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Wen jing Gao
Hunan Agricultural University
Xue ning Luo
Hunan Agricultural University
Lin Mu
Hunan Agricultural University
Zhi fei Zhang
Hunan Agricultural University
Gui hua Chen
chen_gh@hunau.edu.cn

Hunan Agricultural University

Research Article

Keywords: Enterococcus faecalis YMA3, rice straw silage, whole-genome sequencing, microbiome, metabolomics, fermentation quality

Posted Date: March 23rd, 2026

DOI: https://doi.org/10.21203/rs.3.rs-8905101/v1

Additional Declarations: No competing interests reported.

Genomic insights into *Enterococcus faecalis* YMA3 and Its Impact on Rice Straw Silage Quality, Microbial community, and Metabolome

Wenjing Gao[1], Xuening Luo[1], Lin Mu[1,2] , ZhiFei Zhang[1,2,] Corresponding auther: Guihua Chen[1,2]

1 College of Agronomy, Hunan Agricultural University, Changsha 410128, China; 18547620509@163.com(W. G.); a651564994@163.com(X. L.);

2 Yuelushan Laboratory, Changsha 410128, China; linmu331@outlook.com(L. M.); zhangzf@hunau.edu.cn(Z. Z.); chen_gh@hunau.edu.cn(G. C.).

Abstract

Background

Rice straw is an abundant agricultural byproduct whose use as ruminant feed is constrained by its recalcitrant lignocellulosic structure and low digestibility. Here, we evaluated a novel autochthonous strain, *Enterococcus faecalis* YMA3, as a silage inoculant to improve rice straw fermentation quality and nutritive value.

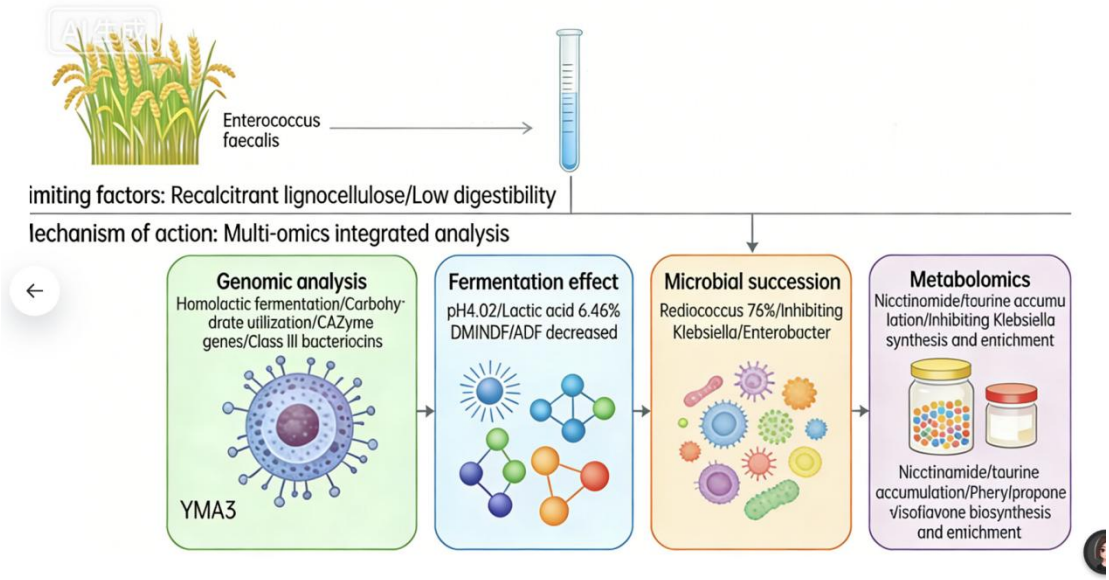
Results

A multi-omics framework integrating whole-genome sequencing (WGS) of *E.faecalis* YMA3 with full-length 16S rRNA gene sequencing and untargeted metabolomics of silage were applied. Genomic analysis indicated genetic capacity for homolactic fermentation, carbohydrate utilization and putative fiber-related substrate degradation (carbohydrate-active enzyme genes), and antimicrobial activity including a Class III bacteriocin. YMA3 significantly accelerated acidification (final pH 4.02), increased lactic acid (6.46% DM), associated with reductions in neutral detergent fiber (NDF) and acid detergent fiber (ADF). *E.faecalis* YMA3 reshaped microbial succession, enriching *Pediococcus* (76% relative abundance) and suppressing undesirable *Enterobacteriacea*e such as *Klebsiella* and *Enterobacte*r. Metabolomics confirmed extensive

biochemical remodeling with marked accumulation of nicotinamide and taurine and enrichment of phenylpropanoid and isoflavonoid biosynthesis pathways.

Conclusions

E.faecalis YMA3 acted as an effective starter culture that coordinates rapid acidification, community restructuring, and functional metabolite formation.



Keywords

Enterococcus faecalis YMA3; rice straw silage; whole-genome sequencing; microbiome; metabolomics; fermentation quality

1 Introduction

Rice straw is produced in large quantities in rice-based systems, yet the conversion of rice straw into high-value ruminant feed remains limited by its high lignin and silica contents, low water-soluble carbohydrates, and poor digestibility(Sarnklong C et al., 2010; Wang Bet et al., 2022). Ensiling is a practical route to stabilize rice straw and reduce environmental burdens associated with open-field burning or disposal, but successful fermentation can be constrained by insufficient

epiphytic lactic acid bacteria (LAB), limited fermentable substrates, and the proliferation of undesirable Enterobacteriaceae during early fermentation (Oladosu Y et al., 2016; Sun Y et al., 2023). Accordingly, LAB inoculants are widely used to accelerate pH decline, improve nutrient preservation, and steer microbial succession toward desirable fermentative communities (Adesogan A T, 2008; Sun L et al., 2023). Most commercial inoculants are dominated by *Lactobacillus/Lactiplantibacillus* species; however, *Enterococcus* and *Pediococcus* are also frequently detected as early-stage silage colonizers and have been incorporated into combination inoculants. Autochthonous strains isolated from local forage environments may exhibit superior ecological fitness and performance in a given substrate compared with allochthonous strains, potentially improving fermentation reliability and reducing inoculant costs (Carvalho B F et al., 2021; Burns P et al., 2023). Recent work on rice straw silage has demonstrated that targeted inoculation can simultaneously improve fermentation indices and drive broad metabolite remodeling, highlighting the value of integrating microbiome and metabolome profiling to elucidate mechanism. More broadly, advances in microbiome and metabolome tools have enabled a shift from descriptive fermentation endpoints to mechanistic understanding of how LAB shape community functions and silage chemistry (Sun Y et al., 2023; Wang Y et al.,2024). In this study, we characterize a novel rice-associated strain, *Enterococcus faecalis* YMA3, which displayed rapid acidification and antioxidant activity in preliminary screening (Huang L et al.,2024). Whole-genome sequencing of *E. faecalis*YMA3 was performed using a combination of second- and third-generation sequencing technologies, followed by bioinformatic analysis to predict genes and potential mechanisms associated with acid production and antioxidant activity. *E. faecalis*YMA3 was inoculated into rice straw for ensiling. After 60 days of ensiling, the effects of YMA3 on fermentation quality, nutritional

composition, microbial diversity, and metabolomic profiles were evaluated. This study aims to provide a scientific basis for the potential use of *E. faecalis*YMA3 as a silage inoculant.

2 Materials and Methods

2.1 Strain origin and whole-genome sequencing

The experimental strain *Enterococcus faecalis* YMA3 was revived from a glycerol stock stored at -80°C in our laboratory. Genomic DNA for sequencing was extracted from bacterial cultures harvested during the mid-exponential growth phase to ensure high quality and integrity. This strain was originally isolated from the phyllosphere of rice seedlings (variety: Zhuo 1S/6W1622) during the nursery stage at the Agricultural experiment farm of Hunan Agricultural University. The strain was selected for its rapid growth, high acid-production capability, and pronounced antioxidant activity in preliminary screenings.

2.2. Genome sequencing and antimicrobial susceptibility profiling

The complete genome of the strain was sequenced using a combination of the PacBio Sequel (for long reads) and Illumina NovaSeq PE150 (for short reads) platforms. De novoassembly was performed using Hifiasm (Cheng H et al., 2021;). The resulting assembly was circularized and the origin of replication was adjusted using Circlator v1.5.5. Protein-coding genes were predicted using Prodigal v2.6.3 (Hyatt D et al., 2010;). The assembly was subsequently polished with Illumina short-read data using Pilon v1.22 to enhance base-level accuracy. The sequence was aligned against the NCBI non-redundant database to confirm the chromosomal structure. Functional annotation was performed by comparing the predicted coding sequences against a series of specialized databases.

Antimicrobial susceptibility was determined using the disk diffusion method according to Shi et al.(Shi, M., et al,2022） with slight modifications. Briefly, an overnight culture was centrifuged

at 8,500 rpm for 5 min. The pellet was washed three times with 0.85% sterile saline and resuspended to an optical density (OD_{600}) of 0.6 ± 0.02. Subsequently, 100 μL of the bacterial suspension was spread evenly onto the surface of MRS agar plates. Eleven antibiotic disks were then aseptically placed on the agar surface. After diffusion at room temperature for 20 min, the plates were incubated at 37 ℃. The diameters of the inhibition zones were measured with a vernier caliper. The results were interpreted according to the Chinese Health Industry Standard 《Technical specification for antimicrobial susceptibility testing (WS/T 639-2018).》

2.2 Silage preparation and treatments

Rice straw (variety Xinxang S/9WH336) was harvested in Liuyang County, Hunan Province, China (28°17′04″ N, 113°25′19″ E), chopped to 2–3 cm, and ensiled in vacuum-sealed bags (600 g per bag). A completely randomized design included six inoculation treatments: *Enterococcus faecalis* YMA3 (Y), a commercial *Enterococcus faecalis* strain (F), *Lactobacillus acidophilus* (S), *Lactobacillus buchneri* (B), *Lactobacillus plantarum* (Z), and a non-inoculated control (CK). The commercial inoculants were purchased from Asia Core Biotechnology Co., Ltd. (Taiwan, China), with a viable bacterial count of 1.0×10^{11}CFU/g. All treatments were inoculated at a rate of 1×10^{6}CFU/g of fresh matter, with three replicates per treatment. Each replicate (600 g) was vacuum-sealed in a polyethylene bag and stored for 60 days at room temperature under light-protected conditions.

To evaluate the independent effect of E.faecalis YMA3 in the absence of epiphytic microbiota, a subset of samples was sterilized before ensiling using 15 kGy electron-beam irradiation. The irradiation treatment was performed using a DZ-10Mev/20KW electron accelerator (China General Nuclear Power Group Jinwo Technology Co., Ltd., Ningxiang, Changsha, Hunan Province, China).

The electron energy was set at 10 MeV with a beam current intensity of 2 mA.Microbiome and metabolomic analyses were performed at two time points: day 3 and day 60.

2.3 Fermentation characteristics and nutritional quality

After 60 days of ensiling, the silage bags were opened. Silage extract was prepared by homogenizing 20 g of sample (quartered) with 180 mL of distilled water in a sealed plastic bag, followed by storage at 4°C for 24 h. The mixture was filtered through two layers of cheesecloth to remove coarse residues, and then through rapid qualitative filter paper. The resulting extract, prepared in triplicate, was used to determine pH, organic acid concentrations, and ammonia nitrogen (NH_3-N/TN) content. pH was measured at room temperature using a Model SI400 pH meter (Spectrum Technologies). The ratio of ammonia nitrogen to total nitrogen (NH_3-N/TN) was determined by the phenol–sodium hypochlorite colorimetric method. The fermentation quality (LA, AA, PA) was analyzed using standard methods (HPLC for organic acids). Chromatographic column: Agilent TC-C18 column (specifications: 250 mm × 4.6 mm × 5μm). The injection volume was 20 μL, with a flow rate set at 0.7 mL/min and the column temperature maintained at 50°C. The UV detection wavelength was set at 210 nm, and the total run time was 35 min. Mobile phase A was methanol, and mobile phase B was a 0.01 mol/L potassium dihydrogen phosphate aqueous solution (pH adjusted to 2.70 using phosphoric acid). The gradient elution program was set as follows: at 0 min, the volume ratio of A:B was 3:97; at 10 min, it was adjusted to 10:90. Nutritional parameters ： dry matter (DM) , crude protein (CP) , Neutral Detergent Fiber (NDF) : Acid Detergent Fiber, (ADF) , Water Soluble Carbohydrates (WSC) , Crude Ash, were determined with reference to "*Feed Analysis and Feed Quality Testing Techniques*" (Zhang L Y et al., 2007).

2.4 Microbiome sequencing and untargeted metabolomics

Microbial DNA was extracted from silage samples collected at day 3 and day 60 from CK and *E.faecalis* YMA3 treatments, with and without irradiation. Full-length 16S rRNA gene (V1-V9) sequencing was performed using the PacBio platform. Community diversity metrics, ordination analyses, and differential taxon analyses were conducted using standard bioinformatics workflows(Community DNA fragments were sequenced using Single Molecule Real-Time sequencing on the PacBio Sequel third-generation sequencing platform). Functional profiles were inferred using 16S-based prediction and mapped to KEGG pathways . For metabolomics, day 3 and day 60 samples were analyzed using UPLC-Q mass spectrometry in positive and negative ionization modes. Differential metabolites were identified using VIP > 1 and $P \leq 0.05$ and annotated to KEGG pathways.

2.5 Statistical analysis

Data were analyzed in IBM SPSS Statistics 27. Differences among treatments were evaluated using one-way ANOVA followed by LSD multiple comparisons ($P < 0.05$). The membership function method was applied for comprehensive evaluation of silage quality. The assumptions of normality (assessed by Shapiro–Wilk test) and homoscedasticity (assessed by Levene's test) were verified prior to ANOVA.

3 Results

3.1 Genome features and functional potential of *Enterococcus faecalis* YMA3

The strain was sequenced using PacBio HiFi mode. After filtering out reads shorter than 2 kb, a total of 94,447 high-quality sequences were obtained, yielding a total of 792,078,919 base pairs. The reads exhibited an N50 length of 9,069 bp, an N90 length of 5,913 bp, an average read length of 8,386 bp, and a maximum read length of 32,172 bp, with an average read quality value of 31.92.

Assembly using Hifiasm yielded a circular chromosome of 2,678,952 bp (Fig. 1), with a G+C content of 37.73%. No plasmids were detected. Genome annotation predicted 2,449 protein-coding genes, along with 12 rRNA genes, 60 tRNA genes, and 3 CRISPR sequences.

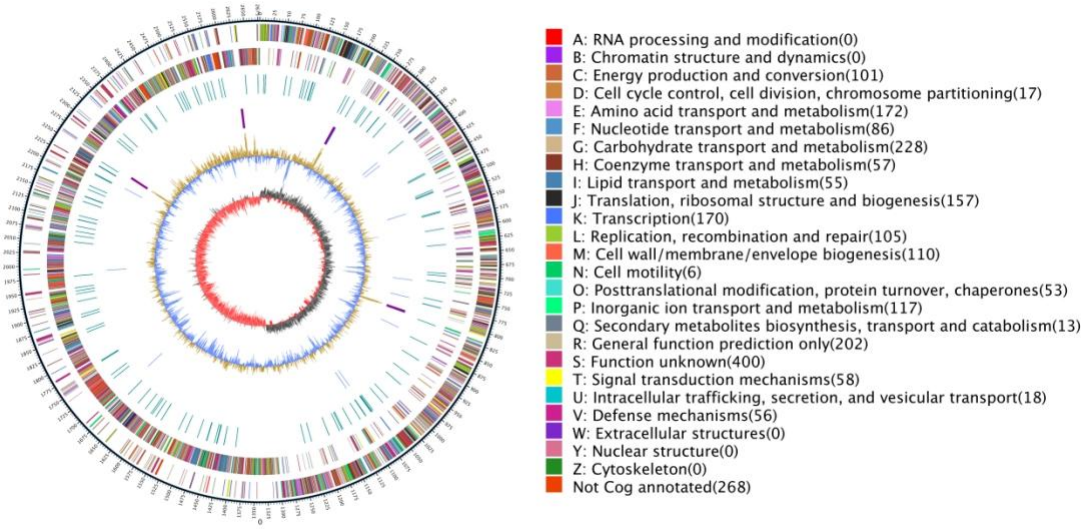


Fig. 1 Genome circle diagram

Note: The outermost circle is indicated by the size of the genome, and each scale is 5 kb; The second and third circles are genes on the positive and negative strands of the genome, respectively, and different colors represent different COG functional classifications. The fourth circle is a repeating sequence; The fifth circle is tRNA and rRNA, blue is tRNA, purple is rRNA; The sixth circle is the GC content, the light yellow part indicates that the GC content in the region is higher than the average GC content of the genome, the higher the peak, the greater the difference from the average GC content, and the blue part indicates that the GC content in the region is lower than the average GC content of the genome. The innermost ring is GC-skew, dark gray represents areas with G content greater than C, and red represents areas with C content greater than G.

Genomic annotation identified key enzymes involved in acid production, such as L-lactate dehydrogenase and pyruvate dehydrogenase (directly participating in pyruvate metabolism), as well

as antioxidant enzymes, including superoxide dismutase, NADH oxidase, and the thioredoxin system.Analysis using antiSMASH v5.0.0 predicted two biosynthetic gene clusters (BGCs) within the genome. Functional annotation revealed that the second BGC encodes a bacteriocin exhibiting 100% sequence identity to the Class III bacteriocin Enterolysin A (EnIA). This finding suggests the strain possesses antimicrobial potential, which may enhance its competitive fitness during the ensiling process (Delpech et al., 2019; Nilsen et al., 2003)

3.1.6 Functional annotation by Pfam database

A total of 2,210 genes were annotated, corresponding to 1,944 protein families. The core pathway for acid production (primarily lactic acid) in Enterococcus faecalisis glycolysis. A total of 12 protein families were associated with acid production, including dehydrogenase, kinase, and carboxylase families. Lactate dehydrogenase, which catalyzes the reduction of pyruvate to lactate, is a hallmark enzyme of homolactic fermentation and directly determines lactic acid yield. Phosphofructokinase, a rate-limiting enzyme in glycolysis, regulates carbon flux and acid production efficiency. Pyruvate kinase generates pyruvate and ATP, representing a key energy-yielding step in glycolysis. Pyruvate carboxylase modulates the tricarboxylic acid (TCA) cycle.

Antioxidant capacity primarily relies on the pentose phosphate pathway, which generates NADPH and supports the glutathione/thioredoxin systems. Thirteen protein families were linked to antioxidant functions, including thioredoxin and glutathione system-related enzyme families. Thioredoxin reduces disulfide bonds to repair oxidatively damaged proteins, relying on NADPH for regeneration. Glutathione reductase converts oxidized glutathione (GSSG) to reduced glutathione (GSH), maintaining the intracellular reducing environment (Table 1).

Table 1 Statistics and analysis of Pfam protein families related to acid production and

Function	Pfam Family	Family Name	Family Description	Functional Association
Acid Production-Related Protein Families	PF00056	Ldh_1_N	Lactate/malate dehydrogenase, NAD binding domain	NAD binding domain of lactate dehydrogenase (LDH), catalyzing pyruvate → lactate
	PF00069	Pkinase	Protein kinase domain	Phosphofructokinase (PFK) regulating glycolytic flux
	PF00070	Pyr_redox	Pyridine nucleotide-disulphide oxidoreductase	Pyruvate dehydrogenase complex (aerobic metabolic branch)
	PF00089	Trypsin	Trypsin protease	Potential peptidase, indirectly involved in metabolic regulation
	PF00121	TIM	Triosephosphate isomerase	Triosephosphate isomerase (TIM), a key glycolytic enzyme
	PF00122	E1-E2_ATPase	E1-E2 ATPase	Pyruvate kinase (PK), generating ATP and

				pyruvate
	PF00128	Alpha-amylase	Alpha amylase, catalytic domain	Starch decomposition into glucose, providing glycolytic substrates
	PF00224	PK	Pyruvate kinase, barrel domain	Pyruvate kinase, a key enzyme in the final step of glycolysis
	PF00365	PFK	Phosphofructokinase	Phosphofructokinase, the rate-limiting enzyme of glycolysis
	PF00456	Transketolase_N	Transketolase, thiamine diphosphate binding domain	Transketolase, involved in the coordination of PPP and glycolysis
	PF02866	Ldh_1_C	Lactate/malate dehydrogenase, alpha/beta C-terminal domain	C-terminal catalytic domain of lactate dehydrogenase (LDH)
	PF03942	PGI	Phosphoglucose isomerase	Glucose-6-phosphate isomerase, linking glycolysis and PPP
Antioxidant-	PF00005	ABC_tran	ABC transporter	May be involved in the

Related Protein Families				transport of oxidative stress response substances
	PF00044	Gp_dh_N	Glyceraldehyde 3-phosphate dehydrogenase, NAD binding domain	Glyceraldehyde-3-phosphate dehydrogenase, generating NADH (indirectly affecting NADPH)
	PF00070	Pyr_redox	Pyridine nucleotide-disulphide oxidoreductase	Glutathione reductase (Gor), maintaining GSH/GSSG balance
	PF00085	Thioredoxin	Thioredoxin	Thioredoxin, repairing oxidatively damaged proteins
	PF00089	Trypsin	Trypsin protease	Potential protease for clearing oxidative damage
	PF00107	ADH_zinc_N	Zinc-binding dehydrogenase	Alcohol dehydrogenase, involved in NAD^+ regeneration

PF00430	ATP-synt_B	ATP synthase B/B' CF(0)	Related to oxidative phosphorylation, maintaining energy homeostasis
PF00480	ROK	ROK family	Transcription factor regulating carbon metabolism flux to PPP
PF00667	5_3_exonuc	5'-3' exonuclease, C-terminal SAM fold	DNA repair, responding to oxidative damage
PF00724	Oxidored_FMN	NADH:flavin oxidoreductase / NADH oxidase family	Scavenging reactive oxygen species (ROS)
PF01396	zf-C4_Topoisom	Topoisomerase DNA binding C4 zinc finger	DNA repair enzyme, responding to oxidative stress
PF03441	FAD_binding_7	FAD binding domain of DNA photolyase	Photolyase, repairing UV or oxidatively damaged DNA
PF07650	Sigma70_ECF	ECF sigma factor	Regulating the expression of oxidative stress-related

genes

3.1.8 Functional annotation by VFDB database

A total of 443 virulence factors were identified in strain YMA3 by alignment against the Virulence Factors Database (VFDB). In accordance with established research on enterococcal pathogens, the pathogenic potential of this strain is primarily attributed to the following five categories of virulence factors. Screening and analysis revealed 26 major virulence factor genes, including: genes encoding cytolysin (cylA, cylI, cylR2); genes within the Cps operon involved in capsular polysaccharide synthesis, which confers antiphagocytic properties (cpsA, cpsB, cpsC, cpsD, cpsE, cpsF, cpsG, cpsH, cpsI, cpsJ, cpsK); genes encoding extracellular enzymes (gelatinase gelE, hyaluronidases EF0818 and EF3023, serine protease SprE); genes associated with biofilm formation (bopD, fsrABC); and genes encoding adhesion-related proteins (Ace, EbpA, EbpB, EbpC, EfaA, and SrtC). This virulence profile is consistent with the known genomic background of the Enterococcus genus (Panthee et al., 2021; Prichula et al., 2020).

3.1.10 Prediction of Acid-Producing Functional Genes and Preliminary Mechanism in Strain YMA3

Lactic acid is a major component of total acids during silage fermentation, and its concentration influences both silage quality and the fermentation process. Therefore, genes involved in lactic acid metabolism in Enterococcus faecalisYMA3 were annotated; the corresponding gene information (Table 2). Based on the gene list and associated enzyme functions, YMA3 lacks the gene encoding phosphoketolase in the obligate heterolactic fermentation pathway, thus preventing the use of the phosphoketolase (PK) pathway to metabolize fructose-6-phosphate or pentoses into mixed products (e.g. acetate and CO_2). However, the strain possesses a complete glycolytic pathway (Embden–

198 Meyerhof–Parnas pathway), allowing the clear conclusion that its core acid-production pathway is
199 homolactic fermentation driven by glycolysis (Fig. 2).The xfpgene encodes phosphoketolase,
200 enabling lactic acid fermentation via the PK pathway. Phosphoketolase genes (xfp) are present in
201 Leuconostoc mesenteroidesand Bifidobacteriumspecies such as B. adolescentis, facilitating
202 heterolactic fermentation through the phosphoketolase pathway (Liu J, 2022). Suppressing xfpgene
203 expression can block the acetate synthesis pathway, reduce acetate by-production, and increase
204 lactic acid yield. This metabolic feature may offer advantages in acid production efficiency,
205 environmental adaptability, and potential applications such as probiotics and food fermentation (Shi
206 L.,2020) .

207 3.1.11 Prediction of Antioxidant Functional Genes and Preliminary Mechanism in Strain
208 YMA3

209 Annotation results indicate that Enterococcus faecalisYMA3 contains a fully functional
210 oxidative phase of the pentose phosphate pathway (PPP), enabling efficient NADPH generation,
211 which is central to its antioxidant capacity(Table 3), the presence of key PPP enzymes suggests
212 metabolic coordination between PPP and glycolysis, supporting both NADPH production for redox
213 balance and flexible carbon utilization for energy supply.

214 The strong antioxidant activity observed in preliminary experiments may be attributed to: (1)
215 high activity of the oxidative PPP phase, where elevated expression of glucose-6-phosphate
216 dehydrogenase and 6-phosphogluconate dehydrogenase could enhance NADPH generation and
217 reinforce reactive oxygen species (ROS) scavenging; (2) synergistic action of the antioxidant
218 enzyme system—although superoxide dismutase (SOD) and catalase were not detected, abundant
219 NADPH may indirectly support glutathione peroxidase (GPx) in eliminating lipid peroxides using

reduced glutathione, and the thioredoxin system in repairing oxidatively damaged proteins; and (3) optimized carbon flux redistribution under oxidative stress, potentially increasing PPP activity at the expense of glycolysis to prioritize NADPH production and improve oxidative stress tolerance.

Table 2 Information on the main genes involved in lactate metabolism

Gene ID	Enzyme Name	EC Number	Function
GE002036	Glucokinase	2.7.1.2	Catalyzes the phosphorylation of glucose to glucose-6-phosphate (G6P)
GE001096	Glucose-6-phosphate isomerase	5.3.1.9	Converts G6P to fructose-6-phosphate (F6P)
GE000738	6-phosphofructokinase 1	2.7.1.11	Rate-limiting enzyme that catalyzes F6P to fructose-1,6-bisphosphate (F1,6BP)
GE000855	Fructose-1,6-bisphosphate aldolase	4.1.2.13	Cleaves F1,6BP into glyceraldehyde-3-phosphate (G3P) and dihydroxyacetone phosphate (DHAP)
GE001513	Triosephosphate isomerase	5.3.1.1	Converts DHAP to G3P, ensuring two molecules of G3P enter subsequent metabolism
GE001137	Glyceraldehyde-3-phosphate dehydrogenase	1.2.1.12	Catalyzes G3P to 1,3-bisphosphoglycerate (1,3-BPG), generating NADH

GE000133	Phosphoglycerate mutase	5.4.2.11	Converts 3-phosphoglycerate (3-PG) to 2-phosphoglycerate (2-PG)
GE001512	Enolase	4.2.1.11	Catalyzes the dehydration of 2-PG to phosphoenolpyruvate (PEP)
GE000739	Pyruvate kinase	2.7.1.40	Key energy-producing enzyme that catalyzes PEP to pyruvate,伴随 ATP generation
GE000189	L-lactate dehydrogenase	1.1.1.27	Catalyzes the reduction of pyruvate to lactate, regenerating NAD^+ to sustain glycolysis
GE001034	Pyruvate dehydrogenase E1α subunit	1.2.4.1	Converts pyruvate to acetyl-CoA under aerobic conditions (enters TCA cycle)
GE001036	Pyruvate dehydrogenase E2 component	2.3.1.12	Same as above, but Enterococcus faecalis is primarily anaerobic fermentative, so this pathway contributes minimally
GE001261	Dihydrolipoamide dehydrogenase	1.8.1.4	Component of the pyruvate dehydrogenase complex, related to aerobic metabolism
GE000592	Aldehyde dehydrogenase/Alcohol dehydrogenase	1.2.1.10	May catalyze pyruvate to acetate or ethanol (a marker of heterofermentation), but with extremely low activity in Enterococcus faecalis

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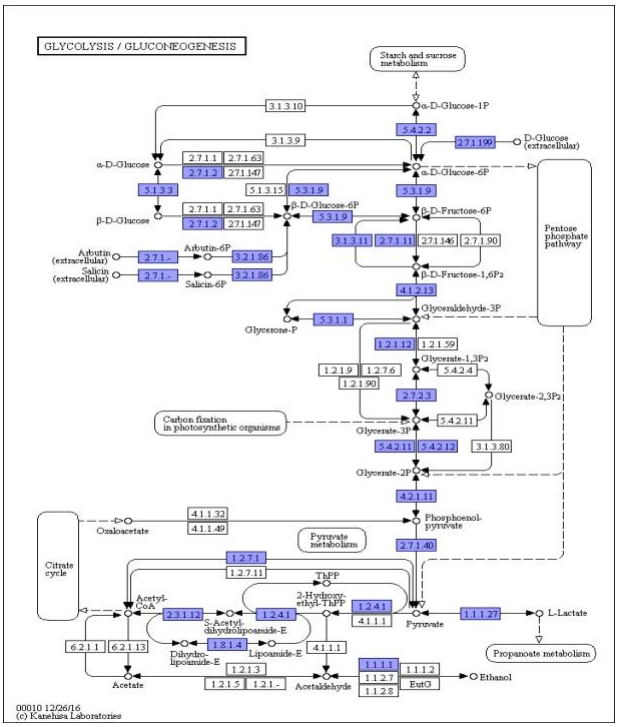


Fig. 2 Glycolytic pathway

Note: The purple part in the image represents the gene carried by *E.faecalis* YMA3

225 **Table 3 Information on the main genes involved in the antioxidant pathway**

Gene ID	Enzyme Name	EC Number	Function
GE000699	Glucose-6-phosphate dehydrogenase (G6PDH)	1.1.1.49 1.1.1.363	Catalyzes: Glucose-6-phosphate (G6P) → 6-phosphogluconolactone, generating NADPH (key antioxidant coenzyme)
GE000742	6-phosphogluconate dehydrogenase (6PGDH)	1.1.1.44 1.1.1.343	Catalyzes: 6-phosphogluconate → ribulose-5-phosphate**, generating NADPH again
GE000135	Ribulose-5-phosphate isomerase (RPI)	5.3.1.6	Converts ribulose-5-phosphate to ribose-5-phosphate (nucleotide precursor)
GE002267	Ribulose-5-phosphate	5.1.3.1	Generates xylulose-5-phosphate (enters

	epimerase (RPE)		non-oxidative phase)
GE001189	Transketolase	5.3.1.1	Recombines pentoses and heptoses to generate glycolytic intermediates (e.g., F6P, G3P)

3.1.12 Antimicrobial Susceptibility of *E.faecalis*YMA3

Strain *E.faecalis* YMA3 is susceptible to Penicillin, Chloramphenicol, Rifampicin, Cefazolin, and Cefuroxime; it shows intermediate susceptibility to Tetracycline, Ofloxacin, and Meropenem; and is resistant to Kanamycin, Vancomycin, and Ciprofloxacin (Table 4).Genomic annotation and phenotypic susceptibility results showed partial concordance. For instance, the presence of the efrA and efrB genes may underlie the observed resistance phenotype to agents such as ciprofloxacin, while the lsaA gene could potentially explain a resistance mechanism towards lincosamides. Conversely, although resistance genes such as dfrE, associated with the folate metabolic pathway, were identified, the strain remained susceptible to multiple β-lactam antibiotics. This suggests that these genes may not be expressed or their expression could be suppressed by regulatory mechanisms.

Table 4 Antibacterial circle diameter and test results of Enterococcus on drug sensitivity (mm)

Antibiotic		Pen	Chl	Rif	Tet	Cef	Cxm	Ofx	Mem	Kan	Van	Cip
Strai nNu mber	*Staphyl ococcus aureus* YMA3	36.33± 0.97 (S)	20.37 ±0.95 (S)	23.15 ±0.60 (S)	20.77 ±0.11 (S)	33.68 ±0.11 (S)	29.21 ±0.35 (S)	22.62 ±0.31 (S)	30.78 ±0.49 (I)	21.65 ±0.12 (S)	13.79 ±0.16 (R)	20.92 ±0.28 (S)
		31.14± 0.35	23.78 ±1.98	21.48 ±0.31	16.26 ±0.41	24.93 ±0.74	20.66 ±0.33	12.00 ±0.21	20.71 ±0.45	— (R)	— (R)	— (R)

(S)	(S)	(S)	(I)	(S)	(S)	(I)	(I)

Note: "S": Susceptible;"I": Intermediate;"R": Resistant;"—": No inhibition zone.

3.2 Fermentation characteristics of rice straw silage

Across 60 d of fermentation, inoculation affected acidification and organic acid profiles (Fig. 3). *E.faecalis* YMA3 (Y) produced a low final pH (4.02) and the highest lactic acid concentration (6.46% DM), indicating strong fermentative performance under the tested conditions. No butyric acid was detected, and ammonia-N/total N ratios were reduced relative to the control, suggesting inhibited proteolysis (Zhao J et al., 2021; Wang Y L et al., 2022).

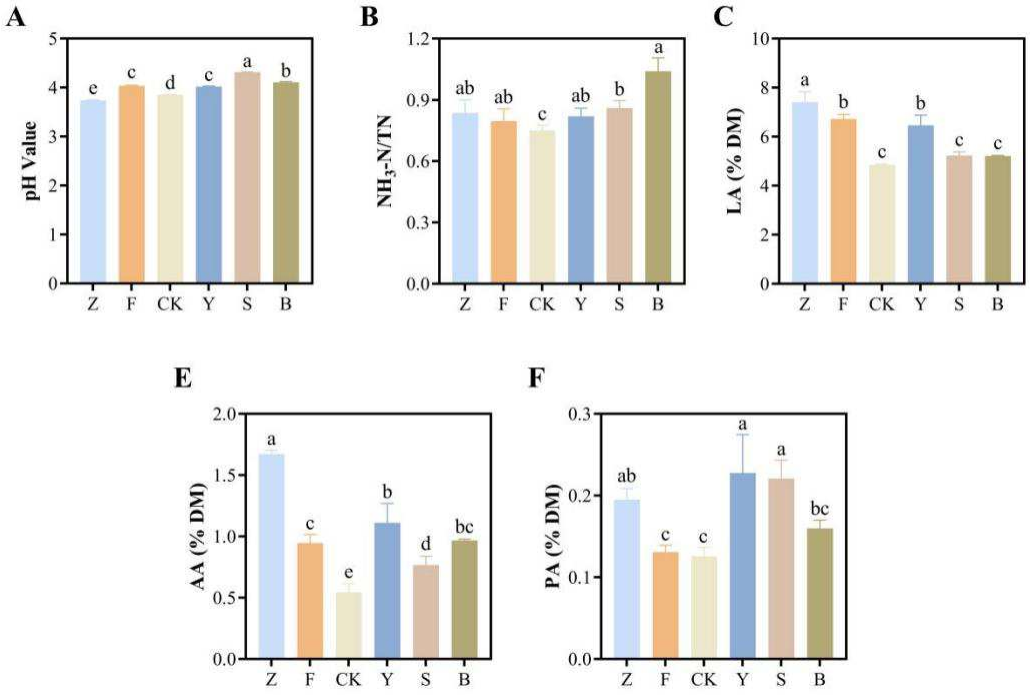


Fig. 3 Effects of lactic acid bacteria inoculation on fermentation quality of rice straw silage (60 d)

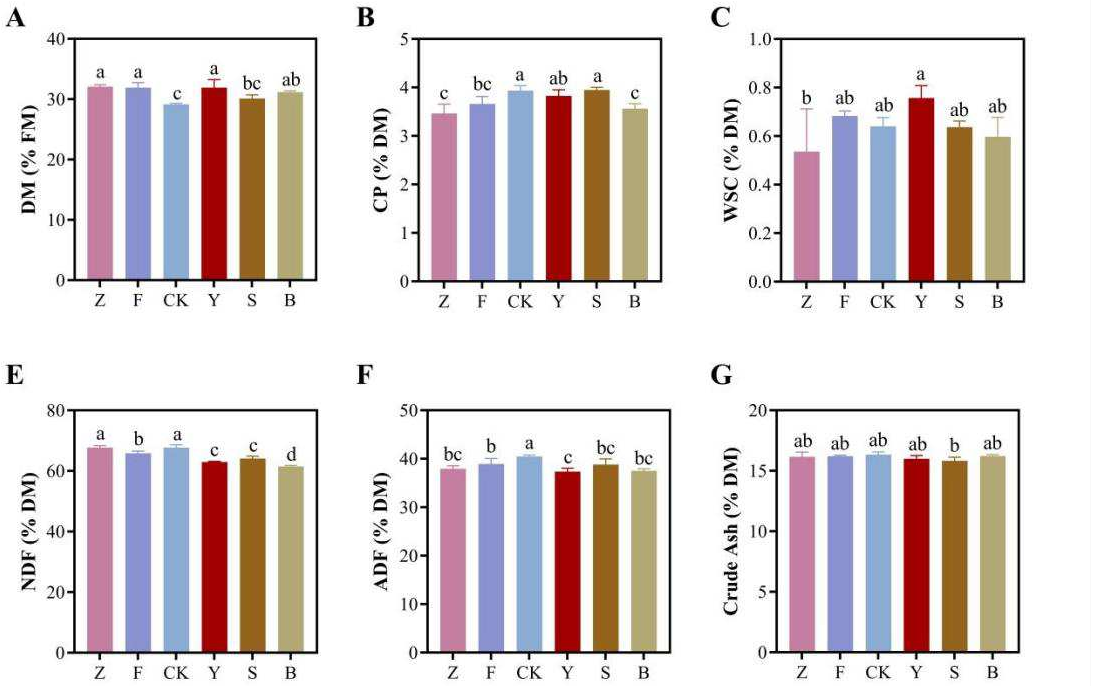
Note: The letters on the x-axis (Z, F, CK, Y, S, B) represent commercial *Lactiplantibacillus plantarum*, commercial *Enterococcus faecalis*, control group, *Enterococcus faecalis*YMA3, commercial *Lactobacillus acidophilus*, and commercial *Lentilactobacillus buchneri*, respectively;

the same abbreviations apply to the figures below. The y-axis indicates different fermentation parameters after 60 days of ensiling: (A) pH, (B) NH_3-N/TN, (C) lactic acid, (E) acetic acid, (F) propionic acid. Different lowercase letters above the bars within the same parameter indicate significant differences among treatments *(P* < 0.05); the same convention applies to the figures below.

3.3 Nutritional composition and feed value indices

Inoculation also influenced the chemical composition and calculated feed value (Fig. 4). Compared with the control group, *E.faecalis* YMA3 maintained higher dry matter and crude protein content while reducing NDF and ADF levels, indicating improved predicted intake potential and digestibility (Zhao J et al., 2021; Wu Y et al., 2022).

From the perspective of the comprehensive evaluation results based on the membership function, Group Y ranked second, and *E.faecalis* YMA3 demonstrated significant effects in improving overall nutritional quality, showing potential to partially replace commercial inoculants(Table 5).



265

266 **Fig. 4 Effect of Lactobacillus Addition on the Nutritional Quality of Rice Straw Silage**

267 **Note:** The y-axis indicates different fermentation parameters after 60 days of ensiling: (A) dry

268 matter , (B) crude protein , (C) water-soluble carbohydrates, (E) neutral detergent fiber (%), (F) acid

269 detergent fiber, (G) ash.

270 **Table 5 Comprehensive Evaluation of 60 Day Weighted Membership Functions for Rice**

271 **Straw Silage**

项目		Z	F	CK	Y	S	B	权重
隶属函数值	pH	0.996	0.866	0.780	0.872	0.742	0.833	0.872
	DM	0.461	0.464	0.785	0.550	0.516	0.518	0.550
	CP	0.122	0.232	0.388	0.326	0.394	0.179	0.326
	WSC	0.045	0.084	0.073	0.104	0.072	0.061	0.104
	NDF	0.481	0.613	0.479	0.810	0.731	0.914	0.810
	ADF	0.810	0.710	0.552	0.869	0.724	0.854	0.869

	LA	0.927	0.839	0.594	0.805	0.645	0.642	0.805
	AA	0.983	0.523	0.268	0.627	0.409	0.536	0.627
	PA	0.773	0.847	0.851	0.733	0.741	0.812	0.733
D 值		0.613	0.577	0.495	0.593	0.526	0.571	
Rank		1	3	6	2	5	4	

3.4 Microbial community dynamics in response to YMA3

To clarify how *E.faecalis* YMA3 modulated fermentation ecology, the bacterial community was profiled at day 3 and day 60 in CK and *E.faecalis* YMA3 silages, with and without irradiation pretreatment. Alpha-diversity metrics are summarized in (Table 6). *E.faecalis* YMA3 shifted community structure toward LAB dominance and reduced the prevalence of undesirable Enterobacteriaceae during fermentation. In non-sterilized silage, Pediococcus became highly enriched (up to 76% relative abundance at 60 d), while potential spoilage and proteolytic taxa such as Klebsiella and Enterobacter were suppressed(Fig. 5) (Bai J et al., 2021; Liu J et al., 2025).

In the Enterococcus faecalisYMA3 treatment group (D60_YW), the microbial community was predominantly constituted by the genus Pediococcus. The predicted metabolic potential of this community showed strong agreement with the complete metabolic capabilities inferred from the whole-genome sequence analysis of *E. faecalis*YMA3, particularly in key pathways such as glycolysis (homolactic fermentation) and the pentose phosphate pathway (Okoye et al., 2023; Jiang et al., 2020). These results indicate that inoculation with *E. faecalis*YMA3 not only modified the microbial community structure but also steered its metabolic function toward enhanced acid production(Fig. 6).

289 **Table 6 Alpha Diversity of Bacterial Communities on Rice Straw Surface**

Sample Number	Chao1	Observed_species	Shannon	Simpson	Goods_coverage
D3_CK	1176.85	1109.43	4.98	0.80	0.99
D3_Y	1006.99	1002.10	5.53	0.93	1.00
D60_CK	856.31	771.33	3.47	0.80	0.99
D60_Y	1471.59	1360.90	5.33	0.91	0.99

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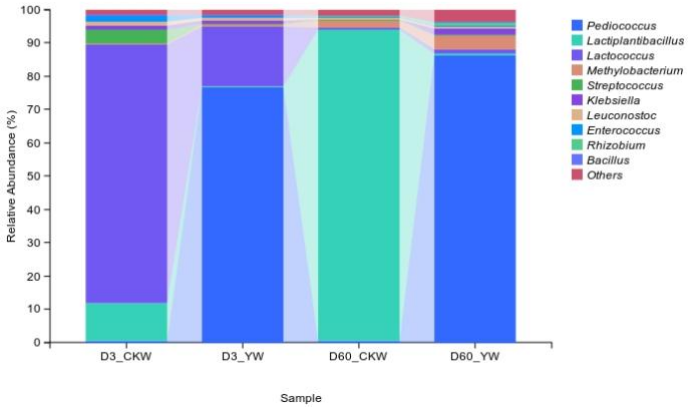

Fig. 5 Species composition (genus level)

Note： Different colors in the figure represent different microorganisms

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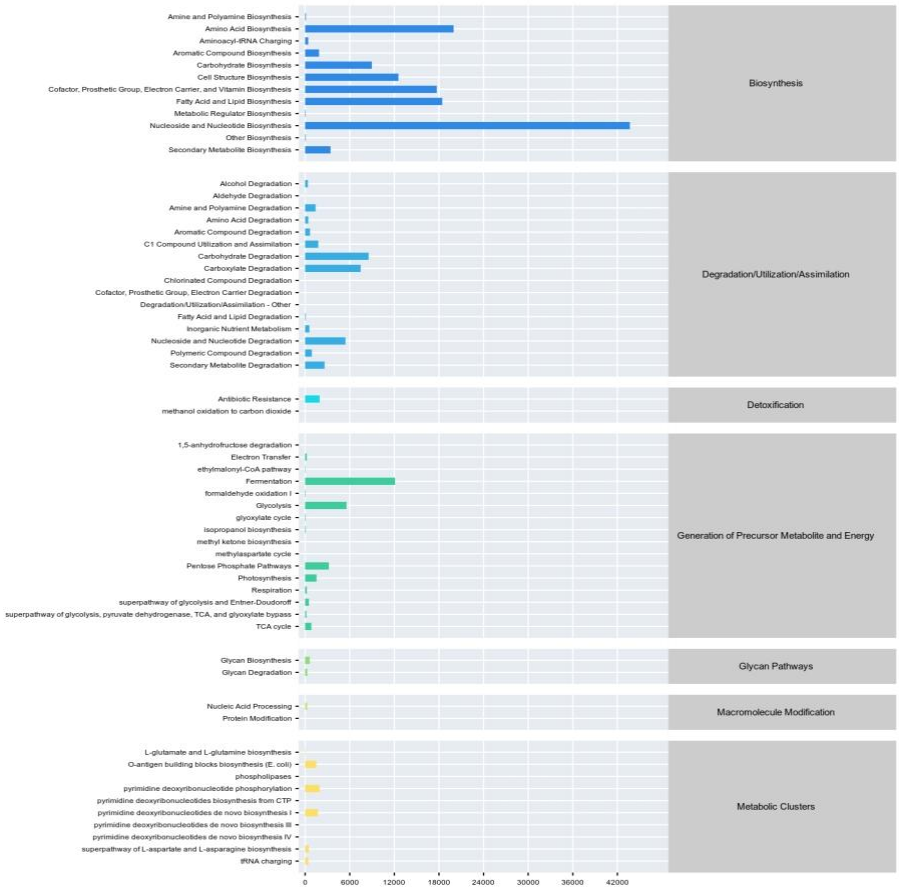

Fig. 6 KEGG Functional Annotation

3.5 Metabolomic remodeling induced by YMA3 inoculation

Untargeted metabolomics revealed substantial remodeling of the silage metabolome following *E.faecalis* YMA3 inoculation. Multivariate analyses separated *E.faecalis* YMA3 from control groups, indicating consistent treatment effects. Among the most strongly upregulated metabolites were nicotinamide and taurine, along with phenylpropanoid-related compounds; pathway enrichment highlighted phenylpropanoid and isoflavonoid biosynthesis among the most responsive pathways (Table 7;Fig.7) (Liu Y et al., 2024; Gao Y et al., 2024).

Table 7 Top 20 upregulated differential metabolites in silage at day 60

Name	Superclass	VIP	Log_2FC

2-Hydroxyisobutyric acid	Organic acids and derivatives	2.42	4.13
Nicotinamide	Organoheterocyclic compounds	2.05	4.01
Taurocholic acid	Lipids and lipid-like molecules	2.09	3.84
1,4-Cyclohexanedione	Organic oxygen compounds	2.11	3.68
Biochanin A	Phenylpropanoids and polyketides	1.50	3.58
Indole-3-lactic acid	Organoheterocyclic compounds	2.05	3.35
2-Hydroxy-3-(4-hydryphenyl)propanoic acid	Phenylpropanoids and polyketides	1.69	2.83
2,4-Dihydroxy-3,6-dimethylbenzoic acid	Benzenoids	2.35	2.67
Hydroxybenzotriazole	Organoheterocyclic compounds	1.44	2.13
4-(Azaniumylmethyl)-5-(hydroxymethyl)-2-methylpyridin-3-olate	Organoheterocyclic compounds	1.89	2.10
Ferulicacid	Phenylpropanoids and polyketides	1.43	2.09
Indole-3-acetaldehyde	Organoheterocyclic compounds	1.93	2.05
Coumarin	Phenylpropanoids and polyketides	2.10	2.01
(S)-Suspensaside	Organic oxygen compounds	1.63	1.99
Chrysoeriol	Phenylpropanoids and polyketides	1.94	1.96
2-(6-Aminopurin-9-yl)-5-(hydroxymethyl)oxolane-3,4-diol	Nucleosides, nucleotides, and analogues	1.67	1.95
Flavone base +3O, 2MeO,O-guaiacylglycerol	Phenylpropanoids and polyketides	1.91	1.94
Primulin	Phenylpropanoids and polyketides	2.02	1.80

(2-Hydroxy-3-octadec-9-enoyloxypropyl)2-(trimethylazaniumyl)ethyl phosphate	Lipids and lipid-like molecules	1.53	1.75
4-Vinylphenol	Benzenoids	2.17	1.63

Note: Due to the large number of differential metabolites, this table only displays the top 20 differential metabolites of Log_2FC in descending order.

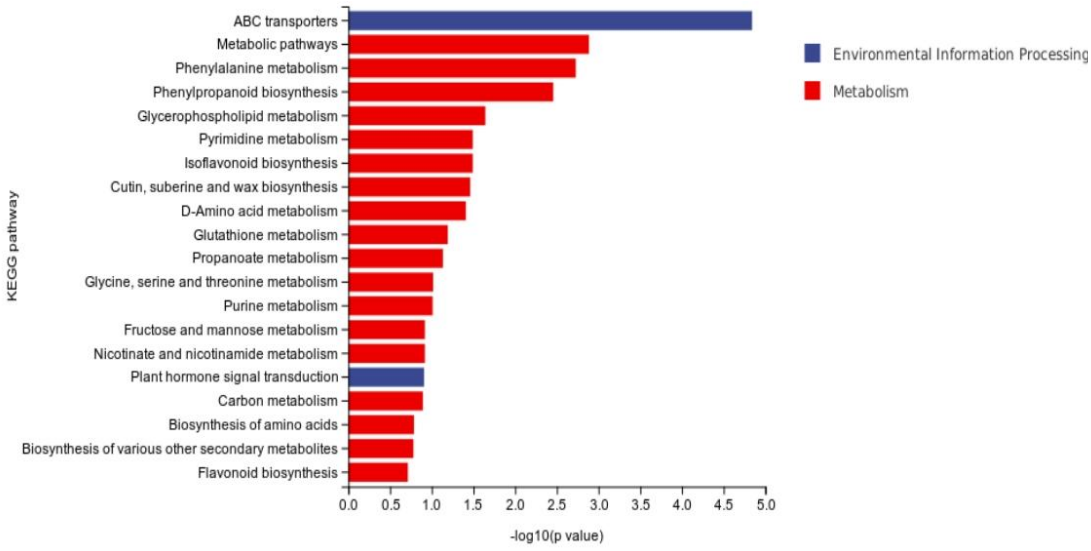

Fig. 7 Differential Metabolite Pathway Classification Diagram

Note: The abscissa of Figure is significant, the ordinate is KEGG pathway

4 Discussion

This study establishes a crucial link between the genomic characteristics of the inoculant Enterococcus faecalis YMA3 and its impact on fermentation parameters, microbial succession, and metabolic profiles in rice straw silage. Rapid pH decline and lactic acid accumulation are fundamental determinants of silage stability (Wang Y et al., 2024; Silva Y A et al., 2025), as they effectively inhibit proteolytic and spoilage microorganisms such as Enterobacteriaceae, thereby reducing proteolysis and dry matter loss. Consistent with this principle, *E. faecalis* YMA3 induced

309 the most pronounced acidification and lactic acid accumulation among all tested inoculants,
310 concurrently resulting in a lower NH_3-N/TN ratio, which indicates improved protein preservation.
311 Rice straw, characterized by its limited water-soluble carbohydrate content and complex
312 lignocellulosic structure, typically exhibits inferior fermentation stability compared to maize or
313 high-sugar forages (Wang X et al., 2022; Chen X et al., 2022). Previous research has demonstrated
314 that targeted inoculation with lactic acid bacteria (LAB) can enhance the quality of rice straw silage
315 while simultaneously reshaping its microbial community structure and metabolome (Sun Y et al.,
316 2023). In the present study, *E. faecalis* YMA3 not only improved key fermentation indicators but
317 also reduced fiber components, suggesting a potential enhancement in predicted feed intake and
318 digestibility (Jiao T et al., 2021; Andrada E et al., 2024).

319 Genus-level analysis of the bacterial community composition revealed distinct successional
320 patterns of key functional microbiota. Pediococcus became the dominant genus in the YMA3-
321 inoculated silage (76.73%–99.59%), which aligns with its rapid acid-producing capability. This
322 dominance was likely facilitated by the rapid pH decrease in the early ensiling phase and the inherent
323 tolerance of Pediococcus to low-moisture and acidic environments (Buxton D R et al., 2003; Du G
324 et al., 2021; Sun L et al., 2023). In contrast, the relative abundance of Lactiplantibacillus increased
325 significantly in the 60-day control group (89.72%–93.54%), indicating the presence of naturally
326 occurring, acid-tolerant Lactiplantibacillus strains within the rice straw microbiota that are adapted
327 to long-term fermentation conditions. This observation is consistent with findings reported in
328 naturally fermented peanut vine silage (Mao K et al., 2025). The relative abundances of
329 Methylobacterium and Klebsiella were minimal and detected only in the non-irradiated groups. Ma
330 (Ma Y S, 2022) reported that mixed silage of alfalfa and whole-plant maize increased the relative

abundance of LAB and reduced harmful microorganisms such as Klebsiella and Enterobacter, which is consistent with the microbial shifts observed in our study.

Our findings share similarities with observations by Guo et al. (Guo X S et al., 2018) and Bai et al. (Bai J et al., 2021). Guo et al. found that inoculating alfalfa silage with Lentilactobacillus buchneri could promote the growth of Lactiplantibacillus plantarum. Bai et al. observed that in alfalfa silage inoculated with *E. faecalis* and ensiled for 60 days, *Weissella* and *Lactobacillus* were the most abundant genera at the genus level, while the relative abundance of *Enterococcus* was low. Furthermore, in a treatment co-inoculated with *E. faecalis, Pediococcus pentosaceus*, and *Lactiplantibacillus plantarum*, the relative abundances of *Weissella* and *Lactobacillus* were higher compared to the single inoculation, and the relative abundance of Enterococcus was even lower. Similarly, Tang et al. (Tang Y et al., 2024), in a study on ensiled alfalfa from different regions of Gansu Province, found that after 48 days of ensiling, the relative abundances of *Pediococcus acidilactici*, *Lactiplantibacillus plantarum*, *Pediococcus pentosaceus*, and *Lacticaseibacillus rhamnosus* increased compared to the raw material, while the relative abundances of *Enterococcus faecalis*, *Weissella cibaria*, *Lentilactobacillus brevis*, *Lacticaseibacillus paracasei*, and *Lentilactobacillus buchneri decreased*, suggesting that Pediococcus might be more suitable than Enterococcus for long-term ensiling.

E. faecalis proliferates rapidly and produces acid during the initial stages of ensiling (1–3 days), lowering the pH below 4.5 and inhibiting other microorganisms. Given the ecological resilience and adaptability of *E. faecalis* within its niche (Díaz A M, 2018), we hypothesize that it creates an ecological niche that facilitates the subsequent dominance of acid-tolerant *Pediococcus*, leading to an increased relative abundance of the latter (Xiao Y et al., 2023; Oskoueian E et al., 2021). As a

353 common commensal in the animal gut (Liu J H et al., 2019), Enterococcus was predicted to possess
354 a Class III bacteriocin in the YMA3 genome. We speculate that the produced bacteriocin may inhibit
355 competitors such as *Klebsiella*, reducing resource competition and indirectly promoting the growth
356 of Pediococcus. Future studies should validate this interaction mechanism by monitoring the
357 dynamics of *E. faecalis* and *Pediococcus* abundances on days 1, 3, 7, 15, and 30 of ensiling, and by
358 determining the growth curves and metabolic products of both strains under co-culture conditions.
359 If the facilitative role of *E. faecalis* is confirmed, designing a combined inoculant (e.g., *E. faecalis*
360 + *Pediococcus*) could be explored to accelerate silage acidification and improve preservation
361 outcomes.

362 Whole-genome sequencing of *E. faecalis* YMA3 predicted the presence of acid-producing
363 metabolic pathways (e.g., glycolysis, pentose phosphate pathway, tricarboxylic acid cycle). These
364 predictions align well with the metabolic functional profiling inferred from the rice straw silage
365 system, indicating the strain's potential activity on complex substrates such as cellulose and
366 hemicellulose. The metabolic pathway analysis suggests that *E. faecalis* contributes to rapid
367 acidification and energy supply in rice straw silage. Our results are consistent with existing literature
368 demonstrating that rapid acid production via the glycolytic pathway during ensiling lowers the pH,
369 thereby inhibiting the growth of spoilage microorganisms (Arriola K G et al., 2011). Furthermore,
370 activity in the pentose phosphate pathway may provide NADPH, supporting resistance to oxidative
371 stress and enhancing the ecological competitiveness of the bacterium (Wen Z Y et al., 2025).

372 The remodeled metabolome provides a functional readout that complements the observed
373 microbial community shifts. The enrichment of nicotinamide and taurine is consistent with a
374 transition towards LAB-driven amino acid and vitamin-related metabolism under low-pH

conditions (Xu D et al., 2021). The enrichment of phenylpropanoid and isoflavonoid biosynthesis pathways suggests that YMA3 may influence the release, transformation, or preservation of plant secondary metabolites during the ensiling process (Gao Y et al., 2024; Chen X et al., 2025). Comparable multi-omics studies have highlighted that inoculant-driven metabolite changes can indicate improved nutritional value and may help explain the ecological dominance of the inoculant (Xu D et al., 2019; Liu Y et al., 2024). From an animal feed science and technology (AFST) perspective, these metabolites could also be relevant to palatability, antioxidant capacity, and potential functional feed properties associated with animal health; however, verification through targeted quantification and animal feeding trials is necessary. The significant upregulation of functional metabolites such as nicotinamide (a vitamin B3 precursor) and taurine (an antioxidant) in the YMA3-inoculated silage suggests the potential for enhanced vitamin supply and antioxidant capacity in animals consuming this feed, which may contribute to improved immune function and metabolic health (Zhang Y et al., 2024).

The safety assessment of *E. faecalis* YMA3 addressed concerns regarding antibiotic resistance and virulence-associated genes. The primary risks for silage inoculants involve horizontal gene transfer and intrinsic pathogenicity. In strain YMA3, the chromosomal location of the resistance genes, as opposed to plasmid-borne, significantly reduces the potential for horizontal transfer. Furthermore, *E. faecalis* is a commensal organism in the microbiomes of many animals, and numerous strains have Generally Recognized as Safe (GRAS) status for use in food fermentation (Graham K et al., 2020; Hanchi H et al., 2018). The detected virulence factors form part of the core genome and do not inherently confer pathogenicity, which is typically regulated by multifactorial genetic and physiological pathways (Vebø H C et al., 2010; Bakshi U et al., 2016). Critically, the

acidic ensiling environment (pH < 4.2) acts as an intrinsic safety barrier by inhibiting bacterial survival in the final feed product. Combined with its plant (phyllosphere) origin and selection based on fermentation efficacy, these factors support a low-risk profile for E. faecalis YMA3 as a silage inoculant. Future studies evaluating its aerobic stability and in vivo feeding performance will provide further validation of its practical safety.

5 Conclusions

E.faecalis YMA3 improved rice straw silage fermentation by accelerating acidification, increasing lactic acid accumulation, reducing proteolysis, and improving predicted feed value indices. YMA3 reshaped microbial succession toward LAB dominance and promoted a distinct metabolomic profile enriched in nicotinamide-, taurine-, and phenylpropanoid-related metabolites. To provide a scientific basis for the potential application of Enterococcus faecalisYMA3 as a silage inoculant.

References

Adesogan A T. Recent advances in bacterial silage inoculant technology[J]. Proc. Florida Ruminant, 2008.

Andrada E, Abeijón-Mukdsi M C, Vinderola G, et al. Ferulic acid esterase-producing Lactobacilli as silage inoculants: A review on the efficacy of improving fiber composition and digestibility of forages[J]. Fermentation, 2024, 10(12): 614.

Arriola K G, Kim S C, Adesogan A T. Effect of applying inoculants with heterolacticor homolactic and heterolactic bacteria on the fermentation and quality of corn silage[J]. Journal of Dairy Science, 2011, 94(3): 1511-1516.

Bai J, Ding Z, Ke W, et al. Different lactic acid bacteria and their combinations regulated the

419 fermentation process of ensiled alfalfa: ensiling characteristics, dynamics of bacterial community
420 and their functional shifts[J]. Microbial Biotechnology, 2021, 14(3): 1171-1182.
421 Bai J, Ding Z, Ke W, et al. Different lactic acid bacteria and their combinations regulatedthe
422 fermentation process of ensiled alfalfa: ensiling characteristics, dynamics of bacterialcommunity
423 and their functional shifts[J]. Microbiat Biotechnology, 2021, 14 (3): 171-182.
424 Bakshi U, Sarkar M, Paul S, et al. Assessment of virulence potential of uncharacterized
425 *Enterococcus faecalis* strains using pan genomic approach–Identification of pathogen–specific and
426 habitat-specific genes[J]. Scientific reports, 2016, 6(1): 38648.
427 Burns P, Borgo M F, Binetti A, et al. Isolation, characterization and performance of autochthonous
428 spray dried lactic acid bacteria in maize micro and bucket-silos[J]. Frontiers in Microbiology, 2018,
429 9: 2861.
430 Buxton D R, Muck R E, Harrison J H.Silage Science and Technology[M]. Silage science and
431 technology, 2003.
432 Carvalho B F, Sales G F C, Schwan R F, et al. Criteria for lactic acid bacteria screening to enhance
433 silage quality[J]. Journal of Applied Microbiology, 2021, 130(2): 341-355.
434 Chen X, Ma Y, Khan M Z, et al. A combination of lactic acid bacteria and molasses improves
435 fermentation quality, chemical composition, physicochemical structure, in vitro degradability and
436 rumen microbiota colonization of rice straw[J]. Frontiers in Veterinary Science, 2022, 9: 900764.
437 Chen X, Wang Y, Shan X, et al. Supplements enhance antioxidant activity and improve the quality
438 of whole-plant corn silage by altering secondary metabolic pathways and phenolic metabolite
439 composition[J]. Chemical and Biological Technologies in Agriculture, 2025, 12(1): 1-17.
440 Cheng H, Concepcion G T, Feng X, et al. Haplotype-resolved de novo assembly using phased

441 assembly graphs with hifiasm[J]. Nature methods, 2021, 18(2): 170-175.

442 Delpech G, Hebert E M, Sparo M, et al. Draft genome sequence of *Enterococcus faecalis* strain

443 CECT7121, a corn silage isolate with antibacterial activity against Gram-positive pathogens[J].

444 Microbiology Resource Announcements, 2019, 8(20): 10.1128/mra. 00245-19.

445 Díaz A M, Almozni B, Molina M A, et al. Potentiation of the humoral immune response elicited by

446 a commercial vaccine against bovine respiratory disease by Enterococcus faecalis CECT7121[J].

447 Beneficial Microbes, 2018, 9(4): 553-562.

448 Du G, Shi J, Zhang J, et al. Exogenous probiotics improve fermentation quality, microflora

449 phenotypes, and trophic modes of fermented vegetable waste for animal feed[J]. Microorganisms,

450 2021, 9(3): 644.

451 Gao Y, Zhou H, Wang Y, et al. Insights into fermentation with lactic acid bacteria on the flavonoids

452 biotransformation of alfalfa silage[J]. Chemical and Biological Technologies in Agriculture, 2024,

453 11(1): 73.

454 Graham K, Stack H, Rea R. Safety, beneficial and technological properties of enterococci for use in

455 functional food applications–a review[J]. Critical Reviews in Food Science and Nutrition, 2020,

456 60(22): 3836-3861.

457 Guo X S., Ke W.C., Ding W.R., et al. Profiling of metabolome and bacterial community dynamics

458 in ensiled Medicago sativa inoculated without or with Lactobacillus plantarum or Lactobacillus

459 buchneri[J]. Scientific Reports, 2018, 8(1), 357.

460 Hanchi H, Mottawea W, Sebei K, et al. The genus Enterococcus: between probiotic potential and

461 safety concerns—an update[J]. Frontiers in microbiology, 2018, 9: 1791.

462 Huang L, Zhang Z, Mu L, et al. Dynamic succession of the quantity and composition of epiphytic

463 microorganisms at different growth stages on rice surface[J]. Frontiers in Microbiology, 2024, 15:
464 1451935.
465 Hyatt D, Chen G L, LoCascio P F, et al. Prodigal: prokaryotic generecognition and translation
466 initiation site identification[J]. BMC bioinformatics, 2010, 11(1): 1.
467 Jiang J, Yang B, Ross R P, et al. Comparative genomics of Pediococcus pentosaceus isolated from
468 different niches reveals genetic diversity in carbohydrate metabolism and immune system[J].
469 Frontiers in microbiology, 2020, 11: 253.
470 Jiao T, Lei Z, Wu J, et al. Effect of additives and filling methods on whole plant corn silage quality,
471 fermentation characteristics and in situ digestibility[J]. Animal bioscience, 2021, 34(11): 1776.
472 Liu J, Liu M, Sheng P, et al. Biotechnological Effects of Lactobacillus plantarum, Cellulase, and
473 Xylanase on Nutritional Quality and Microbial Community Structure of Corn Stover Silage[J].
474 Fermentation, 2025, 11(1): 14.
475 Liu J. Rational metabolic engineering of L-glutamate producing strain and optimization of its
476 fermentation control[D]. Tianjin: Tianjin University of Science and Technology, 2022.
477 Liu Y, Du S, Sun L, et al. Volatile metabolomics and metagenomics reveal the effects of lactic acid
478 bacteria on alfalfa silage quality, microbial communities, and volatile organic compounds[J].
479 Communications Biology, 2024, 7(1): 1565.
480 Liu, J H, Wu, F Y, Chen, B Y, et al. Study on the probiotic characteristics and toxic side effects of
481 Enterococcus faecalis [C].2019: 157-161.
482 Ma Y S. Effects of mixed silage with alfalfa on silage quality and microbial diversity. Acta
483 Prataculturae Sinica, 2022, 35(4): 123-134.
484 Mao, K., Xu, Y., Wang, X., et al. Effects of Lactiplantibacillus plantarumand molasses on the

485 fermentation quality, biogenic amine content, and bacterial community of peanut vine silage. Acta
486 Prataculturae Sinica, 2025, 34(5): 146-158.
487 Nilsen T, Nes I F, Holo H. Enterolysin A, a cell wall-degrading bacteriocin from *Enterococcus*
488 *faecalis* LMG 2333[J]. Applied and environmental microbiology, 2003, 69(5): 2975-2984.
489 Okoye C O, Wei Z, Jiang H, et al. Metagenomics analysis reveals the performance of homo-and
490 heterofermentative lactic acid bacteria in alfalfa silage fermentation, bacterial community, and
491 functional profiles[J]. Journal of Animal Science, 2023, 101: skad163.
492 Oladosu Y, Rafii M Y, Abdullah N, et al. Fermentation quality and additives: a case of rice straw
493 silage[J]. BioMed research international, 2016, 2016(1): 7985167.
494 Oskoueian E, Jahromi M F, Jafari S, et al. Manipulation of rice straw silage fermentation with
495 different types of lactic acid bacteria inoculant affects rumen microbial fermentation characteristics
496 and methane production[J]. Veterinary Sciences, 2021, 8(6): 100.
497 Panthee S, Paudel A, Hamamoto H, et al. Complete genome sequence and comparative genomic
498 analysis of *Enterococcus faecalis* EF-2001, a probiotic bacterium[J]. Genomics, 2021, 113(3): 1534-
499 1542.
500 Prichula J, Van Tyne D, Schwartzman J, et al. Enterococci from wild magellanic penguins
501 (Spheniscus magellanicus) as an indicator of marine ecosystem health and human impact[J].
502 Applied and Environmental Microbiology, 2020, 86(19): e01662-20.
503 Sarnklong C, Cone J W, Pellikaan W, et al. Utilization of rice straw and different treatments to
504 improve its feed value for ruminants: a review[J]. Asian-Australasian Journal of Animal Sciences,
505 2010, 23(5): 680-692.
506 Shi L. BeijingConstruction and application of the xylulose cleavage pathway in Escherichia coli[D].

Beijing: University of Chemical Technology, 2020.

Shi, M., Wu, Y., Yang, K., et al. Screening and safety evaluation of potential probiotic Lactiplantibacillus plantarum from Sichuan Paocai [J]. Science and Technology of Food Industry, 2022, 43(22): 165-172.

Silva Y A, Cardoso M V S B, Carvalho B F, et al. Lactic acid bacteria strains isolated from rehydrated corn grain silage enhance the chemical and microbiological characteristics and aerobic stability of these silages[J]. Journal of Applied Microbiology, 2025, 136(8): lxaf185.

Sun L, Xue Y, Xiao Y, et al. Community synergy of lactic acid bacteria and cleaner fermentation of oat silage prepared with a multispecies microbial inoculant[J].

Sun Y, Sun Q, Tang Y, et al. Integrated microbiology and metabolomic analysis reveal the improvement of rice straw silage quality by inoculation of Lactobacillus brevis[J]. Biotechnology for Biofuels and Bioproducts, 2023, 16(1): 184.

Tang Y., Liu X., Zhao Y., et al. Study on the characteristics of lactic acid bacteria community and its driving factors in silaged alfalfa from different regions of Gansu Province. Acta Prataculturae Sinica, 2024, 33(2): 112-124.

Vebø H C, Solheim M, Snipen L, et al. Comparative genomic analysis of pathogenic and probiotic *Enterococcus faecalis* isolates, and their transcriptional responses to growth in human urine[J]. PloS one, 2010, 5(8): e12489.

Wang X, Song J, Liu Z, et al. Fermentation quality and microbial community of corn stover or rice straw silage mixed with soybean curd residue[J]. Animals, 2022, 12(7): 919.

Wang Y L, Wang W K, Wu Q C, et al. The effect of different lactic acid bacteria inoculants on silage quality, phenolic acid profiles, bacterial community and in vitro rumen fermentation characteristic

of whole corn silage[J]. Fermentation, 2022, 8(6): 285.

Wang Y T , She Xinyou, Xie Zhan, et al. Effects of wheat bran addition ratio on the silage quality of hybrid elephant grass 'Guimu No.1' [J]. Feed Research, 2022,45(12):90-93.

Wang Y, Sun Y, Huang K X, et al. Multi-omics analysis reveals the core microbiome and biomarker for nutrition degradation in alfalfa silage fermentation[J]. Msystems, 2024, 9(11): e00682-24.

Wen, Z Y, He, X C, Zheng, Y Y Research progress on energy metabolism disorder in hypertrophic cardiomyopathy [J/OL]. Advances in Cardiovascular Diseases,,2025,46(5):394-398.

Wu Y, Pang X, Wu Y, et al. Enterocins: classification, synthesis, antibacterial mechanisms and food applications[J]. Molecules, 2022, 27(7): 2258.

Xiao Y, Sun L, Xin X, et al. Physicochemical characteristics and microbial community succession during oat silage prepared without or with Lactiplantibacillus plantarum or Lentilactobacillus buchneri[J]. Microbiology Spectrum, 2023, 11(6): e02228-23.

Xu D, Ding W, Ke W, et al. Modulation of metabolome and bacterial community in whole crop corn silage by inoculating homofermentative Lactobacillus plantarum and heterofermentative Lactobacillus buchneri[J]. Frontiers in Microbiology, 2019, 9: 3299.

Xu D, Wang N, Rinne M, et al. The bacterial community and metabolome dynamics and their interactions modulate fermentation process of whole crop corn silage prepared with or without inoculants[J]. Microbial Biotechnology, 2021, 14(2): 561-576.

Zhang L Y. Feed Analysis and Feed Quality Testing Technology [M]. Beijing: China Agricultural University Press, 2007.

Zhang Y, Usman S, Li Q, et al. Effects of antioxidant-rich Lactiplantibacillus plantarum inoculated alfalfa silage on rumen fermentation, antioxidant and immunity status, and mammary gland gene

551 expression in dairy goats[J]. Journal of Animal Science and Biotechnology, 2024, 15(1): 9.
552 Zhao J, Shao T, Chen S, et al. Characterization and identification of cellulase-producing
553 Enterococcus species isolated from Tibetan yak (Bos grunniens) rumen and their application in
554 various forage silages[J]. Journal of Applied Microbiology, 2021, 131(3): 1102-1112.

555 **Declarations**

556 Consent for publication： All authors have read and agreed to the published version of the
557 manuscript.

558 Availability of data and material：The datasets used and/or analysed during the current study
559 are available from the corresponding author on reasonable request.

560 Competing interests：The authors' initials to refer to each authors' competing interests.

561 Funding：Mechanisms of Leaf-Sourced Lactic Acid Bacteria from Rice and Grain Amaranth
562 in Regulating Silage Fermentation (2026JJ3006); Hunan Province Major Scientific and
563 Technological Breakthrough Project under the "Open Competition for Leadership" System (No.
564 2025AQ2034).

565 Authors' contributions： Investigation, data curation, writing—original draft preparation,
566 visual ization, review and editing, resources, W. G.; formal analysis, data curation, X. L.; writing—
567 review and editing, resources, Z.Z.; methodology, writing— review and editing, supervision,
568 project administration, L.M.; conceptualization, methodology, writing — review and editing,
569 resources, supervision, project administration, G.C..