Letters in Applied Microbiology

Letters in Applied Microbiology ISSN 0266-8254

ORIGINAL ARTICLE

Probiotic characterization of *Lactiplantibacillus plantarum* HOM3204 and its restoration effect on antibiotic-induced dysbiosis in mice

S. Zhang[1], T. Wang[2], D. Zhang[1], X. Wang[2], Z. Zhang[2], C. Lim[1,3] and S. Lee[1,3]

1 Coree Beijing Co., Ltd., Beijing, China
2 Beijing Hanmi pharmaceutical Co., Ltd., Beijing, China
3 Coree Pohang Co., Ltd., Pohang, Korea

Significance and Impact of the Study: *Lactiplantibacillus plantarum* HOM3204 was isolated from homemade pickled cabbage. *Lact. plantarum* HOM3204 showed excellent probiotic characterization *in vitro* tests. *Lact. plantarum* HOM3204 significantly restored the gut microbiota composition by increasing the abundance of Lactobacilli and Bifidobacteria and decreasing Enterococci, and improved antioxidative function by raising the concentrations of glutathione peroxidase (GSH-Px) and superoxide dismutase (SOD) in serum of antibiotic-induced dysbiosis in mice. These results suggest that *Lact. plantarum* HOM3204 could be a potential probiotic as a functional food ingredient.

Keywords
antioxidation, gut dysbiosis, *Lactiplantibacillus plantarum*, probiotic, short-chain fatty acids.

Correspondence
Suwon Lee, Coree Beijing Co., Ltd., No. A-7 Tianzhu West Road, Tianzhu Airport Industrial Zone A, Shunyi District, Beijing 101312, China.
E-mail: leesw19@naver.com

2021/0419: received 14 December 2021, revised 22 February 2022 and accepted 23 February 2022

doi:10.1111/lam.13683

Abstract

The purpose of this study was to evaluate the probiotic characteristics of *Lactiplantibacillus plantarum* HOM3204 isolated from homemade pickled cabbage and to examine its restoration effect on antibiotic-induced dysbiosis in mice. *Lact. plantarum* HOM3204 tolerated simulated gastric and intestinal juices with a 99·38% survival rate. It also showed strong adhesion ability (3·45%) to Caco-2 cells and excellent antimicrobial activity against foodborne pathogens *in vitro*. For safety (antibiotic susceptibility) of this strain, it was susceptible to all the tested seven antibiotics. *Lact. plantarum* HOM3204 had good stability during storage, especially in cold and frozen conditions. Furthermore, *Lact. plantarum* HOM3204 significantly restored the gut microbiota composition by increasing the abundance of Lactobacilli and Bifidobacteria and decreasing Enterococci, and improved antioxidative function by raising the concentrations of glutathione peroxidase (GSH-Px) and superoxide dismutase (SOD) in serum of antibiotic-induced dysbiosis in mice. These results suggest that *Lact. plantarum* HOM3204 could be a potential probiotic as a functional food ingredient.

Introduction

There are about 1000 species of bacteria in the human gut with 2000 genes per species, which yields about 2 000 000 genes (Qin *et al.* 2010). It is 100 times higher than human genes, which is approximately 20 000 (Moraes and Goes 2016). More and more studies on humans and germ-free mice showed the important roles of gut microbiota in physiology, metabolism, immunomodulation and protection against pathogens (Jandhyala *et al.* 2015; Andremont *et al.* 2021). The normal human gut microbiota is mainly composed of Bacteroidetes and Firmicutes. The microbial diversity and number gradually increase from birth, and start resembling the adult flora by the age of three (Nagpal *et al.* 2017). This dynamic microbiome exists throughout a person's life (Kundu *et al.* 2017). Recognition about the gut microbiota as another organ in the human body is gaining attention. Whereas, the 'balanced' microbiota is the most vulnerable organ to orally administered antibiotics, which involves substantial changes in the gut microbial community and consequent impacts on their metabolic capacity, and the

spread of antibiotic-resistant pathogenic bacteria (Duan *et al.* 2020). The effect of oral supplementation with probiotics and prebiotics to prevent and ameliorate antibiotic-induced gut dysbiosis was widely demonstrated in clinical studies (Andremont *et al.* 2021). Furthermore, faecal microbiota transplantation was highly beneficial in patients with serious antibiotic-related adverse effects in the gut, especially *Clostridium difficile* infection (Li *et al.* 2018).

Probiotics are defined as 'live microorganisms which when administered in adequate amounts confer a health benefit on the host' (FAO/WHO 2002). As probiotics, *Lactobacillus*, *Bifidobacterium* and *Saccharomyces* are used to prevent and treat gut dysbiosis, which effectively act via production of organic acids and antimicrobial compounds, improving gut barrier integrity, enzyme formation and immunomodulation (Sanders *et al.* 2019). In despite of its benefits, probiotic effects are strain-specific. The beneficial effects of probiotics relate to their physiological characteristics: gastrointestinal transit ability, intestinal epithelial cell adhesion ability and antimicrobial activity (Dunne *et al.* 2001; Rocha-Ramirez *et al.* 2021). Another important factor limiting the efficacy of probiotics is their survivability during processing and storage (Gonzalez-Ferrero *et al.* 2018).

Lactiplantibacillus plantarum (formerly *Lactobacillus plantarum*) exists predominantly in traditional fermented vegetables (Chiou *et al.* 2017). As a probiotic, *Lact. plantarum* has been isolated from and used in various foods fermentation, including meat, dairy products and plant-based products (Dicks *et al.* 2004; Mills *et al.* 2011; Oh *et al.* 2020). Several studies have reported that *Lact. plantarum* strains alleviate gut-related metabolic disorders such as obesity, diabetes and non-alcoholic fatty liver disease and modulate immune response (Yoo *et al.* 2013; Park *et al.* 2020; Kim *et al.* 2021).

In the present study, we determined the *in vitro* probiotic characteristics and survivability during storage and evaluated the restoration effect on ampicillin-induced dysbiosis in mice of *Lact. plantarum* HOM3204, which was isolated from homemade pickled cabbage. Organic acid contents in faeces and antioxidative enzyme concentration in blood were measured to correlate function of tested strain.

Results and discussion

Tolerance to the gastrointestinal tract conditions

The gastrointestinal tract environment is harsh: intragastric pH generally ranges from 1·6 to 4·5 during daytime and rises to 2·4–6·7 after a meal (McLauchlan *et al.* 1989; Koziolek *et al.* 2015); The concentration of bile acids in intestinal tract is 0·1–2% (Amara *et al.* 2019). The transit ability of *L. plantarum* HOM3204 and *Lactiplantibacillus rhamnosus* GG to artificial gastric acids and bile salts is shown in Table 1. The initial cell density in simulated gastric juice was 1×10^8 CFU per ml. *Lact. plantarum* HOM3204 and *Lact. rhamnosus* GG tolerated pH 3·0 for 3 h with 99·63 and 98·31% survival. The final survival rates of *Lact. plantarum* HOM3204 and *Lact. rhamnosus* GG following exposure to simulated gastric juices and simulated small intestinal juices were 99·38 and 90·96% after 24 h, respectively ($P < 0·05$). *Lact. plantarum* HOM3204 showed excellent gastrointestinal tract tolerance which is necessary for colonization to the gut and performing beneficial effects (Table 1).

Lact. plantarum uses several strategies to withstand the stress of acid and bile salt. Huang *et al.* (2016) showed that *Lact. plantarum* ZDY2013 can facilitate the expulsion of protons from intracellular environment to help maintain pH homeostasis. Zhou *et al.* (2019) demonstrated *Lact. plantarum* LP-115 can shift the fatty acid composition of the plasma membrane and induce the expression of a bile salt hydrolase gene to enhance bile tolerance.

Table 1 Tolerance to simulated gastric and intestinal juices and adhesion ability to Caco-2 cells

Treatment	*Lactiplantibacillus plantarum* HOM3204	*Lactiplantibacillus rhamnosus* GG
Tolerance to simulated gastric and intestinal juices (survival rate, %)		
pH 3·0, 3 h	99·63 ± 4·48	98·31 ± 3·83
0·3% bile salt, 3 h	98·50 ± 3·07	95·30 ± 2·15
0·3% bile salt, 21 h	99·38 ± 3·52*	90·96 ± 3·39
Adhesion ability to Caco-2 cells		
Adhesion percentage (%)	3·45 ± 0·38	3·84 ± 0·52
Adhesion index (CFU per cell)	4·14 ± 0·52	4·61 ± 0·41

Survival rate, determined viable cell count/initial viable cell count × 100%; Adhesion percentage, count of adhered bacteria/count of added bacteria × 100%; Adhesion index, count of adhered bacteria/count of Caco-2 cells (by well).
*$P < 0·05$ *vs Lact. rhamnosus* GG group.

Adhesion to Caco-2 cells

Adherence ability to enterocyte is one of the most important factors of potential probiotics. Long-term colonization in gastrointestinal tract entitles probiotics to confer persistent benefits on the host. *In vitro* assessments of bacterial adhesion are generally performed with Caco-2 or HT-29 cells. These studies showed that the adherent ability of *Lactobacillus* genus ranged from 2 to 10% (Van Tassell and Miller 2011). Surface layer proteins, hydrophobicity and electrostatic forces promote bacteria to colonize on gut epithelial cells (Gusils *et al.* 2002; Wakai *et al.* 2021). The adherence ability of *Lact. plantarum* HOM3204 was expressed by adhesion percentage and adhesion index (Table 1). The adhesion percentage of *Lact. plantarum* HOM3204 on Caco-2 cells after 2 h incubation was 3·45%. One Caco-2 cell was adhered by about four bacteria. It is like the commercial strain *Lact. rhamnosus* GG (3·84%), which is one of the most studied probiotics.

Antibiotic susceptibility

The assessment of antibiotic susceptibility is fundamental because probiotics might become a potential source for transferable antibiotic resistance genes, which can transfer to pathogens and gut microbiota in the gastrointestinal tract through horizontal spread (Daniali *et al.* 2020). The minimal inhibitory concentrations (MICs) of seven antibiotics against *Lact. plantarum* HOM3204 were determined: erythromycin 0·5 mg l^{-1}, chloramphenicol 8 mg l^{-1}, tetracycline 16 mg l^{-1}, gentamicin 8 mg l^{-1}, kanamycin 64 mg l^{-1}, ampicillin 1 mg l^{-1} and clindamycin 2 mg l^{-1}. All seven MICs against *Lact. plantarum* HOM3204 were lower than the cut-off values described in EFSA's guidance.

Antimicrobial activity

Antagonistic activity against potentially pathogenic microbes is also one of the important properties of probiotics (Shokryazdan *et al.* 2017). *Lact. plantarum* HOM3204 and *Lact. rhamnosus* GG showed antimicrobial activity against all five foodborne pathogens by presenting clear zone whose diameter were up to 17 mm (Table 2). The clear zone's diameter of *Escherichia coli* was even larger than 23 mm by *Lact. plantarum* HOM3204. The main mechanism of antimicrobial activities of lactic acid bacteria is the production of organic acids such as lactate and acetate. Also other strain-specific components, bacteriocin, exopolysaccharides and polyphenol possess inhibitory activity of pathogenic bacteria (Sharma *et al.* 2018). Further study needs to be carried out on the identification of antimicrobial substances produced by *Lact. plantarum* HOM3204.

Stability during storage at different conditions

A daily intake of at least 10^8–10^9 CFU probiotic bacteria could exert their effect in the human body (Terpou *et al.* 2019). With the extension of time, the survival rates of freeze-dried *Lact. plantarum* HOM3204 (1×10^{11} CFU per g) decreased (Fig. 1). At the end of the storage (6 months), the survival rates of *Lact. plantarum* HOM3204 were 92·39 and 89·57% at −20 and 4°C, respectively, with no significant difference between the two groups. However, the final survival rate significantly reduced to 75·06% at room temperature (25 ± 2°C, 60 ± 10% RH) compared with −20°C and 4°C ($P < 0·05$). Low-temperature storage was more suitable to maintain cell viability. The storage stability of freeze-dried *Lact. plantarum* HOM3204 could meet the requirements of commercialization. In addition, it is necessary to confirm the long-term storage stability of freeze-dried *Lact. plantarum* HOM3204. Preserving the viability of probiotic bacteria is a great challenge that needs to be addressed during the development of probiotic products. Resistance to negative environmental conditions such as heat, water activity, acidity and oxygen content during processing and storage should be a screening criterion for potential probiotics. Fermentation, lyophilization and packaging conditions are factors that also affect the survival of probiotic microorganisms in delivery vehicles (Gonzalez-Ferrero *et al.* 2018).

Restoration effects in antibiotic-induced dysbiosis

Some studies have shown that antibiotics treatment reduced the level of gut bacteria by 90% and decreased the microbial diversity by 25% (Panda *et al.* 2014). The

Table 2 Pathogenic bacteria inhibition ability of *Lactiplantibacillus plantarum* HOM3204

Strains	*Escherichia coli*	*Salmonella* Typhimurium	*Staphylococcus aureus*	*Listeria monocytogenes*	*Pseudomonas aeruginosa*
HOM3204	+++	++	++	++	++
GG	++	++	++	++	++

Indications of inhibition zone diameter: '–' no inhibition; '+' 11–16 mm; '++' 17–22 mm; '+++' ≥23 mm.

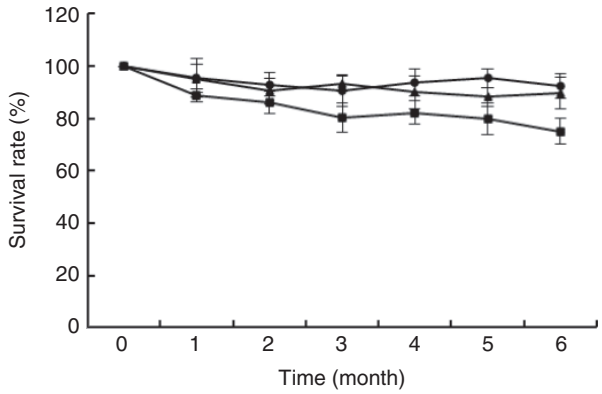

Figure 1 The stability of *Lactiplantibacillus plantarum* HOM3204 during storage at different temperature. Viable cell densities were checked monthly by plate counting. Temperature: (●) −20°C, (▲) 4°C, (■) 25°C. The survival rate was calculated by comparing the viable cell count at each check point with the initial viable cell count.

number of Lactobacilli and Bifidobacteria was greatly inhibited (Willing *et al.* 2011). Significant disruptions to the normal composition and function of the gut microbiome caused by antibiotics persist long after antibiotics are discontinued, and are associated with the development of obesity, asthma and inflammatory bowel disease (Mekonnen *et al.* 2020). Antibiotic treatment is also highly likely to cause antibiotic-associated diarrhoea in as many as 30% of patients (Szajewska *et al.* 2016). The interest in probiotic interventions is increasing, and the evidence for the effectiveness of probiotics in preventing or treating gut microbiome associated diseases is also increasing (Hempel *et al.* 2012). In our study, the viable cell densities of four main intestinal bacteria (Lactobacilli, Bifidobacteria, Enterobacteriaceae and Enterococci) decreased to >1000 CFU per g faeces after 2 weeks of ampicillin gavage. Viable cell densities of four main intestinal bacteria after 4-week probiotics treatment are shown in Fig. 2. *Lact. plantarum* HOM3204 treatment increased the abundance of Lactobacilli and Bifidobacteria significantly ($P < 0{\cdot}05$). In the model group, the abundance of Lactobacilli and Bifidobacteria were not restored to the level of control group. *Lact. plantarum* HOM3204 treatment also decreased the abundance of Enterococci at the end of experiment ($P < 0{\cdot}05$) obviously. No significant differences were observed on the viable count of Enterobacteriaceae among three groups. *Lact. plantarum* HOM3204 intervention resulted in restoring the damage of gut microbiota caused by antibiotic treatment to normal condition.

Antibiotic-induced alteration of intestinal flora also results in significant changes to gut metabolomes (Theriot *et al.* 2014; Jump *et al.* 2017; Patton *et al.* 2020). One of the most important changes is the reduction in short-chain fatty acids (SCFAs) levels. SCFAs, mainly such as acetate, propionate and butyrate, are essential for the health and well-being of the host (Poelaert *et al.* 2019). It has been estimated that approximately 10% of the human daily caloric requirement is provided by SCFAs (Mekonnen *et al.* 2020). Probiotics could directly produce SCFAs or produce organic acids such as lactate and acetate to lower colonic pH and oxygen levels for the proliferation of other SCFAs producing bacteria (Louis and Flint 2017). In our study, the treatment of ampicillin altered the metabolic profile of organic acids (Fig. 3a–d). After 4 weeks of natural recovery, the concentration of lactate and acetate in faecal samples were still significantly lower than the control group. The administration of *Lact. plantarum* HOM3204 elevated the levels of lactate and acetate to normal level. The levels of propionate and butyrate among three groups were similar ($P > 0{\cdot}05$).

Another change along with antibiotics exposure is the rise in reactive oxygen species (ROS) level which leads to damage of cells (Karaman *et al.* 2019). High levels of ROS are usually found in patients who have diseases associated with unbalance of redox system, such as metabolic diseases, neurodegenerative diseases, autoimmune rheumatic diseases and periodontitis (Tune *et al.* 1989; Boia *et al.* 2019). Administration of probiotics could directly remove ROS and elevate the concentrations of antioxidant enzymes (Sannasimuthu *et al.* 2020; Wang *et al.* 2021). To investigate the restoring effect of probiotics on the oxidative stress in ampicillin-induced dysbiosis, two antioxidant enzymes (GSH-Px and SOD) were measured in serum samples as shown in Fig. 3e,f. The concentration of GSH-Px in the model group was remarkably lower than the control group ($P < 0{\cdot}01$). *Lact. plantarum* HOM3204 treatment significantly improved the deficiency of GSH-Px caused by ampicillin use ($P < 0{\cdot}01$) and increased the level of SOD in serum.

There are numerous studies demonstrating that administration of *Lact. plantarum* can have a beneficial impact on human gut health. In a randomized, double-blind, placebo-controlled study, 4-week administration of *Lact. plantarum* 299v significantly improved abdominal pain and bloating in patients with irritable bowel syndrome (Ducrotte *et al.* 2012). Panigrahi *et al.* (2017) assessed the efficacy of an oral synbiotic preparation (*Lact. plantarum* ATCC 202195 plus fructooligosaccharide) on preventing infant sepsis in rural Indian. Seven-day treatment with synbiotic preparation ($n = 2278$) significantly reduced neonatal sepsis and death (42%) compared with the placebo arm ($n = 2278$). The biological and functional characteristics of *Lact. plantarum* make it an ideal probiotic candidate.

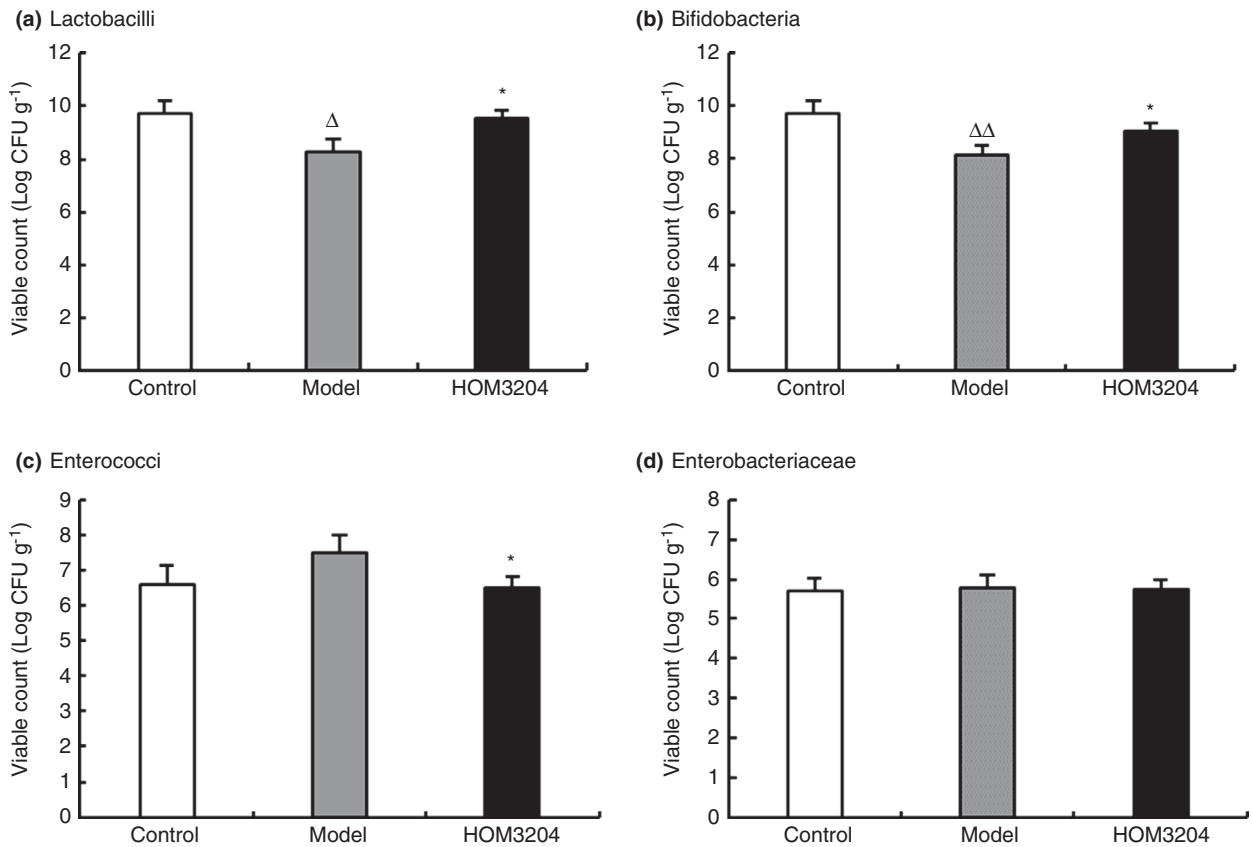

Figure 2 The restorative effect of *Lactiplantibacillus plantarum* HOM3204 following ampicillin-induced dysbiosis via gut microbiota. Control group: daily gavage of 0·9% saline for 6 weeks. Model group: daily gavage of ampicillin (500 mg kg^{-1} body weight) for 2 weeks and daily gavage of 0·9% saline for another 4 weeks. HOM3204 group: daily gavage of ampicillin (500 mg kg^{-1} body weight) for 2 weeks and then daily gavage of *Lact. plantarum* HOM3204 (1×10^9 CFU) for 4 weeks. The abundance of Lactobacilli (a), Bifidobacteria (b), Enterococci (c), and Enterobacteriaceae (d) in faeces were measured by selective agars. *$P < 0{\cdot}05$ *vs* model group, $\Delta P < 0{\cdot}05$ *vs* control group, $\Delta\Delta P < 0{\cdot}01$ *vs* control group.

In conclusion, *Lact. plantarum* HOM3204 significantly increased the abundance of Lactobacilli and Bifidobacteria and decreased the density of Enterococci in faeces, elevated the levels of lactate and acetate in faeces and raised the concentrations of GSH-Px and SOD in serum on antibiotic-induced dysbiosis in mice. *Lact. plantarum* HOM3204 significantly restored the gut microbiota composition and improved antioxidative function in mice with antibiotics induced dysbiosis. It could be a potential probiotic as a functional food ingredient.

Materials and methods

Bacterial strains and growth conditions

Lact. plantarum HOM3204 was isolated from homemade pickled cabbage and deposited in China General Microbiological Culture Collection Center (CGMCC No. 18760). *Lacticaseibacillus rhamnosus* GG (ATCC 53103) and *Lacticaseibacillus casei* ATCC 334 were purchased as reference strains from American Type Culture Collection (ATCC). The indicator strains used in antimicrobial activity assays included *Escherichia coli* ATCC 8739, *Staphylococcus aureus* ATCC 6538, *Salmonella* Typhimurium ATCC 14028, *Pseudomonas aeruginosa* ATCC 9027 and *Listeria monocytogenes* ATCC 19111 were purchased from ATCC. Lactobacilli were cultured in De Man, Rogosa and Sharpe broth (MRS, Oxiod, UK) at 37°C for 20 h aerobically. Antimicrobial indicator strains were cultured in tryptone soya broth (TSB, Oxiod, UK) at 37°C for 20 h aerobically.

Preparation of freeze-dried *Lact. plantarum* HOM3204 bacterial powder

Lact. plantarum HOM3204 was fermented in MRS broth with a 5l fermentation tank (BIOSTAT Bplus, Sartorius

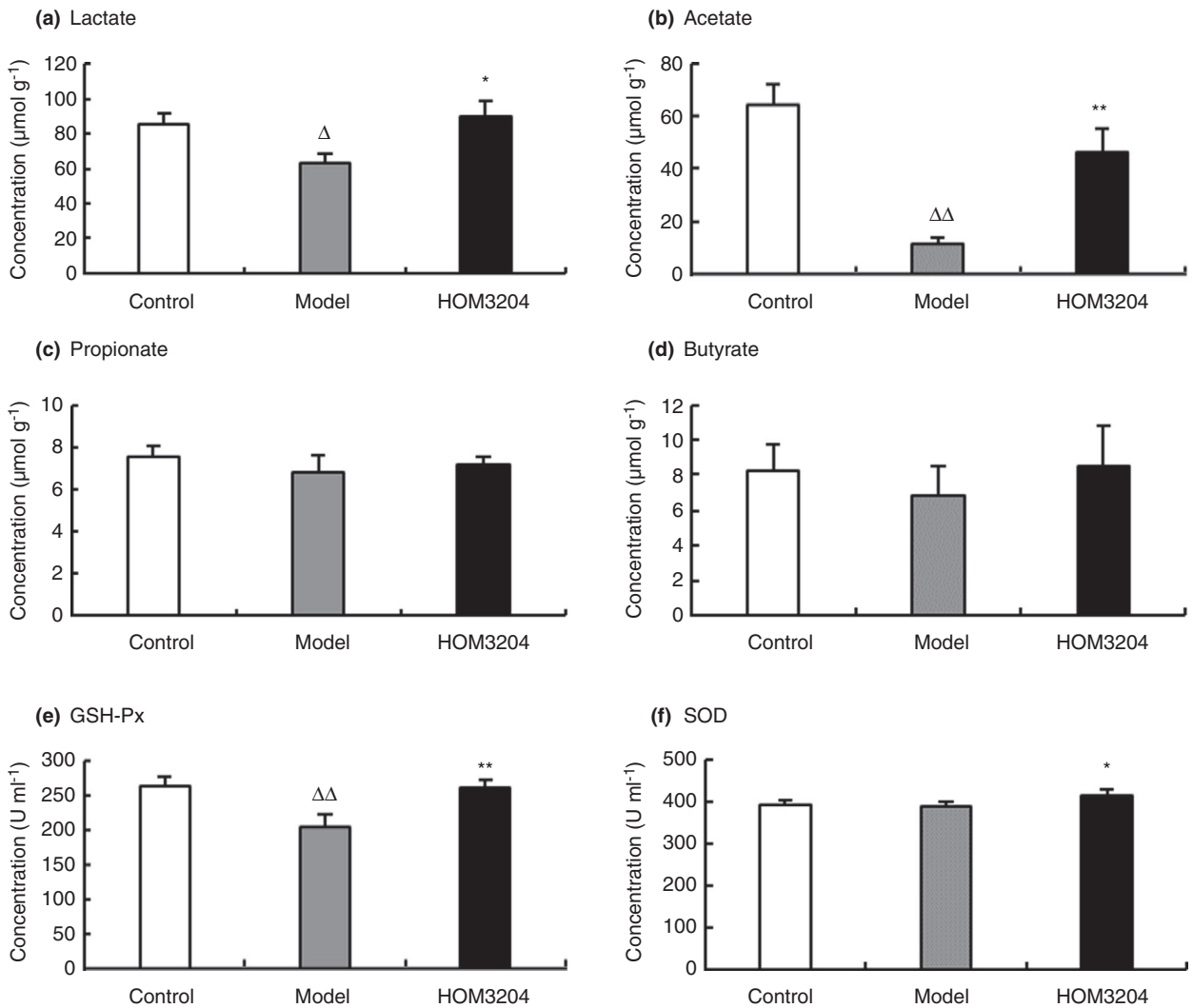

Figure 3 The restoration effect of *Lactiplantibacillus plantarum* HOM3204 after ampicillin-induced dysbiosis on the selected four organic acids and antioxidation. The levels of lactate (a), acetate (b), propionate (c), and butyrate (d) in faeces were measured by gas chromatography. The concentration of GSH-Px (e), and SOD (f) in serum were measured by using commercial kits. *$P < 0{\cdot}05$ *vs* model group, $\Delta P < 0{\cdot}05$ *vs* control group, $\Delta\Delta P < 0{\cdot}01$ *vs* control group.

Stedim, Germany) at a temperature of 37°C with constant pH control at 5·8 and stirring speed of 100 rpm for 16 h. Cell pellet was washed twice with distilled sterile water to remove the exhausted medium and resuspended in 10% reconstituted skimmed milk. After 24 h of freeze-drying, the bacterial cake was ground into powder. Viable cell density of freeze-dried bacterial powder was measured with using MRS agar plate.

Tolerance to the gastrointestinal tract conditions

Tolerance to the gastrointestinal tract conditions was evaluated with simulated gastric and intestinal juices according to the method of Grispoldi *et al.* (2020), with some modifications. One gram of freeze-dried bacterial powder was suspended in 99 ml of MRS broth. Pepsin (Sigma-Aldrich, Saint Louis, MO, USA) was suspended in distilled water at 1 g l^{-1} and the pH was adjusted to 3·0 with HCl to simulate gastric juice. One hundred microliter of MRS-bacteria suspension above was inoculated into 9·9 ml of simulated gastric juice and incubated at 37°C for 3 h. Trypsin (Sigma-Aldrich, Saint Louis, MO, USA) and bile salt (Thermo Fisher Scientific, UK) were suspended in 6·8 g l^{-1} of KH_2PO_4 solution at 10 g l^{-1} and 3 g l^{-1}, respectively. pH was adjusted to 6·8 to simulate small intestinal juice. After 3 h incubation in

simulated gastric juice, bacteria were centrifuged and resuspended in 10 ml of simulated small intestinal juice and incubated at 37°C for 21 h sequentially. The survival rates were determined by comparing the viable cell counts at 3, 6 and 24 h with the initial viable cell count.

Adhesion to Caco-2 cells

The adhesion ability was determined according to the method of Crociani *et al.* (1995), with some modifications. Caco-2 cells were obtained from Cell Bank of the Chinese Academy of Sciences and cultured in Dulbecco's Modified Eagle Medium (DMEM, Thermo Fisher Scientific, UK) supplemented with 10% foetal bovine serum and 1% streptomycin/penicillin solution at 37°C with 5% CO_2. Caco-2 cells at 1×10^5 cells per ml were seeded onto a 24-well cell culture plate and incubated at 37°C for 24 h. The bacterial cells cultured in MRS were harvested by centrifugation and washed three times with sterile phosphate-buffered saline (PBS) and inoculated as 5×10^7 CFU per ml onto the wells with fresh DMEM. After 2 h incubation, the cells were washed three times with sterile PBS to remove non-adhesive bacteria. Adherent bacteria were treated with trypsin-EDTA solution and counted by using MRS plates. The adhesion percentage was calculated as the count of adhered bacteria to the count of added bacteria. The adhesion index was expressed as bacteria per Caco-2 cell.

Antibiotic susceptibility

Antibiotic susceptibility was measured according to the European Food Safety Authority (EFSA) technical guidance and ISO (the International Organization for Standardization) 10932:2010 (EFSA 2012; ISO 10932:2010). Glycerol stocks of *Lact. plantarum* HOM3204 and *Lact. casei* ATCC 334 (as reference) were activated twice in MRS media respectively. Seven antibiotics including erythromycin, chloramphenicol, tetracycline, gentamicin, kanamycin, ampicillin and clindamycin were tested.

Antimicrobial activity

The antimicrobial activity was evaluated by growth inhibition of foodborne pathogens such as *E. coli* ATCC 8739, *Staph. aureus* ATCC 6538, *Salm*. Typhimurium ATCC 14028, *Ps. aeruginosa* ATCC 9027 and *L. monocytogenes* ATCC 19111 by using the well diffusion method (Tejero-Sarinena *et al.* 2012). To evaluate antimicrobial activity, 20 ml of tryptone soya agar (TSA, Oxiod, UK) at about 45°C were mixed with 1 ml of an indicator strain (10^6–10^7 CFU per l) and poured into Petri dish. The agar was left for 30 min at room temperature and three separate 10-mm diameter wells punched into the agar with a sterile metal cylinder. 150 µl of overnight cultured probiotic were dispensed into each well and diffused at 4°C for 2 h, then incubated at 37°C for 12 h. Antimicrobial activity was determined as the diameter (mm) of clear zone around the well.

Stability during storage at different conditions

The lyophilized bacterial powder was packaged into aluminium foil bags and stored at −20°C, 4°C in refrigerator and 25°C with 60% relative humidity for 6 months. Viable cell density was measured regularly by MRS agar after 10-fold dilution series were made with saline. The survival rate was calculated by comparing the viable cell count at each month with the initial viable cell count.

Restoration effects in antibiotic-induced dysbiosis

Eight-week-old male BALB / c mice with body weight 18 to 22 g were purchased from Beijing Vital River Laboratory Animal Technology Co. Ltd (China), and fed distilled water and standard laboratory chow *ad libitum*. The experimental protocol was approved by the Ethics Committee of Beijing Hanmi pharmaceutical Co. Ltd (HM-19032). After 1 week of acclimation, mice were randomly divided into three groups (eight animals/group; one cage/group). Control group: daily gavage of 0·9% saline for 6 weeks. Model group: daily gavage of ampicillin (500 mg kg^{-1} body weight) for 2 weeks and daily gavage of 0·9% saline for another 4 weeks. HOM3204 group: daily gavage of ampicillin (500 mg kg^{-1} body weight) for 2 weeks (Shi *et al.* 2018) and then daily gavage of lyophilized *Lact. plantarum* HOM3204 powder dissolved in saline (1×10^9 CFU per day) for 4 weeks.

Fresh faecal samples were collected 24 h after final gavage and 10-fold dilution series were made with saline to count viable cell densities of 4 kinds of main intestinal bacteria. Selective media used for Lactobacilli was *Lactobacillus* selective (LBs) agar plate added with 50 mg l^{-1} of Nalidixic acid, for Bifidobacteria was blood-liver (BL) agar plate supplemented with 50 mg l^{-1} of Mupirocin, for Enterobacteriaceae was eosin methylene blue agar plate and for Enterococci was bile esculin azide agar plate (Delgado *et al.* 2006; Xia *et al.* 2019). All selective media were purchased from Beijing Landbridge Co. Ltd (China). Bifidobacteria were incubated anaerobically at 37°C for 48 h. The other three bacteria were incubated aerobically at 37°C for 24 h. The typical colonies were selected for microscopic examination after Gram's staining and 16S rRNA gene identification (3500 Genetic Analyzer, Life Technology Corporation). Results were expressed as log10 colony forming units per gram of fresh faeces. Organic acids

(lactate, acetate, propionate, and butyrate) in faeces were measured with gas chromatography (Agilent, America) using column DB-23 (Arrieta *et al.* 2018). The concentrations of glutathione peroxidase (GSH-Px) and SOD were measured from eyeball blood samples using commercial kits (Nanjing Jiancheng Bioengineering institute, China).

Statistical analysis

All experiments were repeated at least triplicate and represented as mean ± standard error of the mean (SEM). Data were analysed using one-way analysis of variance (ANOVA), followed by Tukey's multiple comparisons test in Graphpad Prism 5. Values were considered significant at $P < 0{\cdot}05$.

Author contributions

S.L., S.Z. and C.L. contributed to the experiment design and interpreted all the results. T.W., X.W. and Z.Z. performed animal related experiments. D.Z. and X.W. performed probiotic characterization in vitro tests. S.Z. performed statistical analysis and wrote the manuscript. S.L. and C.L. edited the manuscript. All authors read and approved the final manuscript.

Conflict of Interest

The authors have no conflict of interest to declare.

Data availability statement

The data used to support the findings of this study are available from the corresponding author upon request.

References

Amara, S., Bourlieu, C., Humbert, L., Rainteau, D. and Carriere, F. (2019) Variations in gastrointestinal lipases, pH and bile acid levels with food intake, age and diseases: possible impact on oral lipid-based drug delivery systems. *Adv Drug Deliv Rev* **142**, 3–15.

Andremont, A., Cervesi, J., Bandinelli, P.A., Vitry, F. and De Gunzburg, J. (2021) Spare and repair the gut microbiota from antibiotic-induced dysbiosis: state-of-the-art. *Drug Discov Today* **26**, 2159–2163.

Arrieta, M.-C., Arévalo, A., Stiemsma, L., Dimitriu, P., Chico, M.E., Loor, S., Vaca, M., Boutin, R.C.T. *et al.* (2018) Associations between infant fungal and bacterial dysbiosis and childhood atopic wheeze in a nonindustrialized setting. *J Allergy Clin Immun* **142**, 1–21.

Boia, S., Boariu, M., Baderca, F., Rusu, D., Muntean, D., Horhat, F., Boia, E.-R., Borza, C. *et al.* (2019) Clinical, microbiological and oxidative stress evaluation of periodontitis patients treated with two regimens of systemic antibiotics, adjunctive to non-surgical therapy: a placebo-controlled randomized clinical trial. *Exp Ther Med* **18**, 5001–5015.

Chiou, T.Y., Suda, W., Oshima, K., Hattori, M. and Takahashi, T. (2017) Changes in the bacterial community in the fermentation process of koso, a Japanese sugar-vegetable fermented beverage. *Biosci Biotechnol Biochem* **81**, 403–410.

Crociani, J., Grill, J.P., Huppert, M. and Ballongue, J. (1995) Adhesion of different bifidobacteria strains to human enterocyte-like Caco-2 cells and comparison with in vivo study. *Lett Appl Microbiol* **21**, 146–148.

Daniali, M., Nikfar, S. and Abdollahi, M. (2020) Antibiotic resistance propagation through probiotics. *Expert Opin Drug Metab Toxicol* **16**, 1207–1215.

Delgado, S., Suarez, A. and Mayo, B. (2006) Identification of dominant bacteria in feces and colonic mucosa from healthy Spanish adults by culturing and by 16S rDNA sequence analysis. *Digest Dis Sci* **51**, 744–751.

Dicks, L.M., Mellett, F.D. and Hoffman, L.C. (2004) Use of bacteriocin-producing starter cultures of *Lactobacillus plantarum* and *Lactobacillus curvatus* in production of ostrich meat salami. *Meat Sci* **66**, 703–708.

Duan, H., Yu, L., Tian, F., Zhai, Q., Fan, L. and Chen, W. (2020) Antibiotic-induced gut dysbiosis and barrier disruption and the potential protective strategies. *Crit Rev Food Sci* **60**, 1–26.

Ducrotte, P., Sawant, P. and Jayanthi, V. (2012) Clinical trial: *Lactobacillus plantarum* 299v (DSM 9843) improves symptoms of irritable bowel syndrome. *World J Gastroenterol* **18**, 4012–4018.

Dunne, C., O'Mahony, L., Murphy, L., Thornton, G., Morrissey, D., O'Halloran, S., Feeney, M., Flynn, S. *et al.* (2001) In vitro selection criteria for probiotic bacteria of human origin: correlation with in vivo findings. *Am J Clin Nutr* **73**, 386S–392S.

EFSA (2012) Guidance on the assessment of bacterial susceptibility to antimicrobials of human and veterinary importance. *ESFA Journal* **10**, 1–8.

FAO/WHO (2002) *Working group report on drafting guidelines for the evaluation of probiotics in food*. Ontario, London: Food and Agriculture Organization of the United Nations/ World Health Organization.

Gonzalez-Ferrero, C., Irache, J.M. and Gonzalez-Navarro, C.J. (2018) Soybean protein-based microparticles for oral delivery of probiotics with improved stability during storage and gut resistance. *Food Chem* **239**, 879–888.

Grispoldi, L., Giglietti, R., Traina, G. and Cenci-Goga, B. (2020) How to assess in vitro probiotic viability and the correct use of neutralizing agents. *Front Microbiol* **11**, 1–9.

Gusils, C., Cuozzo, S., Sesma, F. and Gonzalez, S. (2002) Examination of adhesive determinants in three species of *Lactobacillus* isolated from chicken. *Can J Microbiol* **48**, 34–42.

Hempel, S., Newberry, S.J., Maher, A.R., Wang, Z., Miles, J.N., Shanman, R., Johnsen, B. and Shekelle, P.G. (2012) Probiotics for the prevention and treatment of antibiotic-associated diarrhea: a systematic review and meta-analysis. *JAMA* **307**, 1959–1969.

Huang, R., Pan, M., Wan, C., Shah, N.P., Tao, X. and Wei, H. (2016) Physiological and transcriptional responses and cross protection of *Lactobacillus plantarum* ZDY2013 under acid stress. *J Dairy Sci* **99**, 1002–1010.

ISO. (10932:2010). Milk and milk products-determination of the minimal inhibitory concentration (MIC) of antibiotics applicable to Bifidobacteria and non-Enterococcal lactic acid bacteria (LAB). *ISO.10932:2010 (IDF 223:2010)*, Geneve, Switzerland, 2010.

Jandhyala, S.M., Talukdar, R., Subramanyam, C., Vuyyuru, H., Sasikala, M. and Nageshwar Reddy, D. (2015) Role of the normal gut microbiota. *World J Gastroenterol* **21**, 8787–8803.

Jump, R.L., Kraft, D., Hurless, K., Polinkovsky, A. and Donskey, C.J. (2017) Impact of tigecycline versus other antibiotics on the fecal metabolome and on colonization resistance to *Clostridium difficile* in mice. *Pathog Immun* **2**, 1–20.

Karaman, M., Budak, H. and Ciftci, M. (2019) Amoxicillin and gentamicin antibiotics treatment adversely influence the fertility and morphology through decreasing the Dazl gene expression level and increasing the oxidative stress. *Arch Physiol Biochem* **125**, 447–455.

Kim, K.J., Paik, H.D. and Kim, J.Y. (2021) Immune-enhancing effects of *Lactobacillus plantarum* 200655 isolated from Korean Kimchi in a cyclophosphamide-induced immunocompromised mouse model. *J Microbiol Biotechnol* **31**, 726–732.

Koziolek, M., Schneider, F., Grimm, M., Modebeta, C., Seekamp, A., Roustom, T., Siegmund, W. and Weitschies, W. (2015) Intragastric pH and pressure profiles after intake of the high-caloric, high-fat meal as used for food effect studies. *J Control Release* **220**, 71–78.

Kundu, P., Blacher, E., Elinav, E. and Pettersson, S. (2017) Our gut microbiome: the evolving inner self. *Cell* **171**, 1481–1493.

Li, X., Gao, X., Hu, H., Xiao, Y., Li, D., Yu, G., Yu, D., Zhang, T. *et al.* (2018) Clinical efficacy and microbiome changes following fecal microbiota transplantation in children with recurrent *Clostridium difficile* infection. *Front Microbiol* **9**, 1–8.

Louis, P. and Flint, H.J. (2017) Formation of propionate and butyrate by the human colonic microbiota. *Environ Microbiol* **19**, 29–41.

Mclauchlan, G., Fullarton, G.M., Crean, G.P. and Mccoll, K.E. (1989) Comparison of gastric body and antral pH: a 24 hour ambulatory study in healthy volunteers. *Gut* **30**, 573–578.

Mekonnen, S.A., Merenstein, D., Fraser, C.M. and Marco, M.L. (2020) Molecular mechanisms of probiotic prevention of antibiotic-associated diarrhea. *Curr Opin Biotechnol* **61**, 226–234.

Mills, S., Serrano, LMariela, Griffin, C., O'Connor, P.M., Schaad, G., Bruining, C., Hill, C., Ross, RPaul *et al.* (2011) Inhibitory activity of *Lactobacillus plantarum* LMG P-26358 against *Listeria innocua* when used as an adjunct starter in the manufacture of cheese. *Microb Cell Fact* **10** (Suppl 1), 1–11.

Moraes, F. and Goes, A. (2016) A decade of human genome project conclusion: scientific diffusion about our genome knowledge. *Biochem Mol Biol Educ* **44**, 215–223.

Nagpal, R., Tsuji, H., Takahashi, T., Nomoto, K., Kawashima, K., Nagata, S. and Yamashiro, Y. (2017) Ontogenesis of the gut microbiota composition in healthy, full-term, vaginally born and breast-fed infants over the first 3 years of life: a quantitative bird's-eye view. *Front Microbiol* **8**, 1–9.

Oh, Y.J., Kim, T.S., Moon, H.W., Lee, S.Y., Lee, S.Y., Ji, G.E. and Hwang, K.T. (2020) *Lactobacillus plantarum* PMO 08 as a probiotic starter culture for plant-based fermented beverages. *Molecules* **25**, 1–13.

Panda, S., El khader, I., Casellas, F., López Vivancos, J., García Cors, M., Santiago, A., Cuenca, S., Guarner, F. *et al.* (2014) Short-term effect of antibiotics on human gut microbiota. *PLoS One* **9**, 1–7.

Panigrahi, P., Parida, S., Nanda, N.C., Satpathy, R., Pradhan, L., Chandel, D.S., Baccaglini, L., Mohapatra, A. *et al.* (2017) A randomized synbiotic trial to prevent sepsis among infants in rural India. *Nature* **548**, 407–412.

Park, E.J., Lee, Y.S., Kim, S.M., Park, G.S., Lee, Y.H., Jeong, D.Y., Kang, J. and Lee, H.J. (2020) Beneficial effects of *Lactobacillus plantarum* strains on non-alcoholic fatty liver disease in high fat/high fructose diet-fed rats. *Nutrients* **12**, 1–16.

Patton, L., Li, N., Garrett, T.J., Ruoss, J.L., Russell, J.T., de la Cruz, D., Bazacliu, C., Polin, R.A. *et al.* (2020) Antibiotics effects on the fecal metabolome in preterm infants. *Metabolites* **10**, 1–14.

Poelaert, K.C.K., Van Cleemput, J., Laval, K., Descamps, S., Favoreel, H.W. and Nauwynck, H.J. (2019) Beyond gut instinct: metabolic short-chain fatty acids moderate the pathogenesis of alphaherpesviruses. *Front Microbiol* **10**, 1–16.

Qin, J., Li, R., Raes, J., Arumugam, M., Burgdorf, K.S., Manichanh, C., Nielsen, T., Pons, N. *et al.* (2010) A human gut microbial gene catalogue established by metagenomic sequencing. *Nature* **464**, 59–65.

Rocha-Ramirez, L.M., Hernandez-Chinas, U., Moreno-Guerrero, S.S., Ramirez-Pacheco, A. and Eslava, C.A. (2021) Probiotic properties and immunomodulatory activity of *Lactobacillus* strains isolated from dairy products. *Microorganisms* **9**, 1–15.

Sanders, M.E., Merenstein, D.J., Reid, G., Gibson, G.R. and Rastall, R.A. (2019) Probiotics and prebiotics in intestinal health and disease: from biology to the clinic. *Nat Rev Gastroenterol Hepatol* **16**, 605–616.

Sannasimuthu, A., Sharma, D., Paray, B.A., Al-Sadoon, M.K. and Arockiaraj, J. (2020) Intracellular oxidative damage due to antibiotics on gut bacteria reduced by glutathione oxidoreductase-derived antioxidant molecule GM15. *Arch Microbiol* **202**, 1127–1133.

Sharma, V., Harjai, K. and Shukla, G. (2018) Effect of bacteriocin and exopolysaccharides isolated from probiotic on *P. aeruginosa* PAO1 biofilm. *Folia Microbiol (Praha)* **63**, 181–190.

Shi, Y., Kellingray, L., Le Gall, G., Zhao, J., Zhang, H., Narbad, A., Zhai, Q. and Chen, W. (2018) The divergent restoration effects of *Lactobacillus* strains in antibiotic-induced dysbiosis. *J Funct Foods* **51**, 142–152.

Shokryazdan, P., Faseleh Jahromi, M., Liang, J.B. and Ho, Y.W. (2017) Probiotics: from isolation to application. *J Am Coll Nutr* **36**, 666–676.

Szajewska, H., Canani, R.B., Guarino, A., Hojsak, I., Indrio, F., Kolacek, S., Orel, R., Shamir, R. *et al.* (2016) Probiotics for the prevention of antibiotic-associated diarrhea in children. *J Pediatr Gastroenterol Nutr* **62**, 495–506.

Tejero-Sarinena, S., Barlow, J., Costabile, A., Gibson, G.R. and Rowland, I. (2012) In vitro evaluation of the antimicrobial activity of a range of probiotics against pathogens: evidence for the effects of organic acids. *Anaerobe* **18**, 530–538.

Terpou, A., Papadaki, A., Lappa, I.K., Kachrimanidou, V., Bosnea, L.A. and Kopsahelis, N. (2019) Probiotics in food systems: significance and emerging strategies towards improved viability and delivery of enhanced beneficial value. *Nutrients* **11**, 1–32.

Theriot, C.M., Koenigsknecht, M.J., Carlson, P.E., Hatton, G.E., Nelson, A.M., Li, B.O., Huffnagle, G.B., Z. Li, J. *et al.* (2014) Antibiotic-induced shifts in the mouse gut microbiome and metabolome increase susceptibility to *Clostridium difficile* infection. *Nat Commun* **5**, 1–10.

Tune, B.M., Fravert, D. and Hsu, C.Y. (1989) Oxidative and mitochondrial toxic effects of cephalosporin antibiotics in the kidney. A comparative study of cephaloridine and cephaloglycin. *Biochem Pharmacol* **38**, 795–802.

Van Tassell, M.L. and Miller, M.J. (2011) *Lactobacillus* adhesion to mucus. *Nutrients* **3**, 613–636.

Wakai, T., Kano, C., Karsens, H., Kok, J. and Yamamoto, N. (2021) Functional role of surface layer proteins of *Lactobacillus acidophilus* L-92 in stress tolerance and binding to host cell proteins. *Biosci Microbiota Food Health* **40**, 33–42.

Wang, Y., Heng, C., Zhou, X., Cao, G., Jiang, L., Wang, J., Li, K., Wang, D. *et al.* (2021) Supplemental *Bacillus subtilis* DSM 29784 and enzymes, alone or in combination, as alternatives for antibiotics to improve growth performance, digestive enzyme activity, anti-oxidative status, immune response and the intestinal barrier of broiler chickens. *Br J Nutr* **125**, 494–507.

Willing, B.P., Russell, S.L. and Finlay, B.B. (2011) Shifting the balance: antibiotic effects on host-microbiota mutualism. *Nat Rev Microbiol* **9**, 233–243.

Xia, Y., Kuda, T., Toyama, A., Goto, M., Fukunaga, M., Takahashi, H. and Kimura, B. (2019) Detection and isolation of bacteria affected by dietary cumin, coriander, turmeric, and red chili pepper in the caecum of ICR mice. *J Func Foods* **61**, 1–8.

Yoo, S.R., Kim, Y.J., Park, D.Y., Jung, U.J., Jeon, S.M., Ahn, Y.T., Huh, C.S., Mcgregor, R. *et al.* (2013) Probiotics *L. plantarum* and *L. curvatus* in combination alter hepatic lipid metabolism and suppress diet-induced obesity. *Obesity (Silver Spring)* **21**, 2571–2578.

Zhou, Y., Wang, J.Q., Hu, C.H., Ren, L.Q., Wang, D.C. and Ye, B.C. (2019) Enhancement of bile resistance by maltodextrin supplementation in *Lactobacillus plantarum* Lp-115. *J Appl Microbiol* **126**, 1551–1557.