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Saccharomyces cerevisiae strains L7 contribute to flavor and deacidification in *Suanyu*, a Chinese traditional fermented fish

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Saccharomyces cerevisiae L7 was found to be an excellent starter and biological deacidification strain for *Suanyu*, however, the underlying mechanisms remain poorly understood. This study aimed to investigate the acid inhibition mechanism of *S. cerevisiae* L7. The strain enhances the sensory and flavor characteristics of *Suanyu*. The growth of *Lactiplantibacillus plantarum* is inhibited due to competition for carbon sources, resulting in a decrease in cell count from 9.00 Lg CFU/mL at 48 h to 7.70 Lg CFU/mL in co-culture. The addition of yeast reduces acidity, decreasing it from 5.83 g/kg to 0.82 g/kg at 48 h, while increasing sugar utilization to 94.52%. We found that cell contact was the main method of inhibition between the two microbials. Transcriptome analysis revealed that multiple pathways were affected under co-culture, ultimately leading to a decrease in lactic acid production. These findings provide valuable insights into the microbial interactions involved in biological deacidification.

Several traditional fermented foods are manufactured using lactic acid bacteria (LAB), which improve their flavor, nutritional value, and preservation capabilities[1,2]. *Suanyu*, a traditional fermented fish widely consumed in southwest China, is utilized to preserve fish for extended periods, ensuring a stable source of protein[3]. *Suanyu* is fermented from fresh fish, various spices, and cooked grains, containing a rich variety of flavor compounds, free amino acids, and peptides[4]. *L. plantarum* is the major starter in *Suanyu* fermentation[5], and autochthonous LAB contributes to the standardization of *Suanyu*, resulting in improved organoleptic properties for industrial applications[6].

The acidity of fermented foods is challenging to control, resulting in poor taste and low consumer acceptance for most lactic acid bacteria (LAB)-fermented products, such as sourdough[2] and fermented vegetables[7]. This low acceptance also limits their potential to support the growth of probiotics. Similarly, the quality of *Suanyu* is adversely affected by high acidity, making it urgent to reduce the acidity in fermentation. Biological deacidification is the preferred method, as it is not subject to regulatory constraints[8,9]. In general, the use of co-fermentation with yeast and LAB instead of single LAB fermentation is a method of controlling excessive acidity in fermented foods. Cassimiro et al.[10] demonstrated that, in comparison to inoculation with *L. plantarum* CCMA 1065 only, co-inoculation with *L. plantarum* CCMA 1065 and *S. cerevisiae* CCMA 0543 considerably decreased the acetic acid, citric acid, malic acid, and succinic acid in fermented coffee. According to a study from Zhang et al.[11], When *L. plantarum* BC114 and *S. cerevisiae* SC125 are co-fermented, the lactic acid content in mulberries (*Morus alba L.*) decreases from 4.70 g/L (when fermented with BC114 alone) to 2.56 g/L. To produce high-quality foods, it is crucial to be able to screen microbes that could both lower acid and positively impact the quality of fermented foods[11]. This study proposed to screen yeast strains from commercial yeast that could degrade acids in fermented foods with the aim of fermentation to produce more suitable organic acid concentrations and improve the quality of foods.

Biological deacidification involves the use of microbial interactions among different strains to reduce acidity in fermented products. This process encompasses both the consumption of acids and the inhibition of acid production. Yeast could utilize organic acids as a carbon source. The inhibition of acid production occurs through microbial interactions, which include both contact and non-contact inhibition[2,6,9,12–15]. Yeast could inhibit acid production or the growth of LAB through these interactions[8,10,16]. However, there are limited studies focused on acid reduction through

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microbial interrelationships. Therefore, we investigated the potential mechanisms by which yeast inhibits acid production in LAB from the perspective of microbial interactions, aiming to provide new insights for controlling the acidity of fermented foods.

This research aimed to identify safe and effective acid-reducing yeasts and to provide autochthonous starters to address the issue of excessive acidity in the traditional *Suanyu* fermentation process. The method of yeast inhibition of LAB was investigated at both the physicochemical and molecular levels, laying the foundation for the further development of this starter culture. This work offers theoretical support for the identification and application of strains, as well as for the regulation of acidity in fermented foods.

Results

Changes in sensory and flavor

The sensory quality of *Suanyu* plays a vital role in determining its overall quality and consumer acceptance. The results of the sensory evaluation are illustrated in Fig. 1a. Sensory evaluation encompasses four dimensions: color, texture, taste, and aroma. Regarding color scores, there is almost no difference between the two groups. However, Group L scores higher in texture, possibly due to the reduction in acidity, which leads to a firmer fish texture. The taste score also improves as a result of the decrease in acidity. In terms of aroma scores, Group L surpasses Group M.

Flavor is a crucial criterion for assessing food quality. Through HS-SPME-GC-MS analysis of volatile compounds in Groups L and M, we identified a total of 422 volatile compounds, which were classified into 14 categories: Acid, Alcohol, Aldehyde, Amine, Aromatics, Ester, Halogenated Hydrocarbons, Heterocyclic Compounds, Hydrocarbons, Ketones, Nitrogen Compounds, Sulfur Compounds, and Terpenoids. Among these, Terpenoids exhibited the highest content, comprising 32.7% in Group L and 33.4% in Group M, followed by Alcohol, which accounted for 23.7% in Group L and 23.4% in Group M (Fig. 1b). The PCA plot of flavor, illustrated in Fig. 1e, reveals a distinct separation between the two groups. The two principal components (PC) of the PCA model explained 64.2% of the total variance in the volatile compounds, with PC1 and PC2 explaining 35.2% and 29%, respectively. The clustering of Group L was less pronounced compared to Group M, suggesting that the introduction of *S. cerevisiae* L7 contributed to a more stable flavor profile in Suanyu. Based on a significance level of $p < 0.05$ and a $\log_2$ fold change greater than 1 or less than −1, we identified 27 significantly different volatile compounds (Fig. 1c), indicating that the introduction of brewing yeast significantly impacts the composition of volatile compounds in *Suanyu*.

The correlation of flavor substance classes is illustrated in Fig. 1d. The significant correlations were observed among various classes of flavor substances. For instance, alcohol exhibited a negative correlation with aldehydes and amines, while phenol showed a negative correlation with sulfur compounds and a positive correlation with terpenoids.

OAV (odor activity value) is widely used to assess the contribution of individual compounds to the overall aroma of food. Volatile compounds with an OAV greater than 1 are generally considered aroma-active and significantly contribute to the overall aroma characteristics. A quantitative analysis of key aroma-active compounds in two groups of *Suanyu* was conducted using OAVs. In total, 63 compounds with an OAV greater than 1 were identified as key compounds influencing the aroma characteristics of Suanyu (see Table 1). In Group L, only two compounds exhibited an OAV greater than 1. These compounds are both alcohols: Nerol and (±)-2-Pentanol acetate. Nerol imparts lemon and fresh flavors, while (±)-2-Pentanol acetate contributes herbal, weedy, musty, green, vegetable, nut skin, beany, ketonic, and animalic notes.

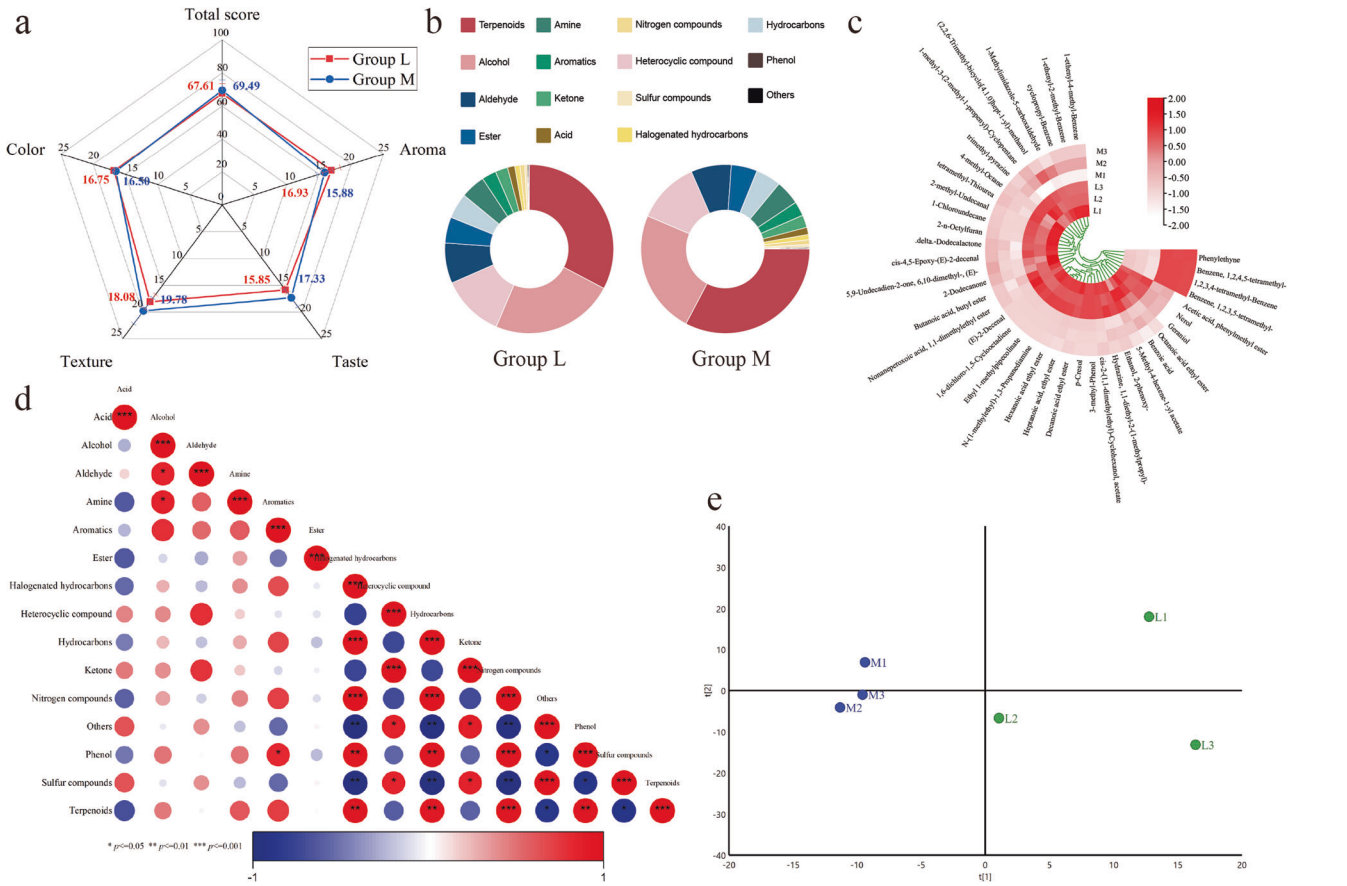

Fig. 1 | Characteristics of *Suanyu*. **a** Sensory evaluation score. **b** The volatile compounds in *Suanyu*. **c** The heatmap of significantly different volatile compounds. **d** The correlation of flavor substances class. **e** PCA based on volatile compounds in *Suanyu*.

Table 1 | Odor-active compounds of two groups of *Suanyu*

Compounds	Type	Odor descriptors	Threshold	Group L	Group M
2-Octen-1-ol	Alcohol	Green, vegetable	0.05	95.15	117.08
3-methyl-1-Butanol	Alcohol	Whiskey, malty, burnt	0.004	102.69	63.33
(E,Z)-3,6-nonadien-1-ol	Alcohol	Fatty, green, cucumber, green pepper, fruity, watermelon	0.003	18.22	16.6
Phenylethyl alcohol	Alcohol	Fruity, rose, sweet, apple	0.14	7.08	7.87
(±)-2-Pentanol acetate	Alcohol	Herbal, weedy, musty, green, vegetable, nut skin, beany, ketonic, animalic	0.015	11.84	0.62
Nerol	Alcohol	Lemon, fresh	0.049	1.62	0.52
10-Undecenal	Aldehyde	Waxy, aldehydic, rose, mandarin, citrus, soapy, fatty	0.0035	95.75	96.06
Nonanal	Aldehyde	Aldehyde, citrus, orange peel	0.001	47.97	95.87
(E)-2-Octenal	Aldehyde	Fresh, cucumber, fatty, green, herbal, banana, waxy, leafy	0.003	76.29	86.16
(E)-2-Decenal	Aldehyde	Waxy, fatty, earthy, green, cilantro, mushroom, aldehydic, fried, chicken, fatty, tallow	0.005	27.83	9.26
Benzeneacetaldehyde	Aldehyde	Floral, honey, rose, cherry	0.0063	9.3	8.46
5-Methyl-2-FurancarboxAldehyde,	Aldehyde	Spice, caramel, maple	0.5	2.62	4.89
2-Undecenal	Aldehyde	Fresh, fruity, citrus, orange, peel	0.00078	4.41	3.44
Naphthalene	Aromatics	Pungent, dry, tarry	0.05	4.41	2.6
5-hexyldihydro-2(3H)-Furanone	Ester	Fresh, oily, waxy, peach, coconut, buttery, sweet	0.0011	29.99	26.2
Propanoic acid, hexyl ester	Ester	Pear, green, fruity, musty, rotten	0.008	13.16	15.19
Hexanoic acid, ethyl ester	Ester	Apple, pear, fruity	0.005	58.93	13.72
Heptanoic acid, ethyl ester	Ester	Fruity, pineapple, cognac, rummy, wine	0.002	26.13	9.72
Methyl salicylate	Ester	Caramel, pepperminty	0.04	8.86	6.91
5-Butyldihydro-2(3H)-Furanone	Ester	Sweet, coconut, waxy, creamy, tonka, dairy, fatty	0.0179	6.63	5.93
5-Heptyldihydro-2(3H)-Furanone	Ester	Fruity, peach, creamy, fatty, lactonic, apricot, ketonic, coconut	0.0021	4.98	5.59
Decanoic acid, ethyl ester	Ester	Sweet, waxy, fruity, apple, grape, oily, brandy	0.005	30.55	5.54
S-Methyl 3-methylbutanethioate	Ester	Sharp, ripe cheese, sulfury, acrid, fermented, tomato, mushroom	0.1	2.93	4.3
3-Hydroxy-4,5-dimethyl-2(5H)-Furanone	Ester	Extremely sweet, strong caramel, maple, burnt, sugar, coffee	0.011	3.3	3.4
Octanoic acid, ethyl ester	Ester	Fruity, banana	0.04	7.6	3.15
Butanoic acid, butyl ester	Ester	Fruity, banana, pineapple, green, cherry, tropical fruit, ripe fruit, juicy fruity	0.028	2.94	1.09
3,5-Diethyl-2-methyl-Pyrazine	Heterocyclic	Nutty, meaty, vegetable	0.05	52.7	56.88
Acetylpyrazine	Heterocyclic	Popcorn, nutty, corn, chip, bread, crust, chocolate, hazelnut, coffee	0.01	8.66	12.46
1-Methyl-1H-Pyrrole-2-carboxaldehyde	Heterocyclic	Roasted, nutty	0.037	2.44	4.67
Pyrazine, trimethyl-	Heterocyclic	Nut skin, earthy, powdery, cocoa, baked, potato, roasted, peanut, hazelnut, musty	0.29	4.76	1.96
4-(2,6,6-Trimethyl-1-cyclohexen-1-yl)-2-Butanone	Ketone	Earthy, woody, mahogany, orris, dry, amber	0.0036	22.94	19.71
3-Methyl-2-(2-pentenyl)-2-Cyclopenten-1-one	Ketone	Woody, herbal, floral, spicy, jasmin, celery	0.00026	3.36	2.73
Tetrahydro-6-methyl-2H-Pyran-2-one,	Ketone	Creamy, fruity, coconut	0.02683	1.83	2.16
2-Methoxy-Phenol	Phenol	Nutty	0.0016	93.78	116.5
Diallyl Sulfur compounds	Sulfur	Sulfury, onion, garlic, horseradish, metallic	0.1	6.37	7.84
1,3,6-Octatriene, 3,7-dimethyl-, (Z)-	Terpenoids	Warm, floral, herbal, flowery, sweet	0.034	51.01	52.39
Trans-.beta.-Ocimene	Terpenoids	Sweet, herbal	0.034	51.01	52.39
1-Methyl-4-(1-methylethenyl)-7-Oxabicyclo[4.1.0] heptane,	Terpenoids	Fresh, citrus, minty, spearmint, herbal	0.1	34.43	32.82
.beta.-Ocimene	Terpenoids	Apple, pear, fruity	0.034	18.19	18.48
5-Methyl-2-(1-methylethyl)-Cyclohexanone	Terpenoids	Minty	0.0047	12.91	17.44
α.-Phellandrene 1	Terpenoids	Citrus, herbal, terpene, green, woody, peppery	0.04	8.36	9.16
Camphor	Terpenoids	Camphor	0.016	5.39	5.5
Citronellol	Terpenoids	Floral, rose, lime	0.04	2.78	5.04
β-Myrcene	Terpenoids	Musty, balsamic, spice	0.015	3.24	4.18
Geraniol	Terpenoids	Sweet, floral, fruity, rose, waxy, citrus	0.0066	12.01	3.86
3,7-Dimethyl-(E)-2,6-octadienal	Terpenoids	Citrus, lemon	0.028	2.02	3.13
β.-Pinene	Terpenoids	Dry, woody, resinous, pine, hay, green	0.14	2.39	2.62
α.-Farnesene	Terpenoids	Citrus, herbal, lavender, bergamot, myrrh, neroli, green	0.087	2.82	1.81

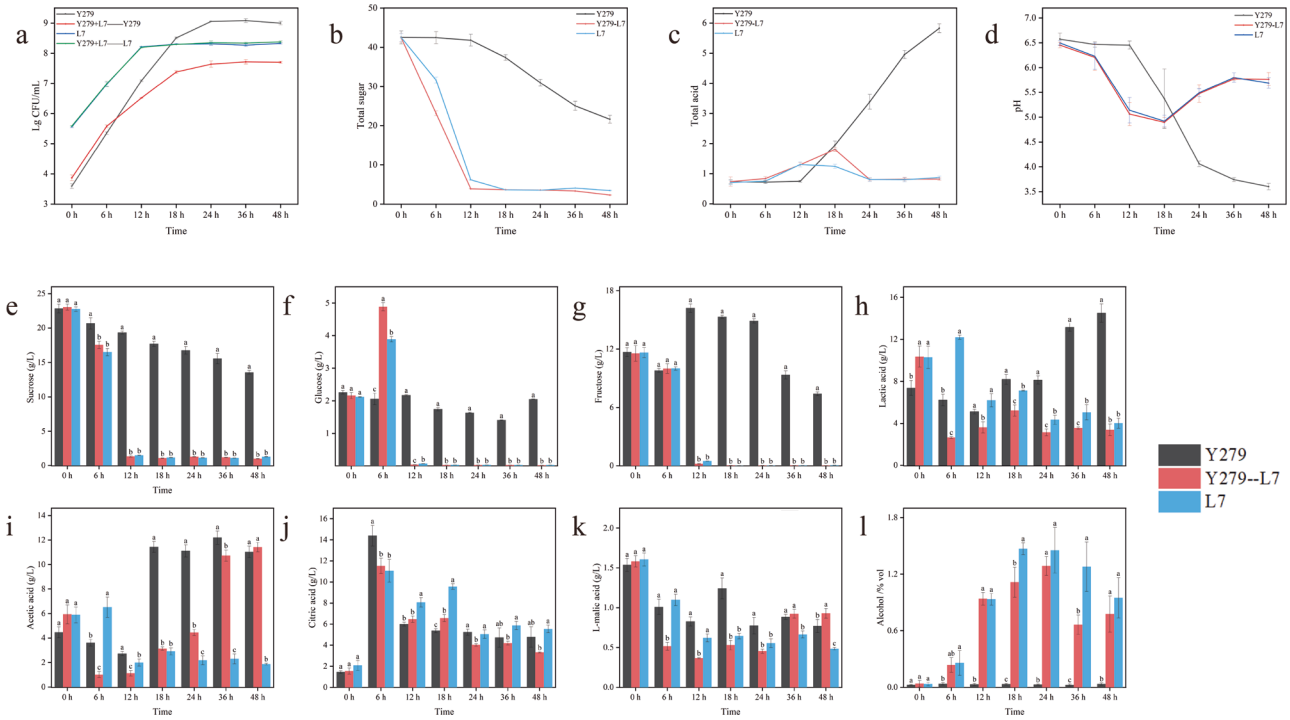

Fig. 2 | Co-culture characteristics of *L. plantarum* Y279 and *S. cerevisiae* L7. a Cell numbers in mono- and coculture systems. Y279 and L7 represent the number of *L. plantarum* Y279 and *S. cerevisiae* L7 in monoculture systems. Y279 + L7–L7 and Y279 + L7–Y279 represented the number of *L. plantarum* Y279 and *S. cerevisiae* L7 in coculture systems, respectively. **b** The total sugar of mono- and coculture systems. **c** The total acid of mono- and coculture systems. **d** The pH of mono- and coculture systems. **e** Contents of sucrose. **f** Contents of glucose. **g** Contents of fructose. **h** Contents of lactic acid. **i** Contents of acetic acid. **j** Contents of citric acid. **k** Contents of L-malic acid. **l** Contents of alcohol.

3-Methyl-1-butanol (OAV = 102.69) and 10-undecenal (OAV = 95.75) exhibited the highest OAVs in Group L. The 3-methyl-1-butanol is known for its whiskey, malty, and burnt flavors, which are commonly reported in various high-protein fermented foods[17,18]. It could be produced by *L. plantarum*[19], and its production decreases with an increase in pH[20], which explains the lower OAV of 63.33 in Group M. In Group M, 2-octen-1-ol (OAV = 117.08) and 2-methoxyphenol (OAV = 116.50) showed the highest OAVs. The 2-octen-1-ol imparts green and vegetable notes, while 2-methoxyphenol has a nutty aroma. The 2-methoxyphenol could be decarboxylated from vanillic acid and ferulic acid[21]. It has been reported to delay spoilage in fish meat[22].

Microbial interaction

The growth dynamics of mono- and cocultures of *L. plantarum* Y279 and *S. cerevisiae* L7 (Fig. 2a) indicated that the growth of *S. cerevisiae* L7 was unaffected by the inoculation of *L. plantarum* Y279 ($p > 0.05$), consistent with the findings reported by Yang et al.[2]. However, the cell counts of *L. plantarum* Y279 in coculture (7.70 lg CFU/mL) was significantly lower than that in monoculture (9.00 lg CFU/mL) at 48 h, suggesting that *S. cerevisiae* L7 may have exerted an inhibitory effect on the growth of *L. plantarum* Y279.

Changes in sugar and acid levels are crucial indicators of microbial metabolic activity during fermentation[23]. The sugar concentration in the SS was 42.50 g/L, comprising approximately 2.23 g/L of glucose, 11.55 g/L of fructose, and 22.93 g/L of sucrose. The sugar consumption rates in the *S. cerevisiae* L7 monoculture system (91.87%) and the coculture (94.52%) were significantly higher than that in the *L. plantarum* Y279 monoculture (49.21%), as yeast utilizes sugar to produce ethanol and glycerol[12]. After 48 h, the remaining sugar concentration was 21.60 g/L in the *L. plantarum* Y279 monoculture system, while the concentrations in the *S. cerevisiae* L7 monoculture and coculture systems were 2.32 g/L and 3.45 g/L, respectively (Fig. 2b). The sugar requirement of yeast was greater than that of LAB. The glycolytic and pentose phosphate pathways in LAB depend on sugar as a substrate, and the competitive disadvantage of sugar in the coculture system may contribute to the reduced acid production by *L. plantarum* Y279. During the first 6 h, sucrose levels remained relatively high across all three culture systems before steadily decreasing (Fig. 2e). This trend aligns with the growth curve of the coculture system. According to a study by Sieuwerts et al.[13], *L. plantarum* and *S. cerevisiae* do not stimulate each other when sucrose is utilized as a carbon source. At 0–6 h, *S. cerevisiae* L7 was unable to influence the growth of *L. plantarum* Y279 because sucrose was still present in the coculture system. After 12 h, the sucrose content in the coculture system was depleted, leading to stimulation between the LAB and yeast. The concentrations of glucose and fructose increased during the initial stage (Fig. 2f, g), which was associated with the hydrolysis of sucrose and other complex polysaccharides[24]. Extracellular sucrose could be metabolized into glucose and fructose through starch and sucrose metabolism. In the monoculture of *L. plantarum* Y279, the fructose concentration suddenly increased from 9.80 g/L (6 h) to 16.21 g/L (12 h), while the glucose concentration did not exhibit a similar trend. This finding suggests that LAB preferentially utilizes glucose over fructose as a carbon source, as reported by Lima et al.[25] and Pereira et al.[26]. Yeast exhibits the same preference, as both sugars are transported by the same carriers, which favor glucose over fructose, and glucose inhibits the uptake of fructose[27].

The total acid levels in the coculture system were significantly higher ($p < 0.01$) than those in the *L. plantarum* Y279 monoculture system after 24 h (Fig. 2c). At 48 h, the total acid concentration of *L. plantarum* Y279 was 5.83 g/kg, while that of the coculture was only 0.82 g/kg. This change may be attributed to the decrease in the population of *L. plantarum* Y279 in the coculture system. Lactic acid is the main acid produced by the metabolism of LAB. The pH also exhibited a trend indicative of inhibition (Fig. 2d).

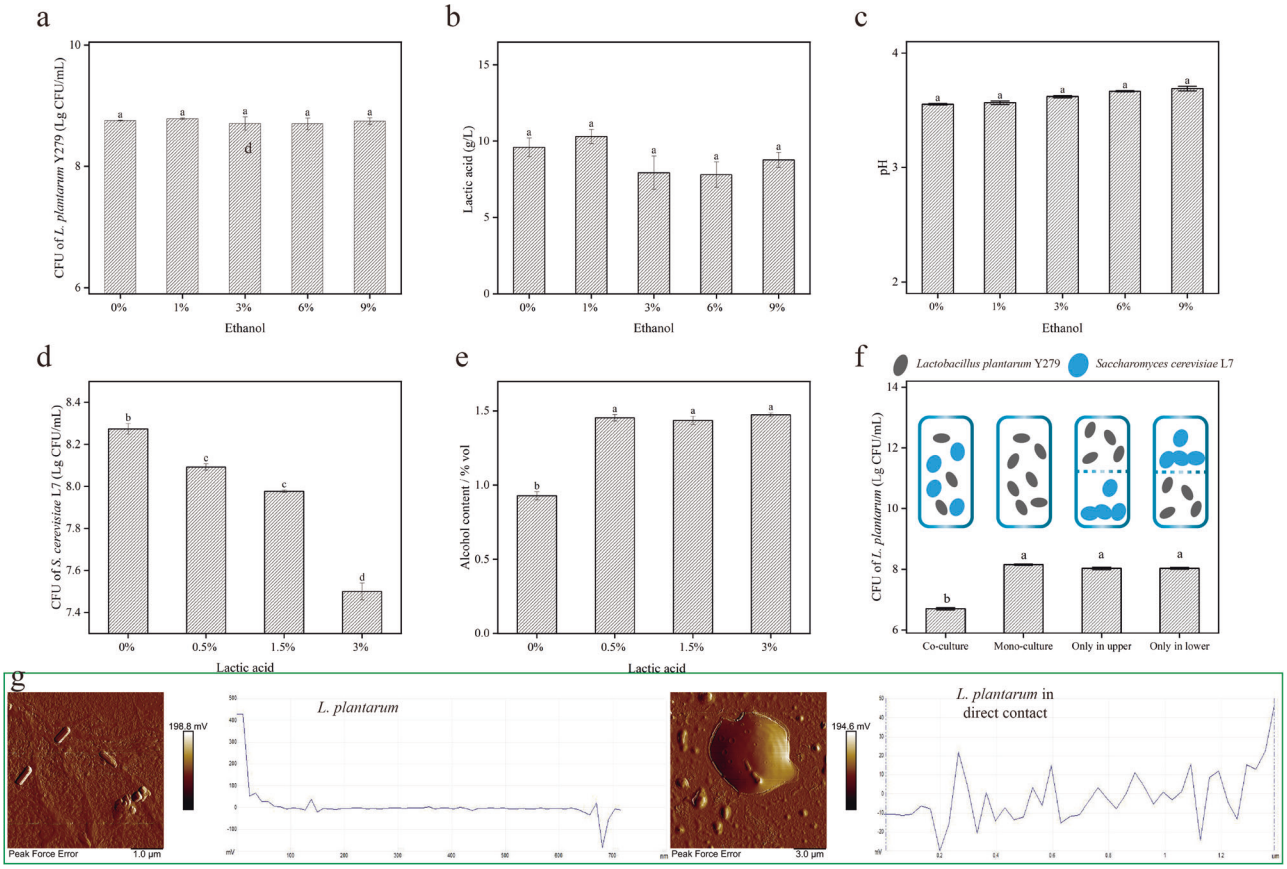

Fig. 3 | The experiment about interactions between *L. plantarum* Y279 and *S. cerevisiae* L7. a Effect of ethanol with different concentrations on the cell numbers of *L. plantarum* Y279. **b** Effect of ethanol with different concentrations on pH in *L. plantarum* Y279 monoculture system. **c** Effect of ethanol with different concentrations on pH in *L. plantarum* Y279 monoculture system. **d** Effect of lactic acid with different concentrations on the cell numbers of *S. cerevisiae* L7 monoculture system. **e** Effect of lactic acid with different concentrations on alcohol content in *S. cerevisiae* L7 monoculture system. **f** Cell numbers in upper and lower chambers of permeable support device under Different combinations. **g** The cell morphology under AFM.

S. cerevisiae L7 significantly inhibited the production of lactic acid by *L. plantarum* Y279 throughout the entire coculture process (Fig. 2h). At 48 h, the lactic acid concentration of *L. plantarum* Y279 was 14.50 g/L, while that of the coculture was only 3.39 g/L. Acetic acid is another major organic acid produced by microbials. The acetic acid levels in the coculture during the process were significantly lower than those in the *L. plantarum* Y279 monoculture (Fig. 2i). At the end of the culture, the acetic acid concentration was not significantly different from that in the *L. plantarum* Y279 monoculture, indicating that *S. cerevisiae* L7 had a significance impact on the accumulation of acetic acid in the system during the pre- to mid-term fermentation period ($p < 0.05$). Viesser et al.[24] demonstrated that the increase in acetic acid at the end of fermentation was associated with ethanol, as the microbes were able to utilize ethanol to produce acetic acid. Citric acid could be transformed into pyruvate by microbes. The citric acid in coculture was significantly lower than in both groups at later stages of fermentation (Fig. 3j). Through the citric acid-pyruvate cycle, microbes could use citric acid as a carbon source[28]. The competition for carbon sources between strains continued to citric acid after sugar was depleted. Malic acid, a key molecule of the citric cycle, has a crucial effect on metabolism[29]. *L. plantarum* showed stronger malic acid production capacity than yeast (Fig. 2k). At the early stage of fermentation, the content of malic acid in coculture was significantly lower than that in the other two groups, and this finding may be caused by the mutual inhibition of the two strains. After 36 h, malic acid accumulated in the coculture, and it was the opposite in the other two groups. *S. cerevisiae* L7, after achieving a dominant position, was unable to process the large amount of malic acid left by the previous LAB. Extracellular malic acid is thought to be poorly metabolized by *S. cerevisiae*, and this has been linked to the absence of a mediated transport system for the acid[30].

L. plantarum Y279 also influenced *S. cerevisiae* L7 in the coculture system. Ethanol production in the coculture system was significantly lower than that in the L7 pure culture system after 18 h (Fig. 2l). The alcohol content decreased from 0.95% to 0.78% in the coculture at 48 h.

In SS, yeast inhibited the growth and acid production of LAB). One method of this inhibition is the competition between yeast and LAB for carbon sources. By enhancing the utilization of sugars, including sucrose, glucose, and fructose, as well as citric acid in SS, yeast gains a competitive advantage for these carbon sources. When LABs are deprived of a carbon source, they struggle to produce lactic acid, which is the primary reason for the observed decrease in total acid production.

S. cerevisiae L7 was the dominant strain in the co-fermentation process. During co-culture, the two strains competed for the available substrate, which influenced their growth.

Inhibition mechanism

Yeast and LAB are known to employ three mechanisms to inhibit other microorganisms: displacement/exclusion, competition for resources, and the synthesis of naturally occurring antimicrobial compounds[31]. In addition to competition for carbon sources, it is also important to explore the potential for metabolite-mediated interactions between the two strains.

Lactic acid and ethanol are the primary metabolites of LAB and yeast, respectively, and their effect on each other needs to be verified. Different concentrations of ethanol were added to the monoculture system to investigate the effect of ethanol on *L. plantarum* Y279. The results showed

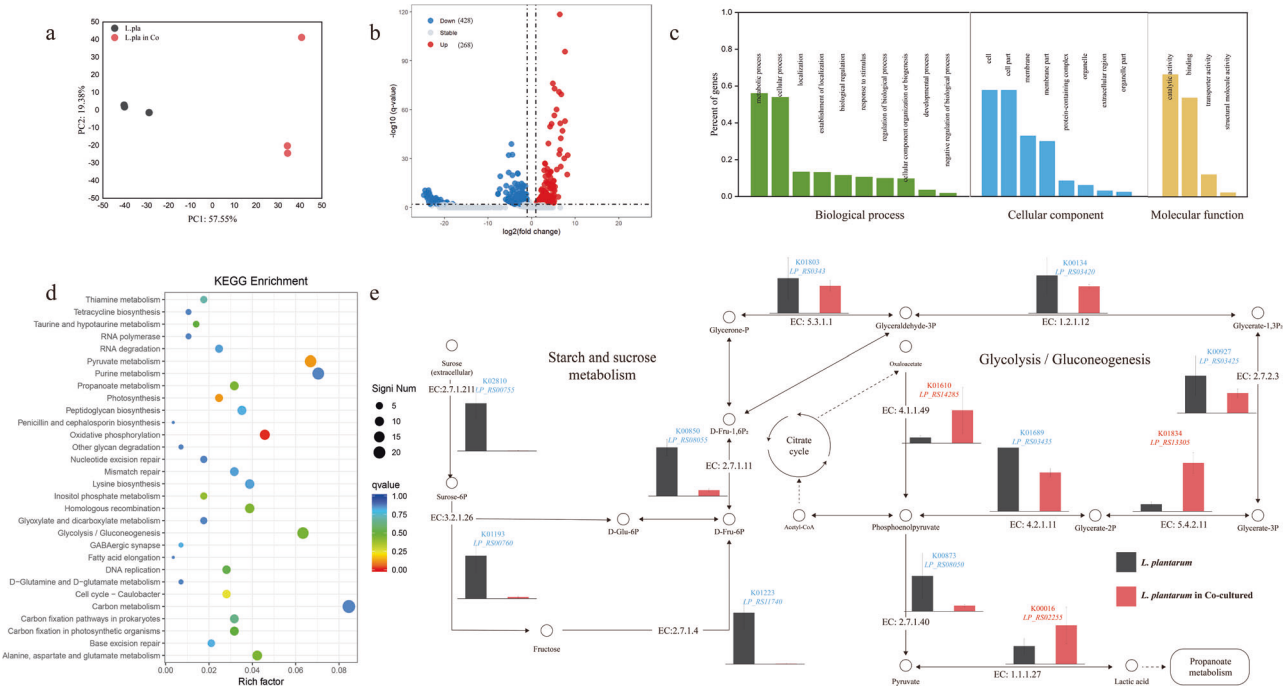

Fig. 4 | Transcriptome analysis. a PCA analysis of prokaryotic transcriptome data revolves around mono- and co-cultures. L.pla and L.pla in Co represented *L. plantarum* Y279 in monoculture systems and coculture systems, respectively. **b** Volcano plot of prokaryotic transcriptome data around monocultures and co-cultures. **c** GO classification of all DEGs of *L. plantarum* Y279 in mono- and co-cultures. The green bar represents biological processes, the blue bar represents cellular components, the yellow bar represents molecular functions, and the *Y*-axis represents the percentage. **d** KEGG enrichment analysis. The corresponding size of the point and colors. **e** Map of differences in the metabolic pathways of *L. plantarum* Y279 in mono- and co-cultures, about the starch and sucrose metabolism and Glycolysis/Gluconeogenesis. The bars represent transcripts per million (TPM) of DEGs.

that ethanol had no significant effect ($p > 0.05$) on the cell number of *L. plantarum* Y279 (Fig. 3a), lactic acid production (Fig. 3b), nor the pH (Fig. 3c), which remained stable around 3.6, across all the ethanol concentrations. These findings suggested that ethanol did not inhibit the growth of *L. plantarum* Y279, and thus, yeast was unlikely to inhibit LAB acid production by the main metabolite, ethanol. Moreover, the presence of lactic acid was able to inhibit yeast growth (Fig. 3d). Specifically, the addition of 3% ethanol decreased the cell number of *S. cerevisiae* L7 from 8.27 Lg CFU/mL to 7.5 Lg CFU/mL, and 0.5% and 1.5% ethanol also influenced yeast. However, with the addition of lactic acid, the ethanol yield of yeast increased from 0.93% to approximately 1.45% (Fig. 3e). This phenomenon is consistent with the results of previous studies demonstrating that *S. cerevisiae* could produce significant amounts of ethanol from lactic acid under acidic conditions[32].

In addition to ethanol, yeast could secrete various metabolites, including short-chain fatty acids, sulfur dioxide, peptides, and proteins, which could also inhibit the growth of other microorganisms[33]. A permeable support device was employed to investigate whether the metabolites of strains Y279 and L7 affected one another (Fig. 3f). This system consists of two chambers (upper and lower) separated by a 0.4 μm septum that permits only the metabolites of the microbes to pass through, while preventing the passage of the cells[34]. This design effectively eliminates the effects of direct contact interactions. To ensure that the influence of carbon source competition on strain growth was excluded, additional sugar was incorporated into the medium. This strategy aimed to guarantee that competition for sugar within the system would not hinder strain growth. Following testing in SS with an additional 20 g/L of glucose, both Y279 and L7 demonstrated the ability to continue growing, with residual unconsumed sugar remaining after fermentation. In co-culture, the cell counts of *L. plantarum* showed no significant difference, regardless of whether it was cultured in the upper or lower chamber (Fig. 3f). This finding suggests that the metabolites of S. cerevisiae passing through the membrane could not inhibit the growth of *L. plantarum*.

AFM was utilized to observe cell morphology (Fig. 3g). When *L. plantarum* Y279 is co-cultured, its morphology appears curved compared to individual cultivation. This observation suggests that contact interactions influence the cells, as the surface roughness of the cells is positively correlated with their metabolic activity[35]. Consequently, non-contact interactions did not inhibit LAB acid production, indicating that substrate competition is not the primary inhibitory mechanism. Instead, the primary inhibitory mechanism was found to be the contact interaction between yeast and LAB.

Transcriptome analysis

In our previous study, we conducted a transcriptome analysis on *S. cerevisiae* in co-cultures[36]. In this study, we performed transcriptome analysis on the victim, L. plantarum, co-cultured in the same environment. PCA was employed to illustrate the differences in gene expression among the samples (Fig. 4a). The two datasets were clustered within groups and separated between groups. PC1 accounted for 57.55% of the variance. Gene expression of *L. plantarum* Y279 was indeed influenced by the co-culture conditions. Based on the criteria of $\log_2$ fold change greater than 1 and a *p*-value of less than 0.01, differentially expressed genes (DEGs) were identified. In total, there were 3551 annotated genes, of which 428 DEGs were down-regulated and 268 were up-regulated (Fig. 4b). These DEGs were categorized into three groups and quantified by percentage to assess their distribution (Fig. 4c). Four molecular function categories, ten biological process categories, and eight cellular component categories were selected for investigation. The "cellular component category" had the highest representation of cells and cell parts. The category of "molecular function" included a representation of catalytic activity. The "response to stimulus" proved that the LAB received external stimulus. The "localization", "cellular component organization or biogenesis", "cell" "cell part", "membrane" and "membrane part", all of them demonstrate the impact of contact interactions on the cell and the membrane. These functional differences may result from cell contact.

KEGG enrichment analysis of the DEGs revealed the 20 most significant pathways, as shown in Fig. 4d. The competition for sugars between the two strains was supported by transcriptome results (Fig. 4e). Starch and sucrose metabolism can utilize the decomposition of extracellular sucrose, maltose, and cellobiose. Their secondary metabolites serve as raw materials for glycolysis/gluconeogenesis, as well as amino sugar and nucleotide sugar metabolism. In the starch and sucrose metabolism pathway, the genes *LP_RS00755* (coding for scrA, sucrose phosphotransferase), *LP_RS00760* (coding for sacA, fructofuranosidase), and *LP_RS00750* (coding for scrK, fructokinase) were downregulated. Multiple genes associated with sugar metabolism were downregulated under co-culture conditions, indicating that the sugars in the medium had been consumed by yeast as a carbon source. This consumption is also the reason for the inhibition of LAB growth. LAB produces lactic acid through glycolysis/gluconeogenesis[37]. The glycolysis/gluconeogenesis pathways were annotated and analyzed based on the transcriptome data. During co-culture, the expression of several genes involved in the glycolysis/gluconeogenesis pathways of LAB was repressed, ultimately leading to a decrease in lactic acid content. This result demonstrates that the disadvantage in substrate competition was the reason for the inhibition of LAB growth.

Discussion

This study aimed to identify a potential acid-reducing starter for the *Suanyu* industry and to investigate the interaction between *S. cerevisiae* and *L. plantarum*, the main starter used in traditional Suanyu production. *S. cerevisiae* L7 demonstrated excellent tolerance to various stress conditions and was identified as a promising acid-reducing starter for *Suanyu*. Following the addition of S. cerevisiae L7, the sensory properties of Suanyu improved significantly. The primary flavor compounds shifted from 3-methyl-1-butanol and 10-undecenal to 2-octen-1-ol and 2-methoxyphenol. The co-culture of *S. cerevisiae* L7 with L. plantarum was examined from both physicochemical and molecular perspectives. The results indicated that the metabolites of *S. cerevisiae*, including ethanol, did not inhibit the growth of *L. plantarum*. Instead, the inhibition was primarily attributed to substrate competition, with direct cell-to-cell contact also contributing to the process. Transcriptome analysis revealed the metabolic pathways affected in *L. plantarum* during co-culture. This study provides valuable insights into the biological deacidification process in Suanyu production and the microbial interactions between *S. cerevisiae* and *L. plantarum*. These findings may inform the industrial production of *Suanyu*.

Methods

Preparation of samples

Whole Carp (*Cyprinus carpio L.*) were purchased at the local supermarket (Guiyang, Guizhou, China). *L. plantarum* Y279, an excellent starting strain for *Suanyu*, provided by Guizhou Provincial Key Laboratory of Agricultural and Animal Products Storage and Processing, School of Liquor and Food Engineering, Guizhou University. *L. plantarum* Y279, is an excellent starting strain for *Suanyu*. *S. cerevisiae* L7, isolated from dry yeast, is a yeast strain with excellent acid-reducing ability and tolerance to spice. It is also capable of improving the microbial community and metabolic products during the *Suanyu* fermentation process[3,36].

Carps were cut into 8–10 cm pieces and combined with finely ground spices, including salt (3% *w*/*w*), cinnamon (0.3% *w*/*w*), star anise (0.3% *w*/*w*), coriander leaves (0.3% *w*/*w*), and dried chili (0.3% *w*/*w*). After that, the fish were marinated at 3–4 °C for 8 h, followed by being dried at 70 °C for 5 h. Each fish was positioned in a small-sized jar with a lid and tightly sealed with water. These samples were fermented at 30 °C for 48 h after adding starter.

After fresh Carp was taken and ground, the meat was boiled with water at a ratio of 30% (*v*/*v*) for 20 min, and then the fish broth was obtained by filtering the above mixture through four layers of gauze. Finally, the obtained fish broth was mixed with water at a ratio of 20% (*v*/*v*), which contained 2% sucrose and 1% peptone, and the pH was adjusted to 6.5 as the simulated *Suanyu* system (SS). All SS were sterilized at 121 °C for 20 min before use.

L. plantarum Y279 and *S. cerevisiae* L7 were cocultured into the SS at 30 °C and 160 r/min for 48 h according to the inoculation amount of 1% (*v*/*v*), based on the results of previous studies, the inoculation ratio of the two strains was set at 10^7:10^5 CFU/mL. In the control group, *S. cerevisiae* L7 and *L. plantarum* Y279 were monocultured under the same conditions. The fermentation broths were centrifuged at 8000 r/min for 10 min at 4 °C and the supernatants were stored at −20 °C for further analysis. The samples for 48 h were stored at −80 °C after quick freezing with liquid nitrogen.

In traditional Suanyu, the count of lactic acid bacteria is approximately 7.21 log CFU/g, while the yeast count is approximately 6.21 log CFU/g[38]. After experimenting with different ratios for mixed inoculation[39], we ultimately chose a 10^7:10^5 ratio for inoculation. A mixed solution of 1 mL of *L. plantarum* Y279 at 10^7 CFU/mL and 1 mL of *S. cerevisiae* at 10^5 CFU/mL were added to the *Suanyu* (Group M). Meanwhile, an *L. plantarum* Y279 solution (1 mL of *L. plantarum* at 10^7 CFU/mL, mixed with 1 mL of 0.28% saline solution) was used as the control group (Group L). These samples were used for sensory evaluation and flavor analysis.

Flavor analysis

The 0.2 g of the sample was transferred immediately to a 20 mL head-space vial, containing 0.2 g NaCl powder. Each vial was placed at 60 °C for 5 min, then a 120 μm DVB/CWR/PDMS fiber was exposed to the headspace of the sample for 15 min at 60 °C.

Agilent 88890 gas chromatography with a 7000D mass spectrometer with a 30 m × 0.25 mm × 0.25 μm DB-5MS (5% phenyl-polymethylsiloxane) capillary column was used. Carrier gas: helium, 1.2 mL/min. The injector temperature: is 250 °C. The oven temperature program: 40 °C, 3.5 min, increasing at 10 °C/min to 100 °C, at 7 °C/min to 180 °C, at 25 °C/min to 280 °C, hold for 5 min. Electron impact: 70 eV. The quadrupole mass detector temperature: 150 °C. ion source temperature e: 230 °C. Transfer line temperatures: 280 °C.

Sensory evaluation

The sensory evaluation members with expertise in assessing the five fundamental tastes: sweetness, sourness, saltiness, bitterness, and umami. Thirty individuals without an aversion to *Suanyu* were randomly chosen for the evaluation that encompassed various aspects, including color, texture, aroma, and taste (each dimension is scored out of 25, with a total score of 100.). All sensory evaluations were conducted in a controlled sensory room (temperature: 23 ± 2 °C, humidity: 55%).

Growth monitoring

The fermentation samples were counted on YPD agar medium with 0.5 g/L chloramphenicol for *S. cerevisiae* L7 colony, and on MRS agar medium with 2 g/L potassium sorbate for *L. plantarum* Y279. Plates were incubated at 37 °C for 48 h.

The pH values were measured with a pH meter. Total acids were analyzed using a titration method with 0.10 M NaOH. Organic acid content was analyzed by high-performance liquid chromatography (HPLC). The supernatant was thawed at −20 °C, diluted at an appropriate amount, and then injected into HPLC (Waters, e2695 separation module, USA) with a 0.22 μm membrane filter. The determination was performed on ZORBAX SB-Aq (4.6 mm × 250 mm, 5.00 μm, Agilent Technologies, Santa Clara, USA) column with a 20 mm mixture of potassium dihydrogen phosphate (pH 2.0) and methanol as mobile phase. The volume ratio was 95:5 (*v*/*v*), the injection volume was 20 uL, the flow rate was 0.60 mL/min, the detection wavelength was 210 nm, and the column temperature was 35 °C.

Total sugars were measured by the phenol sulfuric acid method. Sucrose was determined by ultraviolet spectrophotometry. A 250 uL sample was sucked into a 1.5 mL centrifuge tube, and 300 uL of 4.8 mol/L HCl was added. The volume was fixed to 1 mL with deionized water and heated in a water bath for 8 min. After cooling, the absorbance was measured at OD_{291nm}. Fructose was measured by the cysteine–carbazole–sulfuric acid

method. Glucose was measured by o-methaniline colorimetry. The sample (100 uL) and 400 uL of o-methaniline reagent were absorbed.

Organic acid content was analyzed by high-performance liquid chromatography (HPLC, Waters, e2695 separation module, USA). The column: ZORBAX SB-Aq (4.6 mm × 250 mm, 5.00 μm, Agilent Technologies, Santa Clara, USA).The mobile phase: potassium dihydrogen phosphate (pH 2.0) and methanol. The volume ratio: is 95:5. The injection volume: is 20 μL. The flow rate: is 0.60 mL/min. The wavelength: is 210 nm. The column temperature: is 35 °C.

Five microliters of SSs were added onto a fresh silicon wafer (1 cm×1 cm) and dried at 50 °C. Place the processed silicon wafer in an Atomic force microscope (AFM) to observe the surface morphology of cells. The AFM analysis uses the Scouldasyst mode for imaging, observed in the air at room temperature, with an elastic modulus of 0.4 N/m, a resonance frequency of 300 kHz, a scalding rate of 0.9–1 Hz, and a resolution of 512 × 512. The AFM images are processed and analyzed using NanoScope 1.5 (Bruker AXS, Germany).

Transcriptome analysis

Samples were sent to Bioengineering (Shanghai) Co., Ltd. for total RNA extraction and cDNA library construction. For RNA-seq analysis, paired-end sequencing was performed using Illumina Hiseq™ on top of the standard Illumina RNA-seq protocol with paired ends of 100 bp or less. Raw reads were filtered by using Trimmomatic to trim poor-quality reads with Q-values < 20. The *L. plantarum* WCFS1 genome (GenBank accession number AL935263) was used as the reference genome, and the sequenced sequences after quality control were compared with the reference genome using HISAT2, and the comparison results were counted by RSeQC. Gene expression levels were counted using StringTie software and quantified by TPM. Differences in gene expression were analyzed with DESeq (version 1.18.0) software. Enrichment analysis of genes with significant differential expression was performed according to the Gene Ontology (GO) annotation and the Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway using clusterProfiler.

Statistical analysis

Each treatment was performed in triplicate. All statistical analyses and data plotting were performed using OriginPro 2021 (OriginLab, Northampton, MA, USA). $p < 0.05$ was considered significant using a two-tailed *t*-test.

Data availability

Data available under request to the corresponding author.

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Author contributions

H.W.: methodology, visualization, and writing—original draft; L.J.: validation, investigation, and writing—review and editing; D.X.: visualization and writing—review and editing; R.Y.: data curation; L.M.: conceptualization; formal analysis; S.T.: data curation and writing—original draft; T.F.: Investigation; Z.X.: funding acquisition, resources, and project administration. All authors have read and approved the manuscript.

Competing interests

The authors declare no competing interests.

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