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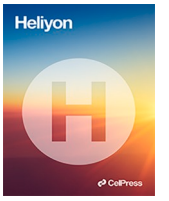

Research article

Unlocking fungal quorum sensing: Oxylipins and yeast interactions enhance secondary metabolism in *monascus*

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ABSTRACT

Exploring the symbiotic potential between fungal and yeast species, this study investigates the co-cultivation dynamics of *Monascus*, a prolific producer of pharmacologically relevant secondary metabolites, and *Wickerhamomyce anomalous*. The collaborative interaction between these microorganisms catalyzed a substantial elevation in the biosynthesis of secondary metabolites, prominently Monacolin K and natural pigments. Central to our discoveries was the identification and enhanced production of oxylipins (13S-hydroxyoctadecadienoic acid , 13S-HODE), putative quorum-sensing molecules, within the co-culture environment. Augmentation with exogenous oxylipins not only boosted Monacolin K production by over half but also mirrored morphological adaptations in *Monascus*, affecting both spores and mycelial structures. This augmentation was paralleled by a significant upregulation in the transcriptional activity of genes integral to the Monacolin K biosynthetic pathway, as well as genes implicated in pigment and spore formation. Through elucidating the interconnected roles of quorum sensing, G-protein-coupled receptors, and the G-protein-mediate signaling pathway, this study provides a comprehensive view of the molecular underpinnings facilitating these metabolic enhancements. Collectively, our findings illuminate the profound influence of *Wickerhamomyces anomalous* co-culture on *Monascus purpureus*, advocating for oxylipins as a pivotal quorum-sensing mechanism driving the observed symbiotic benefits.

1. Introduction

Monascus purpureus, a filamentous saprophytic fungus, is noted for its robust production of diverse, pharmacologically important secondary metabolites [1–5]. Notably, *Monascus* pigments (MPs) and Monacolin K (MK) are extensively studied for their significant applications [6,7]. MPs, a family of structurally related metabolites, have emerged as critical elements in food biotechnology due to their natural coloring properties and their array of health benefits, including antioxidant, anti-tumor, and anti-inflammatory effects [8,9]. Their utilization as natural food colorants is particularly noted in Asia, where they are integrated into the food industry as color enhancers and preservative alternatives [10]. Concurrently, MK is recognized for its capacity to modulate cholesterol biosynthesis through the inhibition of HMG-CoA reductase, highlighting its therapeutic value in hyperlipidemia management [11–13].

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Within fermented food ecosystems, *Monascus* engages with various microorganisms, such as yeasts and lactic acid bacteria, emulating ecological dynamics that can significantly impact metabolite profiles [14,15]. This investigation focuses on the symbiotic relationship with *Wickerhamomyces anomalus*, an opportunistic yeast with unique morphological and physiological attributes beneficial to fermented food applications. This co-cultivation study aims to shed light on how these interactions influence *Monascus*' secondary metabolism and structural development [16,17].

Expanding the discourse to microbial communication, this research explores fungal quorum sensing (QS) mechanisms mediated by oxylipins. Originating from the lipoxygenase pathway, oxylipins act as essential signaling molecules, coordinating fungal behaviors and interspecies interactions [18–20]. The QS system, traditionally associated with bacteria, is now recognized for its role in fungal communities, where it regulates complex biological functions and metabolic outputs in response to population density [18,19,21,22].

We hypothesize that oxylipins play a crucial role in the co-cultivation of *Monascus* and *W. anomalus* co-cultivation, potentially enhancing metabolite production and altering fungal morphology. By integrating exogenous oxylipins, we aim to decipher their impact on the fermentation process and delineate the G-protein signaling pathways involved in QS responses. The results of this research are expected to deepen our understanding of microbial interspecies communication and provide innovative approaches for enhancing the production of secondary metabolites in *Monascus* fermentations.

Through a detailed examination of the QS effects and the biochemical pathways involved, this study aims to advance the field of microbial interaction research. By elucidating the mechanisms underpinning the enhanced production of MK and other valuable metabolites, we intend to establish a foundation for the use of *W. anomalous* in the development of *Monascus*-fermented products, thereby enriching the biotechnological toolkit available for food and pharmaceutical applications.

2. Material and methods

2.1. Microbial strains and media

The strains employed in this study were *M. purpureus* M1 (CGMCC 3.0568) and *W. anomalus* C22, both preserved in our laboratory. The *M. purpureus* M1 strain was cultured on potato dextrose agar (PDA) medium containing 20 g/L glucose, 3 g/L peptone, 4 g/L yeast extract, 20 g/L malt, 20 g/L agar, 2 g/L KH_2PO_4, 2 g/L $NaNO_3$, and 1 g/L $MgSO_4{\cdot}7H_2O$ for two generations. It was then inoculated into a seeding medium consisting of 30 g/L glucose, 15 g/L soybean meal powder, 10 g/L peptone, 70 g/L glycerol, 2 g/L KH_2PO_4, 2 g/L $NaNO_3$, and 1 g/L $MgSO_4{\cdot}7H_2O$, and incubated for two days at 30 °C with agitation at 200 rpm. The culture was then transferred to a fermentation medium containing 90 g/L glycerol, 20 g/L rice powder, 10 g/L peptone, 2.5 g/L KH_2PO_4, 5 g/L $NaNO_3$, 1 g/L $MgSO_4{\cdot}7H_2O$, and 2 g/L $ZnSO_4{\cdot}7H_2O$, supplemented with a 10 % volume of inoculum and incubated under the same conditions for an additional two days.

W. anomalus C22 was cultured from a single colony on a plate in 50 mL of yeast extract peptone dextrose medium (YPD) at 30 °C for 24 h. The culture was then diluted to concentrations of 10^9 CFU/mL, 10^6 CFU/mL, 10^3 CFU/mL, and 10 CFU/mL, and 100 μL was added to the M1 fermentation medium for two days.

2.2. Determination of pigment

To evaluate pigment production, 5 mL of *M. purpureus* M1 fermentation broth was mixed with 15 mL of 70 % ethanol. The mixture was heated at 60 °C for 1 h in a water bath shielded from light. The absorbance at 410 nm, 448 nm, and 505 nm was measured using a spectrophotometer, and the color value was calculated by multiplying the absorbance value by the dilution factor.

2.3. High-performance liquid chromatography (HPLC) analysis of MK production

The quantification of MK was carried out by mixing the fermentation broth with 70 % methanol and subjecting it to a 30-min sonication (250 W, 40 kHz). After centrifugation at 8000*g* for 5 min, the supernatant was filtered through a 0.22 μm membrane and subjected to HPLC analysis.

HPLC analysis was conducted using an Inertsil ODS-3 C_{18} column (150 mm × 4.6 mm × 5 μm) with a mobile phase of 0.1 % H_3PO_4: methanol (35:65, v/v), a flow rate of 1 mL/min, a column temperature of 30 °C, and UV detection at 237 nm. An injection volume of 10 μL was employed.

2.4. High-performance liquid chromatography-mass spectrometry (HPLC-MS) analysis of 13S-HODE production

For the quantification of 13S-hydroxyoctadecadienoic acid (13S-HODE) production, 1 mL of *M. purpureus* M1 fermentation broth was thoroughly mixed with 3 mL of chromatographic-grade acetonitrile and sonicated for 30 min. The mixture was filtered using a 0.22 μm membrane and analyzed by HPLC-MS under the following conditions: methanol-5% isopropanol solution: 20 mM ammonium acetate solution (9:1, v/v) as the mobile phase, a flow rate of 0.3 mL/min, and a sample intake of 5 μL. The column used was an Eclipse plus C18 (2.1 mm × 50 mm × 3.5 μm) with a column temperature of 30 °C. The MS was operated in negative ion mode using electrospray ionization (ESI), with a quadrupole mass analyzer, an ion source temperature of 100 °C, a drying gas temperature of 350 °C, a drying gas flow rate of 8.0 L/min, an atomized gas pressure of 35.0 psi, a fragmentation voltage of 135 V, and detection in multiple reaction monitoring (MRM) modes using ion pairs of 13S-HODE at *m/z* 195/295.

2.5. Optical microscopy of the hyphal morphology

Hyphal morphology was observed by placing small PDA tablets (approx. 3 cm diameter) on slides, inoculating with a *Monascus* spore suspension, and introducing *W. anomalus* C22 after 48 h. The samples were incubated at 30 °C for 5 days in a moist environment provided by filter paper soaked in 30 % glycerol. Morphological observations were made using light microscopy.

2.6. Scanning electron microscope (SEM) analysis of M. purpureus mycelial

Mycelial morphology was examined using SEM. *Monascus* cells cultured for 8 days were harvested by centrifugation at 12,000 rpm for 5 min, fixed in 2.5 % glutaraldehyde for 12 h, and washed twice with 0.1 M phosphate buffer (pH 7.2). The cells were dehydrated using a graded ethanol series and suspended in isoamyl acetate and ethanol (1:1), followed by isoamyl acetate alone. After drying with hexamethyldisilazane, samples were observed with a Su8020 SEM.

2.7. Real-time quantitative PCR (RT-qPCR) analysis of genes related to hyphal morphology

RT-qPCR was conducted using the CFX96 real-time PCR detection system (Bio-Rad, Hercules, CA, USA). Primers used were listed in Table 1. The protocol included a 15-min denaturation at 95 °C, followed by 40 cycles of 95 °C for 10 s, 52 °C for 20 s, and 72 °C for 30 s. Gene expression levels were quantified using the $2^{(-\Delta\Delta Ct)}$ method, normalized to the GAPDH gene levels, with three replicates per sample.

2.8. Detection of four indexes of the G protein signaling pathway

Concentrations of cyclic Adenosine Monophosphate (cAMP), phospholipase C(PLC), protein kinase A(PKA), and protein kinase C (PKC) were measured using enzyme-free enzyme-linked immunosorbent assay (ELISA) kits from Jiangsu Meimian Industrial Co., Ltd. (Jiangsu, China). Mycelium was collected, washed, dried, and ground to a fine powder in liquid nitrogen. After mixing with deionized water, the samples were centrifuged, and the supernatant was analyzed using the ELISA kits.

Table 1
Primer sequences for the key genes in *M. purpureus M1*.

Genes	Primer sequences (50–30)	bp	Tm Value
mokA F	GACCTCGGTCATCTTGGC	18	57
mokA R	TTGTTCCAAGCGGTCTTC	18	54
mokB F	TTGTTCCAAGCGGTCTTC	18	53
mokB R	AAACATCGTCACCAGTCT	18	53
mokC F	CAGTCCTCGTCCCTTCCAGT	20	62
mokC R	CCACGGTGAAGGATGTCGAG	21	60
mokD F	TCAACACGGGAGATGCTGTC	21	53
mokD R	GCCAAAGGACAGGAGCAGATCAGGAAATCTGGACTTACCCCATTG	25	52
mokH F	TGTTGGATTGTTGTTGGAGATATAC	23	58
mokH R	GAGATCATAGTGGCCGACTGAA ACCGTCTCATCCAACCTCACGA	25	55
mokF F	CCAGGTAACCAACGGATTA	23	60
mokF R	GATCAGAGCAGTCACCAG	23	61
mokG F	ATGTTGAATGGCAATGATGG	19	51
mokG R	CAGCGTGGGTGATGTATC	18	54
mokI F	TCCCGTTTCTTGGACGTGAG	19	60
mokI R	ACGTGCCATGGTTCTGTCTT	17	57
mppA-F	CGTCTCGCCCGATAACTTCA	19	59
mppA-R	TTGACAGACGGGTCGAAGTC	19	59
mppB-F	CAGTCCTCGTCCCTTCCAGT	19	60
mppB-R	CCACGGTGAAGGATGTCGAG	20	58
mppC-F	ATGTCAGGGTGGCGTGAAGT	19	60
mppC-R		20	59
brIA F		20	60
brIA R	CCTGAACTGTACCTGCTTGAT	20	56
wetA F	ATGTGTTATATTCCCCGGGA	20	60
wetA R	TTAGCAGAGTGCGGCCTCGAG	21	62
laeA F	ACTCGTAGCGGATGTAAGA	19	55
laeA R	CCGTGCTTGGTAGATGTG	18	55
GAPDH F	CCGTATTGTCTTCCGTAAC	19	55
GAPDH R	GTGGGTGCTGTCATACTTG	19	56

3. Results

3.1. Detection of MPs and MK yield in the co-culture system

The levels of red, orange, and yellow MPs in both the fermentation and co-culture broths were quantified on days 3, 5, 7, 9, and 13 using a UV spectrophotometer (Fig. 1A–C). The data indicated that the trends in pigment concentration were consistent across all pigments, with a significant and steady rise in the levels of monascus red, orange, and yellow pigments during the initial 7 days of fermentation by *M. purpureus* M1. The peak in red pigment occurred on day 5 with a yeast concentration of 10^6 CFU/mL. The highest growth rates for the red and orange pigments were observed on day 3, at 77.7 % and 66.4 %, respectively. After day 11, pigment concentrations declined in the control samples, while the introduction of yeast at other concentrations led to decreased pigment levels, except for the continued positive effect of 10^6 CFU/mL yeast on pigment concentrations.

MK production, analyzed via HPLC on days 3, 5, 7, 11, and 15 (Fig. 1D), displayed a generally increasing trend throughout the fermentation. The highest MK yield, with a 52.32 % increase over the control, was achieved on day 15 day following the inoculation of 10^9 yeast cells. The introduction of yeast at varying concentrations from day 3 of fermentation significantly enhanced MK production, especially in later stages, with notable increases at yeast concentrations of 10^6 and 10^9 CFU/mL, resulting in MK levels of 48.80 mg/L and 67.95 mg/L, respectively.

3.2. Effect of co-culture on the transcription level of monascus-related genes

The addition of medium-density yeast to the *Monascus* fermentation culture affected the transcription level of genes in the MK synthesis pathway (Fig. 2). The transcription levels exhibited an initial rise followed by a decline, consistent across most genes. *mokA-mokI*, regulating MK synthesis, showed increased expression by day 15. Conversely, *mokC* levels decreased, while *mokH* and *mokI* remained stable. The expression of *mmpB* and *mmpC* increased, whereas *mmpD* decreased. Notably, the expression of the spore

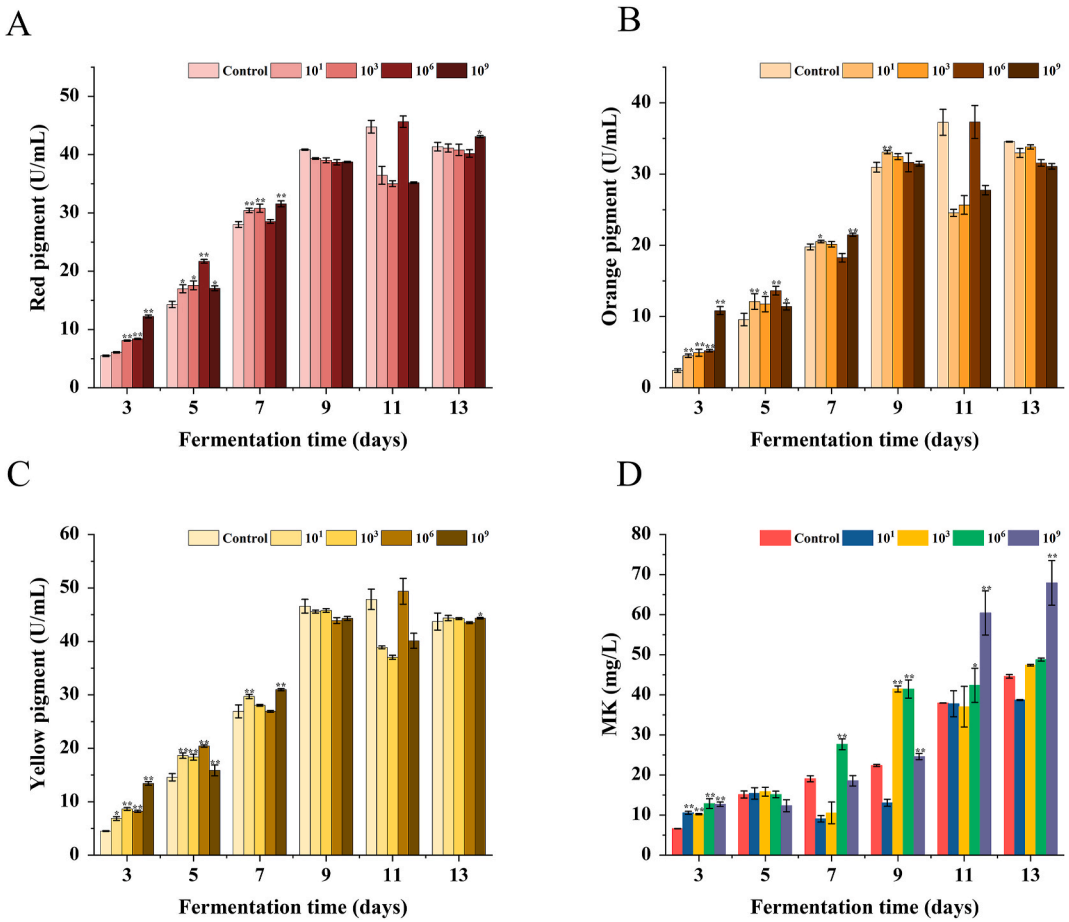

Fig. 1. Effects of different concentrations of *W.nomalus C22* on *M. purpureus M1* pigment and MK.(A) Red pigment. (B) Orange pigment. (C) Yellow pigment. (D) MK content. Data are expressed as the mean ± SD ($n = 3$). *$p < 0.05$ and **$p < 0.01$, compared to the control. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

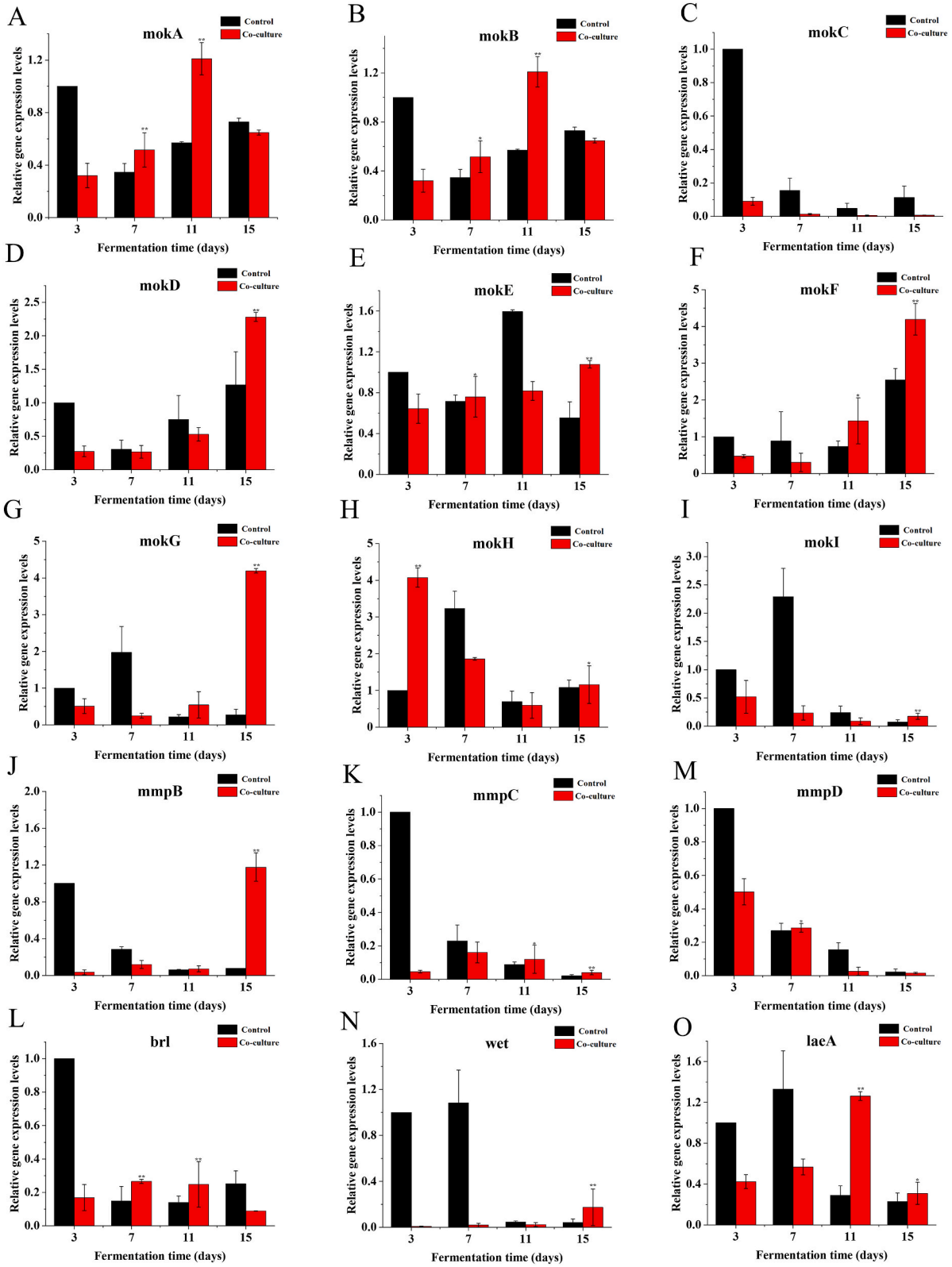

Fig. 2. Effects of different densities of *W.omalus C22* on the expression of *M. purpureus M1* biosynthetic pigment and MK and spore-forming genes. (A–I) *mokA-mokI* gene expression level. (J–K) Expression level of *Monascus* pigment gene. (L–O). Expression levels of morphology-related genes in *Monascus* spore. $^{*}p < 0.05$, $^{**}p < 0.01$, compared with control group. Data are expressed as mean ± SD ($n = 3$).

formation regulatory genes *brlA* and *wetA* increased by 2.82-fold and 1.84-fold, respectively. The global regulatory gene *laeA* was upregulated later in the fermentation process. The introduction of *W. anomalus* C22 appeared to regulate genes in the *M. purpureus* M1 metabolite synthesis pathway, enhancing MK and pigment production, consistent with QS mechanisms.

3.3. Qualitative and quantitative analysis of oxylipins in the co-culture system

Analysis of the *Monascus* fermentation broth via HPLC-MS identified a significant peak at a molecular weight of 295.2, aligned with oxylipins-13S-HODE (Fig. 3A). Further examination confirmed a daughter ion at 195.1 (Fig. 3B–C), establishing *Monascus*' ability to synthesize 13S-HODE during fermentation, indicative of microbial quorum-sensing molecules (QSMs). Additionally, the levels of oxylipins at various stages of fermentation were quantified using HPLC-MS to observe changes in 13S-HODE production in *M. Purpureus*, *W. anomalous*, and co-cultured samples. As shown in Fig. 4A, the amount of oxylipin 13S-HODE in the co-culture medium progressively increased over the fermentation period. In contrast, oxylipins were not detected in the *W. anomalous* fermentation liquid alone, and the concentration of 13S-HODE was minimal in solo *Monascus* fermentation, suggesting that co-culture stimulates *Monascus* to enhance oxylipin production, supporting their use as QSMs.

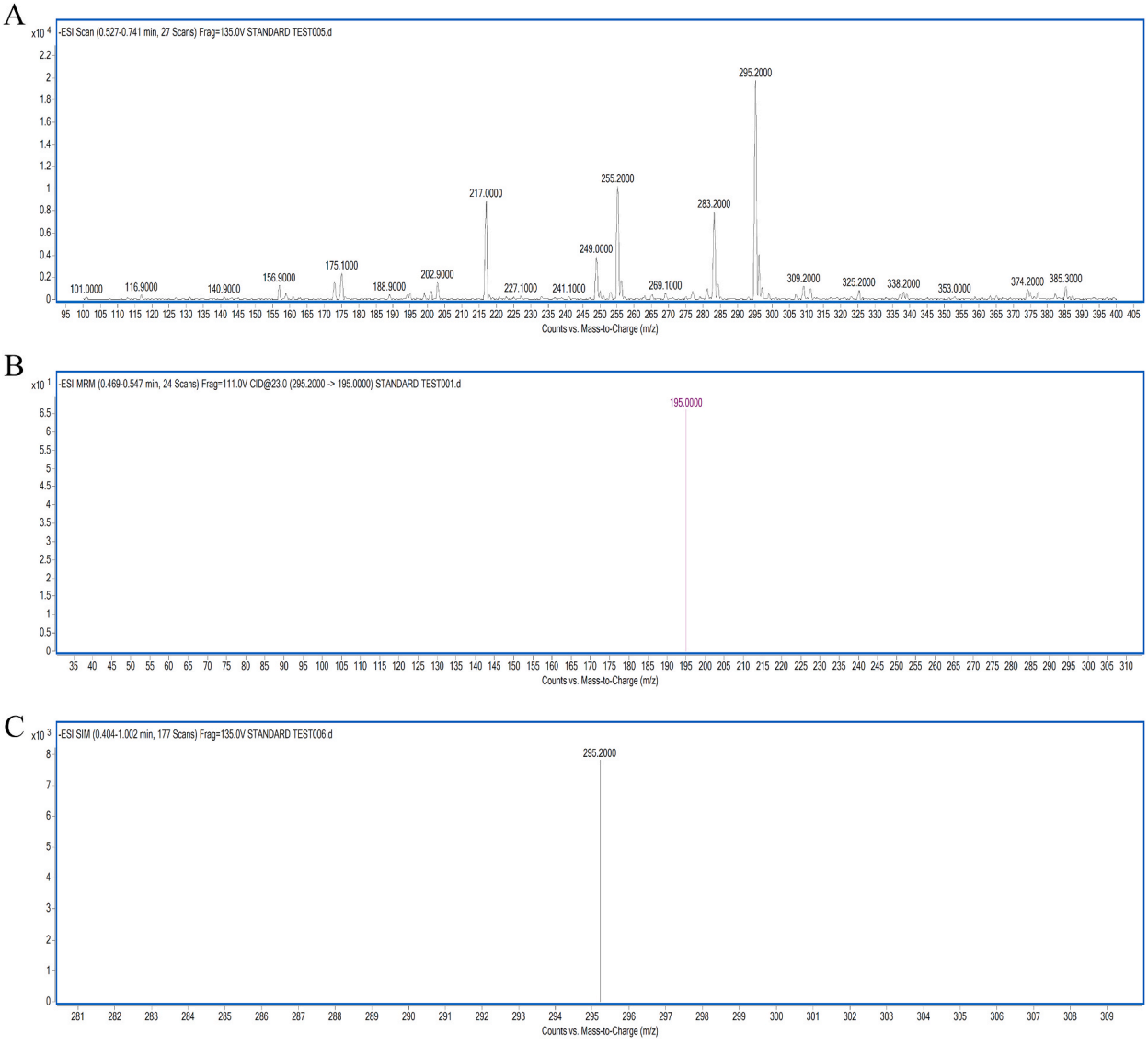

Fig. 3. Determination of 13S-HODE in co-culture *M. purpureus M1* and *W.nomalus C22* system. (A) TIC of *Monascus* by HPLC-MS. (B) HPLC-MS analysis of the daughter ions of oxylipin 13S-HODE. (C) HPLC-MS analysis of the parent ion of oxylipin 13S-HODE. *$p < 0.05$, and **$p < 0.01$, compared to the control. Data are expressed as the mean ± SD ($n = 3$).

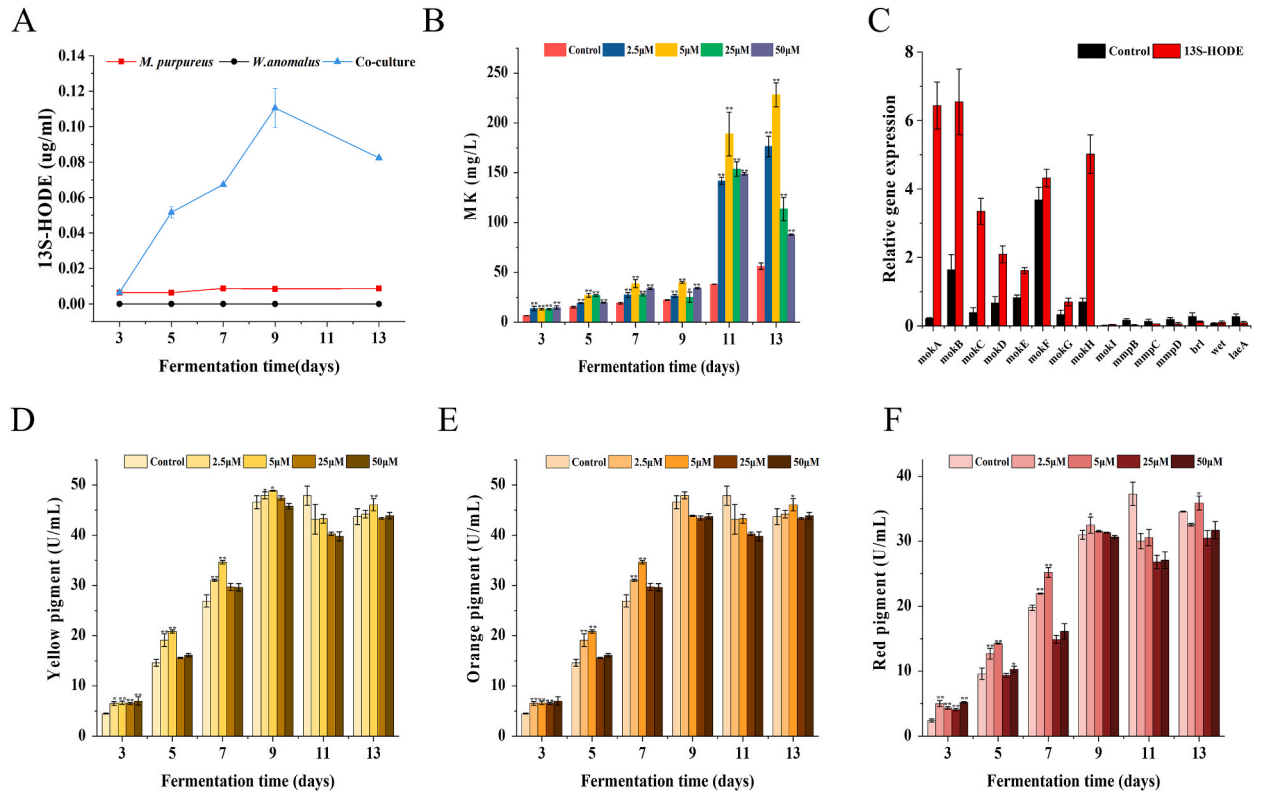

Fig. 4. Effect of Exogenous Oxylipin on *Monascus*. (A) Oxylipin 13S-HODE content in control and co-culture samples (3, 5, 7, 11, and 13 days).(B–C) Effects of different concentrations of 13S-HODE on *M. purpureus* MK and Gene expression levels. Effects of 13S-HODE on *M. purpureus* pigment. (D) Red pigment. (E) Orange pigment. (F) Yellow pigment. Data are expressed as the mean ± SD ($n = 3$). *$p < 0.05$ and **$p < 0.01$, compared to the control. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

3.4. Effect of Exogenous Oxylipin on Monascus

External 13S-HODE was added to *Monascus* monocultures to mimic co-culture effects (Fig. 4B). With the addition of 13S-HODE, there was a steady increase in MK production from day 3 to day 13, compared to the control group (Fig. 4B). Notably, the concentration of MK in the fermentation broth containing red pigment and treated with 5 μM 13S-HODE peaked at 228.26 mg/L by day 13, marking a threefold increase over the control.

Gene expression in the MK biosynthesis pathway was significantly upregulated with 25 μM of 13S-HODE, especially for genes *mokA*, *mokB*, and *mokH*, which showed increases of 26.63, 9.45, and 12.33 times, respectively, over the control. Unlike these, genes involved in MP synthesis did not show a marked upregulation. Additionally, the spore development gene *wetA* also showed enhanced expression.

The results (Fig. 4D–F) demonstrated that, akin to the control group lacking 13S-HODE, the introduction of various concentrations of 13S-HODE initially enhanced but later diminished the intensity of red pigment coloration, typically commencing from day 9. Notably, the influence of external oxylipin concentrations on the color intensity of red MPs was less pronounced compared to the changes observed in co-cultures with *W. anomalus* C22.

3.5. Effects of yeast co-culture and 13S-HODE on the micromorphology of monascus mycelium

Microscopic and SEM techniques were employed to evaluate the variations in mycelial differentiation and spore morphology between the co-culture with *W. anomalus* C22 and the control group. As depicted in Fig. 5A–B, the co-culture group exhibited significantly enhanced mycelial differentiation at 48 h compared to the control samples. In the control, conidia emergence at mycelial tips was sparse, averaging 4–5 conidia per apex. In contrast, the experimental setup with *W. anomalus* C22 showed a three to fourfold increase in conidia counts compared to the control. Furthermore, the ascospores in the experimental group were significantly larger, with diameters approximately twice those observed in the control group.

Further SEM analysis of *M. purpureus* M1's morphological and surface structures (Fig. 5D–F) revealed that the mycelium in the control group had a more uniform structure. Conversely, the mycelium in the experimental group was denser and more compactly arranged, with a noticeably higher spore count and more nodules present on the surface compared to the control.

Observations from both the untreated control and the 13S-HODE supplemented group (Fig. 5A and C) revealed significant

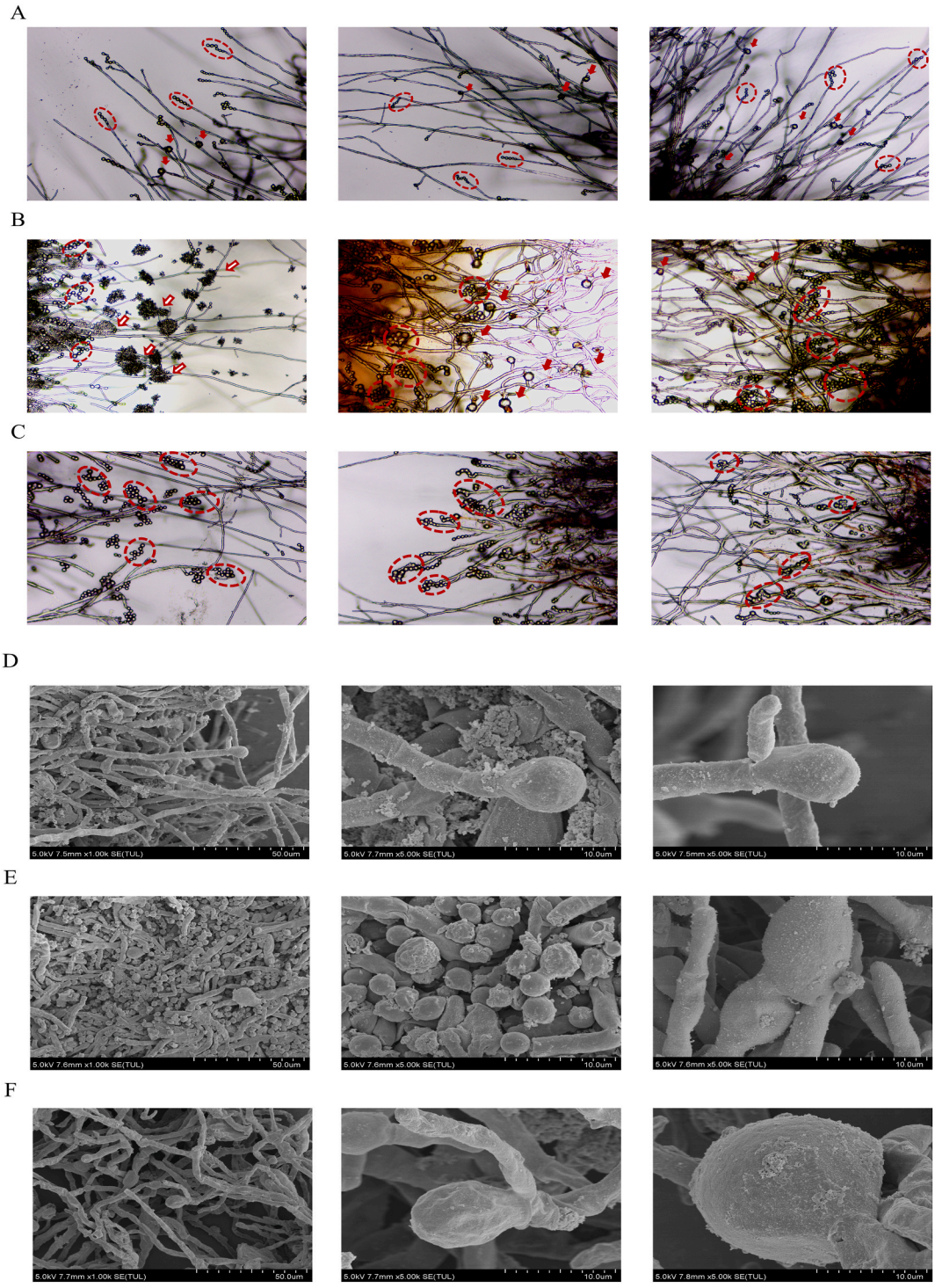

Fig. 5. Morphological changes of *M. purpureus M1* after being cultured by co-culture and exogenous addition of 13S-HODE. (A)Morphology of *M. purpureus M1* at × 100 magnifications by optical microscopy. (B) Morphology of co-cultured *M. purpureus M1* and *W.anomalus C22* at 100 magnifications by optical microscopy. (C) Morphology of *M. purpureus M1* with 13S-HODE added at × 100 magnification by optical microscopy. Solid red arrows point to monascus spores, hollow arrows point to yeast, and red circles represent conidia. (D) Scanning electron microscope images of the *M. purpureus M1* mycelium and spore at 5000 × ,10,000 × . (E) Scanning electron microscope images of the co-cultured *M. purpureus M1* and *W.anomalus C22* mycelium and spore at 5000 × ,10,000 × . (F) Scanning electron microscope images of the *M. purpureus M1* with 13S-HODE added mycelium and spore at 5000 × ,10,000 × . (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

differences. The group treated with 13S-HODE showed more pronounced mycelial differentiation and a higher conidium count than the control. SEM further elucidated the morphology and surface structure differences in *M. purpureus* M1 between the control and experimental groups (Fig. 5C and F). The experimental group's mycelium displayed reduced fullness and roundness, characterized by aggregation and a collapsed, folded appearance, indicative of additional product secretion.

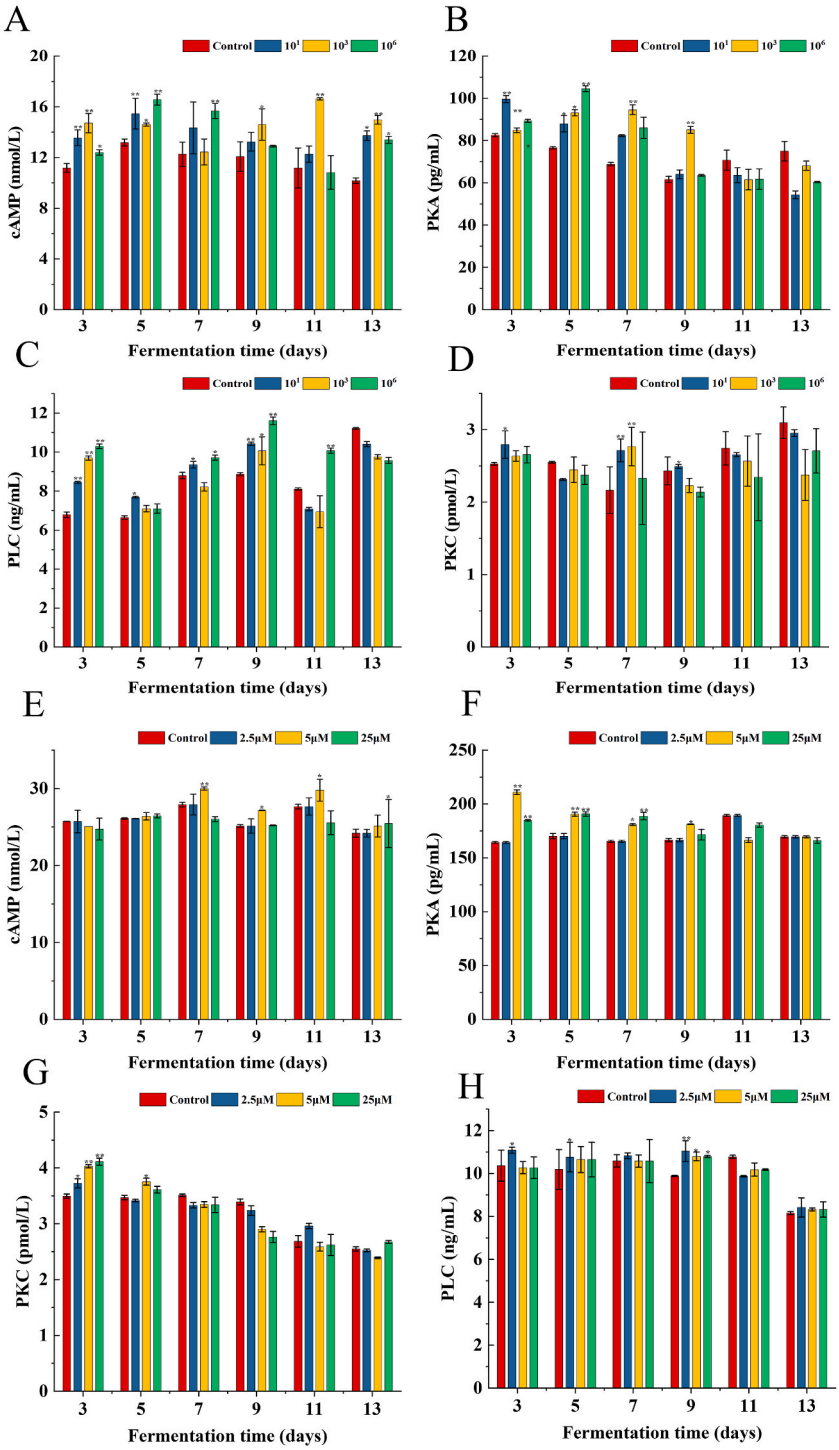

Fig. 6. Co-culture with *W. nomalus C22* and exogenous addition of oxylipins to cAMP signaling pathway and phosphatidyl inositol pathway of cAMP, PKA, PKC, and PLC. (A–D) Co-culture with *W. nomalus C22*. (E–H) Exogenous addition of oxylipins. $*p < 0.05$, $**p < 0.01$, compared with control group. Data are expressed as mean ± SD ($n = 3$).

3.6. Exploration of G protein signaling pathway in monascus

This study explored the impact of quorum sensing on the G protein-coupled receptor-mediated cAMP signaling pathway in *Monascus* (Fig. 6A). From day 3, a marked increase in cAMP levels was observed, with the highest concentration seen on day 11 in the yeast-supplemented groups. Specifically, the introduction of yeast led to a noticeable elevation in the intracellular concentration of cAMP, with the group receiving 10^3 CFU/mL yeast showcasing a peak increase of 16.63 on day 11, which is a 13.9 % rise compared to the control group.

The addition of yeast significantly boosted the activities of PKA and PKC, particularly impacting PKA more strongly. However, from day 11 onwards, the levels of PKA and PKC in the yeast-supplemented groups decreased below those observed in the control group. This pattern suggests that PKA activity correlated with the shifts seen in cAMP levels. Furthermore, the activity of PLC in the co-culture was more pronounced than that of PKC. Interestingly, the concentration of cAMP also appeared to be influenced by the oxygen content in Oxylipins, especially noticeable at a 25 μM concentration of 13S-HODE, where the peak cAMP concentration was recorded on day 11, suggesting an oxygen-dependent interaction.

When compared with the control, the experimental group treated with 13S-HODE demonstrated significant enhancements in the activities of PKA, PKC, and PLC, underscoring the profound impact of yeast addition and oxylipin presence on the signaling pathways within *Monascus*.

4. Discussion

This research delved into the effects of co-culturing *Monascus* with *W. anomalus* on the production of MPs and MK, highly regarded metabolites with extensive applications in the food and pharmaceutical sectors [23]. Our experimental results led us to propose and analyze a model of co-culture effects mediated by QS, as depicted in Fig. 7. The inclusion of *W. anomalus* influenced the production of secondary metabolites in *M. purpureus* M1, increasing the generation of oxylipins, which act as QSMs. These oxylipins were not only found to elevate the production of MPs and MK but also to influence the number and structure of conidia and ascospores. During co-culture, *W. anomalus* significantly enhanced the production of oxylipins, stimulating *Monascus* to produce these compounds through a QS mechanism. This interaction was primarily facilitated by the G protein signaling pathway, leading to increased levels of cAMP, PLC, PKA, and PKC, crucial components in cellular signaling.

Previous research has shown that compounds like tyrosol and farnesol can boost the production of valuable secondary metabolites by affecting the secondary metabolism and modifying fungal morphology through gene regulation [24]. Many of these compounds function as QSMs, essential for microbial development and metabolic processes [20,25–29].

Underscored the significant role of co-cultivation in enhancing *Monascus*'s secondary metabolic activity, echoing findings from prior interactions with *Saccharomyces cerevisiae* or *A. niger* that led to notable increases in pigment production. Specifically, brewing yeast was found to be particularly effective in this capacity [30]. The low-alcohol-producing characteristics of *W. anomalus* made it an effective partner for *Monascus*, facilitating the generation of group-sensing molecules such as oxylipins during co-culture (Fig. 7).

The presence of oxylipins, correlated with cell density, was found to regulate both the morphological and metabolic characteristics of various fungi. Despite the natural occurrence of trace oxylipins in *Monascus*, the introduction of *W. anomalus* in a co-culture setup significantly boosted their production, markedly affecting the morphology of *Monascus* spores and the synthesis of key metabolites [31–34].

The study also delved into the broader context of microbial symbiotic interactions and QS [35–37], emphasizing its importance in both intra- and inter-species communication [18,38,39]. Oxylipins, identified as interspecies QSMs, are prevalent across various organisms and play a pivotal role in QS regulation [40,41].

The ability of *Monascus* to produce the oxylipin 13S-HODE, influencing the production of polyketide secondary metabolites and fungal development, was a key discovery of this study. This influence extends to the regulation of genes involved in the synthesis of MK and MPs, underscoring the regulatory impact of oxylipins on *Monascus* biology. The research further explored the fungal quorum sensing regulation mechanisms, particularly focusing on the G protein-coupled signaling pathways, which are crucial for environmental sensing and response [25]. The role of oxylipins in mediating aflatoxin production in other fungi underscored the interconnectedness of QS, GPCRs, and G-protein-mediated signaling pathways, with this study reinforcing the impact of oxylipins on elevating cAMP levels and influencing MK synthesis gene transcription [41–43].

In conclusion, our research provides a deeper understanding of interspecies quorum sensing in *Monascus*, offering pathways to optimize MK and pigment production, which have significant implications for the food and pharmaceutical sectors [44–46]. Future research will focus on delineating the specific mechanisms of oxylipins as signaling molecules and their extensive impact on *Monascus*'s morphological and metabolic pathways. This investigation not only advances our comprehension of *Monascus*-related metabolism but also contributes broadly to the field of microbial QS regulation, presenting new avenues for practical applications in biotechnology.

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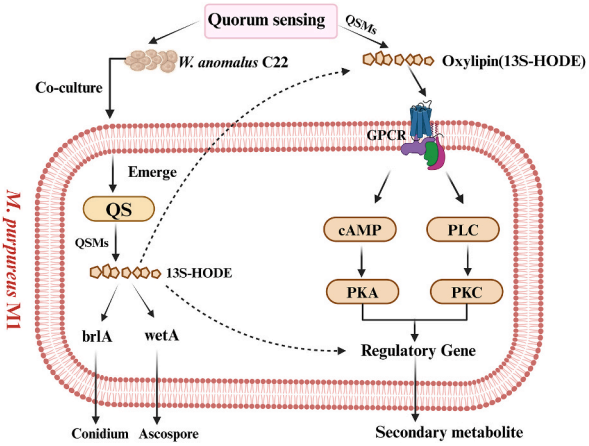

Fig. 7. The result model of the interaction of *W. anomalus* and *M. purpureus* co-culture by quorum induction.

Data availability

All raw data and materials will be made available following a reasonable request.

CRediT authorship contribution statement

Huiqian Liu: Writing – review & editing, Writing – original draft, Validation, Methodology, Investigation, Formal analysis, Conceptualization. **Mengyao Zhang:** Investigation, Data curation. **Linlin Xu:** Validation, Methodology. **FuRong Xue:** Data curation. **Wei Chen:** Writing – review & editing, Supervision, Resources, Conceptualization. **Chengtao Wang:** Writing – review & editing, Resources, Project administration, Funding acquisition, Conceptualization.

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.

References

[1] S.A.S. Mapari, U. Thrane, A.S. Meyer, Fungal polyketide azaphilone pigments as future natural food colorants? Trends Biotechnol. 28 (2010) 300–307, https://doi.org/10.1016/j.tibtech.2010.03.004.
[2] M.K. Endo A, A new hypocholesterolemic agent produced by a *Monascus* species, J. Antibiot. 32 (1979) 852–854, https://doi.org/10.7164/antibiotics.32.852.
[3] P. Dutar, R.A. Nicoll, Pre-and Postsynaptic GABAB Receptors in the Hippocampus Have Different Pharmacological Properties 1 (1988) 585–591, https://doi.org/10.1016/0896-6273(88)90108-0.
[4] W.H. Hsu, T.M. Pan, Treatment of metabolic syndrome with ankaflavin, a secondary metabolite isolated from the edible fungus Monascus spp, Appl. Microbiol. Biotechnol. 98 (2014) 4853–4863, https://doi.org/10.1007/s00253-014-5716-5.
[5] S. Tachibana, M. Yasuda, Purification and characterization of heterogeneous glucoamylases from *Monascus purpureus*, Biosci. Biotechnol. Biochem. 71 (2007) 2573–2576, https://doi.org/10.1271/bbb.70285.
[6] P. Jůzlová, L. Martínková, V. Křen, Secondary metabolites of the fungus *Monascus*: a review, J. Ind. Microbiol. 16 (1996) 163–170, https://doi.org/10.1007/BF01569999.
[7] S.N. Adin, I. Gupta, B.P. Panda, M. Mujeeb, *Monascin* and ankaflavin—biosynthesis from *Monascus purpureus*, production methods, pharmacological properties: a review, Biotechnol. Appl. Biochem. 70 (2023) 137–147, https://doi.org/10.1002/bab.2336.
[8] S.S. Feng, W. Li, Y.J. Hu, J.X. Feng, J. Deng, The biological activity and application of *Monascus* pigments: a mini review, Int. J. Food Eng. 18 (2022) 253–266, https://doi.org/10.1515/ijfe-2021-0235.
[9] C. Agboyibor, W.B. Kong, D. Chen, A.M. Zhang, S.Q. Niu, *Monascus* pigments production, composition, bioactivity and its application: a review, Biocatal. Agric. Biotechnol. 16 (2018) 433–447, https://doi.org/10.1016/j.bcab.2018.09.012.
[10] J. Qiao, X. He, C. Wang, H. Yang, Z. Xin, B. Xin, J. Wang, R. Dong, H. Zeng, F. Li, Transcriptome analysis revealing molecular mechanisms of enhanced pigment yield by succinic acid and fluconazole, Prep. Biochem. Biotechnol. 52 (2022) 990–1000, https://doi.org/10.1080/10826068.2021.2023822.
[11] J.A. Pfefferkorn, D.M. Bowles, W. Kissel, D.C. Boyles, C. Choi, S.D. Larsen, Y. Song, K.L. Sun, S.R. Miller, B.K. Trivedi, Development of a practical synthesis of novel, pyrrole-based HMG-CoA reductase inhibitors, Tetrahedron 63 (2007) 8124–8134, https://doi.org/10.1016/j.tet.2007.06.005.
[12] Y. Zhang, Z. Chen, Q. Wen, Z. Xiong, X. Cao, Z. Zheng, Y. Zhang, Z. Huang, An overview on the biosynthesis and metabolic regulation of monacolin K/lovastatin, Food Funct. 11 (2020) 5738–5748, https://doi.org/10.1039/d0fo00691b.
[13] A. Mazza, L. Schiavon, G. Rigatelli, G. Torin, F. Montanaro, S. Lenti, The short-term supplementation of monacolin K improves the lipid and metabolic patterns of hypertensive and hypercholesterolemic subjects at low cardiovascular risk, Food Funct. 9 (2018) 3845–3852, https://doi.org/10.1039/c8fo00415c.
[14] X.Y. Peng, J.T. Wu, C.L. Shao, Z.Y. Li, M. Chen, C.Y. Wang, Co-culture: stimulate the metabolic potential and explore the molecular diversity of natural products from microorganisms, Mar Life Sci Technol 3 (2021) 363–374, https://doi.org/10.1007/s42995-020-00077-5.
[15] D. Arora, P. Gupta, S. Jaglan, C. Roullier, O. Grovel, S. Bertrand, Expanding the chemical diversity through microorganisms co-culture Current status and outlook, Biotechnol. Adv. 40 (2020), https://doi.org/10.1016/j.biotechadv.2020.107521.
[16] C.P. Kurtzman, Phylogeny of the ascomycetous yeasts and the renaming of Pichia anomala to *Wickerhamomyces anomalus*, Antonie van Leeuwenhoek, International Journal of General and Molecular Microbiology 99 (2011) 13–23, https://doi.org/10.1007/s10482-010-9505-6.

[17] G. Gellissen, G. Kunze, C. Gaillardin, J.M. Cregg, E. Berardi, M. Veenhuis, I. Van Der Klei, New yeast expression platforms based on methylotrophic Hansenula polymorpha and Pichia pastoris and dimorphic Arxula adeninivorans and Yarrowia lipolytica - a comparison, FEMS Yeast Res. 5 (2005) 1079–1096, https://doi.org/10.1016/j.femsyr.2005.06.004.

[18] A. Mehmood, G. Liu, X. Wang, G. Meng, C. Wang, Y. Liu, Fungal quorum-sensing molecules and inhibitors with potential antifungal activity: a review, Molecules 24 (2019), https://doi.org/10.3390/molecules24101950.

[19] S.A. Padder, R. Prasad, A.H. Shah, Quorum sensing: a less known mode of communication among fungi, Microbiol. Res. 210 (2018) 51–58, https://doi.org/10.1016/j.micres.2018.03.007.

[20] N.N. Gessler, S.Y. Filippovich, G.P. Bachurina, E.A. Kharchenko, N.V. Groza, T.A. Belozerskaya, Oxylipins and oxylipin synthesis pathways in fungi, Appl. Biochem. Microbiol. 53 (2017) 628–639, https://doi.org/10.1134/S0003683817060060.

[21] M. Avbelj, J. Zupan, P. Raspor, Quorum-sensing in yeast and its potential in wine making, Appl. Microbiol. Biotechnol. 100 (2016) 7841–7852, https://doi.org/10.1007/s00253-016-7758-3.

[22] M. Qiu, Y. Wang, L. Sun, Q. Deng, J. Zhao, Fatty acids and oxylipins as antifungal and anti-mycotoxin agents in food: a review, Toxins 13 (2021), https://doi.org/10.3390/toxins13120852.

[23] C. Zhang, J. Liang, A. Zhang, S. Hao, H. Zhang, Q. Zhu, B. Sun, C. Wang, Overexpression of monacolin K biosynthesis genes in the *Monascus purpureus* azaphilone polyketide pathway, J. Agric. Food Chem. 67 (2019) 2563–2569, https://doi.org/10.1021/acs.jafc.8b05524.

[24] R. Shi, Q. Luo, Y. Liu, W. Chen, C. Wang, Effect of γ-heptalactone on the morphology and production of *monascus* pigments and monacolin K in *Monascus* purpureus, Journal of Fungi 8 (2022), https://doi.org/10.3390/jof8020179.

[25] H. Liu, X. Zhang, W. Chen, C. Wang, The regulatory functions of oxylipins in fungi: a review, J. Basic Microbiol. 63 (2023) 1073–1084, https://doi.org/10.1002/jobm.202200721.

[26] A.F. Costa, L.D.C. Silva, A.C. Amaral, Farnesol: an approach on biofilms and nanotechnology, Med. Mycol. 59 (2021) 958–969, https://doi.org/10.1093/mmy/myab020.

[27] A. Chang, S. Sun, L. Li, X. Dai, H. Li, Q. He, H. Zhu, Tyrosol from marine Fungi, a novel Quorum sensing inhibitor against Chromobacterium violaceum and Pseudomonas aeruginosa, Bioorg. Chem. 91 (2019), https://doi.org/10.1016/j.bioorg.2019.103140.

[28] M. Christwardana, D. Frattini, K.D.Z. Duarte, G. Accardo, Y. Kwon, Carbon felt molecular modification and biofilm augmentation via quorum sensing approach in yeast-based microbial fuel cells, Appl. Energy 238 (2019) 239–248, https://doi.org/10.1016/j.apenergy.2019.01.078.

[29] H.E. Williams, J.C.P. Steele, M.O. Clements, T. Keshavarz, γ-Heptalactone is an endogenously produced quorum-sensing molecule regulating growth and secondary metabolite production by *Aspergillus nidulans*, Appl. Microbiol. Biotechnol. 96 (2012) 773–781, https://doi.org/10.1007/s00253-012-4065-5.

[30] C.S. Shin, H.J. Kim, M.J. Kim, J.Y. Ju, Morphological Change and Enhanced Pigment Production of *Monascus* when Cocultured with Saccharomyces cerevisiae or Aspergillus oryzae, vol. 59, 1998, pp. 576–581, 2-7.

[31] V. Chaudhary, P. Katyal, A.K. Poonia, J. Kaur, A.K. Puniya, H. Panwar, Natural pigment from *Monascus*: the production and therapeutic significance, J. Appl. Microbiol. 133 (2022) 18–38, https://doi.org/10.1111/jam.15308.

[32] Q. Wen, X. Cao, Z. Chen, Z. Xiong, J. Liu, Z. Cheng, Z. Zheng, C. Long, B. Zheng, Z. Huang, An overview of *Monascus* fermentation processes for monacolin K production, Open Chem. 18 (2020) 10–21, https://doi.org/10.1515/chem-2020-0006.

[33] C. Zhang, H. Zhang, Q. Zhu, S. Hao, S. Chai, Y. Li, Z. Jiao, J. Shi, B. Sun, C. Wang, Overexpression of global regulator LaeA increases secondary metabolite production in *Monascus purpureus*, Appl. Microbiol. Biotechnol. 104 (2020) 3049–3060, https://doi.org/10.1007/s00253-020-10379-4.

[34] C. Zhang, M. Chen, L. Yang, Y. Cheng, Y. Qin, Y. Zang, B. Wang, B. Sun, C. Wang, Effects of *mokF* gene deletion and overexpression on the Monacolin K metabolism yields of *Monascus purpureus*, Appl. Microbiol. Biotechnol. 106 (2022) 3069–3080, https://doi.org/10.1007/s00253-022-11913-2.

[35] Z. Sun, J. Xi, C. Yang, W. Cong, Quorum sensing regulation methods and their effects on biofilm in biological waste treatment systems: a review, Front. Environ. Sci. Eng. 16 (2022), https://doi.org/10.1007/s11783-021-1495-2.

[36] N.C. Reading, V. Sperandio, Quorum sensing: the many languages of bacteria, FEMS Microbiol. Lett. 254 (2006) 1–11, https://doi.org/10.1111/j.1574-6968.2005.00001.x.

[37] A.M. Hardman, G. Sab Stewart, P. Williams, Quorum sensing and the cell-cell communication dependent regulation of gene expression in pathogenic and non-pathogenic bacteria, Antonie Leeuwenhoek 74 (1998) 199–210, https://doi.org/10.1023/A:1001178702503.

[38] A. Hartmann, A. Schikora, Editorial: plant responses to bacterial quorum sensing molecules, Front. Plant Sci. 6 (2015), https://doi.org/10.3389/fpls.2015.00643.

[39] E. Esmaeilishirazifard, A. Dariush, S.A. Moschos, T. Keshavarz, A novel antifungal property for the Bacillus licheniformis ComX pheromone and its possible role in inter-kingdom cross-talk, Appl. Microbiol. Biotechnol. 102 (2018) 5197–5208, https://doi.org/10.1007/s00253-018-9004-7.

[40] D.I. Tsitsigiannis, T.M. Kowieski, R. Zarnowski, N.P. Keller, Three putative oxylipin biosynthetic genes integrate sexual and asexual development in *Aspergillus nidulans*, Microbiology (N. Y.) 151 (2005) 1809–1821, https://doi.org/10.1099/mic.0.27880-0.

[41] K.J. Affeldt, M. Brodhagen, N.P. Keller, Aspergillus oxylipin signaling and quorum sensing pathways depend on G protein-coupled receptors, Toxins 4 (2012) 695–717, https://doi.org/10.3390/toxins4090695.

[42] J.S. Smith, T.F. Pack, A. Inoue, C. Lee, K. Zheng, I. Choi, D.S. Eiger, A. Warman, X. Xiong, Z. Ma, G. Viswanathan, I.M. Levitan, L.K. Rochelle, D.P. Staus, J. C. Snyder, A.W. Kahsai, M.G. Caron, S. Rajagopal, Noncanonical scaffolding of Gαi and β-arrestin by G protein-coupled receptors, Science 371 (2021) eaay1833, https://doi.org/10.1126/science.aay1833, 1979.

[43] J. Gao, X. Xu, K. Huang, Z. Liang, Fungal G-protein-coupled receptors: a promising mediator of the impact of extracellular signals on biosynthesis of ochratoxin A, Front. Microbiol. 12 (2021), https://doi.org/10.3389/fmicb.2021.631392.

[44] R. Shi, P. Gong, Y. Liu, Q. Luo, W. Chen, C. Wang, Linoleic acid functions as a quorum-sensing molecule in *Monascus purpureus*–Saccharomyces cerevisiae co-culture, Yeast 40 (2023) 42–52, https://doi.org/10.1002/yea.3831.

[45] H.D. Moghadam, F. Tabatabaee Yazdi, F. Shahidi, M. Sarabi-Jamab, Z. Es'haghi, Co-culture of *Monascus purpureus* with Saccharomyces cerevisiae: a strategy for pigments increment and citrinin reduction, Biocatal. Agric. Biotechnol. 45 (2022), https://doi.org/10.1016/j.bcab.2022.102501.

[46] R. Dikshit, P. Tallapragada, Bio-synthesis and screening of nutrients for lovastatin by Monascus sp. under solid-state fermentation, J. Food Sci. Technol. 52 (2015) 6679–6686, https://doi.org/10.1007/s13197-014-1678-y.