



Solid-state fermentation of waste herbal medicine residues by a novel rumen-derived probiotic consortium for valorization and microbial-metabolite shifts

Junda Li^a, Jie Xu^a, Piao Wei^b, Hanxuan Gong^c, Min Tian^a, Weijie Yan^d, Caiying Ma^e, Dmitry Il^f, Yuxin Yang^{a,*}

^a College of Animal Science and Technology, Northwest A&F University, Yangling, Shaanxi 712100, China

^b Agricultural and Rural Affairs Bureau, Sandu, Guizhou 558100, China

^c Microbial Research Institute, Chaoyang, Liaoning 122000, China

^d Yasheng Benyuan Biotechnology Co., LTD, Lanzhou, Gansu 730000, China

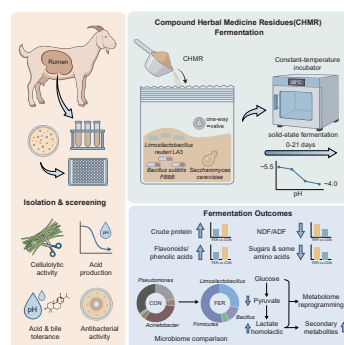
^e Hengshan Industrial Development Institute for Sheep & Goat, Yulin, Shaanxi 719053, China

^f Department of Food Security, Agrotechnological Faculty, Kozybayev University, Petropavlovsk 150000, Kazakhstan

HIGHLIGHTS

- A novel rumen-derived probiotic consortium enabled SSF of CHMR.
- SSF increased CP and reduced NDF and ADF in CHMR.
- Inoculated SSF modulated microbial communities and metabolite profiles.
- Probiotic valorization upgraded CHMR into a functional feed resource.

GRAPHICAL ABSTRACT



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ABSTRACT

Compound herbal medicine residues (CHMR) are an underutilized lignocellulosic by-product with potential for conversion into value-added feed. In this study, a rumen-derived probiotic consortium was developed for the solid-state fermentation (SSF) of CHMR, and the associated microbial and metabolic shifts were characterized. A total of 123 bacterial strains were isolated from goat rumen, of which 58 met the criteria for potential feed additives. *Bacillus subtilis* FB8B and *Limosilactobacillus reuteri* LA3 were selected as core strains based on cellulolytic activity, acidification capacity, stress tolerance, and growth performance in CHMR. The optimized consortium increased crude protein and flavonoids by 10.87% and 17.99%, respectively, while reducing NDF and ADF by 17.27% and 25.97%, respectively. Integrated 16S rRNA sequencing and untargeted metabolomics showed that inoculated fermentation restructured the bacterial community, enriched beneficial fermentative taxa, and suppressed indigenous competitors. Metabolome reprogramming was characterized by depletion of

* Corresponding author.

E-mail address: yangyuxin2002@126.com (Y. Yang).

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sugars and amino acids, accumulation of organic acids and phenolic derivatives, and coordinated shifts in pyruvate, amino acid, and secondary metabolite pathways. These findings demonstrate that rumen-derived probiotic fermentation is an effective strategy for CHMR valorization and provide mechanistic insight into microbiome-driven bioconversion of herbal residues into functional feed resources.

1. Introduction

The rapid intensification of the livestock and poultry industry has raised increasing environmental and ecological concerns, particularly regarding the widespread use of veterinary antibiotics (VAs) and synthetic growth promoters (SGPs). Their continued application contributes not only to the accumulation of pharmaceutical residues in agricultural environments and animal-derived products, but also to the dissemination of antibiotic resistance genes (ARGs) across the human–animal–environment interface. Recent evidence indicates that livestock production systems remain important reservoirs and transmission routes for ARGs, underscoring the urgent need for effective and environmentally sustainable alternatives to conventional growth-promoting strategies (Li et al., 2024a; Wojnarowski et al., 2025).

Probiotics and direct-fed microbials (DFMs) have gained considerable attention as sustainable biological strategies for modulating gastrointestinal microbiota and enhancing feed utilization. Among them, *Bacillus* spp., lactic acid bacteria, and yeasts are widely applied due to their enzymatic versatility and resilience. Recent studies have highlighted probiotic *Bacillus* as promising fermentation agents due to their enzyme secretion capacity, robustness, and contributions to nutrient upgrading in fermented substrates (Liu et al., 2024). Lactic acid bacteria produce organic acids that suppress undesirable microorganisms and reshape microbial community structures (Teleky et al., 2020), whereas yeasts participate in carbon flux redistribution and the synthesis of volatile metabolites (Shi et al., 2020). Importantly, the rumen ecosystem of herbivores represents a highly specialized anaerobic microbial habitat with remarkable lignocellulose-degrading potential and metabolic plasticity. Multi-omic analysis of lignin-degrading fungi has also shown that lignocellulose conversion involves coordinated regulation of carbohydrate-active enzymes, oxidative enzymes, and secondary metabolism, providing a mechanistic basis for microbial valorization of recalcitrant plant biomass (Li et al., 2024b). Exploring rumen-derived microorganisms offers opportunities to harness naturally evolved consortia for biomass valorization and ecological engineering applications.

Compound herbal medicine residues (CHMR) are insoluble by-products generated during the decoction and extraction of herbal medicines. Although often treated as waste, these residues still contain substantial amounts of unused polysaccharides, phenolics, and other bioactive components, and therefore represent an underutilized lignocellulosic biomass resource (Ma et al., 2025). Evaluations of Chinese medicine herbal residues have further highlighted their considerable application potential in feed manufacture, extraction of residual actives, and other value-added utilization pathways within a circular bioeconomy framework (Luo et al., 2023). Conventional disposal practices, such as open accumulation, landfilling, and incineration, may create additional environmental burdens.

Microbial fermentation has emerged as a promising strategy for converting agro-industrial residues into value-added products while reducing environmental burdens. Recent advances in sustainable single-cell protein production from non-grain feedstocks further support the feasibility of converting lignocellulosic and agro-industrial residues into protein-enriched feed resources through microbial biomanufacturing (Gao et al., 2026). Through enzymatic hydrolysis, redox transformation, and secondary metabolite production, microbial consortia can restructure complex organic matrices and improve substrate bioavailability (Wang et al., 2025). Recent studies on fermented herbal residues have shown that fermentation can reduce fiber fractions, enrich functional metabolites, and improve the applicability of such materials as feed

resources (Troiano and Studer, 2025). Fermented CHMR products enriched in beneficial microbes and metabolites also have broader implications for nutritional regulation of intestinal immune homeostasis (Feng et al., 2024). Beyond compositional improvement, fermentation is fundamentally an ecological succession process in which microbial communities dynamically reorganize, driving coordinated metabolic reprogramming (He et al., 2025). However, the mechanisms governing microbial assembly, interspecies interactions, and metabolite network shifts during CHMR fermentation remain poorly understood. This knowledge gap limits the development of targeted strategies for optimizing biomass conversion efficiency and ecological functionality.

In this context, rumen-derived probiotic consortia may provide a biologically robust strategy for CHMR valorization due to their intrinsic lignocellulose-degrading capacity and environmental adaptability. Elucidating the microbial ecological dynamics and metabolic pathways underlying fermentation is essential for advancing sustainable waste management technologies and minimizing ecological footprints. Therefore, this study aims to: (1) isolate and characterize probiotic strains from goat rumen with high fermentation efficiency and environmental tolerance; (2) construct a defined multi-strain consortium composed of *Bacillus subtilis*, *Limosilactobacillus reuteri*, and *Saccharomyces cerevisiae* for the SSF of CHMR; (3) optimize inoculation strategies to enhance biomass degradation and protein enrichment; and (4) elucidate microbial community succession and metabolic reprogramming during fermentation using 16S rDNA sequencing and untargeted metabolomics. By integrating microbial ecology with resource valorization, this work provides mechanistic insights into the sustainable bioconversion of herbal medicine residues and contributes to the development of environmentally sound biomass management strategies.

2. Materials and methods

2.1. Anaerobic and aerobic isolation and purification of bacterial strains

Rumen fluid was collected from fattening Boer goats and filtered through a double layer of cheesecloth. Under anaerobic conditions (<1% O₂), aliquots (100 µL) from serial dilutions (10⁻¹ to 10⁻⁵) were spread onto wide-sieve solid medium plates. The medium consisted of Brain-Heart Infusion (BHI; OXOID, UK) broth (37 g/L), inulin (1 g/L), resazurin (1 g/L), C₃H₁₀ClNO₃S (1.5 g/L), and agar (15 g/L), adjusted to pH 5.5. The plates were incubated anaerobically at 39°C for 48 h. Distinct colonies were selected, inoculated into wide-sieve liquid medium, and returned to the anaerobic incubator for enrichment. Upon turbidity development, the cultures were streaked onto plates for isolation. This purification process was repeated several times to obtain pure cultures, which were stored at 4°C on wide-sieve medium for subsequent use.

For aerobic isolation, aliquots (100 µL) from the same dilution series were spread on deMan, Rogosa, and Sharpe (MRS) solid medium plates (10 g/L peptone, 5 g/L yeast extract, 20 g/L glucose, 10 g/L beef extract, 5 g/L sodium acetate, 2 g/L triammonium citrate, 2 g/L K₂HPO₄, 0.25 g/L MnSO₄, 0.58 g/L MgSO₄, 1 mL/L Tween 80) and incubated at 37°C for 48 h. Colonies with distinct morphologies were picked and repeatedly streaked to obtain pure cultures. Pure strains were stored in maintenance medium at 4°C.

2.2. Identification of isolates

Isolated strains were inoculated into liquid medium and shaken at

39°C for 24 h. Cultures were centrifuged at $12,000 \times g$ for 1 min, and the supernatant was discarded. Genomic DNA was extracted using the sodium dodecyl sulfate (SDS)-proteinase K method. The 16S rRNA gene was amplified via polymerase chain reaction (PCR) with universal primers 27F and 1492R under the following conditions: 94°C for 5 min; 35 cycles of 94°C for 30 s, 56°C for 30 s, and 72°C for 90 s; and a final extension at 72°C for 10 min. Resulting sequences were compared with reference sequences in the NCBI using BLAST.

2.3. Primary screening of *Bacillus* and *Lactobacillus*

Based on the CFA (Catalogue of Feed Additives, China, 2013) and NCBI, potential direct-fed microbes (DFMs) were inoculated onto carboxymethylcellulose sodium (CMC-Na) medium and cultured at 37°C for 48 h. Plates were stained with 1% Congo red for 15 min and destained with 1 M NaCl. The diameters of the hydrolysis zone (D) and the colony (d) were measured using a caliper. Strains with a high D/d ratio were selected as potential fiber degraders.

Putative *Lactobacillus* were inoculated into MRS broth in culture tubes fitted with an inverted Durham tube and incubated at 37°C for 48 h. Final pH was measured to evaluate acid production capacity. To further characterize acid production, organic acids in the culture supernatants were quantified by HPLC. After incubation, samples were centrifuged ($10,000 \times g$, 10 min, 4°C) and filtered (0.22 µm). Analysis was performed using an LC-40 system (Shimadzu, Japan) equipped with an Aminex HPX-87H column under the following conditions: 5 µL injection volume, 0.5 mL/min flow rate, 60°C column temperature, and 5 mmol/L H₂SO₄ as the mobile phase.

2.4. Bacterial growth curves

Growth curves were determined using a microbial growth curve analyzer (Bioscreen C Pro, Finland). *Bacillus* strains were cultured in Luria-Bertani (LB) medium, and *Lactobacillus* in MRS medium. Optical density at 600 nm (OD₆₀₀) was measured every 2 h. The pH of *Lactobacillus* cultures was recorded concurrently. Growth and acid production curves were plotted with culture time as the x-axis and OD₆₀₀ or pH as the y-axis, respectively.

2.5. Functional characterization of *Lactobacillus*

Staphylococcus aureus, *Salmonella Typhimurium*, and *Escherichia coli* (lab stock) were used as indicator strains. They were spread on LB plates, and wells (6.5 mm diameter) were punched and filled with 100 µL of *Lactobacillus* culture supernatant. After 48 h at 37°C, the diameter of the inhibition zone was measured. Acid tolerance was assessed by inoculating *Lactobacillus* (1% v/v) into basal liquid medium adjusted to pH 3.0, 3.5, and 4.0 with glacial acetic acid. After 24 h at 37°C, OD₆₀₀ was measured and compared to the control. Bile salt tolerance was evaluated by supplementing basal liquid medium with 0.1%, 0.3%, and 0.5% (w/v) ox bile salts, inoculating with 1% *Lactobacillus*, incubating for 24 h at 37°C, and measuring OD₆₀₀.

2.6. *Bacillus* cellulase activity assay

Endo-β-1,4-glucanase activity was determined using the Ghose method. A mixture of 50 µL bacterial culture and 400 µL 1% CMC-Na (in acetate buffer, pH 5.0) was incubated at 50°C for 5 min. Absorbance at 540 nm was measured using a microplate reader (Synergy H1, BioTek, USA). Filter paper enzyme activity was assayed by incubating 100 µL bacterial culture with a 1 × 3 cm filter paper strip in 500 µL sodium acetate buffer (0.1 M, pH 5.5) at 50°C for 30 min. The reaction was stopped by adding 100 µL DNS reagent and boiling for 5 min. Controls received boiled culture or sterile medium. Absorbance at 540 nm was measured, and sugar release was quantified against a standard curve.

2.7. Tolerance to CHMR

The supernatant of CHMR extract was used to prepare LB and MRS media in place of distilled water. Media were sterilized (121°C, 30 min), inoculated with bacterial cultures, and incubated for 24 h. Growth was assessed by turbidity and OD₆₀₀ measurement.

2.8. Optimization of fermentation conditions

The tested inoculation-ratio gradients are listed in [supplementary materials](#), including the designed combinations of *L. reuteri*, *S. cerevisiae*, and *B. subtilis* (1:1:1, 1:1:3, 1:3:1, 3:1:1, 3:3:1, 3:1:3, 2:3:1, 3:2:1, 3:3:2). The substrate consisted of a compound CHMR (*Codonopsis pilosula* 10.6 g, *Atractylodes macrocephala* 10.6 g, *Poria cocos* 10.6 g, *Glycyrrhiza uralensis* 5.3 g, *Angelica sinensis* 10.6 g, *Ligusticum chuanxiong* 5.3 g, *Paeonia lactiflora* 10.6 g, *Rehmannia glutinosa* 10.6 g, *Astragalus membranaceus* 10.6 g, *Cinnamomum cassia* 3.2). The initial moisture content was set at 50% (v/w), with the corresponding initial water activity (aw) measured by a water activity meter as 0.97 ± 0.01 . The microbial concentration was determined using the plate count method, yielding 1.0×10^9 CFU/mL.

According to the experimental design, the inoculum suspension or an equivalent amount of sterilized culture medium was added in a volume ratio, with a total addition of 5% (v/w, based on the dry substrate mass). Additionally, 1% glucose was added. The fermentation bags were sealed with breathable caps fitted with one-way exhaust valves, allowing fermentation gases to escape while restricting the entry of external air. Fermentation was conducted in a constant temperature incubator at 28°C for 21 days. Every 72 h, the fermentation bags were gently kneaded and flipped to promote microbial contact. The effects of inoculation ratio on fermentation outcomes, including pH, crude protein (CP), neutral detergent fiber (NDF), acid detergent fiber (ADF), flavonoids, and soluble sugars, were evaluated individually.

To address the safety of CHMR as a potential feed resource, samples before fermentation and after inoculated fermentation were further analyzed for heavy metals, residual alkaloids, glycosides/saponins, and mycotoxins. Metal elements were determined according to GB 5009.268–2025 using inductively coupled plasma mass spectrometry (iCAP RQ, Thermo Fisher Scientific, USA). Total alkaloids, ginsenosides, oleanane-type saponins, aflatoxin B₁, and deoxynivalenol were determined using commercial assay kits (Solarbio, China) according to the manufacturers' instructions.

2.9. Microbial community analysis

The experiment included two groups: a fermentation group (FER) and a control group (CON). The FER group was inoculated with the optimal combination of *L. reuteri* LA3, *B. subtilis* FB8B and *S. cerevisiae* while the CON group received an equivalent amount of sterile culture medium. Due to the limitations of 16S rRNA sequencing, this study focuses on the changes in microbial community and metabolism induced by bacterial strains. Total DNA was extracted from fermented CHMR samples using the CretMag™ PowerSoil DNA Kit. DNA quality was checked by agarose gel electrophoresis and NanoDrop2000. The V4 region of the bacterial 16S rRNA gene was amplified with primers 515F/806R using qualified DNA as template (triplicates per sample). PCR products were pooled, purified, and used to construct sequencing libraries with the VAHTS® Universal Plus DNA Library Prep Kit for MGI V2. Paired-end sequencing was performed on the MGI platform.

2.10. Metabolite extraction and metabolomics analysis

Lyophilized and powdered samples (30 mg) were extracted with 1,500 µL of pre-cooled 70% aqueous methanol (v/v) containing internal standards. Samples were vortexed for 30 s, sonicated in an ice bath for 10 min, and then left to extract at 4°C for 30 min. Extracts were

centrifuged at $12,000 \times g$ for 10 min at 4°C . The resulting supernatant was collected, filtered through a $0.22 \mu\text{m}$ membrane, and stored at -80°C until UPLC–MS/MS analysis. Chromatographic separation was performed on a Waters Corporation ACQUITY UPLC HSS T3 column ($1.8 \mu\text{m}$, $2.1 \times 100 \text{ mm}$) maintained at 40°C . Mobile phase A was water with 0.1% formic acid and mobile phase B was acetonitrile with 0.1% formic acid; a gradient elution was applied. The flow rate was 0.40 mL/min and the injection volume was $4 \mu\text{L}$. Mass spectrometric analysis was carried out on a Q Exactive (QE) platform equipped with an electrospray ionization (ESI) source. Gas flow and pressure were set to 18 L/min and 275 kPa , respectively, and auxiliary gas flow was 3 L/min . UPLC–HRMS data acquisition, alignment, and normalization were performed using ExactFinder 1.0.

2.11. Statistical analysis

Data were analyzed using IBM SPSS Statistics 26. Parametric or non-parametric tests were applied based on data distribution. Results are expressed as mean $\pm$ standard deviation, with false discovery rate (FDR) correction for multiple comparisons. For 16S rRNA data, QIIME 2 was used for analysis, and ASVs were taxonomically annotated against the SILVA SSUrRNA database via the Mothur method. Microbial features with FDR-adjusted $P < 0.05$ were considered significant. In metabolomics, metabolites with $\text{VIP} > 1$, FDR-adjusted $P < 0.05$, and fold change ≥ 2 or ≤ 0.5 were defined as differentially abundant. Metabolites were annotated using the KEGG Compound database and mapped to KEGG pathways. Figures were generated using GraphPad Prism 10.1.2, Adobe Illustrator 2025.

3. Results

3.1. Isolation and functional screening of rumen-derived candidate strains

A total of 123 bacterial strains were isolated (supplementary materials), belonging to genera including *Bacillus*, *Lactobacillus*, *Pediococcus*, and *Clostridium*, among others. 58 strains met feed additive standards according to CFA. Among 20 *Bacillus* strains screened using Congo red and CMC-Na medium, 12 showed cellulolytic activity, with hydrolysis zone-to-colony diameter ratios ranging from 1 to 4 (Fig. 1A). This primary screening narrowed the *Bacillus* candidates to strains with potential to hydrolyze the lignocellulosic fraction of CHMR. In parallel, 28 *Lactobacillus* strains were assessed for acid production. The culture pH declined rapidly in several strains during 48 h of incubation, with LA3, LA9, LA13, and FC1-3 reaching the lowest final pH value of 3.41 (Fig. 1B).

Growth curve analysis further showed that most *Bacillus* isolates entered exponential growth between 2 and 14 h and declined after approximately 32 h, whereas most *Lactobacillus* isolates entered exponential growth between 2 and 12 h and stabilized after 16 h. Lactic acid was the predominant organic acid produced by all selected strains, with LA9 showing the highest production (13.27 g/L), followed by FC1-3 and LA13. In addition to lactic acid, moderate levels of acetic acid were observed, particularly in LA3, which was significantly higher than in other strains ($P < 0.05$; supplementary materials).

3.2. Probiotic properties and substrate adaptability of selected strains

The *Lactobacillus* strains exhibited varied antibacterial activity against pathogens (Fig. 1C). FC1-3 strongly inhibited *Staphylococcus aureus*, and *Escherichia coli*, with inhibition zone diameters of 12.87 and 13.20 mm, respectively. LA13 and LA14 were most effective against *Salmonella typhimurium*, with inhibition zone diameters of 14.50 and 14.60 mm, respectively. ($P < 0.05$).

To determine whether candidate strains could adapt to the CHMR matrix, selected isolates were cultured in CHMR extract medium. Both *Bacillus* and LAB strains were able to grow, but their growth levels

differed significantly. Among *Bacillus*, FB8B was most resistant, while *Lactobacillus* strains LA3 exhibited strong resistance ($P < 0.05$; Fig. 1D).

Fig. 1E–F showed that LA3 had strong overall stress tolerance. Under acid stress, LA3 maintained a survival rate of approximately 88.37% at pH 4.0 and 39.40% at pH 3.5, while survival decreased to about 4.87% at pH 3.0 ($P < 0.05$). In the bile salt assay, LA3 retained approximately 60.57% survival at 0.1% bile salt and 10.17% at 0.3% bile salt.

The cellulase activity assay confirmed the functional advantage of FB8B. Endo- β -1,4-glucanase activity peaked at 48 h in most *Bacillus* strains, with FB8B reaching the highest activity of 12.65 U/mL ($P < 0.05$, Fig. 1G). Filter paper enzyme activity was highest in C3A at 24 h and 48 h, while FB8B showed peak activity of 0.710 U/mL at 72 h ($P < 0.05$, Fig. 1H). Considering cellulase production, acidification, stress tolerance, and CHMR adaptability together, FB8B and LA3 were selected as core strains for subsequent fermentation optimization.

3.3. Fermentation optimization

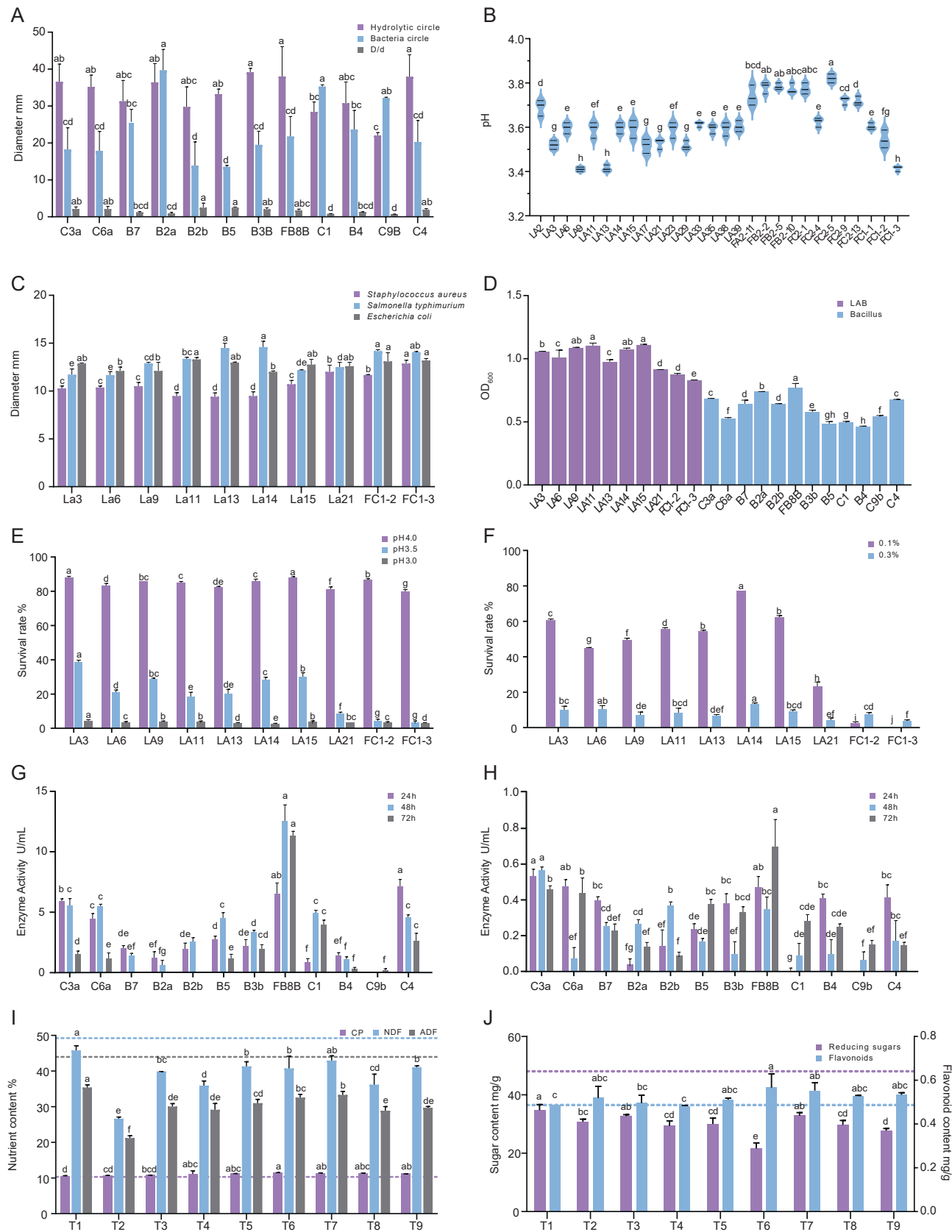
Compared with CON, all fermentation groups exhibited marked reductions in ADF and NDF, along with a decrease in reducing sugar content. Among all treatments, T6 showed the highest CP content, which was 10.87% higher than that of the unfermented product. Flavonoid content was also significantly elevated in T6, with the greatest increase of 17.99% ($P < 0.05$, Fig. 1I). Therefore, T6 was selected as the representative fermentation treatment for subsequent microbial community and metabolomics analyses. No significant differences were observed between the FER and CON groups in As, Cd, Cr, Cu, Hg, Ni, Pb, Zn, aflatoxin B₁, or deoxynivalenol (supplementary materials) and all values complied with the hygienic standard for feeds (GB 13078–2017).

3.4. Bacterial community composition

After denoising, Amplicon Sequence Variants (ASVs) were generated and annotated for each sample (supplementary materials). Principal Coordinates Analysis (PCoA) based on the Bray Curtis algorithm revealed a clear separation in community structure between the FER and CON groups (Fig. 2A). Alpha diversity indices showed that compound bacterial fermentation significantly reduced the bacterial community diversity of the herbal residue ($P < 0.05$, Fig. 2B). Taxonomically, FER was dominated by Firmicutes, with a notable increase in beneficial genera like *Limosilactobacillus* and a marked reduction in potential pathogens such as *Pseudomonas* and *Acinetobacter* ($P < 0.05$, Fig. 2C, D). LEfSe analysis ($\text{LDA} \geq 4$) confirmed that FER was characterized by Lactobacillales, culminating in the enrichment of the inoculated *Limosilactobacillus reuteri*. In contrast, CON was defined by native taxa including *Caulobacter* and *Sphingomonas* (Fig. 2E–2G).

3.5. Untargeted metabolomics analysis

Untargeted metabolomics identified 2,487 metabolites across all samples (Fig. 3A; supplementary materials). Partial Least Squares-Discriminant Analysis (PLS-DA) models effectively distinguished FER from CON, with validation (A permutation test with 200 iterations, $Q^2 = 0.977$, $P < 0.005$) confirming model robustness and significant inter-group metabolic differences (Fig. 3B–3D). Based on $\text{FC} \geq 2$ or ≤ 0.5 , $\text{VIP} \geq 1$, and $\text{FDR} < 0.05$, 317 differential abundance metabolites were identified between FER and CON (supplementary materials; Fig. 3E). The down-regulated metabolites mainly included carbohydrates and amino acids, such as isomaltose, β -D-galactose, L-tryptophan, and L-phenylalanine. In contrast, several organic acids, phenolic acid derivatives, and nitrogen-containing metabolites, including ethyl salicylate, 3-hydroxyisovaleric acid, and N-carbamoylputrescine, were increased in FER, indicating active microbial transformation and secondary metabolite production (Fig. 3F). Correlation analysis further revealed two contrasting metabolite clusters. Up-regulated metabolites, represented by ethyl salicylate and related organic compounds, were



(caption on next page)

Fig. 1. Comprehensive evaluation of candidate strains with fermentation performance and functional characteristics. (A) Comparison of hydrolytic and bacterial zones of different strains, showing hydrolytic activity efficiency. (B) pH variation during fermentation across different strains, indicating acid production ability. (C) Antibacterial activity of selected strains against *S. aureus*, *S. typhimurium*, and *E. coli*. (D) Bacterial growth curves (OD₆₀₀) of the strains in CHMR extract. (E) Acid tolerance test showing survival rate of LAB and *Bacillus* under different pH conditions (pH 3.0, 3.5, and 4.0). (F) Bile salt tolerance test showing survival rate of strains under different bile salt concentrations (0.1%, 0.3%). (G–H) Enzyme activity assays (U/mL) of the screened strains, measured at 24 h, 48 h, and 72 h, showing the dynamic changes of hydrolytic enzymes. (I) Nutrient composition in CHMR samples after fermentation under different inoculation-ratio treatments. (J) Reducing sugar and flavonoid contents in CHMR after fermentation. Abbreviations: CP, crude protein; NDF, neutral detergent fiber; ADF, acid detergent fiber; OD₆₀₀, optical density at 600 nm. CON, control group. Note: T1–T9 represent nine fermentation treatments with different inoculation ratios of *L. reuteri*, *B. subtilis*, and *S. cerevisiae*, as detailed in supplementary materials. The dashed lines indicate the values of CON. Different lowercase letters above the bars indicate significant differences among groups ($P < 0.05$, one-way ANOVA followed by Tukey's test). Error bars represent standard deviations ($n = 3$).

positively correlated with each other but negatively correlated with down-regulated sugars and amino acids (Fig. 3G).

3.6. KEGG pathway and Integration analysis

To elucidate the functional implications of the altered metabolome, KEGG pathway enrichment analysis was performed using the microbial metabolome database. 14 metabolic pathways were significantly enriched between FER and CON ($P < 0.05$, Fig. 4A). Key changes included the down-regulation of pyruvate and citrate, and the accumulation of L-lactate, indicating a shift toward homolactic fermentation. Concurrent consumption of amino acids and accumulation of derivatives aligned with enriched amino acid metabolic pathways. Furthermore, fermentation may enhance the synthesis of bioactive secondary metabolites, including naringenin, matairesinol, and ephedrine (Fig. 4B). Microbiome-metabolome correlation analysis further supported this interpretation. *L. reuteri* and *B. subtilis* were positively associated with beneficial metabolites like lactate, naringenin, and ephedrine, but negatively correlated with pyruvate, aspartate, tryptophan, and other consumed substrates. In contrast, genera enriched in CON, such as *Sphingomonas* and *Caulobacter*, showed the opposite correlation pattern (Fig. 4C).

4. Discussion

4.1. Culture omics screening for optimal fermentation probiotic

In this study, a culture-omics strategy was applied to screen functional microorganisms suitable for CHMR fermentation. Among these isolates, *B. subtilis* FB8B exhibited the strongest cellulose-degrading potential, while LA3 showed sufficient acidification ability and was identified as *L. reuteri*, a species with reported probiotic traits, including acid and bile tolerance, intestinal adhesion, antimicrobial activity, intestinal barrier protection, and immunomodulatory effects (Lasaviciute et al., 2026). Therefore, LA3 was considered more appropriate for subsequent co-fermentation. Recent studies also suggest that *L. reuteri* is suitable for plant-based fermentation, contributing to improved antioxidant activity and flavor quality (Li et al., 2025). For CHMR fermentation, the selection of *B. subtilis* FB8B was mainly supported by its cellulase-producing ability. High CMCase and filter paper enzyme activities indicate that FB8B contribute to the degradation of structural carbohydrates in CHMR. This function is particularly important because CHMR contains abundant fiber components, which can limit palatability and nutrient utilization (Gao et al., 2025).

The optimized inoculation ratio of *L. reuteri*: *B. subtilis*: *S. cerevisiae* = 3:3:1 resulted in the greatest increase in crude protein, suggesting that balanced microbial cooperation was more effective than dominance by a single strain (Maehara et al., 2018). *B. subtilis* may have contributed through extracellular enzyme secretion and substrate degradation, *L. reuteri* through acidification and microbial stabilization, and *S. cerevisiae* through microbial biomass accumulation and nutrient transformation.

In broilers, the study recommended 1.0% as the optimal inclusion level because it improved growth performance, nutrient digestibility, meat quality, and immune indices (Ding et al., 2025). In finishing pigs,

fermented Chinese herbal medicine was supplemented at 0.4%, 0.6%, and 0.8%, with improved growth performance and altered cecal microbiota and metabolites (Luo et al., 2026). Based on these reports and the present safety data, fermented CHMR may be considered as a functional feed ingredient at a conservative preliminary inclusion range of 0.5–1.0% of the diet. For ruminants, which have greater tolerance and utilization capacity for fibrous fermented substrates, a broader exploratory range could be evaluated.

4.2. Microbial community succession driven by inoculated fermentation

In controlled fermentation systems, decreased diversity often reflects successful starter colonization and microbial succession. In the present study, FER was dominated by Firmicutes, especially *Limosilactobacillus* and *Bacillus*, whereas CON retained a more heterogeneous native community, including Proteobacteria, Actinobacteria, Bacteroidota, and other environmental taxa. LEfSe analysis further confirmed that *L. reuteri* and Lactobacillales were the major biomarkers of FER, while *Caulobacter*, *Sphingomonas*, and other native bacteria characterized CON. This pattern aligns with the typical trajectory of fermentation, wherein starter cultures create a selective environment through rapid acidification, bacteriocin production, or other antimicrobial mechanisms, thereby suppressing background microorganisms (Hao et al., 2021).

The microbial succession, from a diverse, environmentally derived community to a specialized, fermentation-oriented consortium, carries important implications. Firstly, it reduces the risk of spoilage and contamination through competitive exclusion of potential pathogens. Secondly, it directs metabolic flux toward desired pathways, potentially enhancing the bioconversion efficiency of herbal residues, promoting the synthesis of beneficial metabolites, and improving the quality and safety of the final product (Wang et al., 2021). The dominance of Firmicutes, specifically lactic acid bacteria and bacilli, is a hallmark of successful SSF, further validating the effectiveness of the applied strategy.

4.3. Metabolic changes and functional enhancement during fermentation

Untargeted metabolomics revealed extensive metabolic remodeling after fermentation. The separation between FER and CON in PLS-DA, together with the identification of 317 differential abundance metabolites, indicates that compound bacterial fermentation caused a systematic change in the chemical composition of CHMR. A major feature of this metabolic shift was the depletion of carbohydrates and amino acids. The reduced abundance of isomaltose, β-D-galactose, β-lactose, L-tryptophan, and L-phenylalanine suggests that these compounds were consumed as carbon and nitrogen sources by fermentative microorganisms. This result is consistent with the enrichment of *L. reuteri* and *B. subtilis*, which are capable of utilizing carbohydrates and amino acids to support growth, acid production, enzyme secretion, and secondary metabolism (Heo et al., 2025).

In contrast, several organic acids, phenolic acid derivatives, and nitrogen-containing metabolites increased after fermentation. The accumulation of 3-hydroxyisovaleric acid, 3-hydroxynonanoic acid, N-carbamoylputrescine, and 5-hydroxylysine indicates active microbial

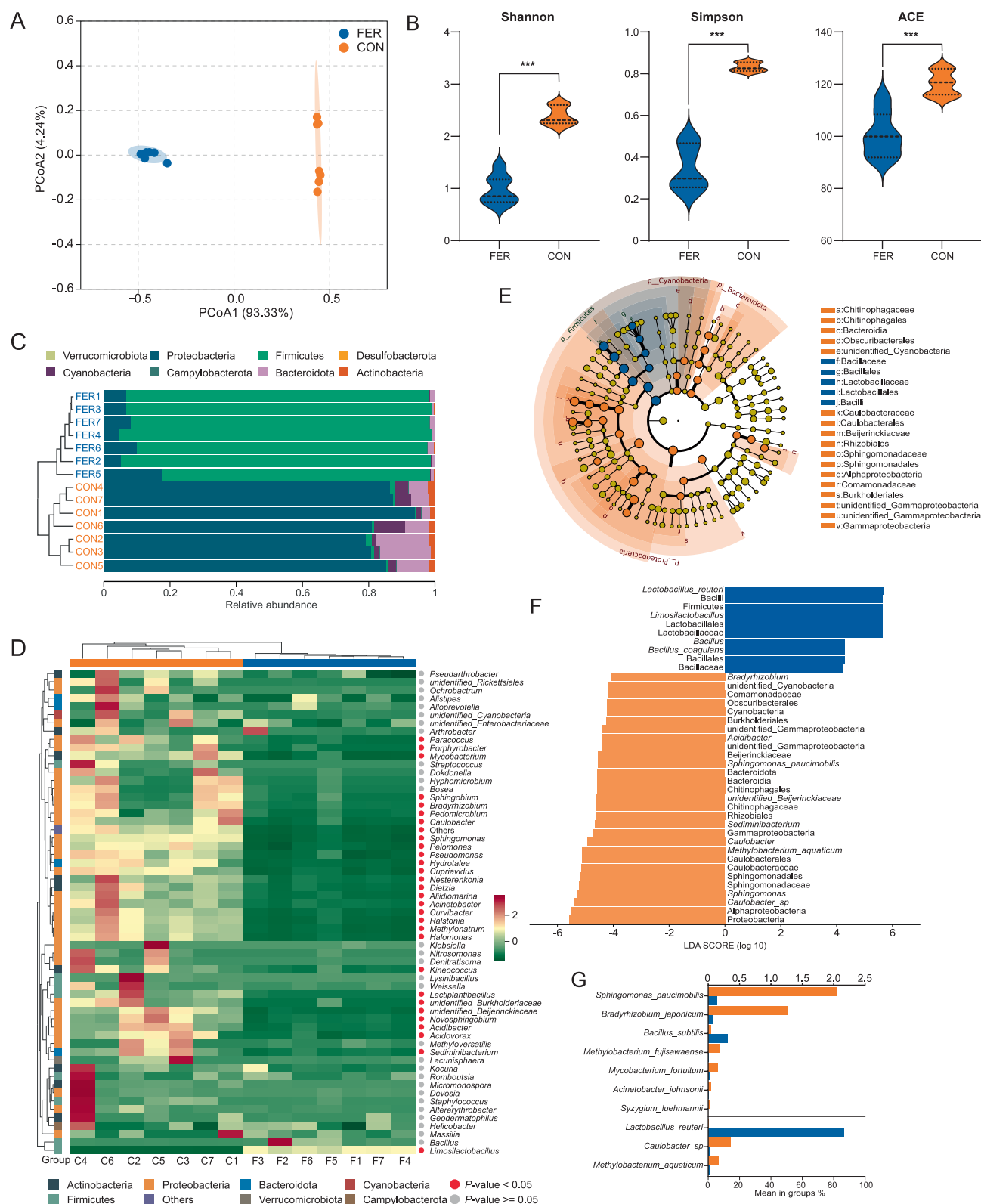


Fig. 2. Gut microbiota composition and diversity analysis between FER and CON groups ($n = 7$ per group). (A) Principal coordinate analysis (PCoA) based on Bray-Curtis distances showing the overall microbial community differences between the fermentation-treated (FER) and control (CON) groups. (B) Alpha diversity indices including Shannon, Simpson, and ACE estimators, comparing species diversity and richness between groups. (C) Relative abundance of major bacterial phyla across samples, highlighting the dominant taxa in each group. (D) Heatmap of the top 50 genera displaying relative abundance patterns across individual samples, with color intensity representing normalized abundance levels. (E) Cladogram generated by LefSe analysis indicating the taxonomic hierarchy of bacterial taxa with significant differences between groups. (F) Linear discriminant analysis (LDA) scores showing taxa enriched in the FER (blue) and CON (orange) groups (LDA score > 2.0, $P < 0.05$). (G) Mean relative abundance of the most discriminative bacterial species between the two groups as identified by LefSe analysis. Abbreviations: FER, fermentation-treated group; CON, control group; PCoA, principal coordinate analysis; LefSe, linear discriminant analysis effect size. Note: Symbols and color codes correspond to those displayed in each subpanel. Asterisks indicating significance (* $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$).

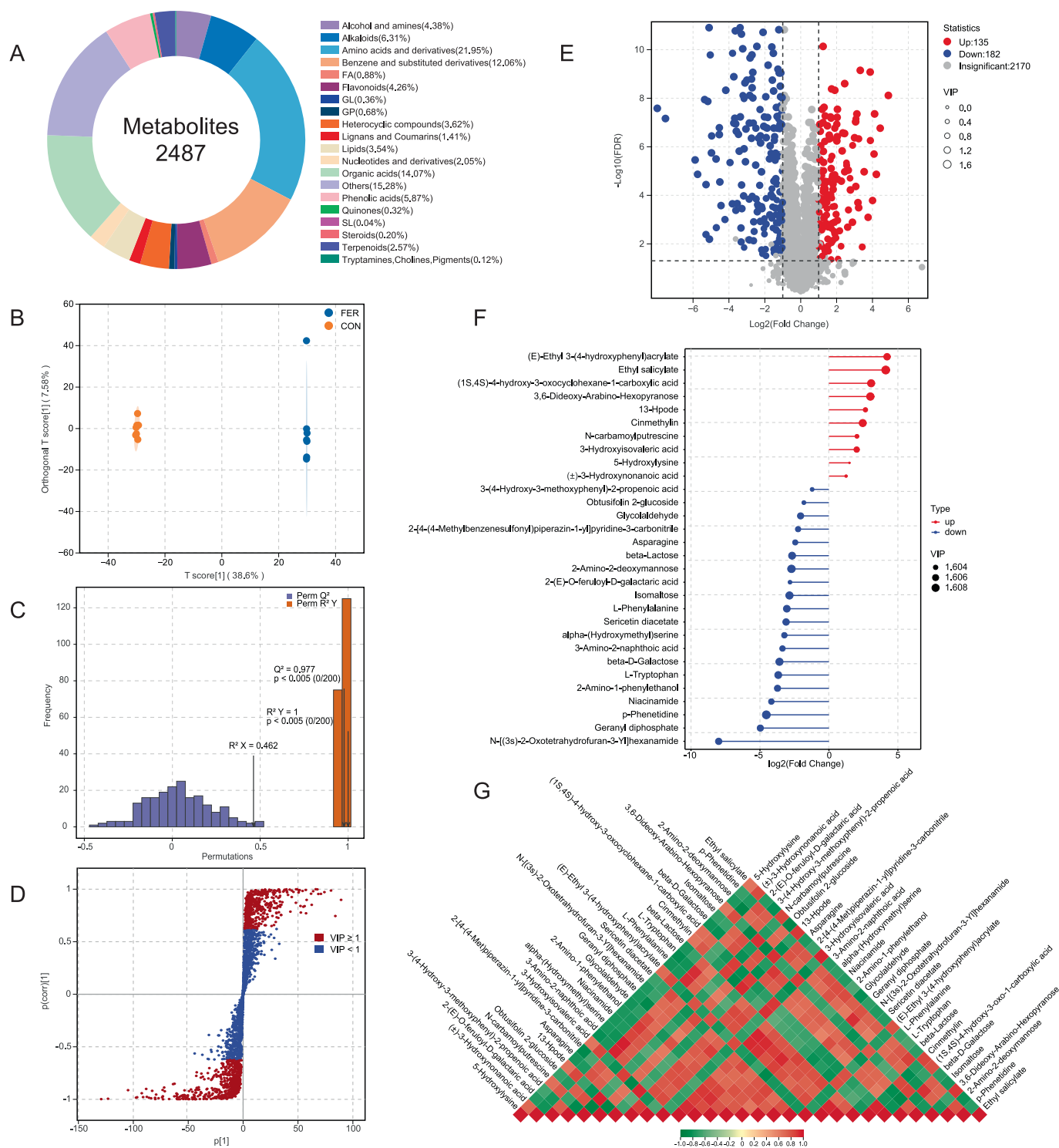


Fig. 3. Metabolomic profiling and differential metabolite analysis between FER and CON groups ($n = 7$ per group). (A) Classification of all identified metabolites based on chemical taxonomy, showing proportions of amino acids, organic acids, alkaloids, flavonoids, lipids, and other metabolite categories. (B) Score plot of orthogonal partial least squares discriminant analysis (OPLS-DA) demonstrating clear separation between fermentation-treated (FER) and control (CON) groups. (C) Permutation test of the OPLS-DA model confirming its reliability and robustness ($R^2 Y = 1$, $Q^2 = 0.977$, $P < 0.005$). (D) S-plot derived from OPLS-DA showing the covariance and correlation of metabolites contributing to group discrimination. (E) Volcano plot depicting significantly upregulated and downregulated metabolites between FER and CON groups ($FC \geq 2$ or ≤ 0.5 , $VIP \geq 1$, $FDR < 0.05$). (F) Variable importance in projection (VIP) plot highlighting the top differential metabolites with $VIP \geq 1.6$. (G) Correlation heatmap of significantly altered metabolites illustrating pairwise Spearman correlation coefficients between key biomarkers. Abbreviations: FER, fermentation-treated group; CON, control group; OPLS-DA, orthogonal partial least squares discriminant analysis; VIP, variable importance in projection; FDR, false discovery rate; FC, fold change. Note: Red and blue colors indicate upregulated and downregulated metabolites, respectively. Asterisks denote statistical significance ($P < 0.05$).

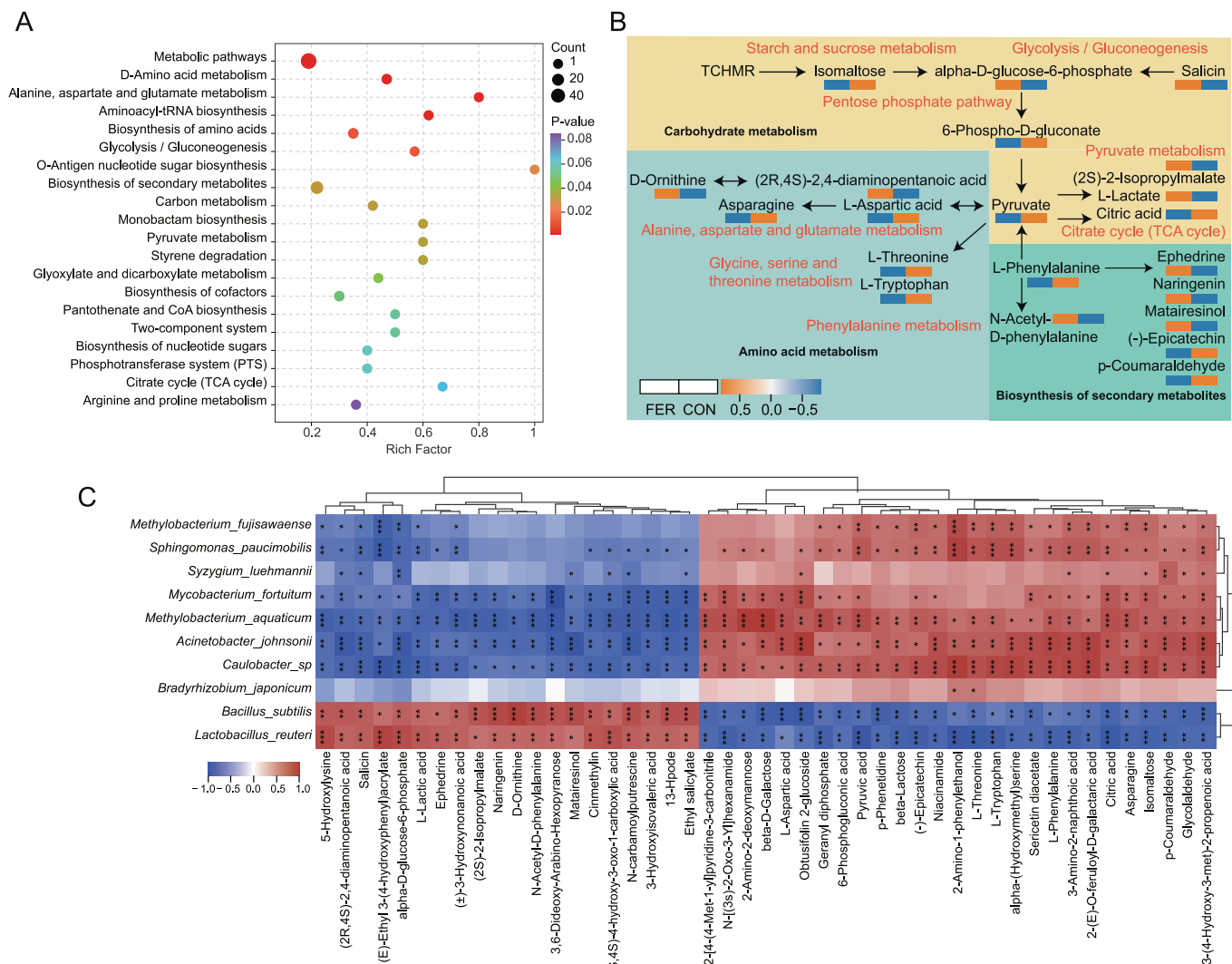


Fig. 4. KEGG-based joint pathway analysis integrating microbiome and metabolome data. (A) KEGG pathway enrichment analysis of differential metabolites showing significantly enriched pathways (Rich factor, P -value, and metabolite count indicated by dot size and color). (B) Integrated KEGG map illustrating the distribution of significantly altered metabolites within key metabolic pathways, including carbohydrate metabolism, amino acid metabolism, and biosynthesis of secondary metabolites. The color gradient represents relative abundance differences between fermentation-treated (FER) and control (CON) groups. (C) Heatmap of Spearman correlations between differential bacterial species and key metabolites enriched in KEGG pathways. Red and blue denote positive and negative correlations, respectively, with asterisks indicating significance (* $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$).

transformation of primary nutrients into organic acids and nitrogenous derivatives. Similar functional evaluations of selenium-enriched *Aspergillus oryzae* have demonstrated that microbial fermentation can enrich bioactive nutritional components and improve the functional value of fermented products (Gao et al., 2024).

The up-regulation of phenolic acid derivatives, particularly ethyl salicylate and related compounds, is noteworthy. These compounds, often associated with antioxidant and anti-inflammatory activities, likely result from microbial esterification of parent phenolics, a common biotransformation pathway mediated by lactic acid bacteria (Rodríguez et al., 2009). Their accumulation underscores microbial esterification as a defining chemical feature of fermented herbal residues, contributing to both aroma and bioactivity. Furthermore, the accumulation of organic acids reflects ongoing microbial fermentation, contributing to product acidification and preservation. The elevation of 13-HPODE suggests the occurrence of lipid peroxidation during fermentation, which may influence oxidative stability and sensory properties (Faizo et al., 2021).

The metabolite correlation network further supports the idea of coordinated substrate consumption and product accumulation. Down-regulated sugars and amino acids formed a positively correlated

cluster, suggesting their simultaneous utilization during fermentation. Conversely, the strong positive correlations within the up-regulated cluster suggest coordinated accumulation. The negative correlation between these two clusters reflects a metabolic trade-off: resources are redirected from primary nutrients toward the synthesis of secondary metabolites and organic acids (Dinglasan et al., 2025). This reflects a classic microbial strategy wherein cells initially consume available resources to support growth before transitioning to specialized metabolite production (Zhang et al., 2022).

4.4. KEGG pathway analysis reveals coordinated metabolic shifts

The accumulation of L-lactic acid, accompanied by the depletion of pyruvate, signifies *Lactobacillus*-driven homolactic fermentation (Gänzle, 2015). This process not only acidifies the environment to suppress competitors but also promotes the breakdown of the plant matrix. Concurrently, *Bacillus* species contribute to polysaccharide deconstruction, as evidenced by the rise in phosphorylated sugars such as α -D-glucose-6-phosphate and the reduction in oligosaccharides like isomaltose and mannito. A shift from oxidative to fermentative

metabolism is indicated by the coordinated down-regulation of TCA cycle intermediates and the rise in lactic acid. The concurrent activation of the pentose phosphate pathway, inferred from glucose-6-phosphate accumulation and 6-phosphogluconate decline, supplies NADPH for reductive biosynthesis and generates precursors for nucleotide and secondary metabolite synthesis, thereby sustaining microbial proliferation and compound output (Zhou and Hua, 2024). Therefore, the carbon metabolic profile suggests a cooperative process involving fiber breakdown, sugar utilization, and organic acid production.

Nitrogen metabolism was also substantially altered. The depletion of proteinogenic amino acids indicates that amino acids were consumed or converted during SSF. The accumulation of D-ornithine and other nitrogen-containing derivatives suggests that amino acid metabolism may contribute to the formation of functional metabolites. More notably, the conversion of aromatic amino acids, including phenylalanine and tryptophan, into flavonoids and alkaloids constitutes a value-adding biotransformation that augments product bioactivity (König et al., 2023). The marked up-regulation of naringenin, a pivotal flavonoid intermediate with recognized anti-inflammatory and antioxidant properties, highlights the potential of fermentation to enhance the functional value of herbal residues (He et al., 2023). Similarly, the accumulation of ephedrine-type alkaloids suggests the *de novo* generation of bioactive compounds not originally present in the substrate (Wu et al., 2024).

5. Conclusion

In conclusion, this study established a compound microbial fermentation strategy for the value-added utilization of CHMR. Through culture-omics screening, *B. subtilis* FB8B and *L. reuteri* LA3 were identified as functionally complementary strains for fiber degradation and acidification, respectively. The optimal inoculation ratio of *L. reuteri*: *B. subtilis*: *S. cerevisiae* = 3:3:1 improved CHMR quality by increasing crude protein and flavonoid contents while reducing fiber components. Integrated 16S rDNA sequencing and untargeted metabolomics further showed that fermentation reshaped the indigenous microbiota into a starter-dominated community, promoted the utilization of carbohydrates and amino acids, enhanced lactate accumulation, and enriched potentially bioactive metabolites, including phenolic derivatives, flavonoids, lignans, and alkaloid-related compounds. Overall, these findings demonstrate that compound microbial fermentation is an effective and sustainable approach for converting CHMR into a higher-value functional feed ingredient. E-supplementary data for this work can be found in e-version of this paper online.

CRediT authorship contribution statement

Junda Li: Writing – original draft, Formal analysis, Conceptualization. **Jie Xu:** Methodology, Data curation. **Piao Wei:** Writing – review & editing, Formal analysis. **Hanxuan Gong:** Writing – review & editing, Validation, Investigation. **Min Tian:** Visualization, Software, Data curation. **Wei jie Yan:** Resources, Investigation. **Caiying Ma:** Writing – review & editing, Validation. **Dmitry Il:** Writing – review & editing. **Yuxin Yang:** Writing – review & editing, Supervision, Funding acquisition.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.biortech.2026.134922>.

Data availability

Data will be made available on request.

References

- Ding, P., Wang, X., Jiang, S., Li, M., He, X., Peng, Y., 2025. Effects of bacteria-enzyme co-fermented Chinese herbal medicine on growth performance, apparent nutrient digestibility, meat quality, and immune function in broilers. *Front. Vet. Sci.* 12, 1676951. <https://doi.org/10.3389/fvets.2025.1676951>.
- Dinglasan, J.L.N., Otani, H., Doering, D.T., Udway, D., Mouncey, N.J., 2025. Microbial secondary metabolites: advancements to accelerate discovery towards application. *Nat. Rev. Microbiol.* 23, 338–354. <https://doi.org/10.1038/s41579-024-01141-y>.
- Faizo, N., Narasimulu, C.A., Forsman, A., Yooshep, S., Parthasarathy, S., 2021. Peroxidized linoleic acid, 13-HPODE, alters gene expression profile in intestinal epithelial cells. *Foods (Basel Switz.)* 10, 314. <https://doi.org/10.3390/foods10020314>.
- Feng, Y., Cheang, W.S., Gan, R., Wu, X., 2024. Editorial: Immunometabolism and nutritional regulation of intestinal mucosal immunity. *Front. Immunol.* 15, 1365690. <https://doi.org/10.3389/fimmu.2024.1365690>.
- Gänzle, M.G., 2015. Lactic metabolism revisited: metabolism of lactic acid bacteria in food fermentations and food spoilage. *Curr. Opin. Food Sci.* 2, 106–117. <https://doi.org/10.1016/j.cofs.2015.03.001>.
- Gao, L., Jiang, F., Zhang, Z., Bao, T., Zhu, D., Wu, X., 2025. Unlocking lignin valorization and harnessing lignin-based raw materials for bio-manufacturing. *Sci. China Life Sci.* 68, 994–1009. <https://doi.org/10.1007/s11427-024-2792-x>.
- Gao, L., Khoo, S.C., Zhang, Z., Wu, X., 2026. Trends in sustainable single-cell protein from non-grain feedstocks. *Trends Biotechnol.* 44, 65–78. <https://doi.org/10.1016/j.tibtech.2025.04.018>.
- Gao, L., Li, X., Li, Y., Zhang, Z., Wang, J., Xu, C., Wu, X., 2024. Biochemical characterization, biosynthesis mechanism, and functional evaluation of selenium-enriched *Aspergillus oryzae* A02. *Int. J. Biol. Macromol.* 275, 133714. <https://doi.org/10.1016/j.jbiomac.2024.133714>.
- Hao, F., Tan, Y., Lv, X., Chen, L., Yang, F., Wang, H., Du, H., Wang, L., Xu, Y., 2021. Microbial community succession and its environment driving factors during initial fermentation of maotai-flavor baijiu. *Front. Microbiol.* 12, 669201. <https://doi.org/10.3389/fmicb.2021.669201>.
- He, C., Yang, S., Dong, S., Wang, S., Zhang, P., Yang, Y., Xu, D., Yang, R., Zeng, B., Hu, Y., Zhang, Q., 2025. Fermentation-driven microbial and metabolic shifts in filler tobacco leaves of different grades. *Front. Microbiol.* 16, 1651289. <https://doi.org/10.3389/fmicb.2025.1651289>.
- He, Y., Zhu, Y., Lv, J., Gu, Y., Wang, T., Chen, J., 2023. Effects of lactic acid bacteria fermentation on the bioactive composition, volatile compounds and antioxidant activity of huyou (*Citrus aurantium* 'changshan-huyou') peel and pomace. *Food Qual. Saf.* 7, fyad003. <https://doi.org/10.1093/fqsafe/fyad003>.
- Heo, J., Ham, Y.-K., Choi, A.Y., Yoon, H., Sung, H.G., 2025. Lactic acid bacteria as probiotics improve bioactive compounds in radix angelica gigas (danggui) via solid-state fermentation. *Fermentation* 11, 342. <https://doi.org/10.3390/fermentation11060342>.
- König, A., Sadova, N., Dormmayr, M., Schwarzwinger, B., Neuhauser, C., Stadlbauer, V., Wallner, M., Woischitzschlagger, J., Müller, A., Tona, R., Kofel, D., Weghuber, J., 2023. Combined acid hydrolysis and fermentation improves bioactivity of citrus flavonoids in vitro and in vivo. *Commun. Biol.* 6, 1083. <https://doi.org/10.1038/s42003-023-05424-7>.
- Lasaviciute, G., López Plana, M., Sundberg Örtengren, S., Telli, S., Kourmoulakis, S., Ermann Lundberg, L., Lidberg, K., Peiris, O., Sinha, I., Jonsson, A.-B., Roos, S., Nilsson, A., Mata Forsberg, M., Sverremark-Ekström, E., 2026. Limosilactobacillus reuteri metabolites modulate immune pathways and intestinal barrier repair after 5 fluorouracil exposure. *Sci. Rep.* 16, 11376. <https://doi.org/10.1038/s41598-026-45524-y>.
- Li, H., Liu, B., Li, M., Shen, M., 2024a. Livestock and poultry breeding farms as a fixed and underestimated source of antibiotic resistance genes. *Environ. Sci. Pollut. Res.* 31, 49916–49931. <https://doi.org/10.1007/s11356-024-34413-4>.
- Li, J., Jiang, F., Pi, C., Bao, T., Gao, L., Wu, X., 2024b. Multi-omic profiling of a novel *Myrothecium* species reveals its potential mechanism of lignin degradation. *Int. J. Biol. Macromol.* 282, 137134. <https://doi.org/10.1016/j.jbiomac.2024.137134>.
- Li, X., Li, X., Liu, L., Zhao, J., Zhang, Y., Chen, L., 2025. Antioxidant activity and metabolic profile changes in *Poria cocos* fermented by breast-milk-derived *Limosilactobacillus reuteri* HM-R28. *Front. Nutr.* 12, 1625875. <https://doi.org/10.3389/fnut.2025.1625875>.
- Liu, S., Zhao, L., Li, M., Zhu, Y., Liang, D., Ma, Y., Sun, L., Zhao, G., Tu, Q., 2024. Probiotic bacillus as fermentation agents: status, potential insights, and future perspectives. *Food Chem.: X* 22, 101465. <https://doi.org/10.1016/j.fochx.2024.101465>.

- Luo, J., Yang, R., Ma, F., Jiang, W., Han, C., 2023. Recycling utilization of Chinese medicine herbal residues resources: systematic evaluation on industrializable treatment modes. *Environ. Sci. Pollut. Res.* 30, 32153–32167. <https://doi.org/10.1007/s11356-023-25614-4>.
- Luo, X., Chen, J., Wu, G., Yao, Y., Ye, P., Zhang, S., Zhao, J., Li, R., Shi, Y., Liu, Y., Mo, H., Wang, L., 2026. Exploring the effects of fermented Chinese herbal medicine on growth, cecal microbiota, metabolism, and muscle flavor-related compounds in fattening pigs. *Front. Microbiol.* 17, 1781152. <https://doi.org/10.3389/fmicb.2026.1781152>.
- Ma, J., Wang, J., Wan, Y., Wang, S., Jiang, C., 2025. Probiotic-fermented traditional Chinese herbal medicine, a promising approach to maintaining the intestinal microecology. *J. Ethnopharmacol.* 337, 118815. <https://doi.org/10.1016/j.jep.2024.118815>.
- Maehara, L., Pereira, S.C., Silva, A.J., Farinas, C.S., 2018. One-pot strategy for on-site enzyme production, biomass hydrolysis, and ethanol production using the whole solid-state fermentation medium of mixed filamentous fungi. *Biotechnol. Prog.* 34, 671–680. <https://doi.org/10.1002/btpr.2619>.
- Rodríguez, H., Curiel, J.A., Landete, J.M., de las Rivas, B., López de Felipe, F., Gómez-Cordovés, C., Mancheño, J.M., Muñoz, R., 2009. Food phenolics and lactic acid bacteria. *Int. J. Food Microbiol.* 132, 79–90. <https://doi.org/10.1016/j.ijfoodmicro.2009.03.025>.
- Shi, H., Zhang, M., Wang, W., Devahastin, S., 2020. Solid-state fermentation with probiotics and mixed yeast on properties of okara. *Food Biosci.* 36, 100610. <https://doi.org/10.1016/j.fbio.2020.100610>.
- Teleky, B.-E., Martău, G.A., Vodnar, D.C., 2020. Physicochemical effects of lactobacillus plantarum and lactobacillus casei cocultures on soy–wheat flour dough fermentation. *Foods* 9, 1894. <https://doi.org/10.3390/foods9121894>.
- Troiano, D.T., Studer, M.-H.-P., 2025. Microbial consortia for the conversion of biomass into fuels and chemicals. *Nat. Commun.* 16, 6712. <https://doi.org/10.1038/s41467-025-61957-x>.
- Wang, Y., Wu, J., Lv, M., Shao, Z., Hungwe, M., Wang, J., Bai, X., Xie, J., Wang, Y., Geng, W., 2021. Metabolism characteristics of lactic acid bacteria and the expanding applications in food industry. *Front. Bioeng. Biotechnol.* 9, 612285. <https://doi.org/10.3389/fbioe.2021.612285>.
- Wang, Z., Li, Y., Tang, H., Luo, D., Huang, J., Ye, B., Yan, W., Liu, G., Yang, Y., 2025. Novel fermentation strategy utilizing a probiotic complex to improve the feeding value of distillers' grains. *Ind. Crop. Prod.* 226, 120748. <https://doi.org/10.1016/j.indcrop.2025.120748>.
- Wojnarowski, K., Cholewińska, P., Zhao, D., Pacoń, J., Bodkowski, R., 2025. Antibiotic resistance genes in food animal production: environmental implications and one health challenges. *Environments* 12, 427. <https://doi.org/10.3390/environments12110427>.
- Wu, P., Luo, D., Wang, Y., Shang, X., Wang, B., Deng, X., Yuan, J., 2024. Biosynthesis of diverse ephedra-type alkaloids via a newly identified enzymatic cascade. *Biodes. Res.* 6, 48. <https://doi.org/10.34133/bdr.0048>.
- Zhang, L., Wu, J., Xu, P., Guo, S., Zhou, T., Li, N., 2022. Soy protein degradation drives diversity of amino-containing compounds via bacillus subtilis natto fermentation. *Food Chem.* 388, 133034. <https://doi.org/10.1016/j.foodchem.2022.133034>.
- Zhou, Y., Hua, J., 2024. Current updates on lactic acid production and control during baijiu brewing. *Fermentation* 10, 505. <https://doi.org/10.3390/fermentation10100505>.