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Lactobacillus gasseri LA39 promotes hepatic primary bile acid biosynthesis and intestinal secondary bile acid biotransformation

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Abstract: A growing body of evidence has linked the gut microbiota to liver metabolism. The manipulation of intestinal microflora has been considered as a promising avenue to promote liver health. However, the effects of *Lactobacillus gasseri* LA39, a potential probiotic, on liver metabolism remain unclear. Accumulating studies have investigated the proteomic profile for mining the host biological events affected by microbes, and used the germ-free (GF) mouse model to evaluate host-microbe interaction. Here, we explored the effects of *L. gasseri* LA39 gavage on the protein expression profiles of the liver of GF mice. Our results showed that a total of 128 proteins were upregulated, whereas a total of 123 proteins were downregulated by treatment with *L. gasseri* LA39. Further bioinformatics analyses suggested that the primary bile acid (BA) biosynthesis pathway in the liver was activated by *L. gasseri* LA39. Three differentially expressed proteins (cytochrome P450 family 27 subfamily A member 1 (CYP27A1), cytochrome P450 family 7 subfamily B member 1 (CYP7B1), and cytochrome P450 family 8 subfamily B member 1 (CYP8B1)) involved in the primary BA biosynthesis pathway were further validated by western blot assay. In addition, targeted metabolomic analyses demonstrated that serum and fecal β-muricholic acid (a primary BA), dehydrolithocholic acid (a secondary BA), and glycolithocholic acid-3-sulfate (a secondary BA) were significantly increased by *L. gasseri* LA39. Thus, our data revealed that *L. gasseri* LA39 activates the hepatic primary BA biosynthesis and promotes the intestinal secondary BA biotransformation. Based on these findings, we suggest that *L. gasseri* LA39 confers an important function in the gut–liver axis through regulating BA metabolism.

Key words: *Lactobacillus gasseri* LA39; Liver; Isobaric tags for relative and absolute quantitation (iTRAQ); Bile acid; Germ-free mice

1 Introduction

An increasing number of studies have established a relationship between gut microbiota and host physiology (Tremaroli and Bäckhed, 2012; Sommer and Bäckhed, 2013; Marchesi et al., 2016). The manipulation of gut microbiota has become a promising avenue to mediate host health (Lemon et al., 2012; Bajaj et al., 2022). As a vital organ, important metabolic functions are conferred on the liver, including nutrition, synthesis, and detoxification (Thomson and Knolle, 2010). Recent studies have revealed the important functions of the gut–liver axis in mammals (Silveira et al., 2022). Gut pathogen overload may play a role in non-alcoholic fatty liver disease (NAFLD) and liver inflammation (Scott, 2017; Ringseis et al., 2020). However, the liver also has a regulatory role in gut microbiota through the production of metabolites, such as bile acid (BA) (Brandl et al., 2017). The use of probiotics has been considered as a potential strategy to prevent various hepatic diseases (Maslennikov et al., 2021). Thus, mining the gut microbes that regulate host liver metabolism and deciphering the underlying mechanism can provide important insights into the gut–liver axis.

Several studies have suggested that members of the *Lactobacillus* genus exert potential beneficial functions on host health (Bernardeau et al., 2006; Lebeer

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et al., 2008; Kleerebezem and Vaughan, 2009; Selle and Klaenhammer, 2013; van Baarlen et al., 2013; Liu et al., 2021). This genus belongs to the typical probiotics that play important roles in nutrition metabolism (Ramakrishna, 2013), host immune responses (Ubeda and Pamer, 2012; Rooks and Garrett, 2016), and intestinal barrier maintenance (Natividad and Verdu, 2013). Our previous studies have demonstrated that *Lactobacillus gasseri* LA39 possesses important roles in promoting intestinal epithelial oxidative phosphorylation (Hu et al., 2018a) and preventing diarrhea (Hu et al., 2018b). A previous study showed that *L. gasseri* LA39 produced gassericin A bacteriocin, which is resistant to several pathogens (Kawai et al., 1994, 2001). However, the effect of *L. gasseri* LA39 on host liver metabolism has not been studied. Thus, dissecting the function of this strain in host liver metabolism can contribute to uncovering the mechanism of interplay between the host and *L. gasseri* LA39.

Germ-free (GF) mice are free from microbes (Bhattarai and Kashyap, 2016) and have been widely employed to investigate the interaction between host and microbiota (Yi and Li, 2012; Grover and Kashyap, 2014). In this work, considering the important function of the gut–liver axis in mammals (Tripathi et al., 2018; Tilg et al., 2022) and the good applicability of proteomics in mining the host potential biological events affected by microbes (Wright, 2018; Zhou et al., 2019; Zoued et al., 2021; Yan et al., 2022), we analyzed the hepatic proteomic profiles of GF mice after *L. gasseri* LA39 gavage. Specifically, we transferred the *L. gasseri* LA39 to GF mice by oral gavage and then explored the effect of *L. gasseri* LA39 on the hepatic protein expression profiles using the strategy of isobaric tags for relative and absolute quantification (iTRAQ). Our data showed that the primary BA biosynthesis pathway in the liver was activated by treatment with *L. gasseri* LA39. Additional targeted metabolomic analyses indicated that serum and fecal β-muricholic acid (a primary BA), dehydrolithocholic acid (a secondary BA), and glycolithocholic acid-3-sulfate (a secondary BA) were significantly increased after oral gavage of *L. gasseri* LA39. These results revealed the functions of *L. gasseri* LA39 in host BA metabolism and provide insights into its roles in the gut–liver axis.

2 Materials and methods

2.1 Bacteria

The facultative anaerobe *L. gasseri* LA39 strain was isolated from the feces of piglets as previously described (Hu et al., 2018b) and cultured in de Man, Rogosa, and Sharpe (MRS) medium at 37 ℃ under anaerobic conditions. An optical microscope combined with methylene blue staining was used to count the number of *L. gasseri* LA39.

2.2 Experimental animals

A total of 12 GF male mice (eight weeks of age) with similar body weight were mixed and then randomly divided into two groups (control (Ctrl) group and *L. gasseri* LA39 (LG) group, n=6 each). The mean body weight in the Ctrl group and the LG group was (36.07±1.87) g and (35.57±1.29) g, respectively. There was no significant difference in mean body weight between the two groups (P=0.8299, Student's t-test). Mice in the LG group were orally gavaged with 200 μL phosphate-buffered saline (PBS) suspension containing the *L. gasseri* LA39 (1×10^8 CFU/mL, where CFU is colony forming units) every other day, while mice in the Ctrl group were orally gavaged with 200 μL sterile PBS. After two weeks, all mice were sacrificed and the liver, serum, feces, and intestinal samples were collected.

2.3 Procedures for proteomics based on iTRAQ

The procedures for the iTRAQ analysis were described previously in detail (Hu et al., 2018a; Nie et al., 2019). Briefly, lysis buffer combined with magnetic beads and TissueLyser was used to extract the hepatic proteins. After centrifugation, the supernatant was successively treated with dithiothreitol and iodoacetamide. Trypsin gold was used to digest the protein solution. The peptides were then desalted and dissolved in 0.5 mol/L tetraethylammonium bromide and labeled with iTRAQ reagent kits. The peptides were fractionated on the LC-20AB system (Shimadzu, Kyoto, Japan) and then vacuum-dried. Subsequently, the obtained peptides were redissolved and then treated using the LC-20AD system (Shimadzu). Finally, the peptides were ionized through a nano-electrospray ionization (nano-ESI) resource and loaded into a Q-Exactive mass spectrometer for data detection.

2.4 Proteomic data analyses

The raw data of iTRAQ were changed into the Mascot generic format (MGF). Mascot software (Matrix Science, London, UK) was used to run against the Uniprot (*Mus musculus*) database for protein identification. The quantification of iTRAQ data was conducted using the iQuant software (BGI, Shenzhen, China). Proteins with fold change of more than 1.2 or less than 0.83 and *q*-value of less than 0.05 in at least two replicates were defined as the differentially expressed proteins (DEPs), as described previously (Zhu et al., 2009; Huang et al., 2015; Hu et al., 2018a). WoLF PSORT software (Computational Biology Research Center, Tokyo, Japan) was employed to predict the subcellular localization of proteins. The Cluster of Orthologous Groups (COG) functional analysis was performed based on the COG database. The STRING database was used to analyze the protein–protein interaction (PPI). The Blast2GO software (BioBam, Valencia, Spain) was utilized for Gene Ontology (GO) analysis, including biological process and molecular function classifications. The pathway analysis was performed based on the Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway database.

2.5 Western blot

The total proteins of the livers of GF mice were extracted with the radioimmunoprecipitation assay (RIPA) protein lysis buffer (Sangon Biotech, C500005, Shanghai, China). The detailed procedures for western blot were previously described by Hu et al. (2017). The antibodies used were as follows: cytochrome P450 family 27 subfamily A member 1 (CYP27A1, Proteintech, 14739-1-AP, Chicago, USA), cytochrome P450 family 7 subfamily B member 1 (CYP7B1, Proteintech, 24889-1-AP), cytochrome P450 family 8 subfamily B member 1 (CYP8B1, Absin, abs135064, Shanghai, China), β-actin antibody (Sigma-Aldrich, A5441, St. Louis, USA), and horseradish peroxidase (HRP)-conjugated secondary antibodies (Cell signaling technology, 7074S and 7076S, Danvers, MA, USA).

2.6 Targeted metabolomic analyses of bile acids

The serum and fecal BAs were detected based on the AB SCIEX QTRAP 6500 liquid chromatography-tandem mass spectrometry (LC-MS/MS) platform. Briefly, 200 μL of methanol and 10 μL of internal standard mixed solution (1 μg/mL) were added into 50 μL of serum samples or 20 mg of fecal samples. The proteins were precipitated by refrigeration at −20 °C for 10 min. After centrifugation (12 000 r/min) for 10 min at 4 °C, the supernatant was obtained and then evaporated to dryness. The extracts were reconstituted in 100 μL of 50% (volume fraction) methanol for further LC-MS/MS. The high-performance liquid chromatography (HPLC) conditions mainly included the following parameters: column, C18 (100 mm×2.1 mm, i.d., 1.8 μm); solvent system, double-distilled water (ddH_2O) with 0.01% (volume fraction) acetic acid and 5 mmol/L ammonium acetate (A), acetonitrile with 0.01% acetic acid (B); flow rate, 0.35 mL/min; column temperature, 40 °C; sample size, 3 μL. The ESI-MS/MS conditions included the following main parameters: ESI source temperature, 550 °C; voltage, −4500 V; curtain gas, 35 psi (1 psi=6.895 kPa). Multiple reaction monitoring was conducted to quantify the BAs. The MS data were processed using the Analyst software (AB SCIEX, Framingham, MA, USA) and MultiQuant software (AB SCIEX). The standard curves of BAs were drawn based on the concentration ratio (external standard/internal standard) and peak area ratio (external standard/internal standard). The contents of BAs were calculated based on the standard curves. A total of 50 BA standards were purchased from Toronto Research Chemicals (TRC, Canada), Zzstandard (Shanghai, China), ANPEL (Shanghai, China), IsoReag (Shanghai, China), CMASS (Shanghai, China), and Boc Sciences (Shanghai, China) (the detailed information was shown in Table S1). Orthogonal partial least squares-discriminant analysis (OPLS-DA) was carried out using the package "MetaboAnalystR" in R software. The heatmap and violin plot were drawn by R software.

2.7 Hematoxylin-eosin staining

Hematoxylin-eosin (HE) staining was used to analyze the intestinal morphologic characteristics. The detailed experiments and analyses were performed according to previously described protocols (Hu et al., 2017). The villi heights and crypts depths in the duodenum, jejunum, and ileum were individually measured. Representative images at 40×, 100×, and 200× magnifications for intestinal tissues were obtained using a light microscope.

2.8 Statistical analyses

For Student's *t*-tests, a *P*-value of <0.05 was defined as statistically significant. For two-way analysis of variance (ANOVA), a *P*-value of <0.05 was defined as statistically significant. The DEPs were defined with the fold change of more than 1.2 or less than 0.83 and a *q*-value of less than 0.05 in at least two replicates, as described previously (Zhu et al., 2009; Huang et al., 2015; Hu et al., 2018a). The differential BA metabolites between the two groups were identified using the criteria of variable importance in projection (VIP)≥1 by OPLS-DA, absolute $\log_2$(fold change)≥1, and *P*-value<0.05 by Student's *t*-test.

3 Results

3.1 Identification of DEPs in the liver of GF mice induced by *L. gasseri* LA39

A growing body of evidence has revealed the important role of the gut–liver axis in mammals (Tripathi et al., 2018; Tilg et al., 2022), and proteomics have been commonly utilized in dissecting host–microbe interactions (Wright, 2018; Zhou et al., 2019; Zoued et al., 2021; Yan et al., 2022). GF mice provide a useful model to investigate the interplay between the host and microbiota (Yi and Li, 2012; Grover and Kashyap, 2014). To evaluate the roles of *L. gasseri* LA39 in the liver of GF mice, we administered *L. gasseri* LA39 to the GF mice by oral gavage. The iTRAQ strategy was used to analyze the profiles of hepatic protein expression in GF mice (Fig. 1a). From the results of three biological replicates, a total of 4761, 4793, and 4850 proteins were quantified in each replicate (Fig. 1b). In total, 128 proteins were upregulated and 123 proteins were downregulated by treatment with *L. gasseri* LA39 (Fig. 1c). The heatmap of DEPs was shown in Fig. 1d and the detailed information for DEPs was shown in Data S1.

3.2 Subcellular localization, COG function annotation, and PPI network analyses of the DEPs induced by *L. gasseri* LA39

We next used the WoLF PSORT software to analyze the subcellular localization of DEPs induced by *L. gasseri* LA39 and assessed their organelle specificity. The results showed that the DEPs were mainly localized in the cytosol, extracellular matrix, mitochondria, endoplasmic reticulum, nucleus, and plasma membrane (Fig. 2a). The results of COG function annotation showed that the DEPs were mainly annotated these COG functions, such as "secondary metabolites biosynthesis, transport and catabolism," "lipid transport and metabolism," "signal transduction mechanisms," and "posttranslational modification, protein turnover, chaperones" (Fig. 2b). The PPI network analysis based on the STRING database indicated that, among these 251 DEPs, a total of 70 proteins (including 37 upregulated proteins and 33 downregulated proteins) were shown in the Cytoscape (Fig. 3). The PPI network data suggested that these 70 DEPs might confer biological functions through interacting with each other to form complexes.

3.3 Biological process and molecular function analyses of the DEPs induced by *L. gasseri* LA39

Subsequently, we performed GO function annotation to dissect the potential role of DEPs induced by *L. gasseri* LA39. The biological processes of the DEPs were mainly enriched in the terms involved in lipid metabolism, such as "arachidonic acid metabolic process," "long-chain fatty acid metabolic process," "unsaturated fatty acid metabolic process," "icosanoid metabolic process," "fatty acid derivative metabolic process," and "fatty acid metabolic process" (Fig. 4a). The molecular functions of DEPs were mainly enriched in terms such as "pheromone activity," "steroid hydroxylase activity," "arachidonic acid monooxygenase activity," and "oxidoreductase activity" (Fig. 4b). These results of GO annotation suggested that hepatic lipid metabolism in GF mice may be regulated by treatment with *L. gasseri* LA39.

3.4 Activation of primary bile acid biosynthesis pathway in the liver of GF mice by *L. gasseri* LA39

We further analyzed the KEGG pathways to evaluate the potential functions of *L. gasseri* LA39 on cell metabolism in the liver of GF mice. The top 20 enriched KEGG pathways, such as "primary bile acid biosynthesis," "drug metabolism cytochrome P450," "retinol metabolism," "phenylalanine metabolism," "arachidonic acid metabolism," and "complement and coagulation cascades" were shown in Fig. 5a. Interestingly, "primary bile acid biosynthesis" was the most enriched pathway with the DEPs (Fig. 5a).

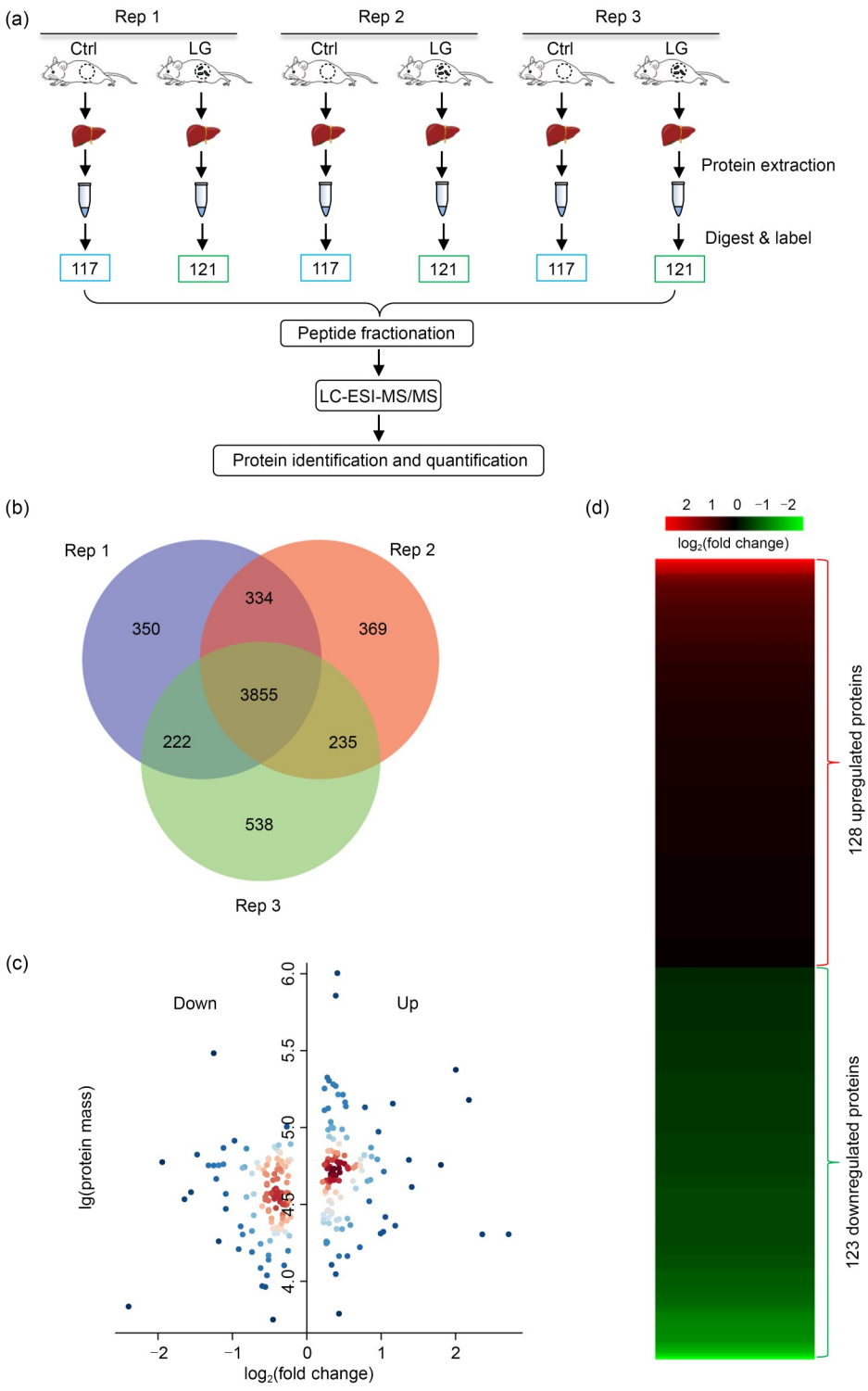

Fig. 1 Proteomics analysis of protein expression profiles in the liver of germ-free (GF) mice using the isobaric tags for relative and absolute quantitation (iTRAQ) strategy. (a) Schematic workflow of proteomics analysis based on the iTRAQ strategy (Rep, replicate; Ctrl, control; LG, *Lactobacillus gasseri* LA39; LC-ESI-MS/MS, liquid chromatography-tandem mass spectrometry). (b) Venn diagram of quantified proteins for three biological replicates. (c) Scatter diagram of the differentially expressed proteins (DEPs). (d) Heatmap of the DEPs. The colors represent the mean iTRAQ ratio (LG/Ctrl, fold change) of three replicates. Proteins with fold change of more than 1.2 or less than 0.83 and *q*-value of less than 0.05 in at least two replicates were defined as DEPs.

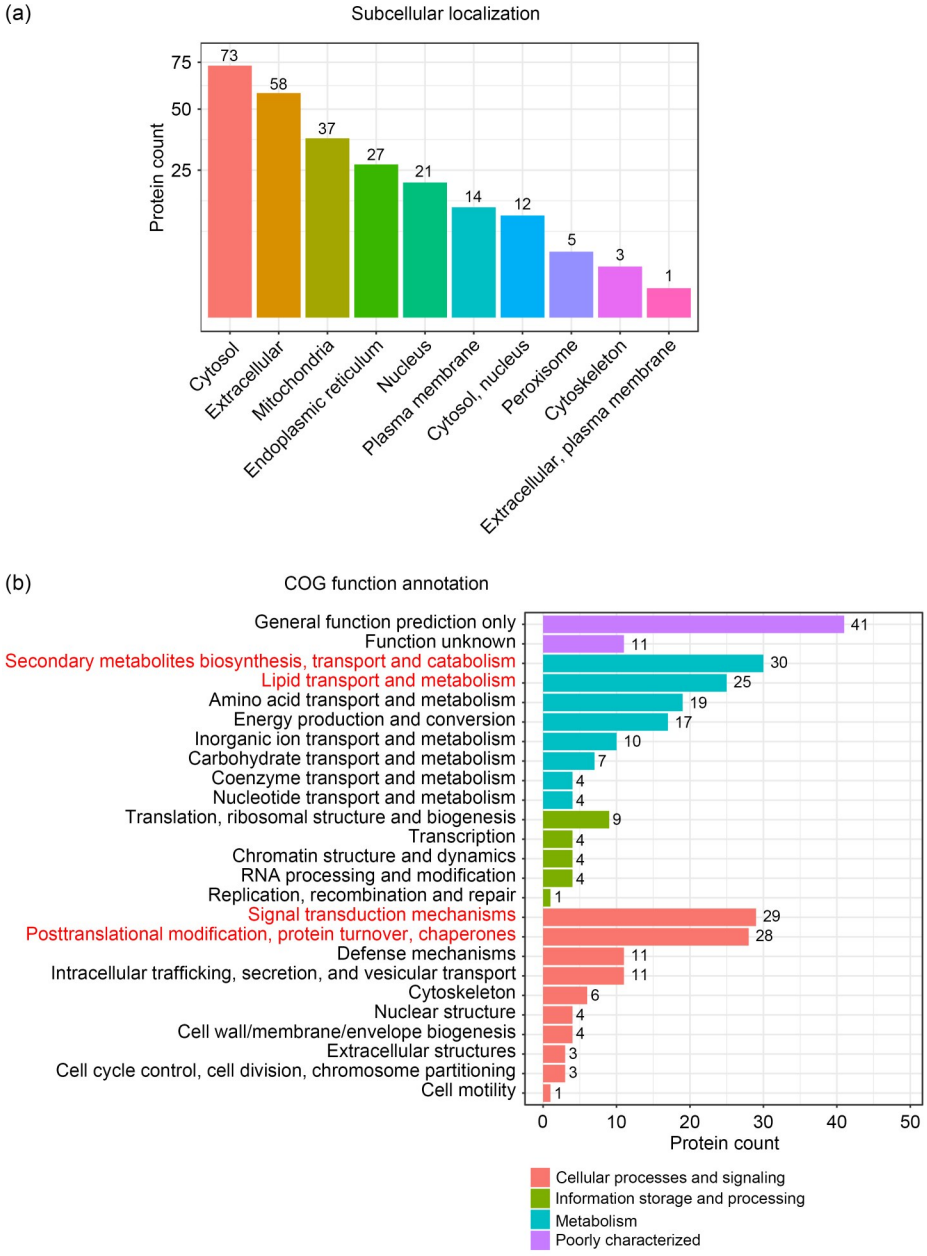

Fig. 2 Subcellular localization (a) and Cluster of Orthologous Groups (COG) function annotation (b) analyses of differentially expressed proteins (DEPs) in the liver of germ-free (GF) mice induced by *Lactobacillus gasseri* LA39.

A total of five proteins (including CYP27A1, CYP7B1, CYP8B1, α-methylacyl-CoA racemase (AMACR), and sterol carrier protein 2 (SCP2)) involved in the primary BA biosynthesis pathway were upregulated by treatment with *L. gasseri* LA39 (Fig. 5b). At the same time, a total of two proteins (including CYP46A1 and aldo-keto reductase family 1 member D1 (AKR1D1)) involved in the primary BA biosynthesis pathway were downregulated by treatment with *L. gasseri* LA39 (Fig. 5b).

We next used western blot assay to further evaluate the levels of three representative DEPs (including CYP27A1, CYP7B1, and CYP8B1). Our results demonstrated that the protein levels of CYP27A1, CYP7B1 and CYP8B1 were significantly increased by treatment with *L. gasseri* LA39 and these data were consistent with the results of iTRAQ analyses (Figs. 5c–5f). Thus, these data suggested that the primary BA biosynthesis pathway in the liver of GF mice was activated by *L. gasseri* LA39.

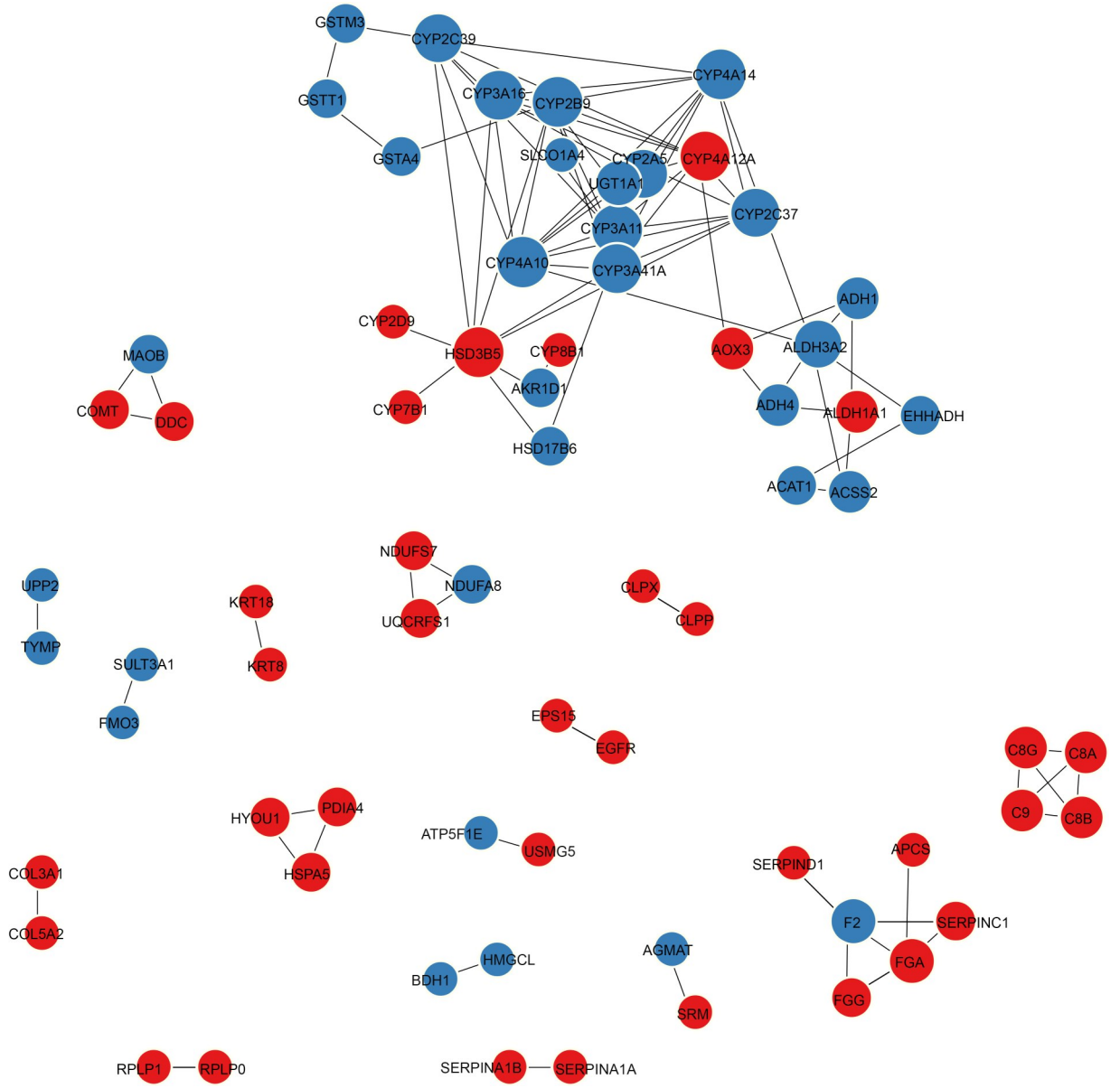

Fig. 3 Protein–protein interaction (PPI) networks of differentially expressed proteins (DEPs) in the liver of germ-free (GF) mice induced by *Lactobacillus gasseri* LA39. The red and blue nodes represent the upregulated and downregulated proteins, respectively. The protein names were shown in the nodes.

3.5 Promotion of hepatic primary bile acid biosynthesis and intestinal secondary bile acid biotransformation by *L. gasseri* LA39

We used BAs-targeted metabolomics to investigate the serum BA profiles in GF mice to further confirm that the primary BA biosynthesis in the liver of GF mice was activated by *L. gasseri* LA39. The results of OPLS-DA showed an obvious distinction between the Ctrl group and the LG group, suggesting that treatment with *L. gasseri* LA39 had an obvious effect on the profiles of serum BAs in GF mice (Fig. 6a). Further heatmap analysis indicated that a total of 17 BAs were identified in the serum of GF mice (Fig. 6b). Among them, the level of serum β-muricholic acid (a primary BA) was significantly increased under treatment with *L. gasseri* LA39 (Fig. 6c). Thus, our data revealed that the primary BA biosynthesis in the liver of GF mice was promoted in response to treatment with *L. gasseri* LA39.

Interestingly, the results of targeted metabolomics demonstrated that the levels of two secondary BAs

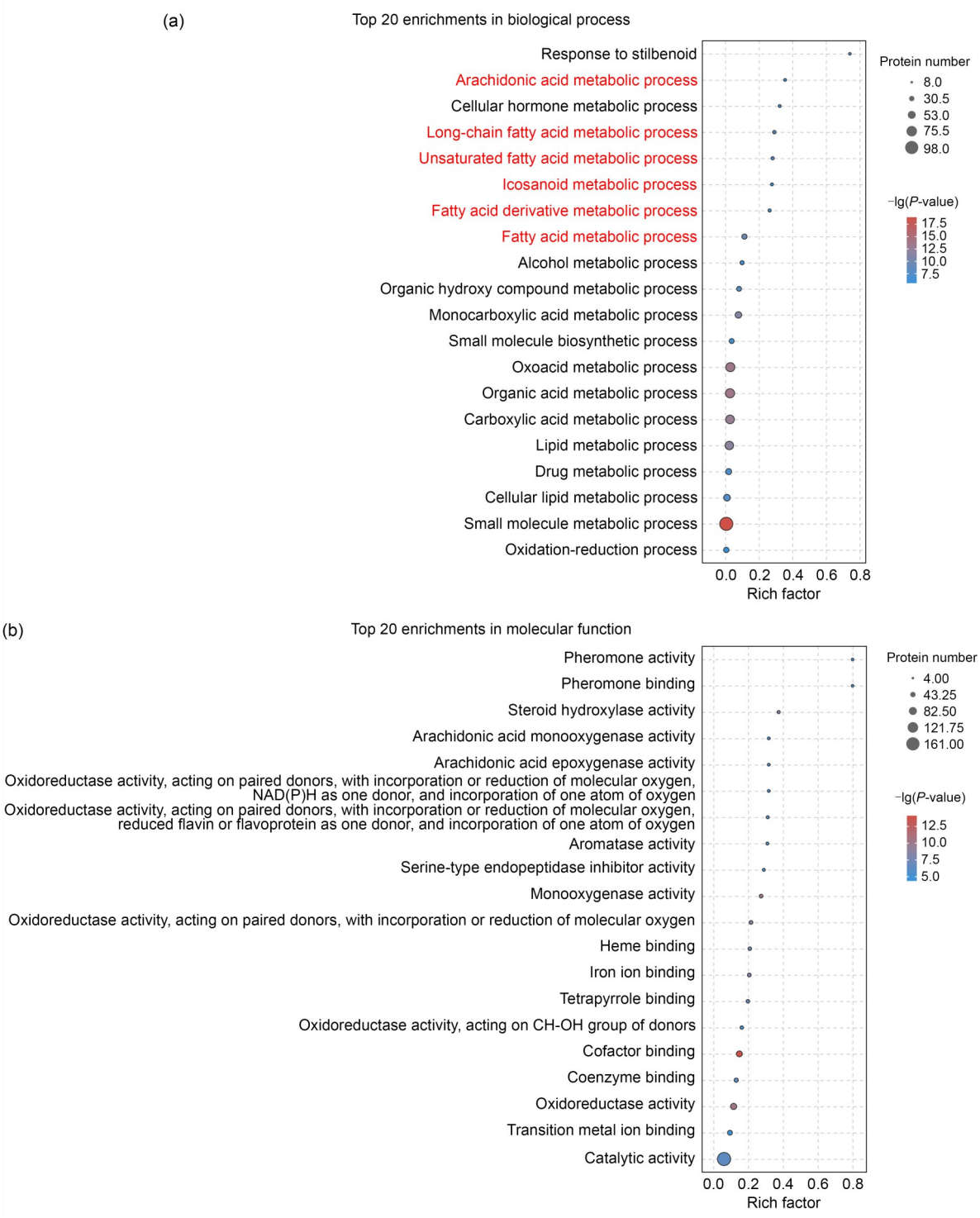

Fig. 4 Biological process and molecular function enrichment analyses of differentially expressed proteins (DEPs) in the liver of germ-free (GF) mice induced by *Lactobacillus gasseri* LA39. (a) The top 20 enriched biological processes for DEPs. (b) The top 20 enriched molecular functions for DEPs.

(including dehydrolithocholic acid and glycolithocholic acid-3-sulfate) in the serum were also significantly up-regulated by treatment with *L. gasseri* LA39 (Figs. 6d and 6e), suggesting that secondary BA biotransformation was enhanced by *L. gasseri* LA39. It is known that the processes of biotransformation from primary

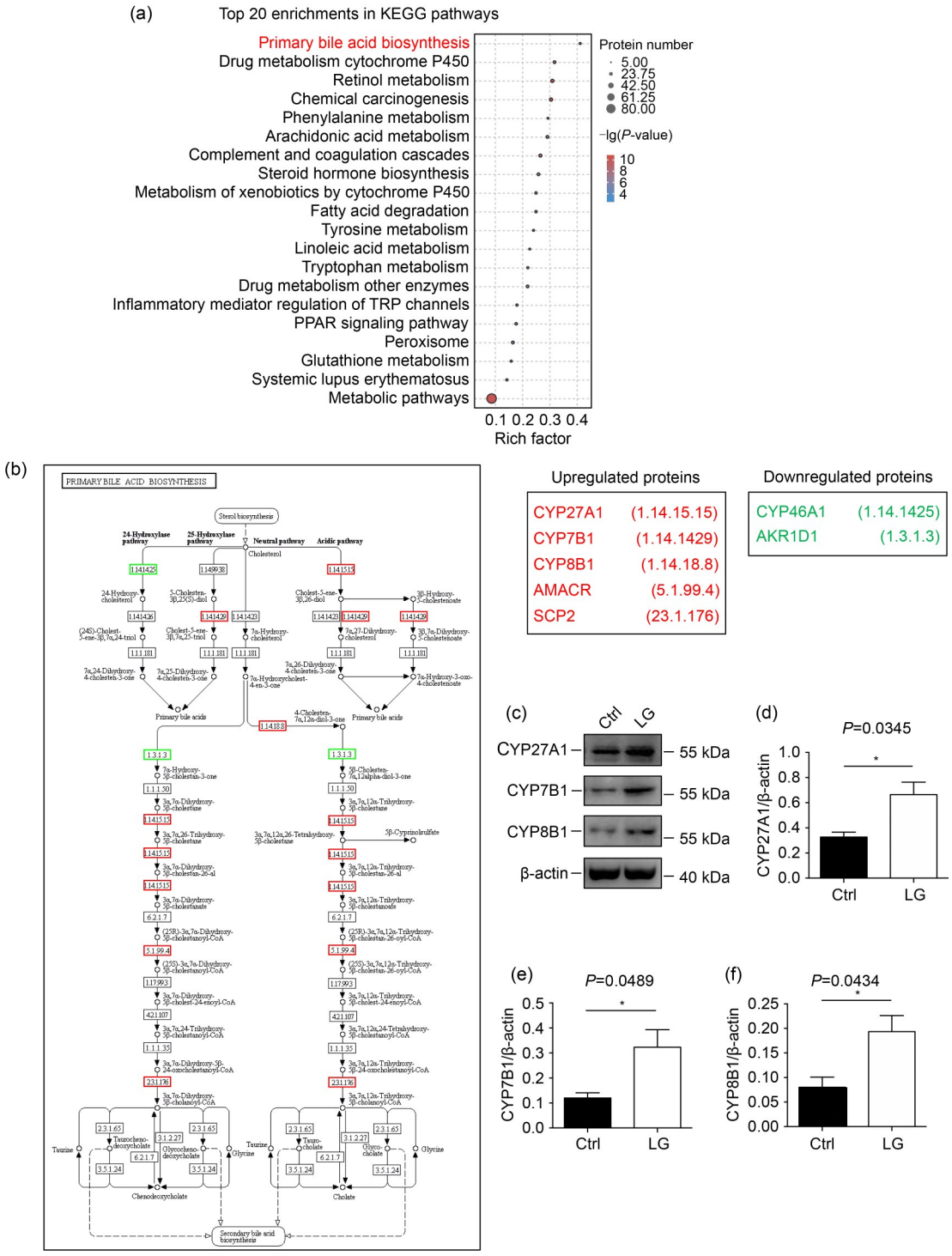

Fig. 5 Primary bile acid (BA) biosynthesis pathway in the liver of germ-free (GF) mice activated by *Lactobacillus gasseri* LA39. (a) The top 20 enriched Kyoto Encyclopedia of Genes and Genomes (KEGG) pathways for differentially expressed proteins (DEPs). (b) DEPs in the primary BA biosynthesis pathway. The red box shows that the corresponding proteins were upregulated by *L. gasseri* LA39, and the green box indicates that they were downregulated by *L. gasseri* LA39. (c) Representative western blots of several proteins, including cytochrome P450 family 27 subfamily A member 1 (CYP27A1), CYP7B1, CYP8B1, and β-actin (Ctrl, control; LG, *L. gasseri* LA39). (d–f) Quantitation of CYP27A1 (d), CYP7B1 (e), and CYP8B1 (f) levels normalized to β-actin levels. All data are presented as mean±standard error of the mean (SEM), (n=3). * P<0.05 evaluated using Student's t-test.

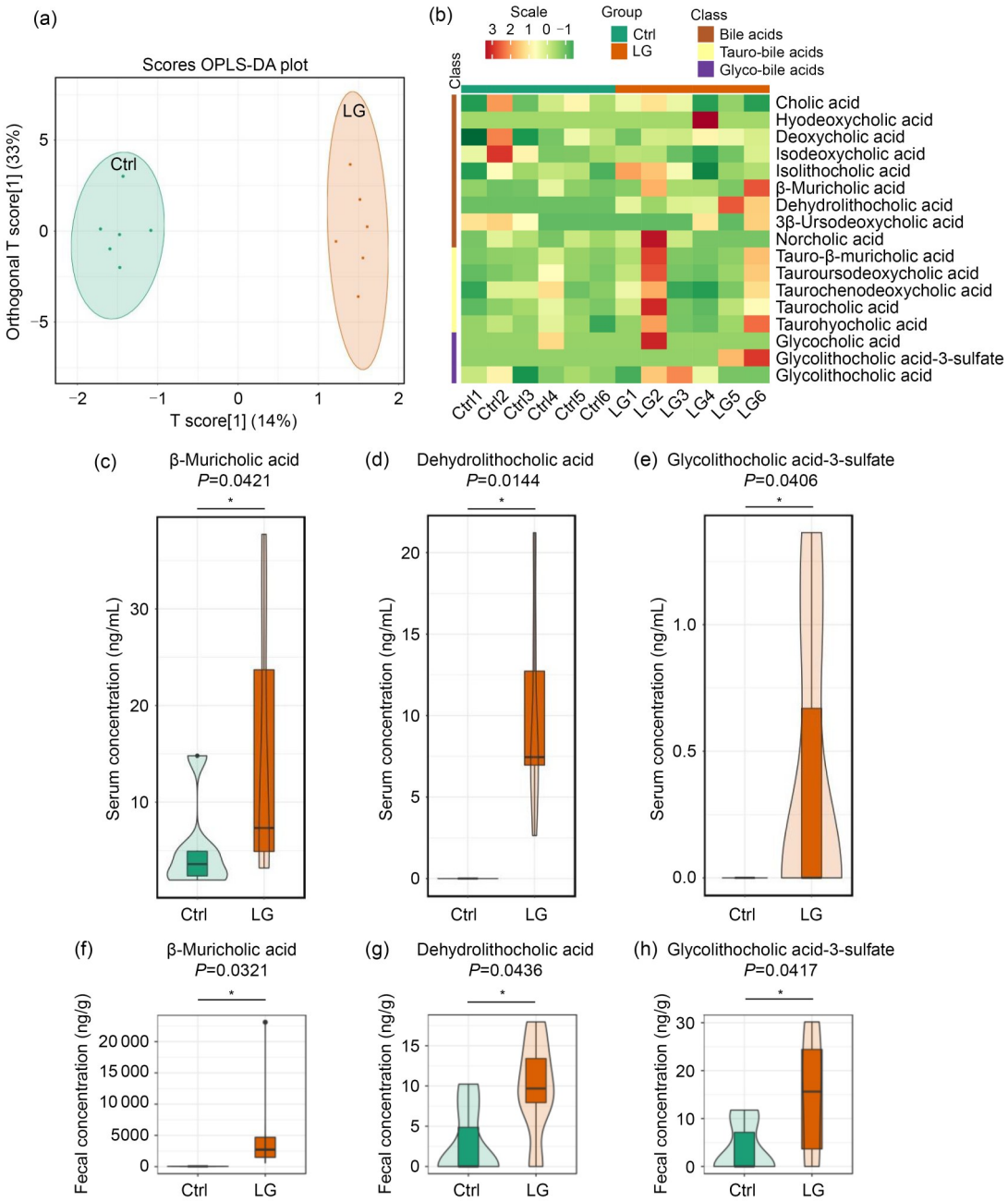

Fig. 6 Targeted metabolomic analysis of serum and fecal bile acid (BA) profiles in germ-free (GF) mice. (a) The orthogonal partial least squares-discriminant analysis (OPLS-DA) of serum BA profiles (Ctrl, control; LG, *Lactobacillus gasseri* LA39). (b) Heatmap analysis of serum BAs identified by targeted metabolomic analysis. (c–e) Comparison analyses of serum β-muricholic acid (c), dehydrolithocholic acid (d), and glycolithocholic acid-3-sulfate (e) levels between the Ctrl and LG groups (n=6). (f–h) Comparison analyses of fecal β-muricholic acid (f), dehydrolithocholic acid (g), and glycolithocholic acid-3-sulfate (h) levels between the Ctrl and LG groups (n=6). The differential metabolites between the two groups were identified using the criteria of variable importance in projection (VIP)≥1 by OPLS-DA, absolute $\log_2$(fold change)≥1, and * P<0.05 by Student's t-test.

BA to secondary BAs are carried out exclusively by gut microbes in the gastrointestinal tract (Nie et al., 2015; Wahlström et al., 2016), and a large proportion of BAs (90%–95%) in the digestive tract are reabsorbed into the portal vein blood (Cai et al., 2022). Thus, the results of serum secondary BA profiles suggested that *L. gasseri* LA39 also conferred the functions of secondary BA biotransformation on the intestinal tract.

We further examined the levels of BAs in the fecal samples of mice using targeted metabolomic analyses. The results indicated that the levels of β-muricholic acid, dehydrolithocholic acid, and glycolithocholic acid-3-sulfate in the feces of mice were increased by the administration of *L. gasseri* LA39 (Figs. 6f–6h), which was consistent with the alteration of serum BAs by *L. gasseri* LA39. There were large individual variations in the serum and fecal BA levels within the LG group (Fig. 6). Therefore, we speculated that the host responses to *L. gasseri* LA39 treatment may be different among GF mice. Previous studies also showed considerable individual variation in the serum BA levels within the treatment group (Liu et al., 2018; Zhang et al., 2018; Xie et al., 2021), further suggesting the presence of individual differences in the response of BA metabolism to stimulation. This diversity in the experimental animals may have a limited impact on the results due to that significance tests have been performed. Our results further showed that the villus height and the ratio of villus height to crypt depth in the ileum of mice were increased by *L. gasseri* LA39 administration, suggesting the potential improvement of this strain in intestinal health status (Fig. S1). Taken together, these findings revealed that the oral administration of *L. gasseri* LA39 promotes hepatic primary BA biosynthesis and intestinal secondary BA biotransformation.

4 Discussion

In the present study, we demonstrated the important function of *L. gasseri* LA39 in promoting hepatic primary BA biosynthesis. A growing number of studies have reported the important function of the gut–liver axis in mammals (Silveira et al., 2022). The liver affects gut microbiota via producing primary BAs that may confer antimicrobial activity (Brandl et al., 2017). However, it was recently indicated that pathogen overload may promote the pathogenesis of NAFLD and hepatic inflammation (Scott, 2017; Ringseis et al., 2020). GF mice are free from all forms of microbial life and have been considered as an ideal model to investigate the interaction between host and microbiota (Bhattarai and Kashyap, 2016). Microbial strains can be introduced into GF mice to study their function on the host metabolism (Yi and Li, 2012; Grover and Kashyap, 2014; Al-Asmakh and Zadjali, 2015; Bhattarai and Kashyap, 2016). In our study, *L. gasseri* LA39, a promising probiotic, was given to GF mice by oral administration. Proteomics analysis revealed that 128 proteins were upregulated and 123 proteins were downregulated in the liver in response to treatment with *L. gasseri* LA39, suggesting that liver metabolism may be mediated by this bacterium. Our data provide fundamental knowledge about the potential functions of *L. gasseri* LA39 on host liver metabolism, and thus contribute to assessing its beneficial roles.

Recently, the iTRAQ strategy has been widely used to study the alteration of protein expression profiles (Treumann and Thiede, 2010). This approach provides accurate and high-throughput information on protein concentration ratios (DeSouza et al., 2005). Our previous studies have implemented the iTRAQ strategy to evaluate the profiles of porcine intestinal epithelial protein expression in response to dietary leucine supplementation (Hu et al., 2017), *L. gasseri* LA39 treatment (Hu et al., 2018a), and *Lactobacillus frumenti* treatment (Nie et al., 2019). In the current study, a total of 251 DEPs were identified in the liver of GF mice between the Ctrl group and the LG group. Thus, the iTRAQ strategy contributes to mining the host biological events that are preferentially altered by gut microbial species.

Our proteomics data revealed that the primary BA biosynthesis pathway in the liver was activated by treatment with *L. gasseri* LA39. BAs-targeted metabolomic analysis further demonstrated that serum β-muricholic acid, a primary BA, was increased by treatment with *L. gasseri* LA39. A previous study has linked the tauro-β-muricholic acid levels with the abundance of *Lactobacillus* genus in the gut and bile salt hydrolase (BSH) activity (Li et al., 2013). A recent study reported that *Lactobacillus rhamnosus* GG inhibited BA synthesis in the liver (Liu et al., 2020). Moreover, one study showed that probiotic mixture VSL#3 promoted the hepatic BA biosynthesis through inhibiting the farnesoid X receptor (FXR)–fibroblast growth factor 15 (FGF15) axis (Degirolamo et al., 2014). The probiotic mixture VSL#3 also increased the expression levels of two key proteins (including CYP7A1 and CYP8B1) involved in primary BA biosynthesis in the liver (Degirolamo et al., 2014). Based on our findings, the expression levels of five proteins (including CYP27A1, CYP7B1, CYP8B1, AMACR, and SCP2)

in the liver were significantly increased by *L. gasseri* LA39 administration. However, the levels of CYP46A1 and AKR1D1 were decreased by treatment with *L. gasseri* LA39, and this result may be caused by the negative feedback mechanism of BA biosynthesis (Nie et al., 2015). These data suggested that the regulatory roles of gut microbes in the hepatic primary BA biosynthesis may be different among microbial species.

As is known, the transformation from primary BA to secondary BA depends on the gut microbiota (Wahlström et al., 2016; Guzior and Quinn, 2021; Cai et al., 2022). Our results indicated that the levels of two secondary BAs (including dehydrolithocholic acid and glycolithocholic acid-3-sulfate) in the serum and feces were significantly increased under treatment with *L. gasseri* LA39. Recently, it was also demonstrated that *Lactobacillus* species contributed to the biotransformation from primary BA to secondary BA (Wong et al., 2022). These genes have also been identified, such as BSHs that were associated with the secondary BA biotransformation in *Lactobacillus* species (de Boever et al., 2000; Foley et al., 2021; Kusada et al., 2021). Although the processes of biotransformation from primary BA to secondary BA are carried out by gut microbes, a large proportion of BAs (90%–95%) in the intestinal tract are reabsorbed into the portal vein blood (Cai et al., 2022). Thus, the profiles of serum secondary BAs can also indicate secondary BA biotransformation in the intestinal tract. In our experiment, *L. gasseri* LA39 facilitated secondary BA biotransformation in the gut, providing important insights into gut microbiota-mediated BA metabolism.

Growing evidence suggests that the enterohepatic FXR–FGF15 axis is involved in hepatic BA biosynthesis mediated by gut microbes (Degirolamo et al., 2014; Liu et al., 2020). Thus, the changes induced by *L. gasseri* LA39 in hepatic primary BA biosynthesis in this study may not have been induced by enhanced intestinal oxidative phosphorylation. Our previous study showed that *L. gasseri* LA39 promoted oxidative phosphorylation in cultured intestinal epithelial cells in vitro (Hu et al., 2018a), suggesting that the enhancement of oxidative phosphorylation induced by *L. gasseri* LA39 may not be mediated by hepatic primary BA biosynthesis. Given that the transformation of primary BAs to secondary BAs in the intestinal tract depends on the gut microbiota through microbial enzymes, such as BSH and 7α/β-dehydroxylases (Cai et al., 2022), *L. gasseri* LA39-induced changes in the intestinal secondary BA biotransformation in this study might not have been the result of enhanced intestinal oxidative phosphorylation. Future studies should also be performed to evaluate the effects of administering microbial species on host BA metabolism not only in GF animals but also in conventional animals.

Accumulating data have suggested that dysregulated BA metabolism is an etiological factor for several diseases (Cai et al., 2022). A previous study indicated that secondary BAs have important roles in inhibiting *Clostridium difficile* growth, thus preventing intestinal *C. difficile* infection (CDI) (Buffie et al., 2015). The activation of Takeda G protein-coupled receptor 5 (TGR5) through secondary BA ligands contributes to blocking liver inflammation and fibrosis progression (Zhang et al., 2022). Thus, the manipulation of *L. gasseri* LA39 that produces secondary BAs may prevent intestinal CDI and liver fibrosis progression. It has been suggested that FXR activation through primary BAs may be a protective strategy to inflammatory bowel disease (IBD) (Gadaleta et al., 2011; Ogilvie and Jones, 2012). Therefore, *L. gasseri* LA39 may be a potential probiotic used to prevent IBD via promoting the biosynthesis of primary BAs, as demonstrated in our study.

5 Conclusions

In sum, the present study revealed that *L. gasseri* LA39 promotes hepatic primary BA biosynthesis and intestinal secondary BA biotransformation. Our findings provide a basis for understanding gut microbiota–BA interplay, and suggest that *L. gasseri* LA39 may become a promising probiotic used to prevent diseases associated with BA metabolism.

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Author contributions

Jun HU designed the study, conducted the experiments, and wrote the manuscript. Qiliang HOU, Wenyong ZHENG,

and Tao YANG conducted the experiments. Xianghua YAN designed the study and wrote the manuscript. All authors have read and approved the final manuscript, and therefore, have full access to all the data in the study and take responsibility for the integrity and security of the data.

Compliance with ethics guidelines

Jun HU, Qiliang HOU, Wenyong ZHENG, Tao YANG, and Xianghua YAN declare that they have no conflict of interest.

All mice procedures were carried out following the guidelines approved by Institutional Animal Care and Use Committee of Huazhong Agricultural University, Wuhan, China (approval No. HZAUMO-2018-067).

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Supplementary information

Table S1; Fig. S1; Data S1