RESEARCH ARTICLE

Influence of *Debaryomyces hansenii* on bacterial lactase gene diversity in intestinal mucosa of mice with antibiotic-associated diarrhea

Yunshan He¹, Yuan Tang¹, Maijiao Peng¹, Guozhen Xie¹, Wenge Li²*, Zhoujin Tan¹*¹

¹ Hunan University of Chinese Medicine, Changsha, Hunan Province, China, ² Hunan Institute of Nuclear Agricultural Sciences and Space-induced Breeding, Changsha, Hunan province, China

* 641386565@qq.com (WL); tanzhjin@sohu.com (ZT)

Abstract

**Aim**

The current study aimed to investigate the effects of *Debaryomyces hansenii* on the diversity of bacterial lactase gene in the intestinal mucosa of antibiotic-associated diarrhea (AAD) mice.

**Methods**

Eighteen mice were randomly divided into three groups (6 mice per group): healthy control group, diarrhea model group and *D. hansenii* treatment group. The antibiotic-associated diarrhea model was established by intragastric administration with a mixture of cephradine and gentamicin sulfate (23.33 mL·kg⁻¹·d⁻¹) twice a day for 5 days continuously. After establishing the AAD model, the mice in the *D. hansenii* treatment group were gavaged with *D. hansenii* for three days, while other groups were gavaged with distilled water. Then, the intestinal mucosa of all three groups was collected and DNA was extracted in an aseptic environment for the following analysis.

**Results**

The difference in the richness and homogeneity of the bacterial lactase gene among all samples were insignificant, as the difference in the Chao1, ACE, Simpson and Shannon indexes among the three groups were insignificantly different (*P* > 0.05). NMDS analysis also showed that the distance of the samples among the three groups was unobvious. Furthermore, the bacterial lactase gene in the mucosa mainly originated from *Actinobacteria*, *Firmicutes* and *Proteobacteria*. Compared with the healthy control group, the abundance of lactase genes originating from *Cupriavidus*, *Lysobacter*, *Citrobacter*, *Enterobacter* and *Pseudomonas* was increased in the *D. hansenii* treatment group, while the lactase gene from *Acidovorax* and *Stenotrophomonas* decreased (*p* < 0.01 or *p* < 0.05) in the diarrhea model group and the *D. hansenii* treatment group.
Conclusion

D. hansenii was capable of improving the growth of some key lactase-producing bacteria like Deinococcus, Cupriavidus and Lysobacter for treating AAD.

Introduction

Lactase, also known as β-galactosidase, is an important enzyme for intestinal function, which can hydrolyze lactose into glucose and galactose, allowing lactose to be absorbed and utilized by human beings and animals. Because of inactivity and deficiency of lactase, lactose is fermented by intestinal bacteria to produce significant amounts of short-chain fatty acid (SCFA) and hydrogen rather than hydrolyzed, which cause diarrhea and is referred to as lactose intolerance (LI) [1,2]. Usually, intestinal lactase is produced by intestinal epithelial cells, microorganisms and obtained from exogenous lactase[3]. Nevertheless, with the widespread use of antibiotics in the clinic, increasing attention has been paid to antibiotic-associated diarrhea (AAD). Currently, it is believed that AAD, persistent diarrhea and lactase-deficiency diarrhea are all related to low lactase activity, which is probably due to a reduced number of lactases, affecting the arrangement and shedding of villi[4]. Fortunately, most diarrhea can be relieved by oral probiotics such as Lactobacillus, Bifidobacterium, Saccharomyces boulardii and by lactase supplementation [5–8].

Approximately 1000 species of bacteria inhabit the human intestinal tract, which plays an important role in metabolism, immunity and other physiological functions[9,10]. The intestinal microbes that inhabit the enteric cavity and epithelial cells of the mucosa establish a complete intestinal mucosal barrier together with intestinal epithelial cells, mucus and secrete so as to play a critical role in maintaining the homeostasis and healthy[11,12]. However, the extensive use of antibiotics pose a risk of improper application, which could cause an imbalance in the intestinal microbiota, destruction of the mucosal barrier related diseases and eventually [13]. Our previous research reported that antibiotics decrease the diversity of bacterial lactase genes in the intestinal contents, and they transforme their community structures[14].

Yeast has been widely used in medicine, food, beverage, alcohol and other industries because of its rapid propagation ability and efficient metabolism[15]. D. hansenii, isolated from the natural environment, food or intestinal tract, is one of the most important unconventional yeasts. A large number of studies verified that D. hansenii plays an important role in the fermentation of cheese and sausage and the production of fuel alcohol. It can produce critical esters in sausage manufacture, as well as the thermophilic lactase necessary to produce fuel alcohol[16–20]. In addition, it can also produce antitoxins to inhibit the growth of the harmful bacteria such as Candida [21]. Our previous studies showed that D. hansenii, which was isolated from the mouse intestine[19], was able to tolerate a high acid and high bile salt environment, and it had high viability in the artificial gastrointestinal fluid environment. In addition, the combination of 25% D. hansenii and 25% Qiweibaizhusan was used to modulate the population of total intestinal bacteria and Escherichia coli, and it can also restore the bacterial diversity in mice with dysfunctional diarrhea[22–25]. The vast majority of previous studies focused on the pathogenesis or treatment of AAD, and our previous studies reported the influence of D. hansenii on AAD based on intestinal bacterial diversity as well. The current research aimed to investigate the effect of D. hansenii on AAD based on the diversity of the bacterial lactase gene in intestinal mucosa by using high-throughput sequencing. It can provide further experimental basis for the development and utilization of D. hansenii as a new microecological preparation.
Materials and methods

Reagents and medicine

Cephradine capsules (batch number: 151101) were purchased from Suzhou Chung-Hwa Chemical & Pharmaceutical Industrial Co. Ltd., and gentamicin sulfate (batch number: 5150307) was purchased from Yichang Pharmaceutical Industrial Co. Ltd. The two antibiotics were then prepared into a mixture at a concentration of 62.5 g·L⁻¹[26]. The DNA extraction reagents (such as Proteinase K, lysozyme, Tris-saturated phenol-chloroform-isoamyl alcohol (25:24:1) and TE buffer) were purchased from Beijing Dingguo Changsheng Biotechnology Co. Ltd., and prepared in the lab (such as 10% SDS, chloroform-isoamyl alcohol (24:1), 5 mol·L⁻¹ NaCl, 0.1 mol·L⁻¹ PBS and CTAB/NaCl). *D. hansenii*, which was provided by the laboratory and shaken at 28˚C for 36 hours after being inoculated into liquid Potato Sucrose medium in a 300 mL erlenmeyer flask. The cells were then gathered by centrifugation at 2000×g for 4 minutes after washed 1~2 times repeatedly with sterile stroke-physiological saline solution. The above cells were diluted to 10¹⁰ mL⁻¹ with sterile stoke-physiological saline solution eventually after being counted by hemocytometer, and stored at 4˚C for subsequent experiments[27].

Animals and procedures

Eighteen specific pathogen-free (SPF) Kunming (KM) mice (nine male and nine female, one month old), weighting 20±2 g, were purchased from Hunan Slaccas Jingda Laboratory Animal Company with license number SCXK (Xiang) 2013–0004. All procedures involving animals were performed according to protocols approved by the Institutional Animal Care and Use Committee of Hunan University of Chinese Medicine. Mice were randomly divided into three groups (6 mice per group, half male and half female): healthy control group (tlcm), diarrhea model group (tlmm) and *D. hansenii* treatment group (tljm). Mice in the healthy control group were gavaged with 0.35 mL of sterile water twice a day for 5 days. To induce diarrhea, mice in both the diarrhea model group and the *D. hansenii* treatment group were injected intragastrically with a mixture of gentamicin sulfate and cefradine capsules (23.33 mL·kg⁻¹·d⁻¹) twice a day for 5 days by following the procedures described previously[28]. After diarrhea symptoms appeared (such as erected coat, reduced intake, watery stool and declined activity), the mice in the *D. hansenii* treatment group were treated with *D. hansenii* by intragastric administration (0.35 mL, 10¹⁰ mL⁻¹), and the other two groups were given aseptic water twice a day for 3 days. Then, the mucosa from the jejunum to ileum were scraped with a cover slip after the contents were extruded and the intestine was washed twice with sterile saline solution in a germ-free environment (each sample contains mucosa from two mice: one male and the other female, which has three samples per group), and then were frozen immediately and stored at -20˚C for further use [29].

Ethical approval was obtained from the Animal Ethics and Welfare Committee of Hunan University of Chinese Medicine.

Total DNA extraction from intestinal mucosa

Total DNA from the intestinal mucosa was extracted following to our previous reports[30,31]. A total of 2.0 g of mucosa was weighed in a germ-free environment and preprocessed with 0.1 mol·L⁻¹ phosphate buffer solution (PBS) and acetone. The cells were collected by the above method and resuspended in 4 mL of TE buffer. Cells walls were disrupted by lysozyme, and total DNA was purified and extracted by proteinase K, CTAB/NaCl, Tris saturated phenol-chloroform-isoamyl alcohol (25:24:1), chloroform-isoamyl alcohol (24:1), absolute ethyl
alcohol and sodium acetate after cells wall-broken by lysozyme. Eventually the DNA was dissolved in 50 μL of TE buffer for further analysis.

**PCR amplification of the mucosal bacterial lactase gene and high-throughput sequencing**

The universal primers for PCR amplification were designed according to the lactase gene sequences of *Lactobacillus* and *Escherichia coli* from NCBI and synthesized by Shanghai Personal Biotechnology Co., Ltd.[32]. The forward primer was 5’-TGGCAACGAATACGGSTG-3’, and the reserve primer was 5’-ACCATGAARTTSGTGGTSARCGG-3’. After PCR mixtures were prepared (25 μL) (including 5 μL of 5 x high GC buffer, 0.25 μL of Q5 high-fidelity DNA polymerase, 1 μL of 10 μmol·L⁻¹ forward primer, 0.5 μL of 10 mmol·L⁻¹ dNTP, 1 μL of 10 μmol·L⁻¹ reserve primer, 1 μL of DNA template and 11.25 μL of sterilized ddH₂O) and added to 0.5 mL PCR tubes, the amplification was carried out as follows: conditions: initial denaturation at 98˚C for 30 s, 32 cycles of denaturation at 98˚C for 15 s, annealing at 46˚C for 30 s and extension at 72˚C for 30 s, then extension at 72˚C for 5 min and holding at 4˚C. PCR products of the bacterial lactase gene were purified and then detected using high-throughput sequencing, which was performed by Shanghai Personal Biotechnology Co., Ltd.

**Gene diversity and statistical analysis**

The software available online, including QIIME (http://qiime.org/)[33] and Mothur (http://www.mothur.org/), were used to analyze the sequencing results. Alpha diversity analysis, including Chao1, ACE, Simpson and Shannon indices, was applied to identify the richness and uniformity of the intestinal mucosa bacterial lactase gene by determining the operational taxonomic units (OTUs)[34–37]. Principle component analysis (PCA)[36] was used to analyze the community difference of lactase-producing bacteria according to the distance among individuals. The source and abundance of the bacterial lactase gene at the specific taxonomic levels are presented in the figure containing species evolution and abundance information and statistics table of relative abundance statistics table. Then, our measurement data were analyzed using the SPSS 21.0 software (IBM Corp, Armonk, NY, USA), of which one-way ANOVA was applied to compare the statistical significance of differences, with p value.

**Results**

**Sequence statistics and OTU analysis**

The diversity and richness of lactase gene can be well studied by measuring and analyzing OTUs (operational taxonomic unit). Fig 1 shows that the numbers of OTUs of the healthy control group, diarrhea model group and *D. hansenii* treatment group was 298, 435 and 326, respectively. The results showed that 45 OTUs were unique to the healthy control group, 202 OTUs were unique to the diarrhea model group and 57 OTUs were unique to the *D. hansenii* treatment group. These results suggested that antibiotic modeling increased the number of OTUs of the lactase gene from the intestinal mucosal bacteria in mice, while the number of OTUs of antibiotic models can be returned to normal following treatment with *D. hansenii*.

**Alpha diversity of the bacterial lactase gene from the intestinal mucosa of AAD mice treated with D. hansenii**

By drawing rarefaction curves, the diversity of each sample could be measured to some extent. Fig 2 shows that each curve tended to flatten with the increase in the number of measured
Fig 1. OTUs of the bacterial lactase genes from intestinal mucosa. tlc, healthy control group; tlmm, diarrhea model group; tljm. D. hansenii treatment group.

https://doi.org/10.1371/journal.pone.0225802.g001

Fig 2. Rarefaction curve of the bacterial lactase gene from the intestinal mucosa in each sample. Note: The abscissa represents the number of lactase gene sequences extracted randomly; the ordinate represents the number of observed OTUs of the bacterial lactase gene. The data indicate that the sequencing tended to be saturated and that increasing the amount of data would have no significant effect on obtaining new OTUs when the curve tended to be flat; tlc1-3, tlmm1-3, tljm1-3 are healthy control group samples 1–3, diarrhea model group 1–3 and D. hansenii treatment samples 1–3, respectively.

https://doi.org/10.1371/journal.pone.0225802.g002
sequences. This result suggested that the sequencing results were more than enough to reflect the current sample containing the intestinal mucosa lactase gene diversity.

The rank abundance curve and alpha indices can be used to determine the richness and uniformity of the bacterial lactase gene from intestinal mucosa to evaluate the therapeutic efficacy of *Debaryomyces hansenii* treatment on AAD. As shown in Fig 3, there was no significant difference in the length on abscissa and the gentleness, which indicated that *D. hansenii* treatment had no significant impact on the richness and uniformity of the bacterial lactase gene among the healthy control group and diarrhea model group. From the alpha indices, we determined that there was no significant difference in the Chao1, ACE, Simpson and Shannon indices among the three groups (Table 1).

![Fig 3. Rank abundance curve of each sample. Note: The abscissa represents the ordinal of the OTU, and the ordinate represents the abundance of the OTU. The larger the curve span, the richer the composition of the species was. The flatter the curve, the higher the evenness the species composition was. tlcm 1–3 represented the healthy control group samples 1–3, tlmm1-3 represented the diarrhea model group samples 1–3, tljm 1–3 represented the *D. hansenii* treatment samples 1–3.](https://doi.org/10.1371/journal.pone.0225802.g003)
Beta diversity of bacterial lactase genes in the intestinal mucosa of AAD diarrhea mice treated with D. hansenii

PCA (principal component analysis) analysis can extract the most important differences between samples from the original data. As shown in Fig 4, that the three samples in the healthy control group and the D. hansenii treatment group were relatively close to each other, while the distance between the groups was relatively far, which can be clearly distinguished. The distribution of the three samples in the diarrhea model group was very scattered and not completely separated from the healthy control group and the D. hansenii treatment group. This finding suggests that antibiotics altered the structure of bacterial lactase-producing genes in the intestinal mucosa, and the percentages attributed to the variations in PC1 and PC2 were 97.32% and 2.66%, respectively.

The differences in the community were measured by distance comparison among individuals through NMDS analysis. The distribution of samples in the healthy control group was more concentrated than that in the other groups, and the distance between the diarrhea model group and the healthy control group was approximately the same distance as that between the D. hansenii treatment group and the healthy control group (Fig 5). The results indicated that D. hansenii treatment had no significant effect on the recovery of the community structure of the bacterial lactase gene from the intestinal mucosa.

Abundance and source of the bacterial lactase gene from the intestinal mucosa of AAD mice treated with D. hansenii

As shown in Fig 6(A), at the genus level, the number of known lactase-producing bacteria detected in the healthy control group, diarrhea model group and D. hansenii treatment group were 16, 12, and 16, respectively. Mesorhizobium was only found in the healthy control group, and Plasmodium was only detected in the D. hansenii treatment group, Sphingomonas and Bordetella were not detected in the diarrhea model group. In addition, a number of other bacterial lactase genes (other, including some genera with low abundance or unclassified) and some new lactase-producing bacterial genera (no Blast hits) were detected. Compared with the healthy control group, the abundance of Cupriavidus was increased significantly after treatment with D. hansenii. The lactase genes originating from Acidovorax and Stenotrophomonas were lower in the diarrhea model group and D. hansenii treatment group than the healthy control group. Conversely, the lactase gene originated from Citrobacter, Enterobacter and Pseudomonas in the diarrhea model group and D. hansenii treatment group was lower than that in the healthy control group. However, the lactase genes of five genera showed no significant difference between the diarrhea model group and the D. hansenii treatment group. Through further analysis with LEfSe, shown in Fig 6(B), it was found that Sphingomonadaceae, Sphingomonas and Sphingomonadales were the key colony members in the D. hansenii treatment group, while no key colony members were found in the healthy control group or diarrhea model group.
The species evolution and abundance information were used for source and abundance analysis of the bacterial lactase gene at different classification levels (Fig 7). The evolution tree showed that the bacterial lactase genes in intestinal mucosa mainly originated from *Actinobacteria, Firmicutes* and *Proteobacteria*. The abundance of the lactase gene in *Actinobacteria* from the *D. hansenii* treatment group was the highest, followed by the healthy control group and the diarrhea model group. Lactase genes from *Aeromonas, Curvibacter* and *Deinococcus* were only detected in the *D. hansenii* treatment group, while those from *Enterobacteriaceae* and

 estal artifact of the bacterial lactase gene from intestinal mucosa. Each point represents a sample, and different color points belong to different groups. The closer the two points are, the smaller the difference of the lactase-producing bacterial community structure between the two samples is. *tlcm, tlmm* and *tljm* were the healthy control group, diarrhea model group and *D. hansenii* treatment group respectively.

https://doi.org/10.1371/journal.pone.0225802.g004

Fig 4. PCA analysis of the bacterial lactase gene from intestinal mucosa. Each point represents a sample, and different color points belong to different groups. The closer the two points are, the smaller the difference of the lactase-producing bacterial community structure between the two samples is. *tlcm, tlmm* and *tljm* were the healthy control group, diarrhea model group and *D. hansenii* treatment group respectively.

https://doi.org/10.1371/journal.pone.0225802.g004
Clostridium were only detected in the diarrhea model group. Compared with the other two groups, the abundance of lactase genes originating from Enterococcus, Lysobacter and Salmonella was the highest in the D. hansenii treatment group.

**Discussion**

The gastrointestinal mucosa is the largest interface where interactions mainly occur between symbiotic bacteria and the host. A dynamic and diverse bacterial community inhabits the mucosa, which is an important part of the intestinal mucosa barrier[38]. As an important metabolic organ, intestinal flora participate in metabolism of human and animal metabolism with various microbial enzymes under gene regulation [39]. Metabolism will be affected as soon as
the enzyme activity is absent or reduced. Furthermore, except for dysbacteriosis, the mechanisms of AAD include intestinal epithelial cilia atrophy, intestinal mucosal damage and a decrease in cellular enzyme activity as well [40]. Fortunately, AAD can be prevented through the rational use of medicines, and microecologics has become one of the common methods for treating AAD. Our previous research has proven that 25% ultramicro Qiweibaizhusan combined with 25% yeast has a relatively better therapeutic effect on AAD [24], which proves that yeast has a certain therapeutic effect on AAD. Based on the chemical nature of proteins, the activities of most enzymes are regulated by coding genes and other physicochemical factors. At the same time, with the development of modern biological technology and continuous research on intestinal microbiology, exploring the interactions between intestinal flora and intestinal diseases from the perspective of bacterial functional enzyme genes has become a new approach to conduct intestinal microbial research. Therefore, the current study was conducted to investigate the effect of \textit{D. hansenii} on the bacterial lactase gene diversity from the intestinal mucosa of AAD mice. The results can be analyzed from three aspects: the variety of lactase-producing bacteria, the abundance of lactase-producing bacteria and the effect of \textit{D. hansenii} on the mucosal barrier.

Intestinal bacteria mainly consist of \textit{Proteobacteria}, \textit{Mycobacteria}, \textit{Actinomycetes}, \textit{Bacteroidetes}, \textit{Mycobacteria}, \textit{Cyanobacteria} and \textit{Fusobacteria}, especially \textit{Bacteroidetes} and \textit{Firmicutes}, which account for approximately 90% of the total [41]. Our results showed that the lactase gene in intestinal mucosa originated from \textit{Actinobacteria}, \textit{Firmicutes} and \textit{Proteobacteria}, among which \textit{Proteobacteria} accounted for more than 90%. There are differences in lactase activities produced by different bacteria in the intestine; therefore, bacterial lactase gene diversity could be reflected through differences in the population and variety of lactase-producing bacteria. NMDS analysis showed that antibiotics caused a large difference in the community structure of lactase-producing bacteria, whereas the recovery effect of \textit{D. hansenii} treatment on the community structure was not obvious. The statistical analysis results of lactase-producing bacteria at different classification levels indicated that the \textit{D. hansenii} had no significant effect on the population of lactase-producing bacteria in the intestinal mucosa at all classification levels except for the order level.

From the high-throughput sequencing results, the relative abundance was used to reflect the quantity difference in lactase-producing bacteria. Of the known genera, the abundance of the lactase gene originating from \textit{Cupriavidus} in the healthy control group was significantly lower than that in the diarrhea model group, while in the \textit{D. hansenii} treatment group, the relative abundance of \textit{Cupriavidus} showed a significant increase compared with the diarrhea model group.
Bacteria lactase gene diversity treated by *Debaryomyces hansenii*
higher than that in the D. hansenii treatment group. The study of Ueatrongchit showed that Cupriavidus was the genus that produced various microbial enzymes and determined that Cupriavidus necator produced L-threonine 3-dehydrogenase that catabolizes L-threonine[42]. Lysobacter, which is a genus of bacteria with fast growth, strong resistance and no pathogenicity[43], not only can secrete a variety of extracellular enzymes such as chitinase and β-1,3-glucanase, but also have significant antagonism against various pathogenic bacteria [44]. Our results showed that the lactase gene in Deinococcus was detected only in the D. hansenii treatment group and that the lactase gene in Lysobacter was highly abundant in the D. hansenii treatment group, which suggested that D. hansenii increased the quantity of potentially valuable lactase-producing bacteria in order to treat AAD.

The extended-spectrum cephalosporin resistant (ESC R) Enterobacteriaceae pose a serious infection control challenge for public health [45]. The appearance of the ESC R phenotype is mainly promoted through plasmid-mediated lateral extended-spectrum β-lactamases (ESBLs) and AmpC gene transfer within Enterobacteriaceae [46]. The current results showed that the lactase gene in Enterobacteriaceae was detected only in the diarrhea model group. The Lactase gene in Enterobacteriaceae in the diarrhea model group was higher than that in the other groups. Moreover, the abundance of the lactase gene in Enterococcus was the highest in the D. hansenii treatment group, followed by the healthy control group and the diarrhea model group. Enterococcus probiotics can increase the expression of small intestinal mucosa tight junction proteins and activity of toll-like receptors 2, 4 and 9 in small intestinal mucosa, inducing an immune response in piglets [47]. All of these studies suggested that antibiotics damaged the mucosa barrier by increasing the population of bacteria with antibiotic resistance and causing dysbacteriosis. And the mucosal barrier could be restored by D. hansenii.

After treatment with D. hansenii, only a few bacterial lactase genes was recovered or increased. The recovery of lactase gene diversity in the intestinal mucosa of AAD mice was not significant. Perhaps our current results can be explained by a recent report, which determined that antibiotics had a long-term impact on intestinal microorganisms[36]. The lactase-producing bacteria in the intestinal mucosa of the diarrhea model could not return to normal levels after a few days, even following treatment with D. hansenii. Moreover, the activity of digestive enzymes present in the brush border of villous epithelial cells in the small intestine mucosa has the function of repairing intestinal mucosa, which is closely related to the structural integrity of the mucosa and probiotics [48,49]. In conclusion, our results suggested that antibiotics disrupted the intestinal mucosa flora in mice, and treatment with D. hansenii may be effective to treat diarrhea by promoting the growth of a few key lactase-producing bacteria or some beneficial bacteria to repair the intestinal mucosa structure.

Supporting information
S1 Dataset. The raw data of healthy control group (tlcm). (ZIP)
S2 Dataset. The raw data of diarrhea model group (tlmm). (ZIP)
S3 Dataset. The raw data of D. hansenii treatment group (tljm). (ZIP)
Author Contributions

Conceptualization: Zhoujin Tan.
Data curation: Yuan Tang.
Funding acquisition: Wenge Li.
Project administration: Zhoujin Tan.
Software: Maijiao Peng, Guozhen Xie.
Writing – original draft: Yunshan He.
Writing – review & editing: Zhoujin Tan.

References

1. Hammer HF, Hammer J. Diarrhea caused by carbohydrate malabsorption. Gastroenterology Clinics of North America. 2012; 41: 611–627. https://doi.org/10.1016/j.gtc.2012.06.003 PMID: 22917167
2. Zhang YY, Zhou LL, Yang SF. The basic research of primary lactase deficiency. International Journal of Pediatrics. 2014; 41: 302–304. https://doi.org/10.3760/cma.j.issn.1673-4408.2014.03.023
3. Tan ZJ, Guo KK, Zeng A, Guo ZH, Wang H. Advances in research of intestinal lactase. World Chinese Journal of Digestology. 2013; 21: 2897–2899. http://dx.doi.org/10.1166/wjcd.v21.i28.2897
4. Li XQ, Zhou F, Li YC, Guo YQ, Wang XH. Clinical significance of 13C exhalation test on lactose intolerance secondary to rotavirus diarrhea. Journal of Chinese Practical Diagnosis and Therapy. 2011; 25: 399–340.
5. Alexandre V, Even PC, Larue-Achagiotis C, Blouin JM, Blachier F, Benamouzig R, et al. Lactose malabsorption and colonic fermentations alter host metabolism in rats. British Journal of Nutrition. 2013; 110: 625–631. https://doi.org/10.1017/S0007114612000557 PMID: 23321004
6. Ojetti V, Gigante G, Gabrielli M, Ainora ME, Mannocci A, Lauritano EC, et al. The effect of oral supplementation with lactobacillus reuteri or tilactase in lactose intolerant patients: randomized trial. European Review for Medical & Pharmacological Sciences. 2010; 14: 163–170. https://doi.org/10.1016/j.eumeuro.2009.11.008 PMID: 20391953
7. Wang W, Yue Y, Hao LL, Yao P, Wei JJ. Clinical effect of the treatment of antibiotic-associated diarrhea. Chinese Journal of Nosocomiology. 2016; 26: 1258–1260. https://doi.org/10.11816/cn.ni.2015-152548
8. Zhu HY. Analysis of the effect of brassella on preventing antibiotic related diarrhea in infants and young children. World Latest Medicine Information. 2017; 17: 59. https://doi.org/10.19613/j.cnki.1671-3141.2017.09.050
9. Zhang X, Guo J. Research progress on association of probiotics with intestinal mucosal immunity. Animal Husbandry and Feed Science. 2017; 38: 58–64. https://doi.org/10.16003/j.cnki.issn1672-5190.2017.11.015
10. Zhai QX, Tian FW, Wang G, Chen W. Progress in research on the role of intestinal microbiota in human health. Food Science. 2013; 34: 337–341. https://doi.org/10.7506/spkx1002-6630-201315069
11. Ding K, Yu ZH, Wang TQ. Progress on gut mucosal immunization. Progress in Veterinary Medicine. 2008; 28: 67–71. https://doi.org/10.3969/j.issn.1007-5038.2007.10.016
12. Li Q, Liu LX. Research progress on the relationship between intestinal mucosal barrier and intestinal endotoxemia. Chinese Journal of Digestion and Medical Imageology[Electronic Edition]. 2012; 2: 291–294. https://doi.org/10.7666/d.y418243
13. Zhang L, Qiao M, Liang QH, Chen XQ, Huang LH, Li CX. Protective effect of lactobacillus rhamnosus GG on the damage of gut barrier function in SD rats with antibiotics induced diarrhea. Chinese Journal of Microecology. 2011; 23: 38–43, 48. https://doi.org/10.13381/j.cnki.cnki.cjm.2011.01.029
14. Long CX, He L, Guo YF, Liu YW, Xiao NQ, Tan ZJ. Diversity of bacterial lactase genes in intestinal contents of mice with antibiotics-induced diarrhea. World Journal of Gastroenterology. 2017; 23: 7584–7593. https://doi.org/10.3748/wjg.v23.i42.7584 PMID: 29204058
15. Yang QX, Wang Z. Distribution and function of yeast in eco-environments. Environmental Science & Technology. 2009; 32: 86–91. https://doi.org/10.3969/j.issn.1003-6504.2009.04.021
16. Gadano M, Almeida JM, Sampaio JP. Assessment of yeast diversity in a marine environment in the south of Portugal by microsatellite-primed PCR. Antonie Van Leeuwenhoek. 2003; 84: 217–227. https://doi.org/10.1023/a:1026036213195 PMID: 14574117
17. Petersen KM, Westall S, Jespersen L. Microbial succession of *Debaryomyces hansenii* strains during the production of danish surfaced-ripened cheeses. Journal of Dairy Science. 2002; 85: 478–486. https://doi.org/10.3168/jds.S0022-0302(02)74098-8 PMID: 11949849

18. Cano-García L, Belloch C, Flores M. Impact of *Debaryomyces hansenii* strains inoculation on the quality of slow dry-cured fermented sausages. Meat Science. 2014; 96: 1469–1477. https://doi.org/10.1016/j.meatsci.2013.12.011 PMID: 24423452

19. Xiao XY, Liu YJ, Deng YL, Guo KK, Tan ZJ. Isolation and identification of a yeast strain from intestine of mice. Journal of Hunan Agricultural University. 2016; 42: 419–423. https://doi.org/10.13331/j.cnki.jhau.2016.04.014

20. Prista C, Michán C, Miranda IM. The halotolerant *Debaryomyces hansenii*, the cinderella of non-conventional yeasts. Yeast. 2016; 33: 523–533. https://doi.org/10.1002/yea.3177 PMID: 27279567

21. Banjara N, Nickerson KW, Suhr MJ, Nickerson, Hallen-Adams HE. Killer toxin from several food-derived *Debaryomyces hansenii* strains effective against pathogenic candida yeasts. International Journal of Food Microbiology. 2016; 222: 23–29. https://doi.org/10.1016/j.ijfoodmicro.2016.01.016 PMID: 26828815

22. Xiao XY, Liu YJ, Deng YL, Tan ZJ. Tolerance experiment of *Debaryomyces hansenii* in the environment of gastric acid or intestinal bile salts. Hunan Agricultural Science. 2016; 6: 6–8. https://doi.org/10.16498/j.cnki.hnnykx.2016.06.003

23. Guo KK, Tan ZJ, Xie MZ, Yu Y, Wang XH. The synergetic effect of ultra-micro powder qiweibaizhuasan combined with yeast on dysbacteriotic diarrhea mice. Chinese Journal of Applied and Environmental Biology. 2015; 21: 61–67. https://doi.org/10.3724/SP.J.1145.2013.10002

24. Long CX, He L, Guo KK, Tan ZJ, Yin KK. Effect of qiweibaizhuasan powder combined with yeast on the intestinal bacteria diversity in dysbacteriotic diarrhea mice. Chinese Journal of Integrated Traditional and Western Medicine. 2018; 38: 66–70. https://doi.org/10.7661/j.cjim.20171207.299

25. Liu YJ, Xiao XY, Deng YL, Guo KK, She Y, Tan ZJ. Effects of qiweibaizhuasan combined with yeast on intestinal lactobacillus diversity in dysbacteriotic diarrhea mice. Space Medicine and Medical Engineering. 2016; 29: 175–180. https://doi.org/10.16289/j.cnki.1002-0837.2016.03.004

26. Zeng A, Zhang HL, Tan ZJ, Cai Y, Cai GX, Zhou SN. The construction of mice diarrhea model due to dysbacteriosis and curative effect of ultra-micro qiweibaizhuasan. Microbiology. 2012; 39: 1341–1348. https://doi.org/10.13344/j.microbiol.china.2012.09.012

27. Jin L, Yang XH, Ren JL, Li JL, Guo XY, Cao P, et al. Effect of dietary compound probiotics on disaccharidase in small intestine mucosa of layer breeders. China Poultry. 2012; 34:14–17. https://doi.org/10.16372/issn.1004-6364.2012.12.007

28. Wu H, Zhou SN, Guo C, Tan ZJ, Cai GX, Zeng A, et al. A metagenome DNA extracting method of intestinal flora in mice for molecular diversity analysis based on PCR technology. Chinese Journal of Microecology. 2012; 24: 648–651. https://doi.org/10.13381/j.cnki.cjme.2012.07.003

29. He L, Long CX, Liu YJ, Guo YF, Xiao NQ, Tan ZJ. Effects of *Debaryomyces hansenii* treatment on intestinal microorganisms in mice with antibiotics-induced diarrhea. 3 Biotech. 2017; 7: 347. https://doi.org/10.1007/s13205-017-0953-9 PMID: 28955644

30. Long CX, He L, Liu YJ, Hui HY, Tan ZJ, Li DD. Universal primer for analysis of the diversity of intestinal bacterial lactase gene. Chinese Journal of Applied and Environmental Biology. 2017; 23: 758–763. https://doi.org/10.3724/SP.J.1145.2016.10008

31. Caporaso JG, Kuczynski J, Stombaugh J, Bittinger K, Bushman FD, Costello EK, et al. QIIME allows analysis of high-throughput community sequencing data. Nature Methods. 2010; 7: 335–336. https://doi.org/10.1038/nmeth.f.303 PMID: 20383131

32. Shannon CE. The mathematical theory of communication. 1963. Bell Labs Technical Journal. 1997; 3: 31–32. https://doi.org/10.1063/1.3067010

33. Mahafef WF, Klopeper JW. Temporal changes in the bacterial communities of soil, rhizosphere, and endorhiza associated with field-grown cucumber (*Cucumis sativus* L). Microbial Ecology. 1997; 34: 210–223. https://doi.org/10.1007/s002489900050 PMID: 9337416

34. Pitta DW, Pinchak E, Dowd SE, Osterstock J, Gontcharova V, Youn E, et al. Rumen bacterial diversity dynamics associated with changing from bermudagrass hay to grazed winter wheat diets. Microbial Ecology. 2010; 59: 511–512. https://doi.org/10.1007/s00248-009-9609-6 PMID: 20037795

35. Pitta DW, Parmar N, Patel AK, Indugu N, Kumar S, Prajapathi KB, et al. Bacterial diversity dynamics associated with different diets and different primer pairs in the rumen of Kankrej cattle. PloS One 2014; 9: e111710. https://doi.org/10.1371/journal.pone.0111710 PMID: 25365522

36. Ramette A. Multivariate analyses in microbial ecology. Fems Microbiology Ecology. 2007; 62: 142–160. https://doi.org/10.1111/j.1574-6941.2007.00375.x PMID: 17892477
37. O’Hara AM, Shanahan F. Gut microbiota: mining for therapeutic potential. Clinical Gastroenterology and Hepatology. 2007; 5: 274–284. https://doi.org/10.1016/j.cgh.2006.12.009 PMID: 17368226

38. Li K, Nie YQ. Research progress on the correlation between intestinal microbial metabolism and drug therapy. Modern Digestion & Intervention. 2017; 22: 756–759. https://doi.org/10.3969/j.issn.1672-2159.2017.05.052

39. Zhang LX, Zhao CP. Antibiotic-associated diarrhea and rational use of drugs. Chinese Community Doctors. 2012; 14: 36–37. https://doi.org/10.3969/j.issn.1007-614x.2012.07.032

40. Ren J, Luo YY. Intestinal microorganism and intestinal diseases. Chinese Journal of Surgery of Integrated Traditional and Western Medicine. 2015; 21: 632–635. https://doi.org/10.3969/j.issn.1007-6948.2015.06.029

41. Ueatrongchit T, Asano Y. Highly selective L-threonine 3-dehydrogenase from Cupriavidus necator and its use in determination of L-threonine. Analytical Biochemistry. 2011; 41: 44–56. https://doi.org/10.1016/j.ab.2010.11.003

42. Liu MJ, Yang S, Cheng KY, Wang LY, Hua YJ. Research progress of deinococcus and Its application. Acta Agriculturae Sinica. 2017; 31: 1723–1729. https://doi.org/10.11869/j.issn.1007-6948.2017.09.029

43. Wang N, Wu KY, Cui LJ, Zhang HW. Advance in bacteria identification and biocontrol mechanism of Lysobacter spp. Journal of Northwest A & F University (Natural Science Edition). 2015; 43: 174–182, 191. https://doi.org/10.13207/j.cnki.jnwafu.2015.05.010

44. Liakopoulos A, van den Bunt G, Geurts Y, Bootsmaj MCJ, Tolemen M, Cecarelli D, et al. High prevalence of intra-familial co-colonization by extended-spectrum cephalosporin-resistant enterobacteriaceae in preschool children and their parents in Dutch households. Frontiers in Microbiology. 2018; 9: 293. https://doi.org/10.3389/fmicb.2018.00293 PMID: 29515562

45. Ghanavati R, Emamieini M, Kalantary-Neyestanaki D, Maraji AS, Dalvand M, Beigverdi R, et al. Clonal relation and antimicrobial resistance pattern of extended-spectrum β-lactamase and AmpC β-lactamase-producing Enterobacter spp. isolated from different clinical samples in Tehran, Iran. Revista da Sociedade Brasileira de Medicina Tropical. 2018; 51, 88–93. https://doi.org/10.1590/0037-8682-0227-2017 PMID: 29513851

46. Guo QP. Study on the regulation of small intestinal mucosa binding proteins, cytokines, and related toll-like receptors by probiotics Enterococcus aerus and pyrethrocystes. Guangxi University. 2017.

47. Palleja A, Mikkelsen KH, Forslund SK, Kashani A, Allin KH, Nielsen T, et al. Recovery of gut microbiota of healthy adults following antibiotic exposure. Nature Microbiology. 2018; 3: 1255–1265. https://doi.org/10.1038/s41564-018-0257-9 PMID: 30349083

48. Liu LY, Xie P. Effects of microcystostoxin-LR on the digestive enzymes activity of intestinal tract in Balc/c mice. Acta Hydrobiologica Sinica. 2014; 38: 333–339. https://doi.org/10.10541/2014.75

49. Wang K, Ma ZM, Chang C, Wang XD, Wu JE. Effect of Saccharomyces boulardii on intestinal enzyme activity of meat ducks with intestinal dysbacteriosis. Hubei Agricultural Science. 2015; 54: 2690–2694. https://doi.org/10.14088/j.cnki.issn0439-8114.2015.11.035.