Letters in Applied Microbiology, 2024, 77, ovae021
https://doi.org/10.1093/lambio/ovae021
Advance access publication date: 23 February 2024
Research Article

LETTERS IN APPLIED MICROBIOLOGY
OXFORD UNIVERSITY PRESS

Isolation of *Lactobacillus acidophilus* strain and its anti-obesity effect in a diet induced obese murine model

Yanyi Zheng[1], Silu Zhang[1], Zhizhu Zhang[1], Tengxun Zhang[2], Xin Teng[2], Guoxun Xiao[1], Song Huang[2,3,4,*]

[1]Wonderlab Innovation Centre for Healthcare, Shenzhen Porshealth Bioengineering Co., Ltd, Shenzhen 518000, China
[2]Bluepha Co., Ltd, Shenzhen 518000, China
[3]Department of Chemical and Biological Engineering, Xiamen University, Xiamen 361102, China
[4]School of Public Health, Lanzhou University, Lanzhou 730000, China
*Corresponding author. Song Huang, Bluepha Co., Ltd, Shenzhen 518000, China. E-mail: songurt@foxmail.com

Abstract

Intestinal microbiota is a potential determinant of obesity, with probiotic bile salt hydrolase (BSH) as one of the key mechanisms in the anti-obesity effects. In this study, we present a *Lactobacillus acidophilus* GOLDGUT-LA100 (LA100) with high BSH activity, good gastric acid and bile salt tolerance, and a potential anti-obesity effect. LA100's anti-obesity effects were evaluated in a high-fat diet-induced, obese mouse model. LA100 administration alleviates high-fat diet-induced pathophysiological symptoms, such as body weight gain, high serum glucose and cholesterol level, hepatic lipid accumulation, and adipose inflammation. These results demonstrate concrete anti-obesity benefit in animal models and show promising applications in future clinical studies.

Impact Statement

This study established a reliable pipeline to screen probiotics with high bile salt hydrolase (BSH) activity, and our results suggested that *Lactobacillus acidophilus* LA100 with high BSH activity has the potential to prevent obesity-associated pathophysiological symptoms.

Keywords: probiotics; *Lactobacillus acidophilus*; obesity; high-fat diet

Introduction

The global prevalence of metabolic syndrome is currently on the rise, exacerbating the risk of severe health ailments such as coronary heart disease and stroke. Metabolic syndrome is influenced by numerous factors, with obesity and a lack of physical activity being the primary contributors. Obesity is associated with various metabolic conditions such as hypertension, insulin resistance, type 2 diabetes mellitus, dyslipidemia, and non-alcoholic fatty liver disease (Jung and Choi 2014, Martin et al. 2015, Gil-Rodríguez and Beresford 2021). Recognized as a worldwide epidemic, obesity is escalating amongst both adults and children (Kobyliak et al. 2018), making it a significant health concern in the 21st century.

Obesity primarily results from an imbalance where energy intake exceeds consumption, predominantly due to overconsumption of fats and refined sugars (Gil-Rodríguez and Beresford 2021). Dietary fats necessitate hydrolysis into smaller molecules via lipases for intestinal absorption (Tucci et al. 2010). Bile, comprising bile acids, cholesterol, phospholipids, and the pigment biliverdin, is a crucial component of fat digestion, functions to emulsify and solubilize lipids (Begley et al. 2006). The bile salt hydrolase (BSH) enzyme catalyze the deconjugation of conjugated bile salts into free bile salts and amino acids (Liong and Shah 2005). The BSHs produced by intestinal bacteria, including Actinobacteria, Bacteroidetes, Euryarchaeota, Firmicutes, and Proteobacteria, as well as intestinal archaea (Jones et al. 2008, Jia et al. 2021). Increased BSH expression leads to a rise in free bile salt in the intestinal lumen, which is less efficiently absorbed than conjugated bile salts (Gil-Rodríguez and Beresford 2021). Moreover, the limited lipid emulsifying capacity in the small intestine increases the excretion of dietary fat and cholesterol with feces (Begley et al. 2006, Gil-Rodríguez and Beresford 2021). Hence, intestinal BSH plays a pivotal role in host fat digestion, lipid metabolism, and dietary energy harvest by reducing total cholesterol (TC) level in serum (Geng and Lin 2016).

The role of probiotics in preventing and reducing obesity has also been widely studied in animal models and humans (Barengolts 2016, Gérard 2016, Kobyliak et al. 2018, Mazloom et al. 2019, Aoun et al. 2020), and enzymatic deconjugation of bile acids by BSH produced by probiotics is one potential mechanism (Klaver and van der Meer 1993, Tahri et al. 1996, 1997, Usman and Hosono 1999). BSH genes are notably abundant in lactic acid bacteria, like *Lactobacilli* and *Bifidobacteria*, recognized as health-promoting probiotics. Recent studies have reported that *Lactobacillus* spp. reduce high-fat diet (HFD)-induced obesity (Naito et al. 2011, Arora et al. 2012, Heo et al. 2016, Ondee et al. 2021, Kang et al. 2022). *Lactobacillus acidophilus* is an indigenous and dominant *Lactobacillus* species that is present in the gastrointestinal tract of most healthy adults (Tannock 1995) and is widely recognized to have probiotic effects as one of the most commonly suggested organism for dietary use (Shah 2007). Many reports have been published on the deconjugation of bile acids by *L. acidophilus* (Gilliland and Speck 1977, Walker and Gilliland 1993, Buck and Gilliland 1994). Therefore,

Received 22 November 2023; **revised** 15 January 2024; **accepted** 22 February 2024

incorporating probiotics like *L. acidophilus* into the diet as a supplement may lead to anti-obesity advantages.

In this work, we aimed to isolate probiotics with high BSH activity and evaluated its anti-obesity effect in an HFD-induced obese murine model. A strain *L. acidophilus* GOLDGUT-LA100 (LA100), isolated from a healthy human fecal sample, displayed high BSH activity *in vitro* and promising anti-obesity effects *in vivo*.

Materials and methods

Sample collection and isolation of LAB strains

Fecal samples were collected from healthy adults and immediately stored at −80°C in 25% glycerol supplemented with 0.5 g l^{-1} L-cysteine HCl for subsequent processing. Single clones were isolated by the pour plate method. The fecal samples were diluted and cultivated on MRS agar plates (Huankai Microbial Sci. & Tech. Co., Ltd, Guangdong, China), plates were incubated at 37°C for 48 h under anaerobic conditions. Smooth and shiny colonies were selected and purified through successive subcultures. Single clones were cultured in MRS broth containing 0.5 g l^{-1} L-cysteine HCl under anaerobic conditions at 37°C for 18 h. Isolated strains were preserved in a sterile glycerol/MRS broth mixture (3:1, v/v) at −80°C.

Taxonomic identification was carried out by 16S rRNA sequence analysis using universal primers 27F and 1492R. The PCR amplification steps were as follows: 98°C, 3 min; 98°C, 10 s, 56°C, 5 s, 72°C, 15 s, 30 cycles; 72°C, 5 min. 16S rRNA sequencing was conducted by Tsingke Biotechnology Co., Ltd, Beijing, China. The obtained sequences were aligned with the NCBI GenBank database to achieve taxonomic classification at the species level. 16S rRNA sequence of *L. acidophilus* LA100 was deposited at GenBank under accession number OR775343.1.

Bile salt tolerance test

The bile salt tolerance ability of isolates was estimated and calculated from its survival rates. In brief, 20 μl overnight culture of strains was mixed with 900 μl MRS supplemented with sodium glycocholate and sodium taurocholate (n/n, 2:1) at a final concentration 10 μmol l^{-1}. Cells were anaerobically incubated at 37°C for 18 h, and the OD600 was measured.

Gastric acid tolerance test

The gastric acid tolerance assays were carried out by the INFOGEST method (Brodkorb et al. 2019). A volume of 100 μl bacterial culture from each strain was mixed with 100 μl artificial human gastric fluid (Xiao Dong Pro-Health Instrumentation Co., Ltd, Suzhou, China) in a 96-well plate. The plate was then incubated under anaerobic conditions at 37°C for 2 h. PBS buffer was used as control. The number of viable cells in the suspension incubated with either artificial human gastric fluid or PBS buffer was assessed using the pour plate method on MRS agar plates. The survival rate for each strain was calculated as follows: Survival rate (%) = $A1/A0 \times 100\%$, where $A1$ represents the number of viable cells in the suspension incubated with artificial human gastric fluid, and $A0$ represents the number of viable cells in the suspension incubated with PBS buffer.

BSH activity assay

The BSH hydrolyzing activities were performed as previously described (Grill et al. 2000). The overnight cultures were harvested (6000 rpm, 10 min), washed twice with equal volume of PBS buffer. The bacterial pellet was then resuspended in 3 ml PBS buffer, and the OD was adjusted to the same value with purified water.

The enzymes from cells were obtained by a tissue grinder at 70 Hz for 60 times (grinding for 20 s, resting for 10 s) with glass beads. The ground cell suspensions were centrifuged at 12 000 rpm for 5 min at 4°C. The supernatant (950 μl) was collected and mixed with 50 μl 0.4 mol l^{-1} bile salts (sodium glycocholate or sodium taurocholate), then incubated at 37°C for 30 min. The reaction was quenched by adding 500 μl of 15% trichloroacetic acid (v/v%), and the mixture was centrifuged at 12 000 rpm for 5 min to remove precipitated proteins. A volume of 200 μl supernatant was then collected and mixed with 1 ml of sodium-citrate buffer with a pH of 5.5 and 1 ml ninhydrin reagent, and boiled for 30 min. The mixture was then cooled with cold water and mixed with 2.8 ml of 70% ethanol. The amount of glycine or taurine released was measured at 570 nm using a spectrophotometer, and standard curves were established with free glycine and taurine.

Acute oral toxicity test

Six-week Kunming mice were used for acute oral toxicity test following the methods described in Standard GB15193.3-2014 (NHFPC, 2014) and approved by the Institutional Animal Care and Use Ethics Committee of Institute of Microbiology, Guangdong Academy of Sciences under protocol GT-IACUC202211163. After fasting for 6 h, 10 male and 10 female mice were orally administrated with *L. acidophilus* LA100 at a dose of 5000 mg kg^{-1} body weight. All mice were observed for 14 days after *L. acidophilus* LA100 administration. The body weight was measured on day 0 and 14.

Animals and experimental design

Specific pathogen-free C57BL/6 mice (4–6 weeks of age) were purchased from Huayuan Shidai Technology Co., Ltd, Beijing, China. Animal experiments were conducted in accordance with the guidelines for laboratory animal use and care under protocol HYSD2022-07 approved by the Animal Ethics Committee of HuaYuan Shidai Biotech Co., Ltd. The mice were housed in a controlled environment (12 h light/dark cycle at 25 ± 2°C) with food and water *ad libitum*. A total of 38 mice were randomly divided into two groups, 8 mice fed with normal diet (ND) (20.6% protein, 67.4% carbohydrate, 12% fat, 3.64 kcal g^{-1}) and 30 mice fed with high-fat diet (HFD) (20% protein, 20% carbohydrate, 60% fat, 5.24 kcal g^{-1}) for 8 weeks. Two mice from the HFD group that had <20% weight gain than ND group were excluded. The ND ($n = 8$): continued feeding with ND for another 6 weeks. For the remaining HFD group mice, the animals were divided into three groups: (i) HFD ($n = 10$): continued feeding with HFD for another 6 weeks and gavage with 500 μl saline solution every day; (ii) HFD + probiotics (HFD + LA100) ($n = 10$): continued feeding with HFD for 6 weeks and gavage with 500 μl 2×10^9 CFU ml^{-1} *L. acidophilus* LA100 in saline solution every day; and (iii) HFD + weight-losing drug orlistat (HFD + orlistat) ($n = 8$): continued feeding with HFD and gavage with 80 mg kg^{-1} orlistat in saline solution every day for 6 weeks. Body weight was measured every week. After

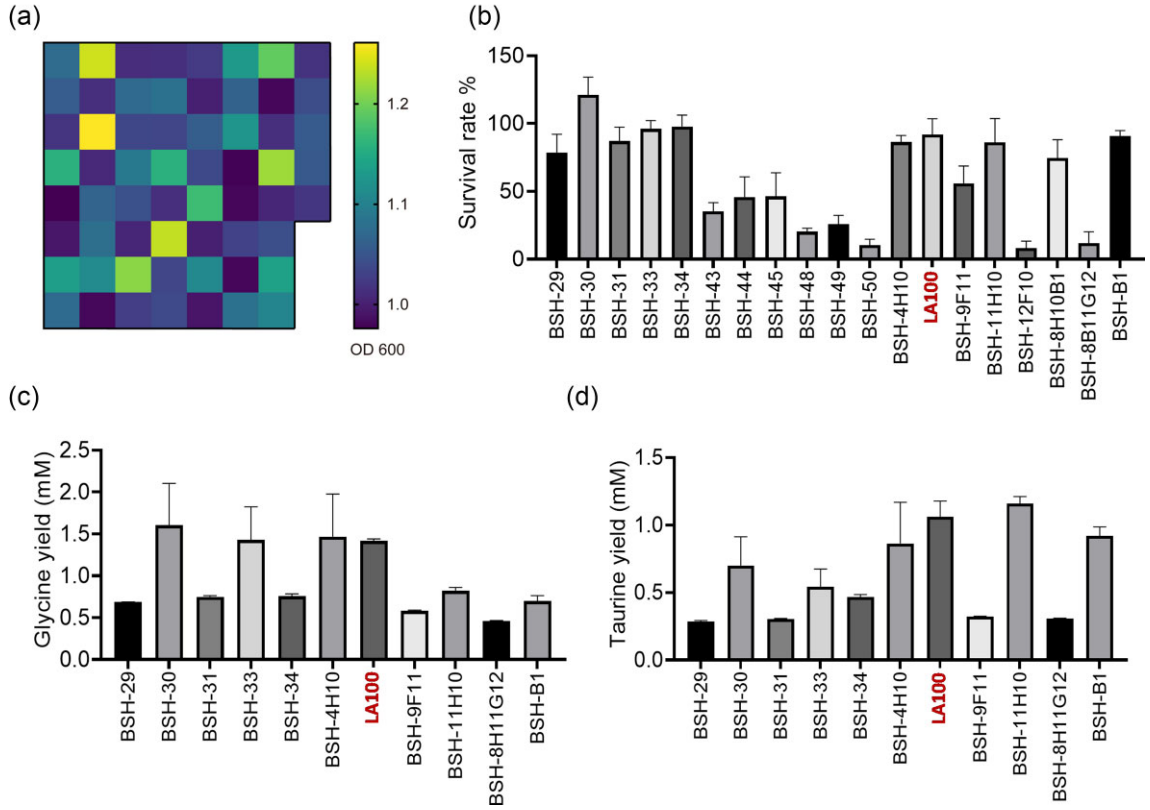

Figure 1. Isolation of potential probiotics with high BSH activity. (a) Cell density of strains after bile salt treatment. (b) Survival rate of strains with high bile salt resistant in artificial gastric juice. Glycine (c) and taurine (d) yield in BSH activity assay. Values are presented as mean ± SEM from three independent experiments.

treatment, animals were euthanized, blood and tissues were collected for further analysis.

Isolation and detection of visceral adipocytes

The epididymal adipose tissue and hepatic adipose tissue were cut into 2 mm × 2 mm pieces with ophthalmic scissors, then collagenase type II was added, and the adipose tissue was digested into chyme for 30 min in a water bath at 37°C. The adipose tissue was centrifuged (1500 rpm, 5 min), the upper layer of the adipose tissue was discarded, and the cells were washed and resuspended in PBS. The resuspended cells were identified by flow cytometry after antibody staining with PE anti-mouse F4/80 Phycoerythrin, PerCP anti-mouse CD11c, and FITC anti-mouse CD11b. The infiltration of macrophages in adipose tissue is calculated according to the results of flow cytometry detection.

Serum biochemical analysis

Mouse serum samples were taken and tested for serum leptin levels using ELISA kit (Millipore). Blood levels of TC were measured by a automated biochemical analyzer and blood glucose level was measured by blood glucose meter.

Histological examination

Liver tissue was fixed in 4% paraformaldehyde and embedded in paraffin to make 5-μm sections. Oil Red O staining was performed and examined by optical microscope to determine the level of lipid accumulation.

Statistical analysis

All analyses were analyzed using GraphPad Prism version 8.0 software, and the results were presented as mean ± standard error of the mean (SEM). The differences between mean values of two groups were evaluated by the Student's t test. The differences between mean values of multiple groups were evaluated by one-way analysis of variance (ANOVA) with Dunnett's method.

Results and discussion

Screening of microbial strain with bile tolerance and high BSH activity

The typical, round, smooth, and shiny colonies were isolated and purified from the fecal samples of healthy adults. To obtain strains with increased resistance to bile salt and gastric acid, 61 isolated stains were inoculated into MRS with 10 μmol l^{-1} bile salts for 18 h. A total of 19 strains exhibited high survival rate after bile salt treatment as determined by OD 600 values (Fig. 1a). These strains were tested for gastric acid tolerance by counting cell numbers of microbes cultured in artificial human gastric fluid vs PBS for 2 h (Fig. 1b). Strains with >50% survival rate in artificial gastric fluid were selected for further analysis.

To determine the BSH activity of potential strains, bacterial proteins extracted with a tissue grinder were incubated with 40 mmol l^{-1} bile salts (sodium glycocholate or sodium taurocholate, respectively) for 30 min and then ninhydrin reagent was used to determine the amino acid produced to compare BSH activity between strains (Fig. 1c and d). Strain LA100,

Table 1. Toxic effects of *L. acidophilus* LA100 on Kunming mice

Sex	Dose (mg kg^{-1} BW)	Body weight (g)		Number of deaths	MTD (mg kg^{-1} BW)	LD_{50} (mg kg^{-1} BW)
		Day 0	Day 14			
Female	5000	19.7 ± 0.1	32.6 ± 0.2	0	>5000	>5000
Male	5000	19.0 ± 0.1	39.4 ± 0.2	0	>5000	>5000

Data are presented as means ± SD, $n = 10$. MTD, maximum tolerated dose; LD_{50}, the half lethal dose.

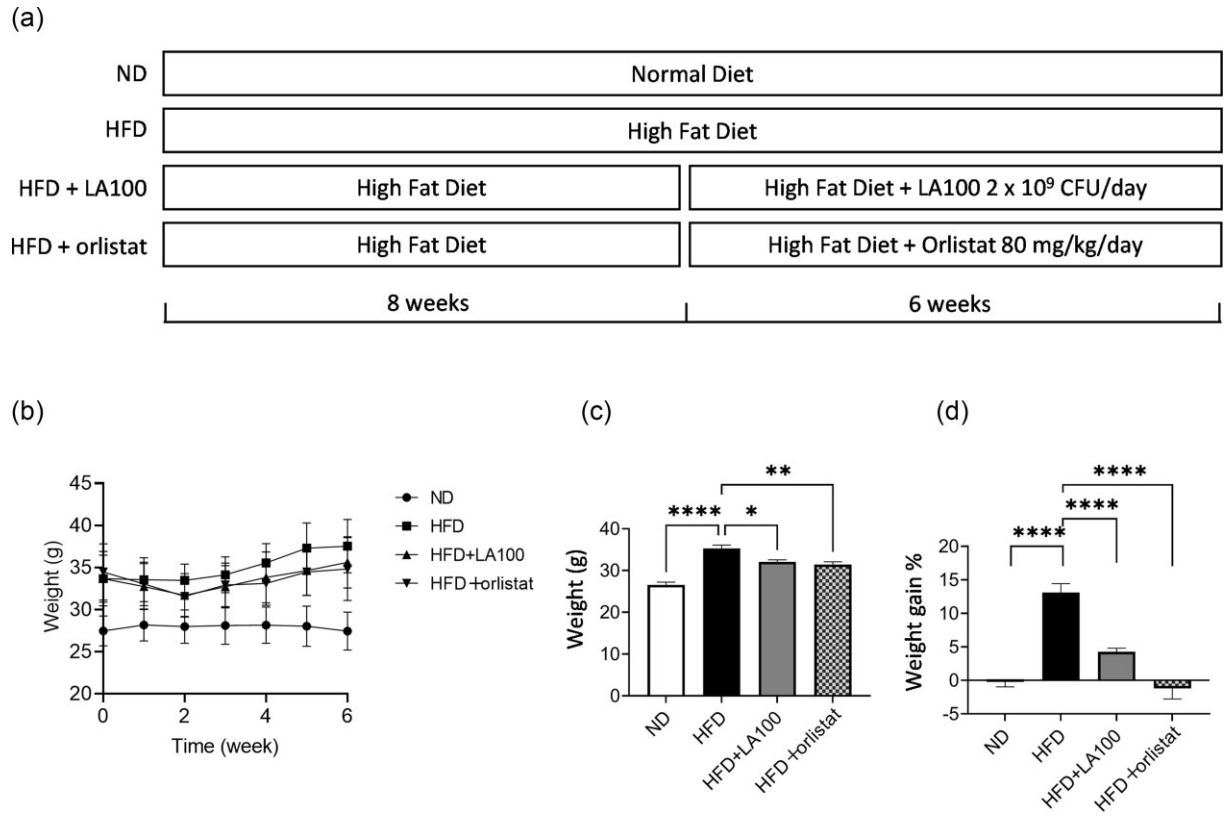

Figure 2. LA100 administration reduced body weight gain induced by HFD. (a) Experimental design of obese animal model and probiotics administration. ND, normal diet group; HFD, high-fat diet group; HFD + LA100, *L. acidophilus* LA100 administration group; HFD + orlistat, orlistat administration group. (b) Body weight change after intervention. (c) Body weight gain and (d) percentage of weight gain at the end of intervention. Values are presented as mean ± SEM.

characterized as *L. acidophilus* by 16S rRNA gene sequencing analysis, demonstrated good gastric acid tolerance and high BSH activity and was selected for animal experiment.

To evaluate the safety of the *L. acidophilus* LA100 using *in vivo* approach, *L. acidophilus* LA100 was orally administered at a dose of 5000 mg kg^{-1} BW to Kunming mice. Body weights during 14-day observation period are shown in Table 1. No weight loss, death, or any abnormal symptoms in the physical appearance and behavior were observed after toxicity test. The maximum tolerated dose and LD_{50} exceeded 5000 mg kg^{-1}. These results demonstrated that *L. acidophilus* LA100 is safe as a probiotic.

LA100 controls weight gain fed with HFD

To examine the effect of LA100 on weight control, obese mice model was established with HFD. Following obese mice model established, LA100, orlistat as positive control, saline solution as negative control were gavaged daily, with same HFD provided for 6 weeks. ND group was used as blank control (Fig. 2a).

Figure 2b shows the body weight changes in the four groups after intervention. Both the LA100 and orlistat interventions significantly reduced the body weight of mice after 6 weeks compared to the HFD control group (Fig. 2c). LA100 intervention was also significantly lower than HFD group in terms of the percentage of weight gain during the intervention (Fig. 2d). These results suggest that *L. acidophilus* LA100 intervention was able to slow down HFD-induced weight gain in obese mice.

LA100 reduced metabolic disorder and adipose tissue inflammation induced by HFD

To investigate how LA100 helps control body weight gain fed with HFD, liver tissues were collected at the end of the intervention, and subjected to oil red O staining to assess hepatic lipid accumulation. As shown in Fig. 3a, HFD induced significant hepatic lipid accumulation; however, this accumulation

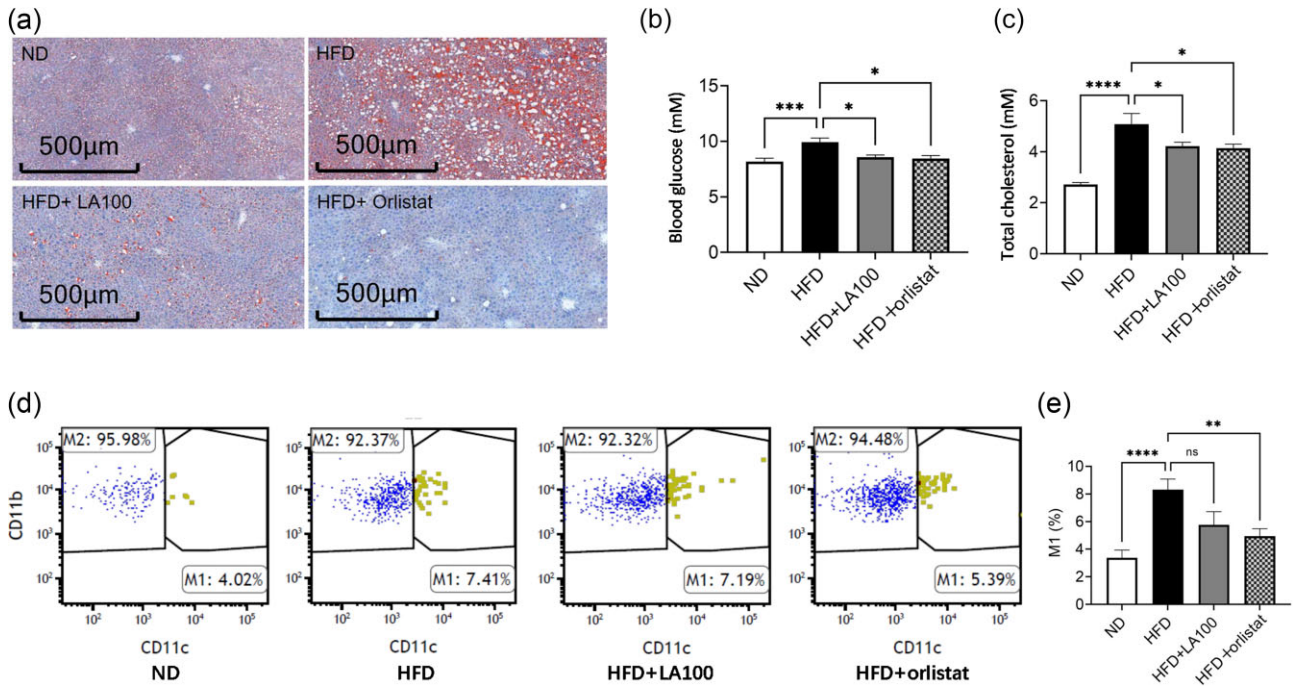

Figure 3. LA100 alleviated metabolic disorder induced by HFD. (a) Representative liver tissue Oil red O staining image and liver accumulation in mice. Blood glucose (b) and total blood cholesterol (c) levels after intervention. Flow cytometry result (d) and calculated (e) M1 macrophage percentage in adipose tissue. Values are presented as mean ± SEM.

was notably reduced following intervention with orlistat and LA100.

To further investigate the effect of LA100 on metabolic regulation, the blood and serum were separated after intervention, blood glucose level and the TC content were measured. The results showed that the probiotic *L. acidophilus* LA100 intervention reduced both blood glucose levels (Fig. 3b) and TC content of the mice (Fig. 3c) compared to HFD group.

Metabolic inflammation in adipose tissue is frequently observed as a result of diet-induced obesity in human and rodent studies. The effect of LA100 on adipose tissue inflammation was investigated. Flow cytometry results (Fig. 3d) and the M1 macrophage quantification results (Fig. 3e) showed that the positive drug orlistat significantly reduced the number of M1 macrophages and decreased adipose tissue inflammation. Probiotic *L. acidophilus* LA100 intervention showed similar trend, indicated that *L. acidophilus* LA100 may also alleviate HFD-induced adipose inflammation.

The prevalence of obesity has continuously increased worldwide over the last few decades. The incidence of metabolic syndrome in humans is also increasing globally, and people are at a higher risk of developing coronary heart disease, stroke, and other serious health problems (Kobyliak et al. 2018). Obesity-induced metabolic conditions include hypertension, insulin resistance and type 2 diabetes, dyslipidemia, and NAFLD. With the improvement of people's living standards and changes in their lifestyles, the incidence of obesity is rising rapidly worldwide and has become a health problem of widespread concern (Jung and Choi 2014, Martin et al. 2015, Gil-Rodríguez and Beresford 2021).

The intestinal microbiota has been reported to be one of the potential determinants of obesity in recent human and animal studies (Gérard 2016, Aoun et al. 2020). BSH produced by intestinal bacteria can hydrolyze conjugated bile salts into free bile salts and amino acid residues. Expression of BSH increases the amount of free bile salts in the intestinal lumen, which are less soluble and less efficiently absorbed compared to conjugated bile salts (Gil-Rodríguez and Beresford 2021). Consequently, the reabsorption and reutilization of bile salts are reduced, thereby increasing the synthesis of bile salts in the liver. This synthesis requires the migration of cholesterol from the blood to the liver, thereby reducing serum TC levels. In addition, the capacity of the small intestine to emulsify lipids is limited, thus increasing the excretion of dietary fat and cholesterol with the feces (Gil-Rodríguez and Beresford 2021). The BSH gene is particularly abundant in lactic acid bacteria such as *Lactobacillus* and *Bifidobacterium*, which are of value to be explored intensively in the direction of lowering cholesterol (Adebola et al. 2020). In our study, we developed a pipeline to isolate probiotics with high BSH activity and successfully isolated *L. acidophilus* LA100 with high BSH activity, high bile salt, and gastric acid tolerance from healthy human feces, which proved that a reliable screening method has been established.

The main cause of obesity is that energy intake exceeds energy expenditure, especially related to excessive intake of fat and refined carbohydrates. Excessive fat intake causes an increase in serum cholesterol levels, which are an important factor in many cardiovascular diseases. Administration of probiotics had been reported to significantly reduce body weight in high-calorie-fed mice (Alard et al. 2016, Kim et al. 2016, Ji et al. 2019, Kong et al. 2019). In our study, we established obesity with HFD in mouse model and evaluated the weight management effect of LA100 with high BSH activity. After obese model was established, *L. acidophilus* LA100, orlistat, and PBS were gavaged. At the beginning of the intervention, body weight dropped and then increased (Fig. 2b), probably due to gavage treatment. After the animals are adapted to gav-

age treatment, body weight started to increase. At the end of the treatment, the body weight (Fig. 2c) and percent weight gain (Fig. 2d) of *L. acidophilus* LA100 group were significantly lower than HFD group, demonstrated the effect of *L. acidophilus* LA100 on weight control.

The impact of an HFD on various pathophysiological symptoms of obesity is well established and supported in current literature (Lee 2013, Chandler et al. 2017, Ludwig et al. 2018). In our animal model, we observed increased body weight (Fig. 2), accompanied by significant increase of blood glucose and cholesterol levels and hepatic fat accumulation, as well as adipose tissue inflammation (Fig. 3). In our study, *L. acidophilus* LA100 can significantly reverse the pathophysiological symptoms, which proved *L. acidophilus* LA100's promising anti-obesity effect. Since we only intervened with *L. acidophilus* LA100 for 6 weeks, we hypothesize that an extended intervention would result in a more significant decrease in obesity as determined by body weight.

Conclusions

We successfully screened a *L. acidophilus* LA100 with high BSH activity, as well as bile salts and gastric acid tolerance. Animal studies further indicated that this strain could significantly mitigate the pathophysiological symptoms caused by HFD. Clinical trials should be further conducted to verify the anti-obesity effect in obese populations.

Conflict of interest: Y.Z., S.Z., Z.Z., and G.X. are employees in Porshealth Bioengineering Co., Ltd.

Funding

This research was funded by Shenzhen Porshealth Bioengineering Co., Ltd.

Author contributions

Yanyi Zheng (Investigation [equal], Methodology [equal]), Silu Zhang (Data curation [equal], Investigation [equal], Validation [equal]), Zhizhu Zhang (Data curation [equal], Validation [equal]), Tengxun Zhang (Investigation [equal], Methodology [equal]), Xin Teng (Project administration [equal], Writing – original draft [equal], Writing – review & editing [equal]), Guoxun Xiao (Funding acquisition [equal]), and Song Huang (Conceptualization [equal], Supervision [equal], Writing – review & editing [equal]).

Data availability

16S rRNA sequence of *L. acidophilus* LA100 was deposited at GenBank under accession number OR775343.1.

References

Adebola OO, Corcoran O, Morgan WA. Prebiotics may alter bile salt hydrolase activity: possible implications for cholesterol metabolism. *PharmaNutrition* 2020;**12**:100182. https://doi.org/10.1016/j.phanu.2020.100182

Alard J, Lehrter V, Rhimi M *et al*. Beneficial metabolic effects of selected probiotics on diet-induced obesity and insulin resistance in mice are associated with improvement of dysbiotic gut microbiota. *Environ Microbiol* 2016;**18**:1484–97. https://doi.org/10.1111/1462-2920.13181

Aoun A, Darwish F, Hamod N. The influence of the gut microbiome on obesity in adults and the role of probiotics, prebiotics, and synbiotics for weight loss. *Prev Nutr Food Sci* 2020;**25**:113–23. https://doi.org/10.3746/pnf.2020.25.2.113

Arora T, Anastasovska J, Gibson G *et al*. Effect of *Lactobacillus acidophilus* NCDC 13 supplementation on the progression of obesity in diet-induced obese mice. *Br J Nutr* 2012;**108**:1382–9. https://doi.org/10.1017/S0007114511006957

Barengolts E. Gut microbiota, prebiotics, probiotics, and synbiotics in management of obesity and prediabetes: review of randomized controlled trials. *Endocr Pract* 2016;**22**:1224–34. https://doi.org/10.4158/EP151157.RA

Begley M, Hill C, Gahan CGM. Bile salt hydrolase activity in probiotics. *Appl Environ Microbiol* 2006;**72**:1729–38. https://doi.org/10.1128/AEM.72.3.1729-1738.2006

Brodkorb A, Egger L, Alminger M *et al*. INFOGEST static *in vitro* simulation of gastrointestinal food digestion. *Nat Protoc* 2019;**14**:991–1014. https://doi.org/10.1038/s41596-018-0119-1

Buck LM, Gilliland SE. Comparisons of freshly isolated strains of *Lactobacillus acidophilus* of human intestinal origin for ability to assimilate cholesterol during growth. *J Dairy Sci* 1994;**77**:2925–33. https://doi.org/10.3168/jds.S0022-0302(94)77233-7

Chandler M, Cunningham S, Lund EM *et al*. Obesity and associated comorbidities in people and companion animals: a one health perspective. *J Comp Pathol* 2017;**156**:296–309. https://doi.org/10.1016/j.jcpa.2017.03.006

Geng W, Lin J. Bacterial bile salt hydrolase: an intestinal microbiome target for enhanced animal health. *Anim Health Res Rev* 2016;**17**:148–58. https://doi.org/10.1017/S1466252316000153

Gérard P. Gut microbiota and obesity. *Cell Mol Life Sci* 2016;**73**:147–62. https://doi.org/10.1007/s00018-015-2061-5

Gilliland SE, Speck ML. Deconjugation of bile acids by intestinal lactobacilli. *Appl Environ Microbiol* 1977;**33**:15–18. https://doi.org/10.1128/aem.33.1.15-18.1977

Gil-Rodríguez AM, Beresford T. Bile salt hydrolase and lipase inhibitory activity in reconstituted skim milk fermented with lactic acid bacteria. *J Funct Foods* 2021;**77**:104342. https://doi.org/10.1016/j.jff.2020.104342

Grill JP, Cayuela C, Antoine JM *et al*. Isolation and characterization of a *Lactobacillus amylovorus* mutant depleted in conjugated bile salt hydrolase activity: relation between activity and bile salt resistance. *J Appl Microbiol* 2000;**89**:553–63. https://doi.org/10.1046/j.1365-2672.2000.01147.x

Heo J, Seo M, Park H *et al*. Gut microbiota modulated by probiotics and garcinia cambogia extract correlate with weight gain and adipocyte sizes in high fat-fed mice. *Sci Rep* 2016;**6**:33566. https://doi.org/10.1038/srep33566

Ji Y, Chung YM, Park S *et al*. Dose-dependent and strain-dependent anti-obesity effects of *Lactobacillus sakei* in a diet induced obese murine model. *PeerJ* 2019;**7**:e6651. https://doi.org/10.7717/peerj.6651

Jia B, Park D, Chun BH *et al*. Diet-related alterations of gut bile salt hydrolases determined using a metagenomic analysis of the human microbiome. *Int J Mol Sci* 2021;**22**:3652. https://doi.org/10.3390/ijms22073652

Jones BV, Begley M, Hill C *et al*. Functional and comparative metagenomic analysis of bile salt hydrolase activity in the human gut microbiome. *Proc Natl Acad Sci USA* 2008;**105**:13580–5. https://doi.org/10.1073/pnas.0804437105

Jung UJ, Choi M-S. Obesity and its metabolic complications: the role of adipokines and the relationship between obesity, inflammation, insulin resistance, dyslipidemia and nonalcoholic fatty liver disease. *Int J Mol Sci* 2014;**15**:6184–223. https://doi.org/10.3390/ijms15046184

Kang Y, Kang X, Yang H *et al*. *Lactobacillus acidophilus* ameliorates obesity in mice through modulation of gut microbiota dysbiosis and intestinal permeability. *Pharmacol Res* 2022;**175**:106020. https://doi.org/10.1016/j.phrs.2021.106020

Kim B, Park K-Y, Ji Y *et al*. Protective effects of *Lactobacillus rhamnosus* GG against dyslipidemia in high-fat diet-induced obese mice. *Biochem Biophys Res Commun* 2016;**473**:530–6. https://doi.org/10.1016/j.bbrc.2016.03.107

Klaver FA, van der Meer R. The assumed assimilation of cholesterol by lactobacilli and bifidobacterium bifidum is due to their bile salt-deconjugating activity. *Appl Environ Microbiol* 1993;**59**:1120–4. https://doi.org/10.1128/aem.59.4.1120-1124.1993

Kobyliak N, Falalyeyeva T, Boyko N *et al*. Probiotics and nutraceuticals as a new frontier in obesity prevention and management. *Diabetes Res Clin Pract* 2018;**141**:190–9. https://doi.org/10.1016/j.diabres.2018.05.005

Kong C, Gao R, Yan X *et al*. Probiotics improve gut microbiota dysbiosis in obese mice fed a high-fat or high-sucrose diet. *Nutrition* 2019;**60**:175–84. https://doi.org/10.1016/j.nut.2018.10.002

Lee CY. The effect of high-fat diet-induced pathophysiological changes in the gut on obesity: what should be the ideal treatment? *Clin Transl Gastroenterol* 2013;**4**:e39. https://doi.org/10.1038/ctg.2013.11

Liong MT, Shah NP. Bile salt deconjugation ability, bile salt hydrolase activity and cholesterol co-precipitation ability of lactobacilli strains. *Int Dairy J* 2005;**15**:391–8. https://doi.org/10.1016/j.idairyj.2004.08.007

Ludwig DS, Willett WC, Volek JS *et al*. Dietary fat: from foe to friend? *Science* 2018;**362**:764–70. https://doi.org/10.1126/science.aau2096

Martin KA, Mani MV, Mani A. New targets to treat obesity and the metabolic syndrome. *Eur J Pharmacol* 2015;**763**:64–74. https://doi.org/10.1016/j.ejphar.2015.03.093

Mazloom K, Siddiqi I, Covasa M. Probiotics: how effective are they in the fight against obesity? *Nutrients* 2019;**11**:258. https://doi.org/10.3390/nu11020258

Naito E, Yoshida Y, Makino K *et al*. Beneficial effect of oral administration of *Lactobacillus casei* strain Shirota on insulin resistance in diet-induced obesity mice. *J Appl Microbiol* 2011;**110**:650–7. https://doi.org/10.1111/j.1365-2672.2010.04922.x

Ondee T, Pongpirul K, Visitchanakun P *et al*. *Lactobacillus acidophilus* LA5 improves saturated fat-induced obesity mouse model through the enhanced intestinal *Akkermansia muciniphila*. *Sci Rep* 2021;**11**:6367. https://doi.org/10.1038/s41598-021-85449-2

Shah NP. Functional cultures and health benefits. *Int Dairy J* 2007;**17**:1262–77. https://doi.org/10.1016/j.idairyj.2007.01.014

Tahri K, Grill JP, Schneider F. Involvement of trihydroxyconjugated bile salts in cholesterol assimilation by bifidobacteria. *Curr Microbiol* 1997;**34**:79–84. https://doi.org/10.1007/s002849900148

Tahri K, Grille JP, Schneider F. Bifidobacteria strain behavior toward cholesterol: coprecipitation with bile salts and assimilation. *Curr Microbiol* 1996;**33**:187–93. https://doi.org/10.1007/s002849900098

Tannock GW. Microecology of the gastrointestinal tract in relation to lactic acid bacteria. *Int Dairy J* 1995;**5**:1059–70. https://doi.org/10.1016/0958-6946(95)00043-7

Tucci SA, Boyland EJ, Halford JC. The role of lipid and carbohydrate digestive enzyme inhibitors in the management of obesity: a review of current and emerging therapeutic agents. *Diabetes Metab Syndr Obes* 2010;**3**:125–43. https://doi.org/10.2147/DMSO.S7005

Usman P, Hosono A. Bile tolerance, taurocholate deconjugation, and binding of cholesterol by *Lactobacillus gasseri* strains. *J Dairy Sci* 1999;**82**:243–8. https://doi.org/10.3168/jds.S0022-0302(99)75229-X

Walker DK, Gilliland SE. Relationship among bile tolerance, bile salt deconjugation, and assimilation of cholesterol by *Lactobacillus acidophilus*. *J Dairy Sci* 1993;**76**:956–61. https://doi.org/10.3168/jds.S0022-0302(93)77422-6

Received 22 November 2023; **revised** 15 January 2024; **accepted** 22 February 2024