Lactobacillus acidophilus CICC 6075 alleviates obesity in mice through modulation of gut microbiota dysbiosis

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Research Article

Keywords: probiotic, Lactobacillus acidophilus CICC 6075, obesity, lipid metabolism, gut microbiota

Posted Date: October 28th, 2022

DOI: https://doi.org/10.21203/rs.3.rs-2195035/v1

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Abstract

Background

Obesity associated with lipid metabolism dysbiosis and intestinal dysbiosis is considered as a major healthcare problem worldwide. In the meanwhile, different probiotics have demonstrated beneficial effects on this condition, thus increasing the interest in the development of probiotic treatments. In this context, the aim of this study is to investigate the anti-obesity effects of potential probiotic Lactobacillus acidophilus CICC 6075.

Methods

C57BL/6J mice on normal chow diet or high-fat feed were treated Lactobacillus acidophilus CICC 6075 by daily oral gavage for 12 weeks. Body weight, adipose tissue weight and HE sections of liver tissue, adipose tissue, and intestine were examined for each group, along with fecal 16S rRNA gene sequences were analyzed.

Results

Overall, L. acidophilus reduced body weight and fat accumulation in obese mice fed with a high-fat diet (HFD). Besides, Sequencing results showed that HFD diet reduced α-diversity and β-diversity, and the relative abundance of Lactobacillus, norank_f_Muribaculaceae was reduced, and significantly increased the relative abundance of ilebacterium. L. acidophilus reversed HFD-induced gut dysbiosis, and decreased Firmicutes-to-Bacteroidetes ratios. In addition, the results of bacterial functional potential prediction using PICRUSt showed that L. acidophilus treatment improved the gut microbiota functions involving metabolism, immune response, and pathopoiesia.

Conclusions

Lactobacillus acidophilus CICC 6075 ameliorated obesity through its alleviation of lipid metabolism dysbiosis and gut dysbiosis. It could be a good candidate for probiotic of ameliorating obesity and associated diseases such as hyperlipidemia, nonalcoholic fatty liver diseases, and insulin resistance.

1 Introduction

Obesity as an important health problem has attracted widespread attention, and the latest statistics show that the number of overweight/obese people worldwide is about 30% of the world's population [1–3]. Obesity is accompanied by changes in body shape during clinical practice and, at the same time, is closely related to disorders of lipid metabolism, chronic inflammation and oxidative stress, and is a risk factor for a range of diseases including diabetes, cancer and cardiovascular disease [4–6]. Drugs
clinically used to combat obesity, such as orlistat, lorcaserin, bupropion and liraglutide, show limited effectiveness and significant side effects [7–11]. Effective and safe treatment strategies are yet to be found.

Gut microbes influence nutrient acquisition, energy regulation and fat storage [12, 13]. There is growing evidence that dysbiosis of the intestinal flora may be a risk factor for obesity [14–16]. Probiotics secrete beneficial substances including short-chain fatty acids and vitamins, as well as products with anti-inflammatory and antioxidant capabilities that regulate host energy metabolic processes and maintain the body's metabolism and energy balance [17–19].

*Lactobacillus* spp. as one of the most commonly used probiotics, orally administered *Lactobacillus acidophilus* has anti-inflammatory, anti-oxidative stress and regulates intestinal flora homeostasis, thus providing health benefits [20, 21]. In terms of improving lipid metabolism, *Lactobacillus acidophilus* NS1 (LNS1) can prevent diet-induced obesity and related metabolic disorders by improving lipid metabolism and insulin sensitivity through the AMPK→SREBP-1c/PPARα signaling pathway [22]. Oral administration of *Lactobacillus acidophilus* NS1 to HFD-fed mice increased Srebp2 and Ldlr expression in the liver or inhibited PEPCK expression in the liver by modulating HNF4α transcriptional activity [23, 24], resulting in lower plasma cholesterol levels. In terms of anti-inflammation, *Lactobacillus acidophilus* KCTC3925 inhibited the increase in body weight and epididymal fat pad weight, as well as inflammatory responses in the liver and spleen of HFD-fed mice. Porcine-derived *Lactobacillus acidophilus* improves obesity through anti-inflammatory properties, alleviation of endothelial dysfunction and intestinal function. Thus, *Lactobacillus acidophilus* possesses the ability to improve lipid metabolism as well as anti-inflammatory properties, however, the effect of *Lactobacillus acidophilus* CICC 6075 as one of them on obesity has not been reported. Thus, the aim of this study was to evaluate the role of *Lactobacillus acidophilus* CICC 6075 in a high-fat diet-induced obesity mouse model as a way to establish its anti-obesity and its effect on intestinal flora.

### 2 Materials And Methods

#### 2.1 Study design

Eight weeks old male C57BL/6J mice (n=12) were fed either a normal chow diet or high fat diet ad libitum for twelve weeks (Figure1). Mice were divided into four groups (n=3), namely normal chow diet (NCD)-fed control, HFD-fed control, HFD-fed *Lactobacillus acidophilus* treated (1.9×10⁷ CFU or 1.9×10⁹ CFU) groups. C57BL/6J mice were purchased from Nanjing Model Animal Research Institute. All mice were retained in a pathogen-free facility of Xuzhou Medical University. Animal experimental procedures were performed in accordance with the ethical guidelines of Xuzhou Medical University (Xuzhou, China).

#### 2.2 Culture and administration of *Lactobacillus acidophilus* CICC 6075
Lactobacillus acidophilus CICC 6075 (L. acidophilus) was purchased from China Industrial Microbial Strain Collection Management Center (CICC). Then, it was grown in De Man, Rogosa and Sharpe (MRS) media for a period of 48 h at 37 °C under anaerobic conditions. The concentration of bacteria was calculated by measuring the absorbance at the wavelength of 600 nm. 1.9×10⁷ CFU or 1.9×10⁹ CFU of Lactobacillus acidophilus in 200 µL of phosphate-buffered saline (PBS) was orally gavaged daily to C57BL/6J mice with HFD. The control C57BL/6J mice (NCD and HFD) were orally gavaged daily with 200 µL PBS.

2.3 Weight monitoring

Measured the body mass of mice every Saturday at 10:00 am until the end of the experiment. The body mass of the HFD group exceeded that of the NCD group by 20% measured in the 12th week, indicating successful modeling.

2.4 Tissue extraction and HE staining

Executed mice at the end of the experiment. Took and weighed their livers, fats and intestinal tissues, and observed the appearance at the meantime. The liver and colon tissues were fixed for 24 h, embedded in paraffin, sliced into 3 µm sections, and stained with hematoxylin for 10 min to stain the nuclei followed by washes in tap water. Transfer the slides to a staining jar with Eosin solution for 3 sec followed by washes in tap water. Dehydration in 80% ethanol for 20 sec, 90% ethanol for 20 sec, 95% ethanol for 20 sec, 100% ethanol for 5 min and xylene for 5 min. Take out slides from xylene and place the slides in a fume hood till the slides are dry. Hematoxylin and Eosin-stained images were captured with Olympus inverted microscope with digital microscope camera.

2.5 DNA extraction and 16S rRNA gene sequencing

Faecal samples were collected at the end of the experiment. Each faecal sample was snap frozen in liquid nitrogen within minutes of donation and then kept at ~80°C. Bacterial genomic DNA was extracted using the QIAamp Fast DNA Stool Mini Kit (Qiagen, California, USA). The 16S rRNA V3-V4 region was amplified by PCR and then sequenced using MiSeq platform (Illumina, San Diego, California, USA). The sequences of the primers were as follows: 338F(5’-ACTCCTACGGGAGGCAGCAG-3’), 806R(5’-GGACTACHVGGGTWTCTAAT-3’).

2.6 Bioinformatic analysis

The 16S rRNA sequencing data were analyzed using Quantitative Insights into Microbial Ecology (QIIME2 V.2021.11) [25]. DADA2 software, wrapped in QIIME2, was used to filter the sequencing reads and construct feature table. We then used search plugin to cluster sequences into operational taxonomic units
(OTUs) at 97% identity and the taxonomy was assigned against the Silva (SSU138) 16S rRNA database (V.13.8). To minimise the effect of spurious sequences, we removed OTUs with a number of sequences <0.005% of the total number of sequences. After filtering, an average of 32708 reads per sample was obtained (min: 47213; max: 56203). The sequences were then aligned using MAFFT and a phylogenetic tree was generated with FastTree plugin. Alpha and beta diversity analyses were performed using q2-diversity at a rarefied sampling depth of 47213. The metagenomes of gut microbiome were imputed from 16S rRNA sequences with PICRUSt (Phylogenetic Investigation of Communities by Reconstruction of Unobserved States) as described previously [26]. Alpha diversity indices were compared by performing the Kruskal–Wallis test followed by pairwise Mann–Whitney U comparison. Beta diversity analysis was conducted to investigate the structural variation of microbial communities across samples using Unweighted UniFrac distance metrics and visualized via principal coordinate analysis (PCoA). Then, resulting pvalues were corrected with the Bonferroni method. Besides, differences in the Unweighted UniFrac distances among groups were determined by analyzing similarities (ANOSIM). To compare the relative levels of abundant taxa at the phylum, family, and genus levels among the groups, the one-way analysis of variance (ANOVA) and post-hoc least significance tests were carried out by GraphPad Prism 6. Other than that, LEfSe (linear discriminant analysis (LDA) effect size) was performed to identify biomarkers for both abundant taxa and functional pathways by calculating the LDA score (more than 3.5) across groups, while the heat map was plotted using R package 4.2.1. Apart from that, Phylogenetic Investigation of Communities by Reconstruction of Unobserved States (PICRUSt) based on OTUs was employed to predict the abundances of functional categories using Kyoto Encyclopedia of Genes and Genomes (KEGG) orthologs (KO), whilst the data are expressed as the mean ± standard error of the mean (SEM), and P < 0.05 was set as the threshold for significance.

2.7 Statistical analysis

In this experiment, all data except those obtained by 16S rRNA gene sequencing were analyzed by GraphPad Prism 8 software for data analysis, and the mean (±SEM) was used. Differences between two groups were assessed by unpaired two-tailed Student's t-test, and the comparison between more than two groups was performed by a one-way analysis of variance (ANOVA), where P<0.05 indicated that the difference was statistically significant.

3 Results

3.1 L. acidophilus administration ameliorated HFD-induced adiposity and fatty liver in mice

To investigate whether L. acidophilus CICC 6075 can ameliorate HFD-induced metabolic disorders, such as obesity and fatty liver. Consequently, 8-week-old male mice were fed a normal diet (NCD) or a HFD
(HFD mice) with different concentrations of *L. acidophilus* CICC 6075 oral supplementation for 12 weeks. Low concentrations of *L. acidophilus* CICC 6075 administrations to mice fed a HFD (HFD-L mice) inhibited body weight gain (~40% decrease), with a reduction in liver weight (20%), total white adipose tissue (45%), total white fat weight/body weight ratio and liver weight/body weight ratio, compared to mice fed a HFD (Figure2A-E). Mice given high concentrations of *L. acidophilus* CICC 6075 with a HFD (HFD-H mice) also showed a similar tendency with HFD-L mice, but the effect of HFD-H on total white adipose tissue weight and liver weight was not statistically significant in mice fed a HFD (Figure3A-C).

To determine the effect of *L. acidophilus* CICC 6075 on lipid deposition in tissues, tissue sections were subjected to histological examination. When the area of lipid droplets in liver sections was analyzed, HFD-L mice showed a significant improvement in liver appearance and a 39% reduction in liver lipid accumulation compared to HFD-fed obese mice (Figure3D). In addition, the number of adipocytes in the epididymal adipose tissue of HFD mice was reduced by 56.4% in the same field of view, compared with control NCD mice. The number of adipocytes was increased by 41.9 and 9% in HFD-L and HFD-H mice, respectively, compared with HFD mice (Figure3E).

Considering intestinal dysbiosis in HFD-fed animals may affect gut permeability and subsequently lead to release of bacterial LPS into the circulation [27], whether *L. acidophilus* modulates gut integrity was identified. Then, HE results showed that HFD feeding dramatically increased intestinal permeability and damaged the intestinal mucosa (Figure3F), which however were completely restored by the *L. acidophilus* treatment.

These results suggest that *L. acidophilus* reduced weight gain and fat accumulation and restored the intestinal barrier in HFD-fed mice, while the group of HFD-L had better results.

### 3.2 *L. acidophilus* reverses HFD-induced gut microbiota dysbiosis

The gut microbiota of obese humans and HFD-fed mice is characterized by reduced α-diversity, altered β-diversity, increased ratios of Firmicutes to Bacteroides, elevated endotoxin-producing bacteria, and reduced immunostable bacterial species [28-30]. With this in mind, the effect of *L. acidophilus* on the composition of the intestinal microbiota was examined by MiSeq sequencing-based analysis of bacterial 16S rRNA gene sequences (V3-V4 region) in feces.

#### 3.2.1 Effects of *L. acidophilus* on gut microbial diversity and richness

Our results showed that all bacterial libraries from our samples represented the bacterial communities well, as the rarefaction curves tended towards saturation (Figure4A). To measure the extent of differences between microbial communities, β-diversity was calculated using principal coordinate analysis (PCoA)
was also performed (Figure 4B). The differences in the overall composition of the gut flora between the different groups were then statistically significant according to ANOVA. We also calculated the amount of gut microbial α-diversity indicators including richness and diversity, such as Sobs index and Shannon index. HFD feeding dramatically decreased gut microbial α diversity (Figure 4C, D), while supplementation with *L. acidophilus* completely restored the effect.

To better understand the shared richness among each group and the degree of variation in the samples, hierarchical clustering and a Venn diagram showing the overlap between groups were performed. The microbial composition of the samples within groups differed less at the genus level (Figure 5A). Then, it was shown that only 152 of the total richness of 995 OTUs were shared among all the groups, and there were more OTUs among three groups, between two groups or in each group (Figure 5B). In addition, *L. acidophilus* treatment significantly increased OTUs in HFD mice.

These data demonstrated that *L. acidophilus* treatment remarkably improved richness and diversity of intestinal microbiota.

### 3.2.2 Effects of *L. acidophilus* on the gut microbiota composition

The ratio of saprophytes to bacteria is a widely used marker of dysbiosis of the gut flora associated with obesity and obesity-related diseases [31]. HFD feeding profoundly affected the relative abundance of Firmicutes and Firmicutes/Bacteroidetes ratio compared with control NCD feeding (Figure 6A, B). Interestingly, under the HFD, *L. acidophilus* treatment, especially HDF-L group, decreased the relative abundance of Firmicutes and Firmicutes/Bacteroidetes ratio, and significantly increased the abundance of Actinobacteria (Figure 7A, B).

In the top 15 families, *L. acidophilus* treatment remarkably increased the abundance of Erysipelotrichaceae and Atopobiacaeae, whereas the relative abundance of Oscilllospiraceae, Lachnospiraceae and Marinilaceaeae decreased. Interestingly, *L. acidophilus* supplement increased Lactobacillaceae (Figure 7C, D). At genus level, *L. acidophilus* treatment, especially HDF-L group, caused increase in *Ileibacterium*(P < 0.05), *Lactobacillus, norank_f_Muribaculaceae* and *Helicobacter*, and caused reduction in *unclassified_f_Lachnospiraceae*(P < 0.05), *Lachnospiraceae_NK4A136, Lachnospiraceae_UCG-006*(P < 0.05), *unclassified_f_Oscilllospiraceae* and *Odoribacter* compared to that in HFD mice(P < 0.05) (Figure 7E, F).

In addition, the bacterial community structure with notable differences among the NCD group, the HFD group, the HFD-L group, and the HFD-H group was further analyzed by adopting the linear discriminant analysis (LDA) effect-size method (LEfSe) (Figure 8A, B). Then, it was figured out that taxa in different levels had differential abundance in the four groups. Besides, *unclassified_f_Prevoeltaceae, Muribaculum, Eubacterium_siraeum_group*, Gammaproteobacteria and Proteobacteria played critical roles and could be taken as a biomarker in the NCD group. However, *unclassified_f_Lachnospiraceae*
and *Lachnospiraceae_UCG-006* functioned importantly and could be used as a biomarker in the HFD group. In addition, Actinobacteriota, Coriobacteriales, Atopobiales, *Faecalibaculum*, Bifidobacteriaceae, *Lachnoloclostridium*, Coriobacteriia and Dubosiella played a crucial part and could be employed as a biomarker in the HFD-L group, whereas Erysipelotrichales, *Ileibacterium*, *Bacilli*, *Romboutsia*, Peptostreptococcaceae, *Turicibacter*, *Blautia*, and Clostridiaceae were vital and could be regarded as a biomarker in the HFD-H group. Furthermore, the number of taxa with differential abundance in the HFD group was lower than that in the HFD-L group and HFD-H groups, which shows that *L. acidophilus* has a recovery effect on the increase of some specific microbiota in the HFD group.

### 3.3 Effects of *L. acidophilus* on the gut microbiota function

The gut microbiota assumes essential physiological functions in the host. Moreover, this huge potential functionality influences whole-body metabolism and is a key factor in the pathology of obesity. Therefore, PICRUSt was employed to predict the functional potential of bacteria in the HFD group, and further analysis was carried out in the context of the Kyoto Encyclopedia of Genes and Genomes (KEGG) database. Then, according to the results, the enzyme, ORTHOLOGY (KO), modules and pathways belonging to KEGG functional categories were identified. Besides, the HFD feeding dramatically affected the four functional categories compared with control NCD feeding. However, these effects were completely restored by the *L. acidophilus* treatment (Figure 9A–F). To sum up, *L. acidophilus* treatment improves gut microbiota functions involving metabolism, immune response, and pathopoiesia.

### 4 Discussion

According to epidemiological surveys, in recent years, with the rapid economic and social development and changes in people's dietary habits and lifestyles, the incidence of obesity has shown a year-on-year increase in the global trend, and the proportion of overweight and obesity among men and women will reach 89% and 85% by 2030 [32], respectively. In addition to increasing the risk of chronic diseases such as diabetes and cardiovascular diseases, obesity also causes impairment of immune system function and becomes one of the important factors threatening people's health [33]. In this experiment, a high-fat diet was given to mice to construct an obesity model. The experimental results showed that the body mass of mice in each model group showed an overall increasing trend compared with the Control group. After 12 weeks of modeling, the body weight of each model group exceeded that of the normal group by 20%, indicating that the obese mouse model induced by high-fat diet was successfully constructed. The mice with different doses of *L. acidophilus* treatment had a significantly lower rate of weight gain than the HFD group, and both the HFD-L group and the HFD-L group could inhibit this trend, with the HFD-L group (1.9 \times 10^7 CFU/ml) being more effective.

The liver is an important site of lipid metabolism, and a long-term high-fat diet not only causes oxidative stress and inflammation in the liver, but also leads to hepatic lipid accumulation and liver function damage [34–36], which is similar to the results of the studies related to this experiment. HE staining
results showed that a high-fat diet induced white fat deposition and hepatic steatosis in the HFD group of mice, and \textit{L. acidophilus} CICC 6075 administration intervention could effectively improve the high-fat diet-induced obesity and non-alcoholic fatty liver in mice.

Obesity leads to a decrease in the Bacteroidetes and an increase in the Firmicutes in the intestine, demonstrating for the first time that obesity is associated with intestinal microorganisms [37, 38]. The results of PCoA analysis (Fig. 4B) showed that high-fat diet did reduce the \( \alpha \)-diversity and \( \beta \)-diversity of intestinal microorganisms in HFD group mice, leading to intestinal dysbiosis. And the results of Bar plot of intestinal flora at all levels and the test of significance of difference between groups (Fig. 7E, F) showed that HFD diet led to the decrease of Lactobacillus, norank\textunderscore f\textunderscore Muribaculaceae, etc. After \textit{L. acidophilus} CICC 6075 administration intervention, the ratio of Firmicutes-to-Bacteroidetes was significantly reduced, \textit{Lactobacillus} and norank\textunderscore f\textunderscore Muribaculaceae abundance was recovered and \textit{Ileibacterium} abundance was higher. It has been shown that \textit{Ileibacterium}, Lactobacillus has the ability to catabolize polysaccharides or produce short-chain fatty acids (SCFAs), which can activate certain \( G \) protein-coupled receptors (GPCRs), thus improving metabolite signaling [39]. Therefore, \textit{Ileibacterium} and norank\textunderscore f\textunderscore Muribaculaceae may be potential beneficial bacteria worth to be further explored.

Contrary to the experimental design expectation, low dose of \textit{L. acidophilus} \( (1.9 \times 10^7 \text{ CFU/ml}) \) was superior to high dose of \textit{L. acidophilus} \( (1.9 \times 10^9 \text{ CFU/ml}) \) in improving liver steatosis and intestinal flora disorders in obese mice induced by high-fat diet. The results of the test for significance of difference between groups of gate levels (Fig. 7B) showed that the abundance of Actinobacteriota in the HFD-L group was significantly higher than the other three groups. Actinobacteria, one of the four major phyla of the intestinal microbiota, are recognized as biosynthetic factories that produce a wide range of secondary metabolites, are a rich source of new bioactive compounds, and have a crucial role in maintaining the stability of the intestinal environment. Indeed, species of this phylum, especially \textit{Bidobacterium}, are widely used as probiotics and show beneficial effects in many pathological conditions [40–42]. Therefore, the superior effect of low-dose \textit{L. acidophilus} may be largely related to the high abundance of Actinobacteriota in the HFD-L group, and its specific mechanism needs to be further explored.

In conclusion, \textit{L. acidophilus} CICC 6075 can inhibit the trend of weight gain in obese mice induced by high-fat diet, improve lipid metabolism, reverse the dysbiosis of intestinal flora, increase the abundance and diversity of intestinal microorganisms, improve the functions of intestinal flora. It provides a new research direction for future treatment of obesity.

\textbf{Abbreviations}

\textit{L. acidophilus}: \textit{Lactobacillus acidophilus} CICC 6075; NCD: normal chow diet; HFD: high fat diet; HFD-L: high fat diet with low concentrations of \textit{L. acidophilus} administration; HFD-H: high fat diet with high concentrations of \textit{L. acidophilus} administration; OTUs: operational taxonomic units.

\textbf{Declarations}
Ethics approval and consent to participate

Animal experimental procedures were performed in accordance with the ethical guidelines of Xuzhou Medical University (Xuzhou, China).

Consent for publication

Not applicable.

Availability of data and materials

The datasets used and analyzed during the current study are available from the corresponding author on reasonable request.

Competing interests

All authors declare that they have no competing interests.

Funding

Key University Science Research Project of Jiangsu Province (22KJA320005); The Jiangsu Planned Projects for Postdoctoral Research Foundation (1501010B); the China Postdoctoral Science Foundation (2016M590506); Jiangsu Province College Students' innovation and entrepreneurship training program (202110313073Y); Jiangsu Key Laboratory of Animal genetic Breeding and Molecular Design (AGBMD2020001); and the Jiangsu Provincial Government Study Abroad Fund.

Author Contributions

Renjin Chen and Zhenzhen Wang conceived the study and designed the experiments. Yun Zhuang, Dan Yang, Xiqun Gu, Yi Wang and Yang Chen performed the experiments. Shuai Yang analyzed the data with suggestions by Renjin Chen, and Shuai Yang wrote the article. All authors read and approved the final manuscript.

Acknowledgments

Not applicable.

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**Figures**
Figure 1

Experimental design timeline.
Figure 2

*L. acidophilus* ameliorates body weight and fat accumulation in HFD-fed mice. Effects of *L. acidophilus* treatment on (A) body weight evolution; (B) body weight gain; (C) total white adipose tissue weight; (D) total white adipose tissue weight/body weight ratio; (E) liver weight/body weight ratio. Data are expressed as means ± SEM (n = 3). * P < 0.05; ** P < 0.01 and *** P < 0.001.
Figure 3

*L. acidophilus* restores morphological changes in HFD-fed mice. Effects of *L. acidophilus* treatment on (A) abdominal photograph; (B) epididymal white adipose tissue (WAT) photograph and weight; (C) liver photograph and weight; (D) photographs of HE-stained sections of livers and steatosis grade; (E) epididymal white adipocyte morphology and the number of adipocytes in the same field of view; (F)
photographs of HE-stained sections of the intestine. Data are expressed as means ± SEM (n = 3). * $P < 0.05$; ** $P < 0.01$ and *** $P < 0.001$.

**Figure 4**

*L. acidophilus* alters gut microbiota richness and diversity in HFD-fed mice. (A) Rarefaction curve; (B) β-diversity was determined using unweighted UniFrac distance based Principal Coordinates Analysis (PCoA); Effects of *L. acidophilus* treatment on α-diversity was determined using (C) Sobs index and (D) Shannon index. Data are expressed as means ± SEM (n = 3). * $P < 0.05$ and ** $P < 0.01$. 
Figure 5

*L. acidophilus* alters gut microbiota richness in HFD-fed mice. (A) The tree diagram shows the microbiome of each group of samples using unweighted UniFrac distance based hierarchical clustering; (B) Venn diagram illustrated overlap of OTUs in intestinal microbiota among the samples. Data are expressed as means ± SEM (n = 3).
Figure 6

*L. acidophilus* reverses Firmicutes/Bacteroidetes ratio in HFD-fed mice. (A) Relative abundance of Firmicutes; (B) The Firmicutes to Bacteroidetes ratio. Data are expressed as means ± SEM (n = 3). *P* < 0.05 and **P** < 0.01.
Figure 7

*L. acidophilus* alters gut microbiota composition in HFD-fed mice. (A) The relative abundance of gut microbiota at phylum level in groups; (B) The relative abundance of the top 10 genera at phylum level; (C) The relative abundance of gut microbiota at family level in groups; (D) The relative abundance of the top 15 genera at family level; (E) The relative abundance of gut microbiota at genus level in groups; (F) The
relative abundance of the top 15 genera at genu level. Data are expressed as means ± SEM (n = 3). * $P < 0.05$; ** $P < 0.01$ and *** $P < 0.001$.

Figure 8

*L. acidophilus* alters on gut microbiota biomarker in HFD-fed mice. (A) Identification of discriminant taxa among the four groups by LDA Effect Size (LEfSe) analysis. (A) Cladogram of the microbiota. Significant
discriminant taxon nodes of the NCD, HFD, HFD-L and HFD-H are represented by blue, red, light blue and green, respectively. While nondiscriminant taxon nodes are represented by yellow. Branch areas are shaded according to the highest ranked variety for that taxon. (B) The LDA score indicates the level of differentiation among the four groups. A threshold value of 3.0 was used as the cutoff level. Horizontal bar chart showing discriminant taxa. Significant discriminant taxa of the NCD, HFD, HFD-L and HFD-H are represented by blue, red, light blue and green, respectively. \( P < 0.05 \).
Figure 9

*L. acidophilus* effects on gut microbiota function basing Kyoto Encyclopedia of Genes and Genomes (KEGG) database in HFD-fed mice. Effects of *L. acidophilus* treatment on (A) enzyme; (B) ORTHOLOGY (KO); (C) module; (D) pathway 1; (E) pathway 2; (F) pathway 3. $P < 0.05$, $P < 0.01$ and $P < 0.001$ versus NCD; *$P < 0.05$; **$P < 0.01$ and ***$P < 0.001$ versus HFD-L and HFD-H.