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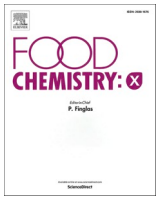

Impact of indigenous *Oenococcus oeni* and *Lactiplantibacillus plantarum* species co-culture on Cabernet Sauvignon wine malolactic fermentation: Kinetic parameters, color and aroma

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A R T I C L E I N F O

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A B S T R A C T

Malolactic fermentation (MLF) is a crucial process to enhance wine quality, and the utilization of indigenous microorganisms has the potential to enhance wine characteristics distinct to a region. Here, the MLF performance of five indigenous *Oenococcus oeni* strains and six synthetic microbial communities (SynComs), were comparatively evaluated in Cabernet Sauvignon wine. In terms of malate metabolism rate and wine aroma diversity, the strain of *O. oeni* Oe114–46 demonstrated comparable MLF performance to the commercial strain of *O. oeni* Oe450 PreAc. Furthermore, the corresponding SynComs (Oe144–46/LpXJ25) exhibited improved fermentation properties, leading to increased viable cell counts of both species, more rapid and thorough MLF, and increased concentrations of important aroma compounds, such as linalool, 4-terpinenol, α-terpineol, diethyl succinate, and ethyl lactate. These findings highlight the remarkable MLF performance of indigenous *O. oeni* and *O. oeni-L. plantarum* microbial communities, emphasizing their immense potential in improving MLF efficiency and wine quality.

1. Introduction

Malolactic fermentation (MLF) is a secondary fermentation process that occurs in the wine-making process following alcoholic fermentation (AF), generally used in the production of red and some white wines in cooler regions. The process is dominated by malolactic bacteria (MLB), which catalyze the decarboxylation of dicarboxylic L-malic acid into monocarboxylic L-lactic acid and CO_2, leading to a noticeable reduction in the total acidity of wines (Cappello, Zapparoli, Logrieco, & Bartowsky, 2017; Sumby, Bartle, Grbin, & Jiranek, 2019). Additionally, MLB also utilize citric acid and residual sugars in wines, thereby eliminating potential carbon or energy sources for spoilage microbiota and enhancing the microbial stabilization of wines (Virdis, Sumby, Bartowsky, & Jiranek, 2021). One other important role of MLF is to enrich the wine flavor through biosynthesis and bioconversion of flavor-active components by MLB. However, the effects of MLF on wine flavor are diverse and complex. Some studies suggest that MLF enhances fruity and buttery aromas while reducing vegetative green and grassy aromas (Lytra, Miot-Sertier, Moine, Coulon, & Barbe, 2020), while some propose that MLF results in a creamier palate, less fruit intensity, and more nutty notes, vanilla, toasty butter, and wet leather aromas (Bartowsky & Henschke, 2004; Lasik-Kurdyś, Majcher, & Nowak, 2018).

Oenococcus oeni and *Lactiplantibacillus plantarum* (formerly *Lactobacillus plantarum*) are commonly used MLB in wine making, due to their efficient acidity degradation ability and excellent stress tolerance to high alcohol and high acidity (Brizuela, Bravo-Ferrada, Pozo-Bayón, Semorile, & Elizabeth Tymczyszyn, 2018). Although commercial starter cultures of both species are widely used, limitations still exist in their application within wineries, particularly in wine regions with challenging environmental conditions, where the stringent conditions in wines (such as high alcohol or high acidity) pose added challenges to MLB (Betteridge, Grbin, & Jiranek, 2015; Krieger-Weber, Heras, &

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Suarez, 2020). One promising avenue to address these challenges is the utilization of synthetic microbial communities (SynComs). Compared to single strain, SynComs usually possess a broader genetic pool, more diverse metabolic pathways, and greater resilience against environmental disturbance. For instance, co-culture with *Tetragenococcus halophilus* has been shown to enhance the ethanol tolerance of *Zygosaccharomyces rouxii* through the up-regulation of genes involved in unsaturated fatty acid biosynthesis, trehalose biosynthesis, various types of N-glycan biosynthesis, inositol phosphate metabolism, MAPK signaling pathway (Yao et al., 2021). While the acid- and ethanol-resistant mechanism of both *O. oeni* and *L. plantarum* have been progressively investigated, studies on the microbial community of MLB response to collaborative wine stressors remain scarce.

Moreover, SynComs are expected to combine different enzyme profiles to produce more intricate flavors. *O. oeni* is known to harbor abundant β-glucosidase, encoded by *bgl* gene family, which hydrolyzes glycosidic-bonded aroma compounds into their free form. The typical aromatic aglycones released by β-glucosidase in wines include 2-ethylphenol, C_{13}-norisoprenoids and terpinols, enhancing floral and fruity notes (Zhang et al., 2021). Variously, a versatile arylesterase *Lp_1002* was characterized firstly in *L. plantarum*, and showed a wide substrate specificity, particularly against phenyl ethyl esters and phenyl acetate (Esteban-Torres, Barcenilla, Mancheño, de las Rivas, & Muñoz, 2014). Additionally, *L. plantarum* can release more acetaldehyde by aldehyde-alcohol dehydrogenase than *O. oeni*, a compound that facilitates the accumulation of polymerized anthocyanins and improve wine color (Wang et al., 2018). Compared to single inoculation, SynComs of *O. oeni* and *L. plantarum* may give more divergent enzyme profiles to modulate the complexity and variety of wine flavors.

Currently, the concept of "microbial terroir" has been demonstrated to be conclusively linked with regional wine characteristics and is increasingly gaining attention in winemaking (Bokulich, Thorngate, Richardson, & Mills, 2014). By using indigenous yeasts or MLB best adapted to specific must or wine environments, winemakers can enhance certain flavors or characteristics in their wines or even create a unique flavor profile that reflects the region's terroir (Aredes Fernández, Farías, & de Nadra, 2010). With many distinctive wine regions, China possesses abundant microbial resources for winemaking. However, the exploration and commercialization of indigenous microbial resources, particularly MLB, remains severely limited in Chinese wine regions. Therefore, it is of great importance to uncover indigenous MLB resources to showcase the unique terroir of Chinese wine regions and produce wines with distinct flavor profiles.

In the present study, we embark on a two-part investigation. Firstly, we comprehensively compare the MLF performance of one commercial *O. oeni* strain and five selected indigenous *O. oeni* strains. Subsequently, we construct six two-species communities consisting of indigenous *L. plantarum* XJ25 and the aforementioned *O. oeni* strains to evaluate their potential.

2. Materials and methods

2.1. Chemicals

Analytical standards for liquid chromatography (LC) analysis were purchased from Shanghai Sigma-Aldrich Biochemical Technology Co., LTD, China, including L-malic acid (PubChem CID:222656), L-lactic acid (PubChem CID:107689), citric acid (PubChem CID:311), acetic acid (PubChem CID:176), tartaric acid (PubChem CID:875), succinic acid (PubChem CID:1110), D-fructose (PubChem CID:2723872), D-glucose (PubChem:5793), glycerol (PubChem:753), and ethanol (PubChem:702). HPLC-grade sulfuric acid, formic acid, and acetonitrile were purchased from Tedia Company Inc., Shanghai, China. Ultrapure water was obtained from a Milli-Q system from Millipore (Milford, MA, USA).

2.2. Strains and acclimation culture condition

A commercial strain *O. oeni* Oe450 PreAc was used as control. The indigenous MLB strains used in this work, including *L. plantarum* (LpXJ25) and *O. oeni* (Oe3–31, Oe9–5, Oe144–46, Oe7–04, Oe28A-1), were isolated from different wine regions in China with great distinction of terroirs, as summarized in Table 1. The five indigenous *O. oeni* strains were identified by Species-specific PCR, and their genetic diversity were analyzed by fluorescent AFLP technique in our previous work (Yu et al., 2018; Yu, Shi, Meng, Liu, & He, 2019).

The culture condition and acclimation process of these strains before inoculation are detailedly described in Fig. 1. The compositions of all culture media used are shown in Tables S1 and S2.

2.3. Wine for MLF and microvinification protocol

Cabernet Sauvignon wine, collected in October 2019 from Tank No.120–48 of Chateau Changyu Moser XV (Ningxia, China), undergone alcoholic fermentation and was immediately transported to the Lab at low temperatures (below 4 °C) without adding sulfur dioxide through. Subsequently, it was filtered through a 0.22 μm nylon membrane to obtain sterile wine for further MLF microvinification experients. The basic physicochemical indexes of the wine included ethanol content of 13.7% (*v/v*), reducing sugar content of 6.2 g/L, total acid content of 7.2 g/L, volatile acid content of 0.25 g/L, free sulfur dioxide (F_{SO2}) content of 11 mg/L, total sulfur dioxide (T_{SO2}) content of 45 mg/L, L-malic acid content of 2.7 g/L, and pH of 3.45. Microvinification experiments were conducted in triplicate using 200 mL anaerobic clamp bottles, with a working volume of 150 mL. CO_2 was added to the headspace of the bottles and the experiments were carried out at 20 °C.

Single-strain inoculations involved the use of LpXJ25, Oe450 PreAc, Oe3–31, Oe9–5, Oe144–46, Oe7–04, and Oe28A-1 with an approximate inoculum size of 1×10^6 colony forming units (CFU) per milliliter. In the case of microbial communities' experiments, LpXJ25 and each *O. oeni* strain were co-inoculated at an initial ratio of 1:1 with a total inoculum size of (1×10^6 CFU/mL) same to the single-strain inoculation. Six SynComs were constructed as follows: Oe450 PreAc/LpXJ25, Oe3–31/LpXJ25, Oe9–5/LpXJ25, Oe144–46/LpXJ25, Oe7–04/LpXJ25, and Oe28A-1/LpXJ25.

During MLF, the total viable counts (TVC) of strains in wine were assessed every 24 h. CFU on agar plates were used to represent TVC of strain. Briefly, appropriate dilutions of the cultures were plated on Addition Tomato juice Broth (ATB) agar or MRS agar plates containing 100 mg/L of cycloheximide and 20 mg/mL of vancomycin (Sigma-Aldrich, China). For single inoculations, MRS agar and ATB agar plates were used to quantify *L. plantarum* and *O. oeni*, respectively. For co-inoculation, ATB agar plates were used to quantify both *L. plantarum* and *O. oeni* based on their different colonial appearances. TVC of strains were assessed every 24 h during fermentation and The CFUs were counted after incubation for 6 days at 26 °C for ATB plates and 40 h at 37 °C for MRS plates.

2.4. HPLC determination methods for compounds in wine

2.4.1. Organic acids, sugars, ethanol, and glycerol

Wine samples were centrifuged at 10000*g* for 3 min. The resulting clarified wine was diluted five times with distilled water and filtered through a 0.22 μm nylon membrane filter. Changes in the concentrations of organic acid (malic acid, lactic acid, citric acid, acetic acid, tartaric acid, and succinic acid), as well as glucose, fructose, ethanol, and glycerol, were monitored in the wine samples during MLF. High-performance liquid chromatography (HPLC) (Waters 1260, Waters Technologies, Massachusetts, USA) equipped with a UV spectrophotometer (G7115A) and a refractive index detector (RID) (G7162A). Aminex HPX-87H column (300 × 7.8 mm, 9 μ, Bio-Rad, USA) was utilized for HPLC seperation. 5 mM H_2SO_4 was served as the mobile phase

Table 1
Selected indigenous strains of *Lactiplantibacillus plantarum* and *Oenococcus oeni* used in this work.

Abbreviation	Species	Strain name	Source	Origin	Chateau	Grape variety	Vine planted	Vintage of wine	Spontaneous MLF period
LpXJ25	*L. plantarum*	XJCS-25	NWAFU[a]	Xinjiang		Cabernet Sauvignon		2010	End-MLF
Oe450 PreAc	*O. oeni*	LACTOENOS 450 PreAc®	France	LAFFORT S.L. , France					
Oe3–31	*O. oeni*	HBCS3–31	NWAFU[b]	Hebei, changli	Huaxia	Cabernet Sauvignon	2006	2016	End-MLF
Oe9–5	*O. oeni*	HBCS9–5	NWAFU[b]	Hebei, changli	Maotai	Cabernet Sauvignon	1999	2016	Middle-MLF
Oe144–46	*O. oeni*	NXCS144–46	NWAFU	Ningxia, Qingtongxia	Ganchengzi	Cabernet Sauvignon	2008	2016	Middle-MLF
Oe7–04	*O. oeni*	NXCS7–04	NWAFU	Ningxia, Yinchuan	Gangxia	Cabernet Sauvignon	2002	2016	Pre-MLF
Oe28A-1	*O. oeni*	NMM28A-1	NWAFU[c]	Neimenggu, Wuhai	Hansen	Merlot		2012	Pre-MLF

NWAFU: wine lactic acid bacteria research group at the College of Enology, Northwest A&F University, Yangling, Shannxi, China.
[a] From (Bu, Xue, Cheng, and Liu, 2017).
[b] From (Yu et al., 2019).
[c] From (Yu et al., 2018).

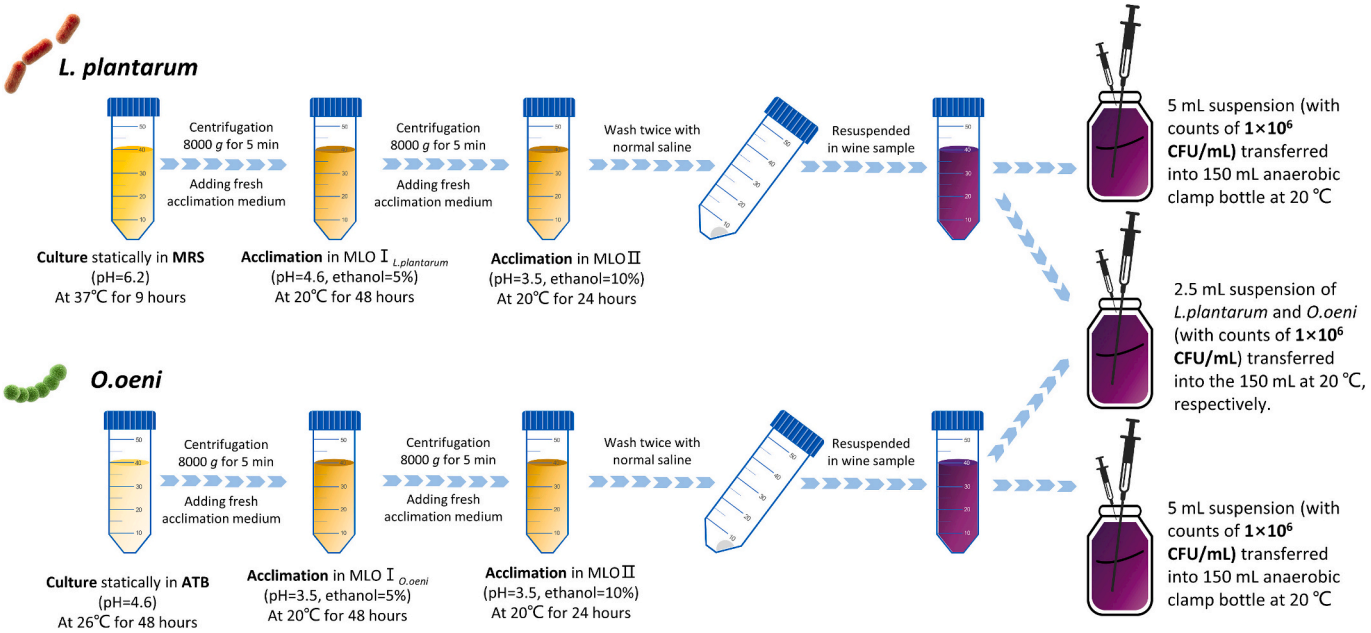

Fig. 1. Protocol of strain acclimation in MLO media and inoculation strategies in wines.

with a flow rate of 0.6 mL/min at 60 °C. Each sample was injected a volume of 20 μL and analyzed for 35 min. The UV detector was set 210 nm for the analysis of organic acids, while the RID was set at 40 °C for quantifying sugars, ethanol, and glycerol. Standards for organic acids, glucose, fructose, ethanol, and glycerol were prepared at various concentrations.

2.4.2. Anthocyanins

Anthocyanins were quantified using Shimadzu HPLC system equipped with a photodiode array detector (PDA) (Shimadzu LC-20AT, Suzhou, China). A Hydro-RP18 column (250 × 4.6 mm, 5 μ, Phenomenex, Torrance, CA, USA) was maintained at a constant temperature of 35 °C. Samples were detected at a wavelength of 520 nm over a 55-min analysis duration with a flow rate was set at 1.0 mL/min. Malvidin 3-*O*-glucoside (M3G) was employed as the external anthocyanin standard for quantification. The elution solvents included 2.5% formic acid (*v/v*) in a mixture of water and acetonitrile (mobile phase A: 8:1, mobile phase B: 4:5, v/v). The linear pump gradient started at 35% B for 45 min, increased to 100% B within 1 min, held at 100% B for 5 min, then reduced to 1% B within 1 min, and finally reduced to 0% B in 1 min and remained for 4 min.

2.5. Volatile compounds determination

Volatile compounds were analyzed using a GC–MS instrument (7890B—5975B, Agilent, Santa Clara, CA, USA) equipped with a PLA RSI 85 automatic sampler and a DB-Wax capillary column (60 m × 0.25 mm × 0.25 μm; Agilent, Santa Clara, CA). The analysis followed modifications according to Li et al. (2021).

Standard solution preparation. Aroma standards were prepared at various concentrations of mother liquor based on their concentration range in wine. The stock solutions at various dilution rates were added to the synthetic wine (containing 2.0 g/L of glucose, 5.0 g/L of tartaric acid, and 12% ethanol (*v/v*), pH 3.5). Subsequently, 10 μL of internal standard solution (4-methyl-2-amyl alcohol, 1.0 g/L, Sigma-Aldrich) was added to each dilution.

Headspace solid-phase microextraction (HS-SPME) procedure and GC–MS analysis. Volatile components in samples were extracted using HS-SPME and analyzed by GC–MS. In detail, 1 g of NaCl and 10 μL of internal standard were dissolved in 5 mL of the wine sample, and the mixture was placed in a 20 mL glass vial and sealed with a silicon septum. It was then vibrated at 400 rpm for 30 min while being heated on a heating platform at 40 °C. A 50/30-μm divinylbenzene−/−carboxen−/− polydimethylsiloxane (DVB/CAR/PDMS) SPME fiber (Supelco in Bellefonte, Pennsylvania) that had been preconditioned was inserted into the extraction headspace and adsorbed at 250 rpm for 30 min. The sample was subsequently injected using PAL after desorbing for 8 min. The carrier gas flow rate (helium, >99.999%) was 1 mL/min,

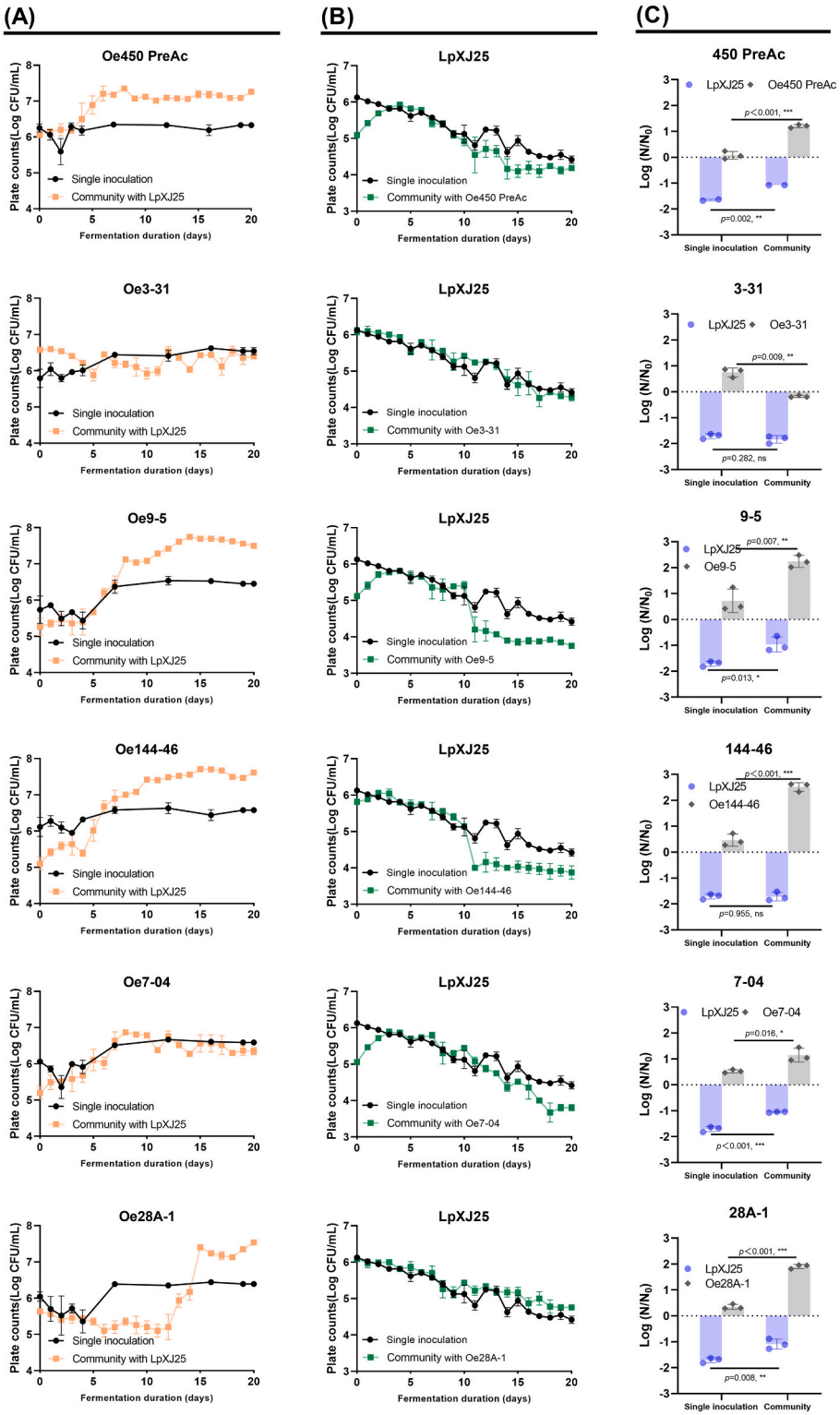

Fig. 2. Population dynamics of LpXJ25 (A) and six *O. oeni* strains (B) during MLF by single inoculation (black filled circle) and two-species microbial communities (colored filled square). (C) Viable cells at the end of MLF is expressed as Log10 (N/N_0), where N_0 is the initial inoculation amount and N is the final amount after 20-day MLF.

and the injection port temperature was 250 °C. The temperature program included a initial maintenance at 50 °C for 1 min, followed by an increase to 220 °C at a rate of 3 °C/min, then a 5-min hold. The temperatures of the MS interface and ionic source were maintained at 230 °C and 280 °C, respectively. Over the scan range of *m/z* 20–350, electron impact spectra at an energy of 70 eV were captured.

Qualification and quantification. Volatile compounds were identified based on chromatographic retention times and the National Institute of Standards and Technology Library (NIST14). Standard curves based on reference materials and an internal standard (as mentioned above) were used for quantification of volatile compounds.

2.6. CIELAB color parameters

CIELAB color parameters of wine samples were determined using an ultraviolet spectrophotometer (UV-1750, Shimadzu, Japan). 1 mm path length glass cuvette was used for scanning the UV–visible spectrum of wine sample with the 400–780 nm range at intervals of 1 nm. The corrected absorbances of wine sample at wavelengths of 450, 520, 570, and 630 nm at 1 cm optical path were used to calculated CIELAB parameters a^* (correlated with red), b^* (correlated with yellow) and L^* (lightness) (Burns & Osborne, 2015). Then the chroma C^*_{ab}, hue H^*_{ab}, and aberration $\triangle E^*_{ab}$ were calculated based on the values of a^*, b^*, and L^*.

2.7. Statistical analysis

Values were presented as the mean ± SD ($n \geq 3$), and all errors were expressed as the standard deviation (SD) of triplicate studies for each treatment group. To identify significant differences among groups, a one-way analysis of variance (ANOVA) was conducted using SPSS 20.0 (SPSS Inc., Chicago, IL). Duncan's test ($p<0.05$) was employed to discern significant differences between the treatments, with different letters implying statistically significant differences. An independent *t*-test was performed to indicate significant differences between two treatments (significant difference: *, $p < 0.05$; extremely significant difference: **, $p < 0.01$). The data were visualized using GraphPad Prism 8.4.2.

3. Results and discussion

3.1. The two-species communities increased TVC of both species

In this study, in addition to single inoculations with individual indigenous MLB strains, six two-species SynComs were also constructed through simultaneous co-inoculation of *O. oeni* strains and *L. plantarum* XJ25 at an initial inoculation ratio of 1:1. The effects of single inoculations and *O. oeni-L. plantarum* microbial communities on the TVC of strains were markedly different.

As shown in Fig. 2A, single inoculation of LpXJ25 in wine with an initial amount of 1.34×10^6 CFU/mL resulted in a declining trend in TVC, dropping by approximately 2 units of Log CFU/mL after 20 days. Similarly, the TVC of *L. plantarum* showed a decline of approximately 4 units after 20-day MLF in sterile Pinot noir wine (14.5% *v/v* ethanol, pH 3.82) (Brizuela et al., 2018). This decline may be attributed to the poor tolerance of LpXJ25 to the harsh wine environment characterized by high acidity and ethanol content. On the contrary, single inoculations of each *O. oeni* strains in wine maintained a stable cell density of approximately 1×10^6 CFU/mL during the 20-day MLF (Fig. 2B). This variation trend in TVC was clearly observed for the strains of commercial Oe450 PreAc and indigenous Oe7–04, which exhibited a significant "V" shape. This observation is consistent with the previous research results (Diez-Ozaeta, Lavilla, & Amárita, 2021). This may be attributed to the fact that cells require time to adapt to the harsh wine environment after they are inoculated into the wine. However, Oe144–46 and Oe3–31 maintained the TVC above the initial inoculation amount throughout the MLF process, indicating their remarkable adaptability and robustness.

Regarding the two-species microbial communities (Fig. 2A&B), the declining trend in the TVC of LpXJ25 differed significantly from that in single inoculation. Co-inoculations with Oe9–5 or Oe144–46 resulted in a significant decrease in the TVC of LpXJ25 after 10 days, while a smooth decrement was observed in co-inoculations with Oe450 PreAc or Oe7–04. Interestingly, in co-inoculations with Oe3–31 or Oe28A-1, the TVC of LpXJ25 showed an almost similar trend or even higher values compared to single inoculation, despite the inoculation size of LpXJ25 in co-inoculation was half of that in single inoculation. The growth trend of the six *O. oeni* strains in communities was found to be completely different from those in single inoculations. Due to the variability in their genome and their ability to adapt to wine-related stress, various indigenous *O. oeni* strains can produce a diverse array of secondary metabolites during MLF. During the cultivation of communities, the direct uptake and utilization of metabolites from one strain may have either a stimulatory or inhibitory effect on the growth of another specie. For instance, *Leuconostoc oenos* 8403 has been shown to be inhibited by *L. plantarum* Lp or *Pediococcus pentosaceus* Yq 791, resulting depressed growth rate and cell density due to the presence of thermostable compounds with a molecular weight of <1000 Da secreted by the two antagonistic strains (Lonvaud-Funel & Joyeux, 1993). However, this inhibitory effect on *Lc. oenos* was not strictly strain specific.

Our results also revealed that *O. oeni* strains isolated from various wine regions had diverse compatibilities with LpXJ25. For example, in the presence of LpXJ25, the growth of Oe28A-1 was inhibited during the early fermentation stage (malic acid >2 g/L); however, the growth of Oe450 PreAc, Oe9–5, Oe144–46 and Oe7–04 was promoted throughout the MLF period (Fig. 2A&B). Meanwhile, the survival rate of LpXJ25 was maintained (co-inoculation with Oe3–31 and Oe144–46) or improved (co-inoculation with other four strains) in the microbial communities during MLF (Fig. 2C, Table 2). A previous study also has indicated such mutualistic relationship between *P. pentosaceus* 12p and *O. oeni* X_2L in their microbial community. The growth of both species was promoted by the amino acids produced through the decomposition of polypeptides by X_2L (Fernández & de Nadra, 2006). However, in *L. hilgardii* 5w- *O. oeni* X_2L microbial community, competition for nitrogen sources, particularly arginine, led to a significant loss of cell viability of X_2L (Aredes Fernández et al., 2010). Furthermore, physical contact between cells of different species in microbial community can directly impact their performance. Membrane-membrane contact has been proved to effectively mediate direct molecule delivery between cells, preventing changes in the structure and nature of the cytoplasmic environment (D'Souza et al., 2018). To sum up, our findings demonstrated that the positive impacts of two-species communities on cell viability compared to single inoculations. Moreover, the compatibility of these *O. oeni* strains with LpXJ25 for interspecific interactions is strain-dependent.

3.2. The two-species communities led to rapid and thorough MLF

As shown in Fig. S1, single inoculation of LpXJ25 consumed only 0.48 ± 0.02 g/L malic acid after 17-day MLF, resulting in a stuck fermentation. However, it produced 1.17 ± 0.01 g/L of lactic acid. This indicates most of the produced lactic acid was derived from sugar metabolism, which will be discussed in the subsequent section. In contrast, single inoculations of commercial Oe450 PreAc and all indigenous *O. oeni* strains (Oe3–31, Oe9–5, Oe144–46, Oe7–04, and Oe28-A-1) completed MLF (final content of malic acid <0.1 g/L) within 11, 20, 20, 11, 20, and 20 days, respectively (Fig. 3A). Among these strains, the indigenous strain Oe144–46 had a similar MLF rate to the commercial strain, indicating superior MLF rate compared to other indigenous strains in the scope of this study.

MLF performance of a strain is also influenced by the initial inoculation amount, the pre-adaption process or not (Lombardi et al., 2020), the expression levels of stress response genes and the malolactic enzyme gene (*mleA*) (Balmaseda, Rozès, Bordons, & Reguant, 2022), as well as the stress conditions in wines (pH, ethanol, SO_2 and deficient nutrition)

Table 2
Effect of two-species microbial consortia on cell viability compared to single inoculation at the end of MLF. (+): positive effect, (0): neutral effect, (−): negative effect.

L. plantarum	LpXJ25					
O. oeni	Oe450 PreAc	Oe3–31	Oe9–5	Oe144–46	Oe7–04	Oe28A-1
Effect on growth (*L. plantarum*, *O. oeni*)	(+,+)	(**0**,−)	(+,+)	(**0**,+)	(+,+)	(+,+)
Best developed strain	Oe450 PreAc	Oe3–31	Oe9–5	Oe144–46	LpXJ25	Oe28A-1

Note: We named the dominant strain (i.e., those with higher relative abundance) were the "best developed strain".

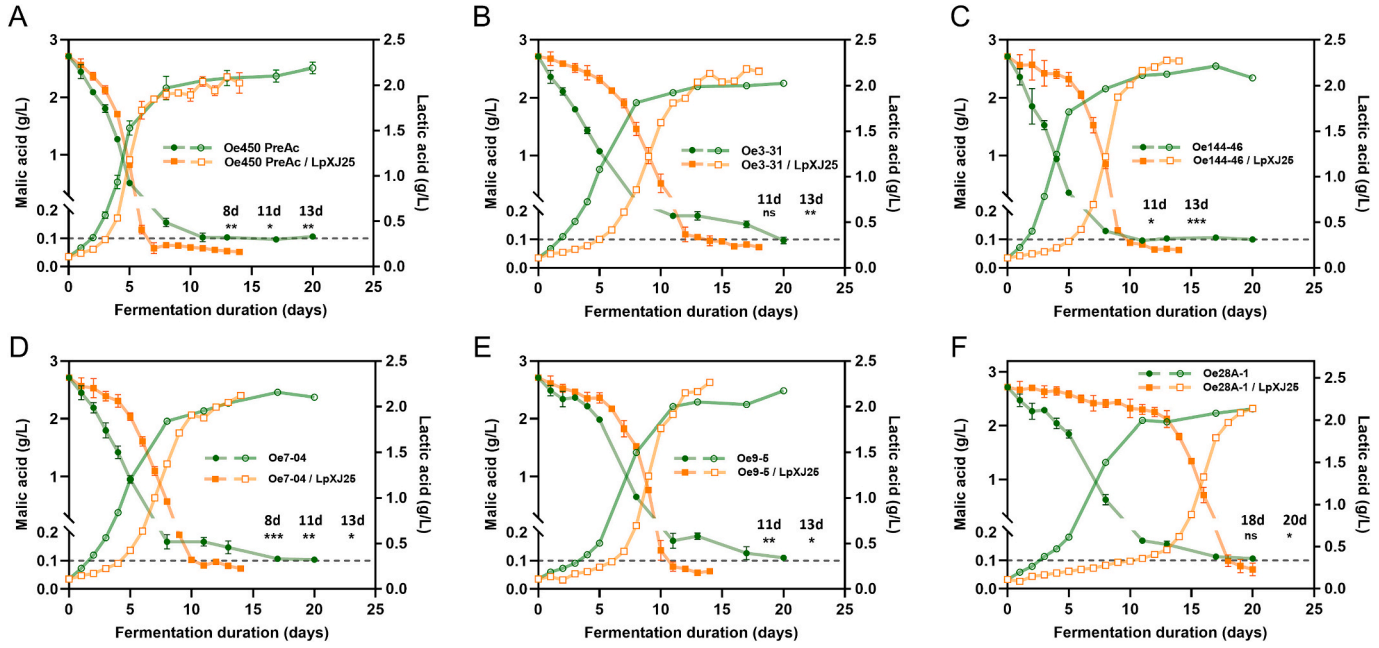

Fig. 3. Dynamic changes of malic acid degradation (filled) and lactic acid production (hollowed) during MLF by single inoculation (green circle) and two-species microbial communities (orange square). Independent t-tests were performed on malic acid degradation between single and co-inoculation containing the same strains of *O. oeni* ($p < 0.05$ as *; $p < 0.01$ as **; $p < 0.001$ as ***). (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

(Sumby et al., 2019). MLF performance of *L. plantarum* has been reported to be highly dependent on nitrogen sources, and nutrient supplements can be used to address the fermentation issues caused by high alcohol and high acidity in wine (13%v/v ethanol content and pH < 3.4) (Ponomarova et al., 2017). In this study, the initial inoculation amount of LpXJ25 was lower than 10^7 CFU/mL, and the removal of wine lees by filtration could result in a lack of nitrogen sources in wine, which may also be responsible for the stuck fermentation of single inoculation of LpXJ25. The genetic diversity of strains also plays a crucial role in determining their different MLF performance. For instance, strains with a faster fermentation rate could upregulate a broader spectrum of genes involved in stress resistance (*hsp18*, *clpP*, *clpX*, *ctsR*), synthesis of extracellular polysaccharides (*dsrO*, *levO*, *wobB*, *wobO*), and malic acid metabolism (*mleA*) (Bastard et al., 2016; Tofalo et al., 2021).

Compared to single inoculations of *O. oeni*, the two-species microbial communities significantly accelerated the MLF process. Specifically, the communities (Oe450 PreAc/LpXJ25, Oe3–31/LpXJ25, Oe9–5/LpXJ25, Oe144–46/LpXJ25, Oe7–04/LpXJ25, and Oe28A-1/LpXJ25) required only 7, 14, 11, 10, 10 and 18 days, respectively, to thoroughly complete MLF (Fig. 3A). However, the microbial communities resulted in a slower degradation of malic acid during the early stage of MLF, followed by a more rapid and thorough degradation. This may be attributed to the adaptation of strains to stress conditions in wine and the competition for nutrition by both *O. oeni* and *L. plantarum* cells during the early stage of MLF. The duration of the initial slow degradation of malic acid varied significantly among these microbial communities, also indicating different compatibility of *O. oeni* strains with LpXJ25 for MLF. Oe450 PreAc showed the best compatibility, with the shortest duration (1 day) of the slow degradation of malic acid, whereas Oe28A-1 exhibited the worst compatibility (or may be a strong competitive relationship) with LpXJ25, resulting in the longest duration (13 days) of the slow degradation of malic acid. The growth of *O. oeni* strains and the survival of LpXJ25 in microbial communities were coupled with malic acid metabolism (Fig. 2A&B and 3A). Once malic acid began to be rapidly metabolized after 5 days by *O. oeni* (Oe450 PreAc, Oe9–5, Oe144–46 and Oe7–04) or 15 days by *O. oeni* (Oe28A-1), the TVC of *O. oeni* strains significantly increased, while the TVC of LpXJ25 significantly decreased once the malic acid was exhausted. This regular phenomenon was consistently observed in the previous report (Diez-Ozaeta et al., 2021). In summary, compared to single inoculations, two-species microbial communities completed MLF more thoroughly and rapidly (an average of 5 days faster). This positive impacts of microbial communities on MLF rate, as well as the abovementioned cell viability, may be closely related to the cell-cell communication and the consequent exchange of metabolites and energy between different species (Liu et al., 2017).

The increase rate of lactic acid was found to be in line with the degradation rate of malic acid (Fig. 3B). The exception is the net production of lactic acid in Oe3–31/LpXJ25 and Oe144–46/LpXJ25 microbial communities, exhibiting a huge difference with single inoculations of the corresponding *O. oeni* strains (Fig. S2A). We observed that Oe144–46/LpXJ25 had a higher yield of lactic acid (the molar conversion ratio of malic acid to lactic acid is theoretically 1:1), which

might be caused by glucose metabolism. In addition to malic acid, the metabolism of citric acid by MLB is also very important for wine quality, as it leads to the production of acetic acid and diacetyl (Cappello et al., 2017). The initial concentration of citric acid in wine was 0.14 g/L, and only a small part of citric acid (<0.03 g/L) was metabolized (Fig. S3). The metabolism of citric acid nearly synchronized with that of malic acid in both single inoculations and the microbial communities. Moreover, the microbial communities gave lower final concentration of acetic acid than single inoculations of the corresponding *O. oeni* strains did. There was no difference in the final concentration of tartaric acid in wine between single inoculations and the microbial communities, while Oe9–5/LpXJ25 and Oe144–46/LpXJ25 communities gave significantly higher final concentration of succinic acid than single inoculations of the corresponding *O. oeni* strains did (Fig. S4).

3.3. Availability of fructose was higher than that of glucose in both single inoculations and two-species communities

Concentrations of glucose, fructose, glycerol, and ethanol in wines before and after MLF are shown in Fig. 4. The major residual fermentable sugars (glucose and fructose) in wine are usually below 4 g/L after AF by yeast. *O. oeni* and *L. plantarum* benefits energetically from the co-metabolism of glucose or fructose with citric acid, which enhances their growth and biomass production (Ramos & Santos, 1996; Salou, Loubiere, & Pareilleux, 1994). The original wine sample after AF in this study still contained 3.97 ± 0.04 g/L glucose and 2.41 ± 0.01 g/L fructose (Fig. 4A&B). After a 20-day MLF in single inoculation of LpXJ25, 36.33% of glucose (8.07 mM) and 70.13% of fructose (9.74 mM) were consumed, which was responsible for the production of 1.17 ± 0.01 g/L (8.07 mM) lactic acid with only 0.48 ± 0.02 g/L of malic acid being consumed (Fig. S1). However, in all single inoculations of *O. oeni* strains, <10% of glucose was consumed, except for Oe28A-1, which exhibited a 25.78% reduction in glucose levels. Among the microbial communities, only Oe144–46/XJ25 consumed 11.52% of glucose, while others consumed <10% glucose. Unlike glucose, >75% of fructose was consumed in all single inoculations and the microbial communities, except for the Oe28A-1/LpXJ25, with only 3.57% fructose consumed. The differences in hexose consumption between *O. oeni* and *L. plantarum* may be attributed to their distinct metabolism pathway. *O. oeni*, being a heterofermentative lactic acid bacterium, metabolizes hexoses by the pentose-phosphate (phosphoketolase) pathway to produce not only lactic acid but also acetic acid, ethanol, CO_2, and other products. In contrast, *L. plantarum*, a facultative heterofermentative lactic acid bacterium, metabolizes hexoses to produce only lactic acid and CO_2 by the Embden-Meyerhof-Parnas (EMP) pathway (Zúñiga, Pardo, & Ferrer, 1993).

The limited ability of Oe28A-1/LpXJ25 community to metabolize hexoses may be attributed to the poor compatibility between these two strains. In single inoculations, the final glycerol content in wines was notably higher compared to that in the corresponding microbial

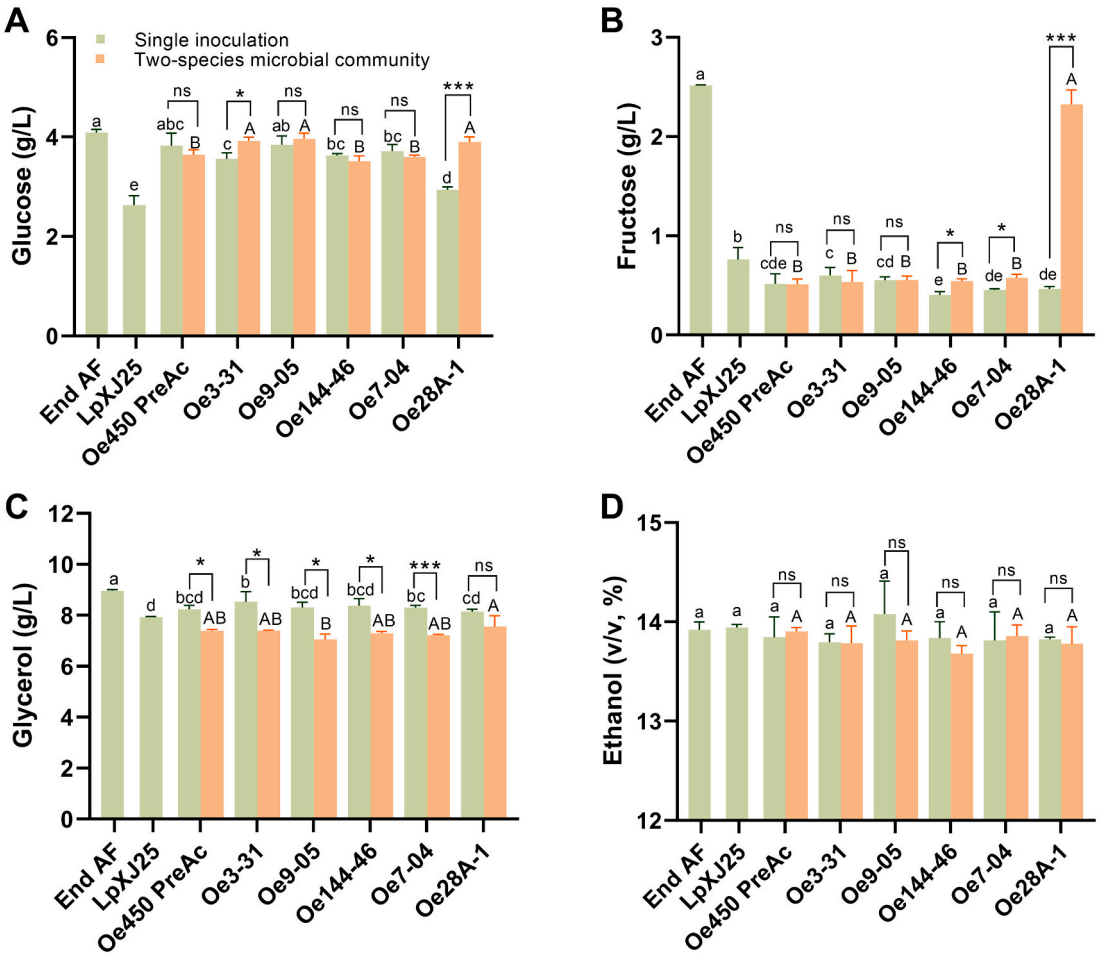

Fig. 4. The residual concentration of (A) glucose, (B) fructose, (C) glycerol, and (D) ethanol in wines after MLF by single inoculation and two-species microbial communities. Control (EndAF) and single inoculations are green columns, while two-species microbial communities are orange columns. Columns with different lowercase letters indicate statistical differences among EndAF and all single inoculation samples ($p < 0.05$), while columns with capital letters indicate statistical differences among all microbial communities' samples ($p < 0.05$). Independent *t*-tests were performed between single and co-inoculation containing the same strains of *O. oeni* ($p < 0.05$ as *; $p < 0.001$ as ***). (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

communities, with an average difference of 1.00 ± 0.25 g/L (Fig. 4C). However, the final ethanol content in wines of all treatments showed no significant difference from that in the original wine sample after AF (Fig. 4D). In summary, the availability of fructose for both species is higher than that of glucose, and the availability of glucose and fructose for microbial communities may be related to the compatibility between the two species.

3.4. The two-species communities gave wines smaller color difference values ($\triangle E^*_{ab}$)

Anthocyanins are the most important color compounds in wine and determine its hue. These compounds, primarily present in the form of glycosides in grape skins and wine, can be converted into unstable aglycons (anthocyanidins) by the hydrolyzation of the glycosidic linkages. However, anthocyanins are susceptible to the fermentation process

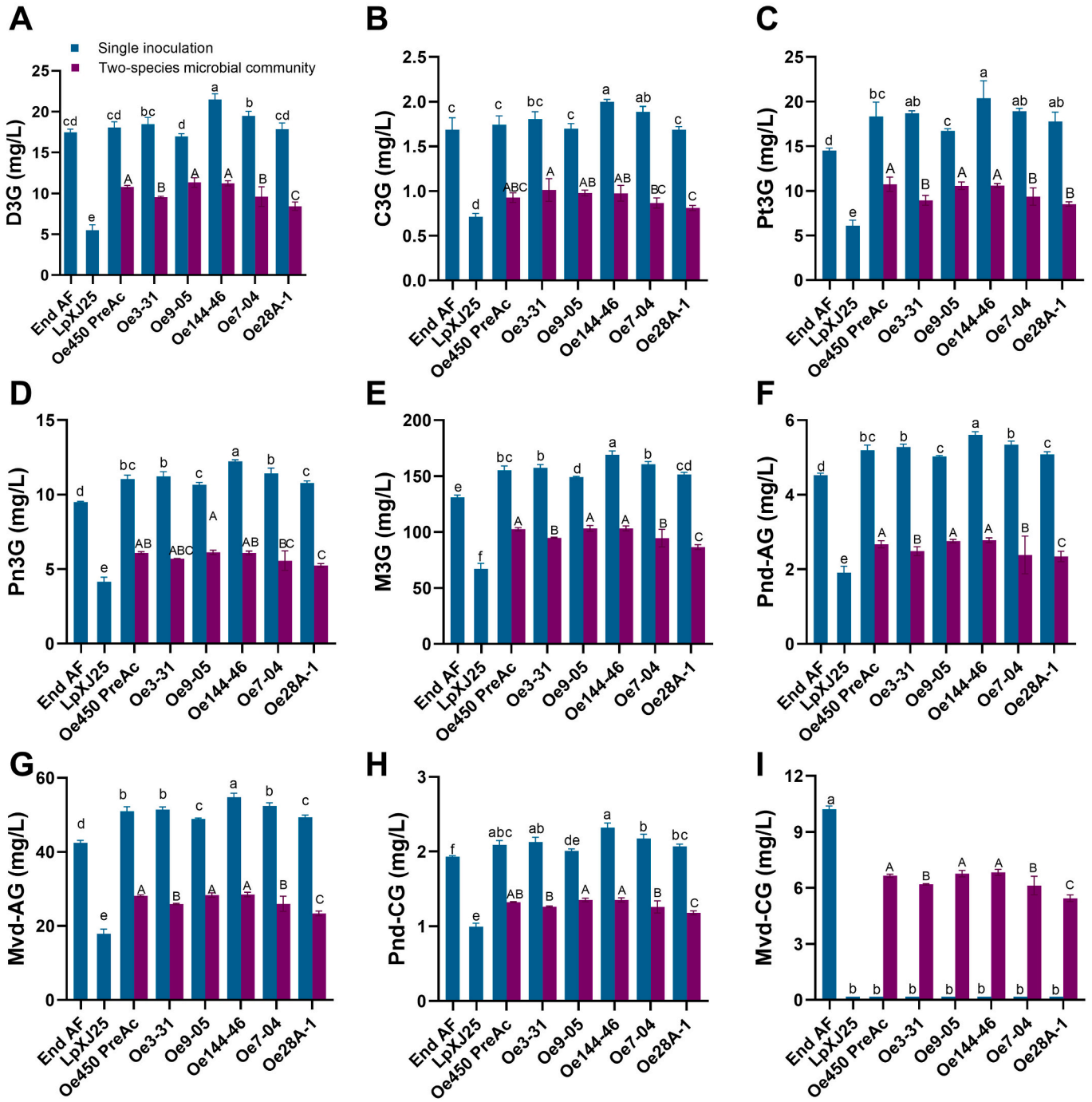

Fig. 5. Nine individual anthocyanins concentration in wines after MLF by single inoculations and two-species microbial communities. (A) Delphinidin 3-*O*-glucoside, (B) Cyanidin 3-*O*-glucoside, (C) Petunidin 3-*O*-glucoside, (D) Peonidin 3-*O*-glucoside, (E) Malvidin 3-*O*-glucoside, (F) Peonidin 3-*O*-(6-*O*-acetyl)-glucoside, (G) Malvidin 3-*O*-(6-*O*-acetyl)-glucoside (H) Peonidin 3-*O*-(6-*O*-trans-*p*-coumaryl)-glucoside, and (I) Malvidin 3-*O*-(6-*O*-trans-*p*-coumaryl)-glucoside. Control (EndAF) and single inoculation are peacock blue columns, while two-species microbial communities are purple columns. Columns with different lowercase letters indicate statistical differences among EndAF and all single inoculation samples ($p < 0.05$), while columns with different capital letters indicate statistical differences among all microbial communities' samples ($p < 0.05$). (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

in winemaking (Li et al., 2021). In the original wine samples, a total of nine individual anthocyanins were identified with a total content of 233.36 ± 3.63 mg/L, including five monomeric anthocyanins (delphinidin 3-*O*-glucoside (D3G), cyanidin 3-*O*-glucoside (C3G), petunidin 3-*O*-glucoside (Pt3G), peonidin 3-*O*-glucoside (Pn3G), malvidin 3-*O*-glucoside (M3G)), two acylated anthocyanins (peonidin 3-*O*-(6-*O*-acetyl)-glucoside (Pnd-AG), malvidin 3-*O*-(6-*O*-acetyl)-glucoside (Mvd-AG)), and two p-coumarylated anthocyanins (peonidin 3-*O*-(6-*O*-trans-p-coumaryl)-glucoside (Pnd-CG), malvidin 3-*O*-(6-*O*-trans-p-coumaryl)-glucoside (Mvd-CG)). Among the monomeric anthocyanins, M3G was the most prevalent type, accounting for 56.16% of the total anthocyanin content, followed by Pt3G (6.22%) and D3G (4.79%). This anthocyanin composition aligns with that reported in Cabernet Gernischt wines by Wang et al. (2018). In single inoculation, LpXJ25 caused over 50% loss in the amount of all individual anthocyanins in wine, especially for Mvd-CG (nearly 100% loss). However, most indigenous *O. oeni* increased the content of these individual anthocyanins, with Oe144–46 and Oe7–04 significantly increasing the content of all individual anthocyanins (except for Mvd-CG) to varying degrees (Fig. 5). M3G and its acylated derivative (Mvd-AG) are the two dominant anthocyanins in the wine samples of all treatments, accounting for 74.36% to 81.27% of the total anthocyanins (Fig. S5A). Oe144–46 gave the highest total anthocyanin content, increasing by 54.71 ± 3.58 mg/L after 20-day MLF, 25.24 mg/L higher than Oe450 PreAc did after MLF (Fig. S5B). Similarly, MLF by *O. oeni* VFO has been reported to significantly increase individual anthocyanin content in wine (Burns & Osborne, 2013). In brief, single inoculations of *O. oeni* can increase the content of these individual anthocyanin in wines. However, the presence of LpXJ25, whether in its single inoculation or as part of microbial community with *O. oeni*, leads to a substantial reduction in the content of these individual anthocyanin in wines.

The impact of MLB on anthocyanin concentration and wine color during MLF has been well-established in previous studies, primarily through the cell adsorption and anthocyanin glucoside cleavage (Virdis et al., 2021). MLB strains with high glucosidase activities, such as *L. plantarum*, have been found to possess stronger anthocyanin affinities compared to *O. oeni* strains (Devi, Konerira Aiyappaa, & Waterhouse, 2020). As observed in this study, both single inoculation of LpXJ25 and the microbial communities decreased the content of detected individual anthocyanins in wines. In addition, acetaldehyde has also been demonstrated to play a crucial role in wine color stabilization by promoting the synthesis of stable ethylene-linked pigments, such as pyranoanthocyanins, which exhibit better colorimetric properties than their monomeric counterparts (Forino, Picariello, Lopatriello, Moio, & Gambuti, 2020). *L. plantarum* has been proved to increase acetaldehyde levels in wine during MLF, resulting in a 2–4-fold increase in total pyranoanthocyanin content, whereas *O. oeni* strains typically decrease acetaldehyde levels (Wang et al., 2018). These likely accounts for the decreased content of individual anthocyanins after MLF in presence of *L. plantarum*.

Anthocyanin alteration in wine is visually manifested through changes in wine coloration. Single inoculation of MLB can lead to noticeable color loss in wine (Burns & Osborne, 2015; Virdis et al., 2021). In this study, all treatments led to a significant increase in L^* and a decrease in a^* and C^*_{ab}, indicating a relatively brighter, less saturated color and a more reddish hue of the wine samples after MLF (Table 3). Importantly, the microbial communities and single inoculations of the corresponding *O. oeni* strains resulted in distinct differences in wine color parameters, particularly in L^*, b^*, H^*_{ab}, and $\triangle E^*_{ab}$. The differences in wine color parameters and anthocyanin content brought by different treatments were highly consistent, suggesting a direct correlation between anthocyanin content and wine color. To be specific, the microbial communities resulted in a greater increase in L^* than single inoculations of the corresponding *O. oeni* strains did, and the corresponding microbial communities either increased or maintained b^* and H^*_{ab}, except for Oe9–5/LpXJ25 and Oe144–46/LpXJ25; wines after MLF by the microbial communities exhibited less color differences ($\triangle E^*_{ab}$) from the original wine sample than those by single inoculations. These results suggest that the microbial communities gave wines smaller color differences, and a relatively brighter and yellower hue compared to single inoculation.

Table 3
Color parameters of wines after MLF by single inoculations and two-species microbial consortia.

Microbial strains	L^*	a^*	b^*	C^*_{ab}	H^*_{ab}	ΔE^*_{ab}
EndAF	57.69 ± 0.01^{c}	44.8 ± 0.01^{a}	7.25 ± 0.01^{b}	45.38 ± 0.01^{a}	0.16 ± 0.01^{c}	0.00 ± 0.00^{e}
LpXJ25	60.85 ± 0.28^{b}	40.46 ± 0.43^{b}	0.26 ± 0.06^{g}	39.73 ± 0.31^{b}	0.01 ± 0.02^{f}	9.32 ± 0.62^{a}
Oe450 PreAc	60.84 ± 2.78^{b}	39.73 ± 2.67^{b}	2.26 ± 0.37^{e}	39.64 ± 2.65^{b}	0.06 ± 0.01^{de}	7.83 ± 3.13^{ab}
Oe450 PreAc/ LpXJ25	66.95 ± 0.17^{a}	38.84 ± 0.13^{b}	7.54 ± 0.40^{b}	39.56 ± 0.07^{b}	0.19 ± 0.01^{b}	5.18 ± 0.12^{cd}
Oe3–31	60.13 ± 0.36^{b}	40.43 ± 0.37^{b}	1.72 ± 0.25^{f}	40.47 ± 0.37^{b}	0.04 ± 0.01^{e}	7.49 ± 0.60^{ab}
Oe3–31/ LpXJ25	66.07 ± 0.11^{a}	39.64 ± 0.17^{b}	8.78 ± 0.02^{a}	40.60 ± 0.17^{b}	0.22 ± 0.00^{a}	4.72 ± 0.14^{d}
Oe9–5	59.86 ± 1.35^{b}	40.69 ± 1.29^{a}	0.66 ± 0.28^{g}	39.83 ± 1.28^{b}	0.04 ± 0.01^{e}	8.32 ± 0.98^{ab}
Oe9–5/ LpXJ25	66.30 ± 0.25^{a}	39.99 ± 0.31^{b}	6.35 ± 0.21^{c}	40.49 ± 0.30^{b}	0.16 ± 0.01^{c}	4.51 ± 0.21^{d}
Oe144–46	60.74 ± 0.83^{b}	39.55 ± 0.70^{b}	2.58 ± 0.12^{e}	39.72 ± 0.70^{b}	0.07 ± 0.00^{d}	7.56 ± 0.50^{ab}
Oe144–46/ LpXJ25	66.18 ± 0.42^{a}	40.21 ± 0.46^{b}	6.21 ± 0.10^{c}	40.68 ± 0.46^{b}	0.15 ± 0.00^{c}	4.43 ± 0.26^{d}
Oe7–04	60.74 ± 0.98^{b}	39.77 ± 0.97^{b}	1.43 ± 0.26^{f}	39.79 ± 0.97^{b}	0.08 ± 0.01^{d}	6.89 ± 0.92^{bc}
Oe7–04/ LpXJ25	66.93 ± 0.60^{a}	38.86 ± 0.66^{b}	8.84 ± 0.77^{a}	39.86 ± 0.56^{b}	0.22 ± 0.02^{a}	5.33 ± 0.60^{cd}
Oe28A-1	60.57 ± 0.68^{b}	39.65 ± 0.63^{b}	3.34 ± 0.30^{d}	39.90 ± 0.64^{b}	0.06 ± 0.01^{de}	7.67 ± 0.74^{ab}
Oe28A-1/ LpXJ25	66.43 ± 0.22^{a}	39.36 ± 0.32^{b}	8.73 ± 0.25^{a}	40.32 ± 0.27^{b}	0.22 ± 0.01^{a}	4.84 ± 0.31^{d}

EndAF: end of alcoholic fermentation. Values of all the samples from mean of three repetitions ± standard deviations. Means in the same row with different letters (a-f) are significantly different.

Correlation analysis between color parameters and anthocyanin content revealed no significant correlation between the color parameters and the content of the nine individual anthocyanins (Fig. S6). It's possible that multiple anthocyanins contribute to changes in color parameters of wine, and our identification of anthocyanin types may be insufficient to establish a relationship with CIELAB. This finding is consistent with previous studies. Han et al. (2008) confirm that wine color is predominantly attributed to polymeric and acylated anthocyanins rather than individual and non-acylated anthocyanins. Similarly, Wang et al. (2018) also observed a significant decline in total anthocyanin content in wine after MLF with *L. plantarum*; however, the redness of wines was maintained. Their results also indicate this preservation of color due to *L. plantarum*'s facilitation in forming of high levels of pyranoanthocyanins (mainly acetaldehyde adduct of anthocyanins) by producing more acetaldehyde, resulting in a deeper red color in wine compared to monomer anthocyanins.

3.5. Advantages of employing two-species communities for improving wine volatile profile

A total of 68 volatile compounds were identified and analyzed in the wine samples. These volatile compounds were categorized into eight major chemical families, including esters (acetate esters, fatty acid ethyl ester, and other ester), phenols, acids, terpenes, higher alcohols, aldehydes, ketones, and furan (Fig. 6A).

As shown in Table S3, MLF resulted in significant changes in the volatile composition of wines. The concentrations of acetate ester and fatty acid ethyl esters in wines significantly increased after MLF. Five acetate esters were identified, with only ethyl acetate and isoamyl acetate exceeding their odor threshold. Single inoculations of *O. oeni* strains increased the total acetate ester concentration from 21.96 mg/L to 28.01–30.14 mg/L, and especially increased the isoamyl acetate concentration nearly three times. Among the tested *O. oeni* strains, Oe28A-1, Oe7–04, and Oe3–31 exhibited similar abilities to commercial Oe450 PreAc in increasing the concentrations of total acetate esters, ethyl acetate, and isoamyl acetate. Furthermore, the microbial communities showed slight increases in the acetate ester concentrations when compared to single inoculations. Nine ethyl fatty acid esters were identified in the wine samples. These medium-chain fatty acid ethyl esters, consisting of 6 to12 carbon atoms and possessing low odor threshold, are known to contribute the fruity aromas of wine (Hu, Jin, Mei, Li, & Tao, 2018). Similar to the abovementioned acetate esters, the concentrations of ethyl butanoate, ethyl hexanoate, ethyl octanoate, ethyl decanoact, ethyl laurate, and total ethyl fatty acid esters in the wine samples were significantly increased in the wine samples after MLF, especially in those after MLF by single inoculations of *O. oeni* strains. Moreover, the concentration of ethyl octanoate increased around twice in wines after MLF by the microbial communities, except for Oe3–31/LpXJ25. The concentration of ethyl decanoate increased by 3–4 times in wines after MLF by single inoculations of *O. oeni* strains, and by 2–3 times in wines after MLF by the microbial communities, except for Oe450 PreAc/LpXJ25.

Ethyl lactate is a characteristic aroma compound produced during MLF that contributes fruity, buttery, and creamy aromas, and a round taste to wines. Its concentration in wines progressively increases through esterification between ethanol and lactic acid produced by MLB during MLF. In this study, both single inoculations and the microbial communities greatly increased the ethyl lactate concentrations in wines, with the microbial communities resulting in higher concentrations than single inoculations of the corresponding *O. oeni* strains, except for Oe450 PreAc/LpXJ25. The concentrations of ethyl lactate in wines of all treatments far exceeded its odor threshold (14 mg/L), except in the case of single inoculation with Oe450 PreAc. Notably, Oe144–46/LpXJ25 community gave the highest concentration of ethyl lactate (78.36 mg/L), which was 18.7 times higher than that in the original wine sample after AF.

Diacetyl, a typical aroma compound from citric acid metabolism with a very low odor threshold (1–4 mg/L), contributes desirable buttery, nutty, or toasty aromas to wines at low concentration (Bartowsky & Henschke, 2004; Li et al., 2018). Interestingly, in this study, it was not detected in any of the wine samples after MLF. This may be due to the low concentration of citric acid in the original wine sample and much less consumption of citric acid after MLF (Fig. S2). The esterification of diacetyl with succinic acid from TCA cycle of yeasts forms diethyl succinate, which contributes a fruity melon aroma to wines (Lasik-Kurdyś et al., 2018). The concentrations of diethyl succinate in all wine samples were well below its odor threshold (6 mg/L). Single inoculation of Oe9–05 and community of Oe9–05/LpXJ25 significantly increased the concentration of diethyl succinate by 1.89- and 2.26-folds, respectively. Acetoin (odor threshold, 15 mg/L) is formed from the reduction of diacetyl by the diacetyl reductase and contributes desirable butter or cream aromas to wines (Cappello et al., 2017). Single inoculations of LpXJ25 or Oe144–46 did not affect the concentration of acetoin, but single inoculations of other *O. oeni* strains significantly increased the concentration by 2–3 times. The microbial communities, except for Oe7–04/LpXJ2, significantly decreased the concentration by 4–7 times. The formation of acetoin might be also responsible for the absence of diacetyl in wines after MLF.

In isolation, higher alcohols and fatty acids are often perceived as having unpleasure odors and are not considered to contribute positively to wine aroma, except for 2-phenylethanol, which provides desirable rose and honey aromas (Ugliano, Bartowsky, McCarthy, Moio, & Henschke, 2006). The original wine sample after AF contained relatively high concentrations of total higher alcohols (282 mg/L). While all single inoculations of *O. oeni* strains significantly increased the concentrations of total higher alcohols and fatty acids in wines, the microbial communities either decreased or had no effects on the concentrations of these compounds.

Terpenes are of great importance to wine aroma due to their desirable flower and fruit aromas. Obviously, MLF increased the concentrations of most terpenes in wines, especially for geraniol and 4-terpinenol. For 4-terpinenol, Oe144–46/LpXJ25 and Oe7–04/LpXJ25 increased the concentration more than single inoculations of the corresponding *O. oeni* strains. Oe144–46/LpXJ25 gave the highest concentration of 4-terpinenol (598.90 μg/L), which was twice as much as what single inoculations of LpXJ25 (250.59 μg/L) and Oe144–46 (287.18 μg/L) did and ten times the concentration in the original wine sample after AF. Oe144–46/LpXJ25 also gave the highest concentration of beta-ionone (0.32 μg/L), nearly twice the amount of what single inoculation of Oe144–46 did or the concentration in the original wine sample. Particularly, single inoculations of *O. oeni* strains did not affect the concentrations of α-terpinenol and linalool, but all the microbial communities significantly increased their concentrations.

To comprehensively overview the potential relationship between the main volatile compounds and different experiment treatments, heatmap and PCA analysis were conducted based on the concentrations of 34 volatile compounds with odor activity values (OAV) > 0.1 (Fig. 6). As shown in Fig. 6B with all treatments loaded, the X-axis represents a 62.41% correlation for PC1, and the Y-axis represents a 15.02% correlation for PC2, collectively explaining 77.42% of the overall variance information. All treatments were distinctly divided into two groups. The original wine sample after AF was located on the positive half axis of PC1 and the negative half axis of PC2, significantly differing from all the wine samples after MLF. Wines after MLF by single inoculations were located on the negative half axis of PC1; in contrast, wines after MLF by the microbial communities were located on the positive harf. Fig. 6C presents PCA analysis among different single inoculations, while Fig. 6D depicts PCA analysis among different microbial communities. Loadings of the 34 volatile compounds on the three PCA graphs were shown in Fig. S7.

4. Conclusion

In this study, the MLF performances of five indigenous *O. oeni* strains and six two-species microbial communities were comprehensively evaluated from the perspectives of fermentation kinetic parameters, wine color and aroma. *O. oeni* Oe144–46 exhibited comparable ability of malate metabolism to the commercial strain of Oe450 PreAc. Compared to single inoculations, the corresponding two-species microbial communities (Oe144–46/LpXJ25) significantly increased the TVC and led to more rapid and thorough MLF. Compared to single inoculations, SynComs gave wines smaller color difference ($\triangle E^*_{ab}$) after MLF, resulting in less color loss in the wines. Moreover, the microbial community of Oe144–46/LpXJ25 either decreased or had no effect on the concentrations of undesirable higher alcohols and fatty acids in wines, and significantly increased the concentrations of some desirable aroma compounds, such as linalool, 4-terpinenol, α-terpinenol, diethyl succinate, and ethyl lactate, thus positively improving the quality of wine aroma. Our study provided the valuable insights into the potential

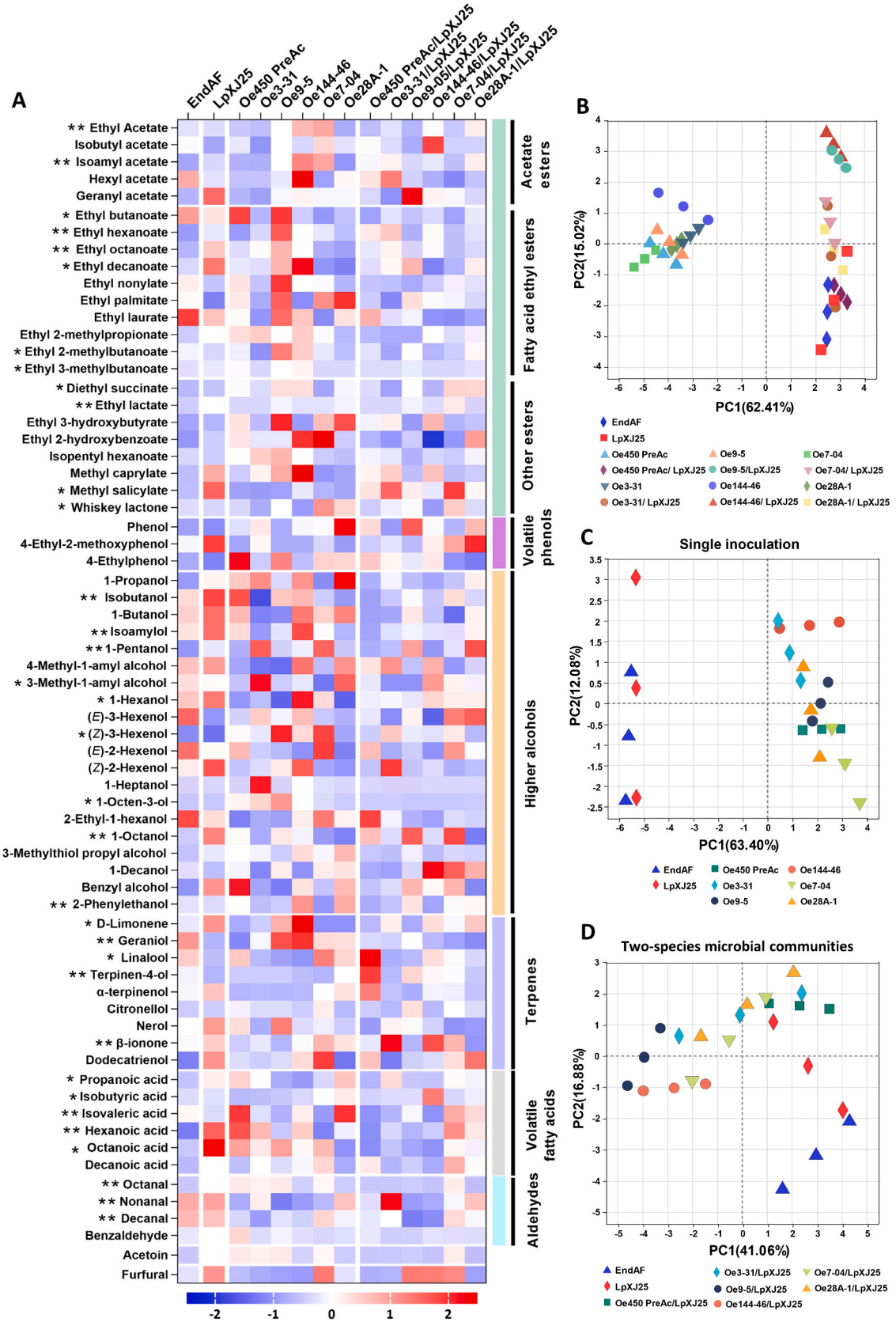

Fig. 6. Heatmap visualization (A) of volatile compounds in wines after MLF by single inoculations and two-species microbial communities. The color scale represents the scaled abundance of each variable, with the red color indicating high abundance and the blue color indicating low abundance. * and ** represents that the compounds have an OAV > 0.1 and > 1, respectively. Principal Component Analysis (PCA) based on the compounds with OAV >0.1 was carried out on (B) all treatments, (C) single inoculation, and (D) two-species microbial communities. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

application of Chinese indigenous *O. oeni* strains and the advantages of *O. oeni-L. plantarum* communities in improving MLF efficiency in winemaking. Future research efforts should focus on refining the robustness of communities *O. oeni-L. plantarum* community to further expend their application in wine industry.

CRediT authorship contribution statement

Biying Zhang: Writing – review & editing, Writing – original draft, Methodology, Investigation. **Doudou Liu:** Writing – review & editing, Methodology, Investigation. **Hui Liu:** Software, Investigation, Formal analysis. **Jiaxuan Zhang:** Software, Methodology. **Ling He:** Investigation, Methodology. **Jin Li:** Formal analysis. **Penghui Zhou:** Formal analysis. **Xueqiang Guan:** Formal analysis. **Shuwen Liu:** Conceptualization, Funding acquisition, Supervision, Writing – review & editing. **Kan Shi:** Conceptualization, Funding acquisition, Supervision, Writing – review & editing.

Declaration of competing interest

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

Data availability

Data will be made available on request.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.fochx.2024.101369.

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