiment

Although this experiment shows that there are differences in the intestinal flora of mice fed in SPF environment and clean environment, the study only revolves around 18-22 g of mouse intestinal flora, because As the age of the rats increases, the weight of the rats increases, and the intestinal flora changes accordingly. In subsequent experiments, mouse feces of different ages raised in SPF and clean environment will be collected to compare intestinal flora changes in mice fed in SPF and clean environment at different ages. And then get a more detailed experimental theoretical basis.

Conclusion

The composition of the colonization flora may affect the individual's immunity. Providing the theory for the researchers to select the corresponding level of mice according to different experimental purposes in the future. Based on, and improve the reliability of the experiment. The difference between SPF-grade and clean-grade mice by comparing the differences in intestinal flora, filling in the gaps in the domestic literature comparing the differences in intestinal flora between SPF mice and clean-grade mice. As the age of the rats increases, the weight of the rats increases, and the intestinal flora changes accordingly. In subsequent experiments, mouse feces of different ages raised in SPF and clean environment will be collected to compare intestinal flora changes in mice fed in SPF and clean environment at different ages. And then get a more detailed experimental theoretical basis.

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