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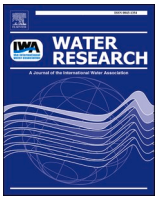

Insights into the interaction mechanisms between Microcystin-degrading bacteria and *Microcystis aeruginosa*

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ABSTRACT

Interactions between bacteria and cyanobacteria influence the occurrence and development of harmful cyanobacterial blooms (HCBs). Bloom-forming cyanobacteria and cyanotoxin-degrading bacteria are essential in HCBs, nonetheless, their interactions and the underlying mechanisms remain unclear. To address this gap, a typical microcystin-LR (MC-LR)-degrading bacterium and a toxic *Microcystis aeruginosa* strain were co-cultivated to investigate their interactions. The cyanobacterial growth was enhanced by 24.8 %-44.3 % in the presence of the bacterium in the first 7 days, and the cyanobacterium enhanced the bacterial growth by 59.2 %-117.5 % throughout the growth phases, suggesting a mutualistic relationship between them. The presence of the bacterium increased cyanobacterial intracellular MC-LR content on days 4, 8, and 10 while reducing the extracellular MC-LR concentration, revealing the dual roles of the bacterium in enhancing cyanotoxin production and degrading cyanotoxins. The bacterium alleviated the oxidative stress, which may be crucial in promoting cyanobacterial growth. Critical functional genes related to cyanobacterial photosynthesis and MC-LR synthesis, and bacterial MC-LR degradation were up-regulated in the presence of the bacterium and cyanobacterium, respectively. Moreover, extracellular polymeric substances (EPS) were produced at the cell interface, implying EPS play a role in cyanobacterial-bacterial interactions. This study is the first to unveil the interaction mechanisms between cyanotoxin-degrading bacteria and bloom-forming cyanobacteria, shedding light on the dynamics of HCBs.

1. Introduction

The extensive outbreak of harmful cyanobacterial blooms (HCBs) has raised global concern (Kruk et al., 2023; Ren et al. 2024; Zhang et al. 2022; Zhang et al. 2023). HCBs pose a serious threat to ecosystems, leading to water quality deteriorating and a decline in aquatic organism diversity (Huisman et al. 2018; Paerl et al. 2001; Paerl and Paul 2012). HCBs also result in unpleasant smells, negatively affecting the landscape function of lakes (Huisman et al. 2018). Moreover, bloom-forming cyanobacteria can produce a wide range of toxic secondary metabolites, with microcystins (MCs) being the most widely distributed toxins released by >30 cyanobacterial species (Svirčev et al. 2019; Tan et al. 2023). These toxins can enter the animal and human bodies through drinking water or can accumulate along the food chain (Massey and Yang 2020; Mohamed 2016; Tamele and Vasconcelos 2020). Among the microcystins, MC-LR (L represents leucine, R represents arginine), is a causative agent for metabolism disorders and damage to multiple organs such as the liver, kidney, stomach, and intestine (Du et al. 2022; Feng et al. 2022; Yang et al. 2023; Yang et al. 2022; Yi et al. 2019; Zheng et al. 2017).

The dynamics of HCBs are influenced not only by environmental conditions like nutrients, temperature and pH (Cook et al. 2020; Yang et al. 2018), but also by biological factors such as the interactions between cyanobacteria and bacteria (Pound et al. 2021). Cyanobacteria

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secrete organic molecules around their cells, creating a microhabitat known as the "phycosphere," which plays a crucial role in shaping the associated bacterial community (Gao et al. 2023; Seymour et al. 2017). The relationships between cyanobacteria and associated bacteria can be mutualistic or antagonistic. Cyanobacteria offer oxygen and dissolved carbon sources for the epiphytic bacteria, and bacteria provide cyanobacteria with CO_2, phosphorus, or growth factors such as vitamins (Cook et al. 2020; Dziallas and Grossart 2012; Zhao et al. 2023). On the other hand, some bacteria exhibit inhibitory or algicidal activities (Berg et al. 2009; Bi et al. 2019; Morón-López et al., 2023), and toxins such as MCs can negatively affect bacterial diversity and richness (Wu et al. 2019; Zhang et al. 2019). These interactions can either facilitate or suppress the proliferation of cyanobacteria, thus influencing the formation, strength, and maintenance of HCBs.

MCs-degrading bacteria are widely distributed in HCBs-contaminated water bodies (Li et al. 2017; Mohamed et al. 2022; Mohamed and Alamri 2012). Some studies reported the composition of species and community structure dynamics of MCs-degrading bacteria during HCBs. For instance, the MCs-degrading bacterium *Sphingomonas* dominated the particle-attached bacterial communities during the decomposition of *Microcystis* blooms (Shao et al. 2014). Gao et al. (2022) revealed that *Sphingopyxis* sp. was the most prominent MC-degrader in water samples of Lake Taihu. Cyanobacteria, particularly *Microcystis* spp. often form colonies where microorganisms are embedded in the mucilage matrix surrounding the cyanobacterial cells (Le et al. 2022). These findings suggest a close association between MCs-degrading bacteria and bloom-forming cyanobacteria. Nonetheless, knowledge of the interactions between MCs-degrading bacteria and bloom-forming cyanobacteria and the underlying mechanisms is still lacking.

Sphingopyxis sp. YF1 was previously isolated from Lake Taihu during the outbreak of HCBs. The strain is a typical MC-LR degrading bacterium and can use MC-LR as its sole carbon and nitrogen sources (Yang et al. 2020). The present study aims to investigate the interplays between *Sphingopyxis* sp. YF1 and the toxic cyanobacterium *Microcystis aeruginosa* FACHB-905. The growth of both strains in the cyanobacterial and bacterial monocultures and their co-culture, the production and degradation of MC-LR were explored. Additionally, the study investigated the biochemical and molecular characteristics underlying these interactions. By understanding the relationships between the MCs-degrading bacteria and toxic bloom-forming cyanobacteria, this study would provide further insights into the emergence and progression of HCBs and the dynamics of cyanotoxins.

2. Materials and methods

2.1. Strains and culture conditions

Sphingopyxis sp. YF1 was cultivated using nutrient broth (NB) medium containing 10.0 g/L peptone, 3.0 g/L beef extract and 5.0 g/L NaCl, pH 7.2 ± 0.2. The NB medium was autoclaved at 121°C for 20 min. The culture was oscillated at 180 rpm at 30°C. *Microcystis aeruginosa* FACHB-905 was purchased from the Freshwater Algae Culture Collection of the Institute of Hydrobiology, Chinese Academy of Sciences (Wuhan, China). The culture medium of *M. aeruginosa* FACHB-905 is BG11 medium (Doppler et al. 2021). The culture was incubated at 28 ±2°C under a 12:12 light: dark cycle, at a light intensity of 150 μmol m^{-2} s^{-1} in a light incubator. In the culture, *Microcystis aeruginosa* FACHB-905 cells occurred as unicellular forms.

2.2. Co-culture of Sphingopyxis sp. YF1 and M. aeruginosa FACHB-905

Cells of *Sphingopyxis* sp. YF1 and *M. aeruginosa* FACHB-905 at the logarithmic growth stage were collected by centrifugation at 12,000 × *g* and 5000 × *g* for 10 min, respectively. Collected cells of both strains were washed twice with sterilized fresh BG11 medium, respectively. Cells of *Sphingopyxis* sp. YF1 and *M. aeruginosa* FACHB-905 were co-cultured with an initial cell density ratio of 10:1. Meanwhile, *Sphingopyxis* sp. YF1 and *M. aeruginosa* FACHB-905 were cultivated separately as controls. All three groups were cultured with 1 L BG11 medium at 28 ±2°C in a light incubator. Bacterial and cyanobacterial cell density was counted with a hemocytometer under a microscope (Wei et al. 2020; Zhang et al. 2020).

2.3. Measurement of chlorophyll a, MDA, and SOD activity

At different time points (on days 0, 2, 4, 6, 8, and 10), cells in the monoculture and co-culture were harvested by centrifugation at 8000 × *g* for 10 min and washed with PBS three times. To determine the chlorophyll *a* (Chl*a*) concentration, the collected cells were soaked in 90 % acetone overnight in darkness. Then Chl*a* concentration was measured as described in a previous study (Xu et al. 2022).

MDA is an indicator of lipid peroxidation intensity and is usually considered a critical index for reactive oxygen species (ROS) production in organisms (Qian et al. 2012). Organisms possess an antioxidant defense system to reduce the levels of ROS, and superoxide dismutase (SOD) is one of the most common antioxidants. To determine the malondialdehyde (MDA) content and SOD activity, the collected cells were washed twice with PBS. Each sample was ground in liquid nitrogen until the cells were broken. The resulting cell debris was collected, suspended in pre-cooled PBS, and centrifuged at 8000 × *g* for 10 min. The supernatant was withdrawn to measure the MDA content and SOD activity according to the instructions of the MDA and SOD Detection Kit (Jiancheng Institute of Bioengineering, Nanjing, China).

2.4. Detection of extracellular and intracellular MC-LR

The extracellular and intracellular MC-LR concentrations in *M. aeruginosa* were measured every two days. Fifteen mL of solutions in the co-culture and the monoculture of *M. aeruginosa* FACHB-905 were withdrawn under aseptic conditions and centrifuged at 8000 × *g* for 10 min. The extracellular MC-LR (ExMC-LR) concentration in the supernatant was determined by the Microcystin Plate Kit (Beacon Analytical Systems, Saco, USA). To determine the intracellular MC-LR (InMC-LR) concentration, the collected cells were ground in liquid nitrogen until broken, and the resulting cell debris was collected and re-suspended in PBS. To prevent the bio-degradation of MC-LR by enzymes of *Sphingopyxis* sp. YF1 in the co-culture during subsequent treatments, the MC-LR degrading enzymes were inactivated by adding 25 μL of 5 mol/L hydrochloric acid (final pH 3.5 ~ 4). As reported previously, MC-LR at this pH range is very stable (Harada et al. 1996). The suspension was centrifuged at 12,000 × *g* to remove the cell debris, and then the concentration of InMC-LR was determined using an ACQUITY ultra-performance liquid chromatograph (UPLC) (Waters, Milford, United States) equipped with an ACQUITY UPLC BEH C18 column. A mixture of methanol (63 %, v/v) and trifluoroacetic acid (0.05 %, v/v) was used as mobile phase with a flow rate of 0.6 mL/min. MC-LR was detected with a photodiode array (PDA) detector at a wavelength of 238 nm. The InMC-LR content was calculated as the ratio of InMC-LR concentration to cyanobacterial cell density.

2.5. RNA extraction, cDNA synthesis and quantitative real-time-PCR

The expression of genes involved in photosynthesis and MC-LR biosynthesis of *M. aeruginosa*, and genes associated with MC-LR degradation of *Sphingopyxis* sp. YF1, was investigated. The gene *psaB* encodes one of the reaction center proteins of photosystem I and *psbB* encodes the core antenna CP47 protein of photosystem II, respectively (Shi et al. 2004). The gene *rbcL* encodes the large subunit of ribulose bisphosphate carboxylase, which is a key enzyme involved in the dark reaction of photosynthesis (Liu et al. 2015). The gene *mcyB* is crucial in MC-LR synthesis. The genes *mlrA, mlrB, mlrC,* and *pAAase* are critical genes involved in MC-LR biodegradation (Cai et al. 2022; Wei et al. 2023). The

target genes and their primers are shown in Table S1. Solutions in the co-culture and the monocultures were withdrawn and centrifuged at 8000 × *g* for 10 min to harvest bacterial and cyanobacterial cells, and the harvested cells were ground in liquid nitrogen. RNA was extracted using the RNAprep Pure Plant Plus Kit (TianGen, Beijing, China) and cDNA was synthesized using the HiScript® IIQ RT SuperMix for qPCR (Vazyme, Nanjing, China). Quantitative real-time PCR (qPCR) was performed using the ChamQ Universal SYBR qPCR Master Mix (Vazyme) on a qTOWER3 Real-Time PCR System (Analytikjena, Jena, Germany). The 16S rRNA genes of *Sphingopyxis* sp. YF1 and *M. aeruginosa* FACHB-905 were used as internal references. The relative expression of target genes was calculated according to $2^{-\Delta\Delta Ct}$ method (Livak and Schmittgen 2001).

2.6. Scanning electron microscopy and energy dispersive spectroscopy

At different time points, bacterial and cyanobacterial cells in the co-culture were harvested and then fixed overnight at 4°C with 2.5 % glutaraldehyde. The samples were washed thrice using phosphate buffer (0.1 M, pH 7) and subsequently fixed with a 1 % osmic acid solution for 1–2 h. After the osmic acid solution was carefully removed, the samples were rinsed and dehydrated with different concentrations of ethanol. The samples were then sprayed with platinum by a coating machine (Quorum Q150T ES plus, East Sussex, United Kingdom) under 10 mA current for 10 s and examined under a Nova Nano 450 scanning electron microscope (FEI, Hillsboro, United States) equipped with an X-Max^{N}50 energy dispersive spectrometer (Oxford Instruments, Abingdon, UK).

2.7. Statistical analysis

All assays were performed in triplicate and the results were reported as means ± standard deviation (SD). The data in this study, including cell density, Chl*a*, oxidative stress, concentration of InMC-LR and ExMC-LR, and functional gene expression, were analyzed using *t*-test (SPSS 26.0). Differences were considered as statistically significant if p-value < 0.05. Correlation between concentrations of InMC-LR and ExMC-LR was evaluated using linear regression.

3. Results

3.1. Growth of *M. aeruginosa* FACHB-905 and *Sphingopyxis* sp. YF1 in the monocultures and the co-culture

The cell density of *M. aeruginosa* FACHB-905 increased from 5.80 × 10^6 cells/mL to 6.45 × 10^7 cells/mL and 6.55 × 10^7 cells/mL in the monoculture and co-culture on day 11, respectively (Fig. 1A). Moreover, the cell density of *M. aeruginosa* FACHB-905 from day 1 to day 7 was promoted by 24.8 % (day 1) to 44.3 % (day 5) in the presence of *Sphingopyxis* sp. YF1 compared to that in the absence of YF1. After day 7, the cyanobacterial cell density in the co-culture and in the monoculture

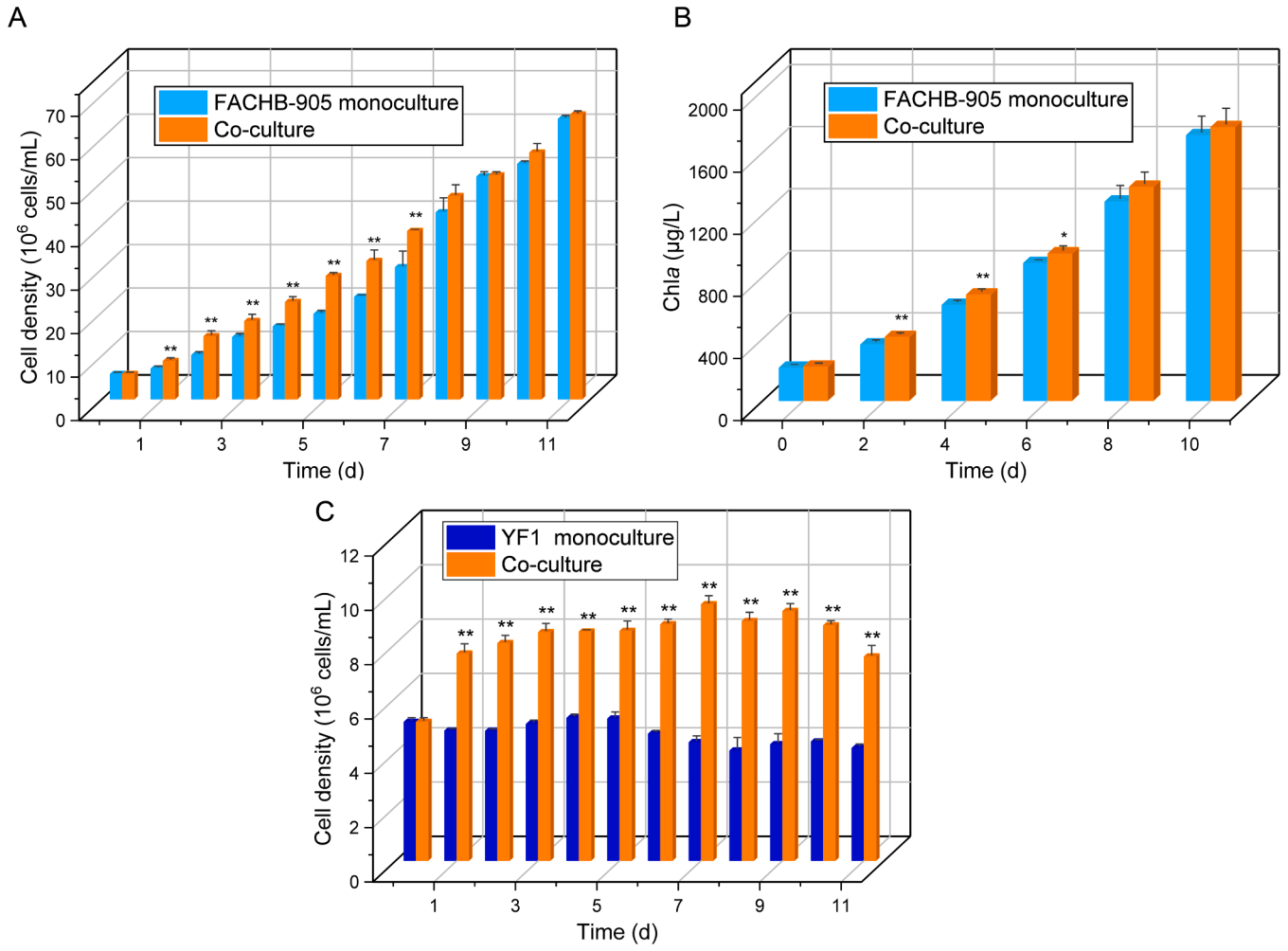

Fig. 1. Variations of (A) cell density of *M. aeruginosa* FACHB-905 in the cyanobacterial monoculture and in the co-culture of *M. aeruginosa* FACHB-905 and *Sphingopyxis* sp. YF1, (B) Chl*a* content of *M. aeruginosa* FACHB-905 in the cyanobacterial monoculture and in the co-culture, and (C) cell density of *Sphingopyxis* sp. YF1 in the bacterial monoculture and in the co-culture. *, $p < 0.05$, **, $p < 0.01$.

became close. These results indicate that strain YF1 is beneficial to the growth of *M. aeruginosa* FACHB-905 during the initial 7-day period.

Chl*a* is one of the major light-capturing pigments in *Microcystis aeruginosa*, responsible for absorbing and transferring light. It serves as an indicator of photosynthetic activity and is closely correlated to cyanobacterial growth (Liu et al. 2015; Zhang et al. 2019). According to Fig. 1B, the concentration of Chl*a* increased from 213.09 μg/L (day 0) to 1711.14 μg/L (day 10) in the monoculture of *M. aeruginosa* FACHB-905, and from 219.72 μg/L (day 0) to 1763.54 μg/L (day 10) in the co-culture. The concentration of Chl*a* in the co-culture of *M. aeruginosa* FACHB-905 and *Sphingopyxis* sp. YF1 was higher than that in the monoculture of strain FACHB-905 on days 2, 4, and 6 ($p < 0.05$). The dynamic of Chl*a* concentration was consistent with the cell density of strain FACHB-905.

The growth of *Sphingopyxis* sp. YF1 in the monoculture and the co-culture was determined. The BG11 medium used in this study has limited organic substances (such as 6 mg/L citric acid and 6 mg/L ammonium ferric citrate). Strain YF1 did not show apparent growth in the monoculture (Fig. 1C), indicating its poor utilization of citric acid and citrate as a carbon source. In the co-culture, the cell density of YF1 increased fast during the first 2 days, from 5.11×10^7 cells/mL to 8.01×10^7 cells/mL. Until day 7, the cell density increased to 9.44×10^7 cells/mL. From day 1 to day 11, the cell density of *Sphingopyxis* sp. YF1 in the co-culture was increased by 59.19 % (day 1) to 117.51 % (day 8) compared to that in the monoculture. Since the BG11 medium can not provide available carbon sources for strain YF1, it should be *M. aeruginosa* FACHB-905 that provides the necessary carbon sources to support the growth of YF1 in the co-culture. Therefore, *M. aeruginosa* FACHB-905 benefited the growth of *Sphingopyxis* sp. YF1. Taken together, the findings in Fig. 1 suggest a mutualistic relationship between *M. aeruginosa* FACHB-905 and *Sphingopyxis* sp. YF1 during the initial 7 days.

3.2. Dynamics of InMC-LR and ExMC-LR

The concentration of InMC-LR increased from the onset to day 10, both in the *M. aeruginosa* FACHB-905 monoculture and in the co-culture (Fig. 2A). In the FACHB-905 monoculture, the concentration of InMC-LR on days 2, 4, 6, 8, and 10 was 32.18, 69.98, 94.28, 117.25 and 174.27 μg/L, respectively. In the co-culture, the concentration of InMC-LR increased by 52.53 %, 50.58 %, 31.09 %, 70.17 %, and 91.15 % on days 2, 4, 6, 8, and 10, respectively. The content of InMC-LR (cellular quota) in the co-culture is higher than in the *M. aeruginosa* FACHB-905 monoculture on days 4, 8, and 10, increased by 12.9 %, 54.8 %, and 82.8 %, respectively (Fig. 2B). This suggests that the bacterial strain YF1 enhanced the MC-LR biosynthesis of *M. aeruginosa*.

The concentration of ExMC-LR was much lower than the concentration of InMC-LR, especially after day 4, the former was ~40 to ~100 times less than the latter. The concentration of ExMC-LR in the cyanobacterial monoculture was 2.84, 2.02, 2.46, 1.77, 2.68 and 4.58 μg/L

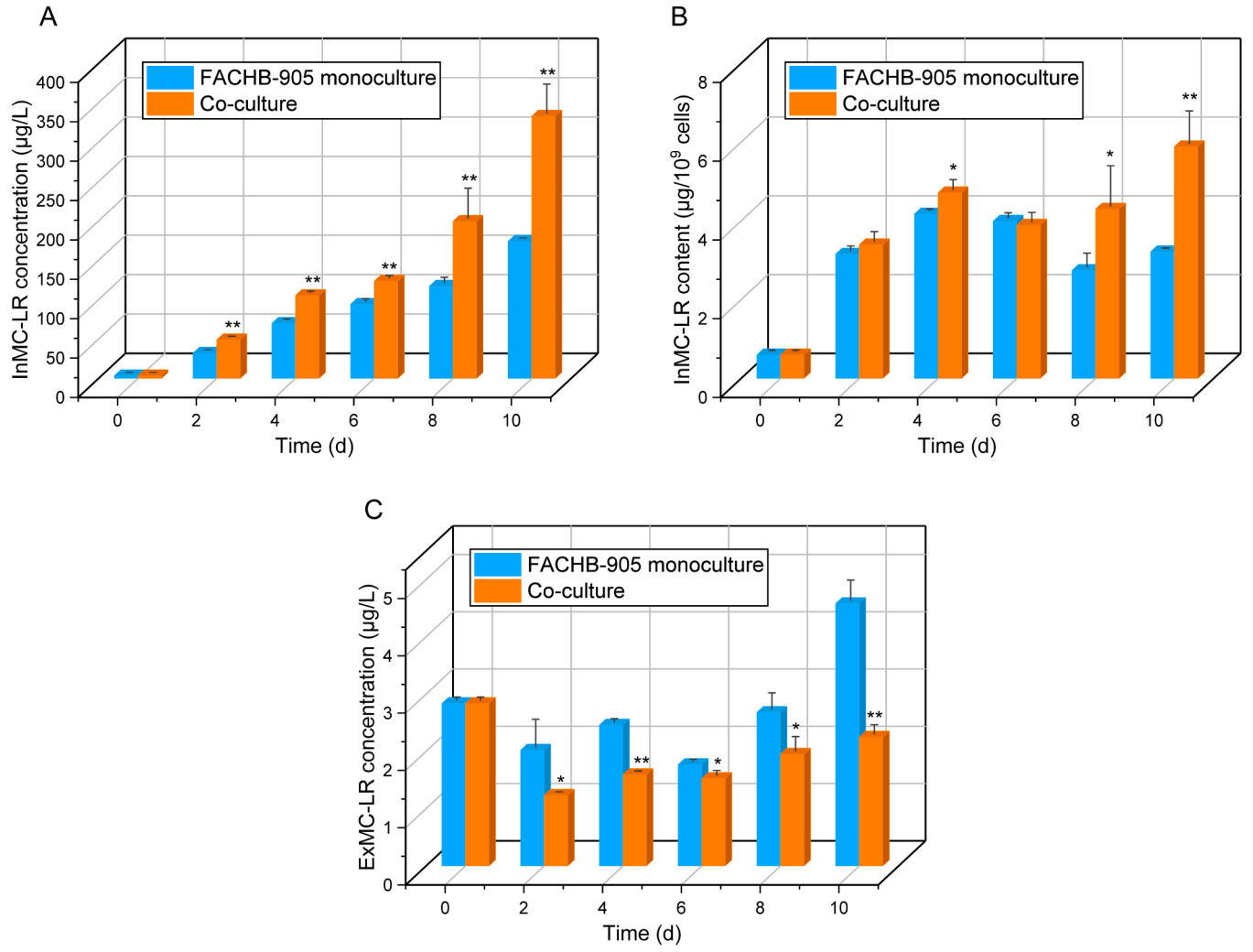

Fig. 2. Variations of (A) InMC-LR concentration in the cyanobacterial monoculture and the co-culture, (B) InMC-LR content in the cyanobacterial monoculture and the co-culture, and (C) ExMC-LR concentration of *M. aeruginosa* FACHB-905 in the cyanobacterial monoculture and the co-culture. *, $p < 0.05$, **, $p < 0.01$.

(Fig. 2C). In the cyanobacterial monoculture, the concentration of ExMC-LR was positively correlated to that of InMC-LR ($r = 0.603$, $p < 0.05$). There was a rapid increase in ExMC-LR during days 8–10, possibly due to that strain FACHB-905 began entering the stationary phase and more lysed cells released MC-LR. The concentration of ExMC-LR in the co-culture was 13.72 % - 50.89 % lower than in the monoculture since day 2, especially on day 10. The results imply strain YF1 effectively reduced the content of ExMC-LR (Fig. 2C). In the co-culture, the concentration of ExMC-LR was not significantly correlated to that of InMC-LR ($r = -0.115$, $p > 0.05$). The concentration of ExMC-LR in the co-culture exhibited a rapid decrease from day 0 to day 2, consistent with the fast growth of *Sphingopyxis* sp. YF1 (Fig. 1C). These findings highlight the critical role of strain YF1 in degrading ExMC-LR.

3.3. Interactions at the interface of *Sphingopyxis* sp. YF1 and *M. aeruginosa* FACHB-905 cells

SEM was performed to better understand the interactions of *Sphingopyxis* sp. YF1 and *M. aeruginosa* FACHB-905. In the beginning, only a few cells of *Sphingopyxis* sp. YF1 attached to the cells of *M. aeruginosa* FACHB-905 (Fig. 3A and 3B). On day 4, more cells of *Sphingopyxis* sp. YF1 adhered to the cells of *M. aeruginosa* FACHB-905 (Fig. 3C and 3D). Exudates were produced at the interface of the cyanobacterial and bacterial cells (arrows 1–3). EDS was used to characterize the

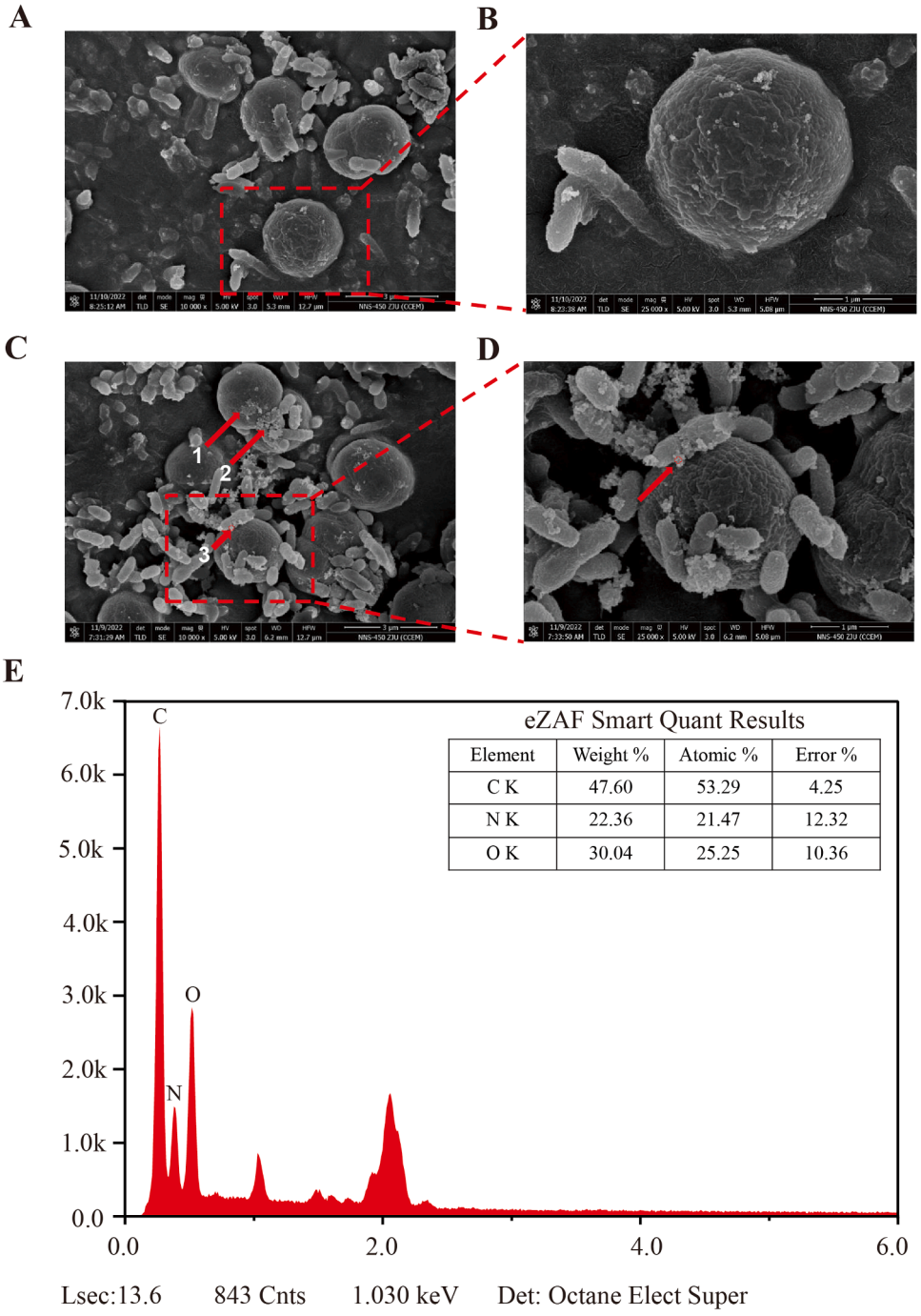

Fig. 3. SEM and EDS analyses of *Sphingopyxis* sp. YF1 and *M. aeruginosa* FACHB-905 cells in the co-culture. A and B, SEM images on day 0; C and D, SEM images on day 4; E, EDS spectra of the exudates at the interface of *Sphingopyxis* sp. YF1 and *M. aeruginosa* FACHB-905 cells.
Note: Sodium element is the component of BG11 medium; Aluminum, osmium and platinum elements were added during the preparation of the SEM sample.

components of the exudates at (arrow 3), and the result shows that the exudates contained C, N, and O (Fig. 3E).

3.4. MDA content and SOD activity in the monoculture and co-culture

The content of MDA in both cultures showed a similar trend (Fig. 4A). The content of MDA increased during the first 6 days and reached the maximal value of 98.63 nmol/mg protein in the monoculture and 82.33 nmol/mg protein in the co-culture on day 6. After that, the content of MDA decreased until day 10. The content of MDA in the co-culture was higher than that in the monoculture on days 4, 6, and 10. This suggests *Sphingopyxis* sp. YF1 mitigated the oxidative stress experienced by *M. aeruginosa* FACHB-905.

We detected the activity of SOD in the monoculture of FACHB-905 and the co-culture. From the onset to day 4, the SOD activities in the monoculture and the culture were at low levels, with no significant difference between the two groups. On day 6, the activity of SOD in the co-culture was 1.97 folds higher than that in the monoculture. The SOD activity in the co-culture was lower than that in the monoculture after day 6 (Fig. 4B).

3.5. Expression of photosynthesis-associated genes of M. aeruginosa FACHB-905

The expression of photosynthesis-associated genes is shown in Fig. 5A-C. The results show that the expression of *psaB and psbB* was significantly up-regulated in the co-culture compared to the monoculture of *M. aeruginosa* FACHB-905 and that of *rbcL* was up-regulated in the co-culture on days 2, 4, 6, and 8 ($p < 0.05$). This suggests strain YF1 increased both the light and dark reaction activities of *M. aeruginosa* FACHB-905. The elevated expression of key genes of photosystems, coupled with the higher Chl*a* content from day 0 to day 6 (as shown in Fig. 1B), resulted in a higher photosynthetic activity of *M. aeruginosa* FACHB-905, and provided an increased energy supply for cyanobacterial growth. In addition, the up-regulation of *rbcL* indicates a strengthened carbon fixation capacity and can provide more carbohydrate for cyanobacterial growth. On days 8 and 10, despite the higher gene expression in the co-culture than in the monoculture of *M. aeruginosa* FACHB-905, there was no significant difference in Chl*a* content between the two groups (Fig. 1B), which means there was no more captured light energy for cyanobacterial growth, thus limiting the growth enhancement in the co-culture.

3.6. Expression of a critical gene involved in MC-LR synthesis

The expression of a critical MC-LR synthesis gene *mcyB* was analyzed. The expression of *mcyB* in the presence of *Sphingopyxis* sp. YF1 on days 2, 4, 6, 8 and 10 was 1.31, 1.48, 1.63, 1.74 and 1.75 times as high as that in the absence of *Sphingopyxis* sp. YF1 (Fig. 5D). The up-regulation of *mcyB* in the co-culture implies that *Sphingopyxis* sp. YF1 promoted the MC-LR production of *M. aeruginosa* FACHB-905. The increase in the InMC-LR concentration in the co-culture (Fig. 2A) can be attributed to the dual effects of *Sphingopyxis* sp. YF1, i.e., it not only stimulated the growth of *M. aeruginosa* FACHB-905 (days 2–6) (Fig. 1A) but also enhanced MC-LR synthesis of individual cells of *M. aeruginosa* FACHB-905, as indicated by the elevated expression of *mcyB*.

3.7. Expression of critical genes involved in MC-LR bio-degradation by Sphingopyxis sp. YF1

The expression of *mlrA, mlrB, mlrC,* and *pAAase* was analyzed. The expression of all four genes was significantly higher in the co-culture than in the monoculture of *Sphingopyxis* sp. YF1 ($p < 0.05$) (Fig. 6). The expression of *mlrA, mlrB*, and *mlrC* in the co-culture was at least 12.52 times that in the YF1 monoculture (Fig. 6A-C), consistent with the lower ExMC-LR level in the co-culture (Fig. 2C). PAAase mediates the reactions during the late phase of MC-LR degradation (Wei et al. 2023), which may account for the relatively low expression on day 2 and day 4 and the higher expression level after day 4 in the co-culture (Fig. 6D).

4. Discussion

Epiphytic bacteria can have either positive or antagonistic or no obvious effects on cyanobacterial growth. Nonetheless, to the best of our knowledge, little is known about the influence of MCs-degrading bacteria on the growth of bloom-forming cyanobacteria and the underlying mechanisms. The present study unveiled that the *Sphingopyxis* sp. YF1 enhanced the growth of *M. aeruginosa* FACHB-905 from day 1 to day 7. On the other hand, after day 7, cyanobacterial growth was close in the presence or absence of YF1. The enhancement of cyanobacterial growth stopped after day 7 may be due to that strain YF1 could not obtain enough ExMC-LR as a main carbon source, resulting in slow bacterial growth. But the ratio of cyanobacterial cells to bacterial cells increased, which diluted the effect of strain YF1 on cyanobacterial growth. Therefore, the effect was weakened with time, and the enhancement stopped after day 8. Although the concentration of extracellular MC-LR increased after day 8, there may be a lag effect in the response of

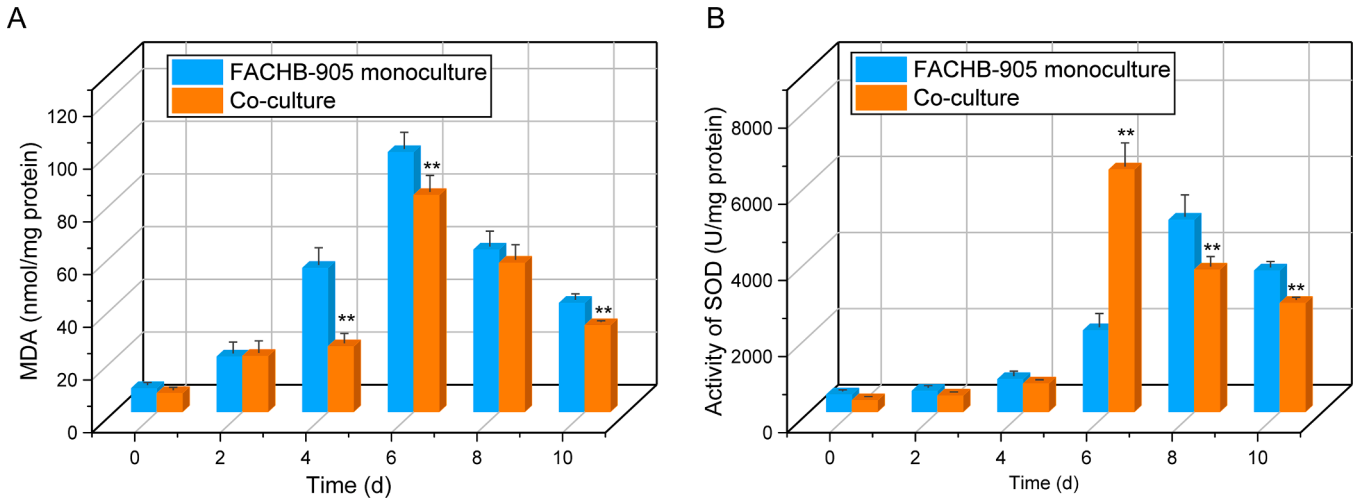

Fig. 4. Variations of MDA content (A) and SOD activity (B) of *M. aeruginosa* FACHB-905 in the cyanobacterial monoculture and the co-culture. *, $p < 0.05$, **, $p < 0.01$.

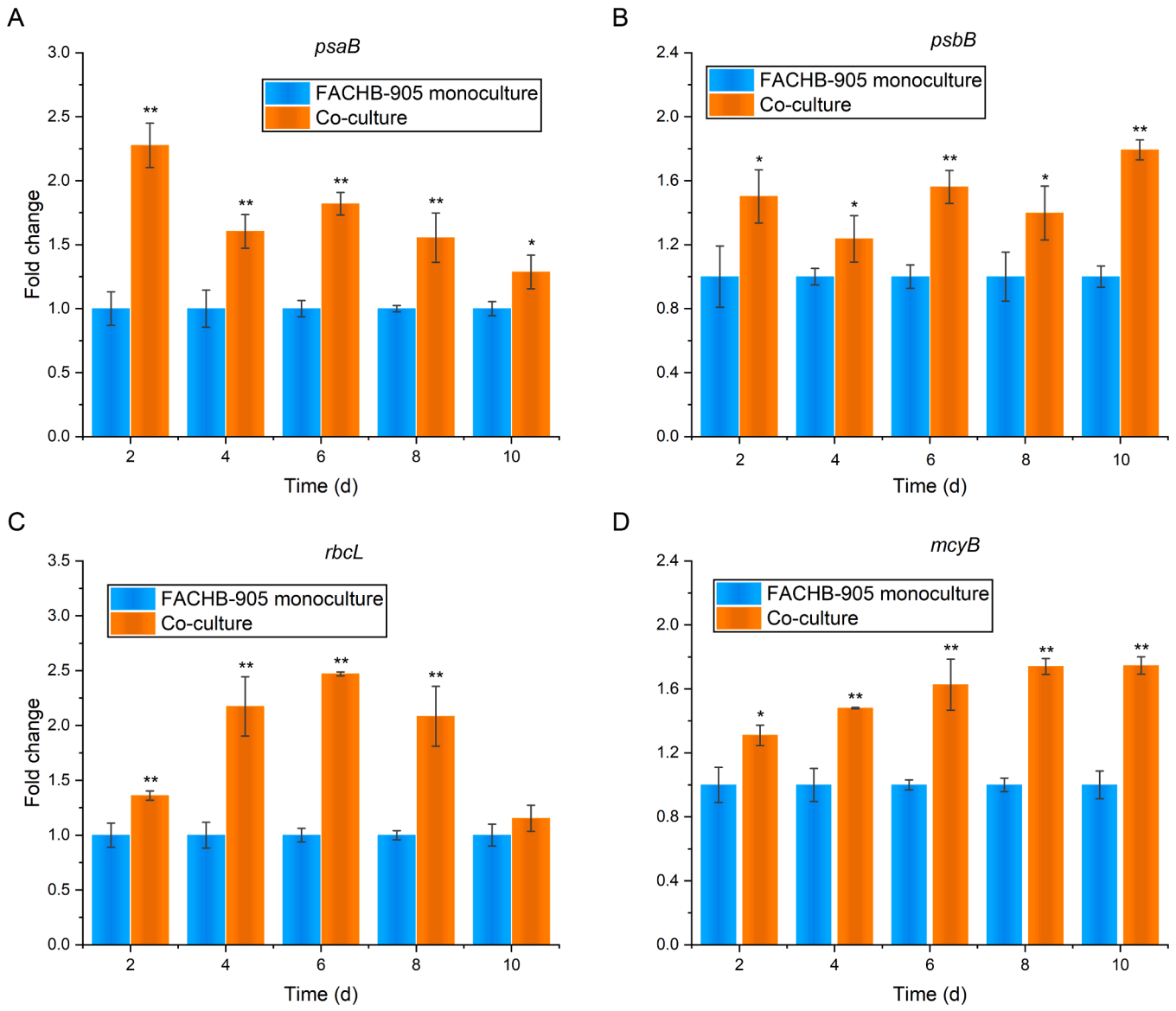

Fig. 5. Expression of photosynthesis-related genes *psaB* (A), *psbB* (B) and *rbcL* (C) and the MC-LR synthesis gene *mcyB* (D) of *M. aeruginosa* FACHB-905 in the presence or absence of *Sphingopyxis* sp. YF1. *, $p < 0.05$, **, $p < 0.01$.

bacterial cells to the change in ExMC-LR concentration, as reported in a previous study (Gao et al. 2022). As a result, bacterial density did not increase at that time. Previous research by Berg et al. (2009) demonstrated that most of the bacterial strains isolated from various aquatic environments enhanced the cyanobacterial growth in one or two weeks, although subsequent cyanobacterial growth was not reported. Kim et al. (2019) revealed that *Rhizobium* sp. MK23 promoted the growth of *M. aeruginosa* PCC7806 from the logarithmic phase to the decline phase, which differs from our findings. Pannard et al. (2016) found that the presence of heterotrophic bacteria did not significantly affect cyanobacterial growth. These discrepancies may be attributed to the differences in the tested cyanobacteria (e.g., toxic or non-toxic) or bacteria (MCs-degrading or non-MCs-degrading). It can be inferred from these studies that different microbes play roles at different stages of cyanobacterial growth and may therefore have varying effects on the occurrence and development of HCBs.

Furthermore, it was found that the MDA content of strain FACHB-905 decreased on days 4, 6, and 10 in the presence of strain YF1, implying the role of strain YF1 in reducing ROS in cells of strain FACHB-905. Excessive ROS are detrimental to cyanobacterial growth due to lipid peroxidation, protein oxidation, damage of nucleic acids and the destruction of cellular components such as phycobilisomes (Liu et al. 2005; Rezayian et al. 2019). Therefore, the alleviation of oxidative stress by strain YF1 may account for the promoted growth of *M. aeruginosa* FACHB-905 in the co-culture. The MDA content was close in the FACHB-905 monoculture and the co-culture on day 2, possibly due to the ROS level being relatively low, which may be not sufficient to induce a response from the cyanobacterial and bacterial antioxidant systems.

Previous studies have shown that H_2O_2-resistant bacteria can provide catalase (CAT) to benefit the growth and photosynthesis of the H_2O_2-sensitive *M. aeruginosa* under H_2O_2 stress or high light (80 μmol m^{-2} s^{-1}) (Kim et al. 2021; Kim et al. 2019). *Sphingopyxis* sp. YF1 has two copies of CAT-coding genes (https://www.ncbi.nlm.nih.gov/datasets/genome/GCF_022701295.1/), while strain FACHB-905 does not have any (https://www.ncbi.nlm.nih.gov/datasets/genome/GCF_000332585.1/). Therefore, strain YF1 is capable of providing CAT to protect FACHB-905 from H_2O_2 attack. H_2O_2 has been shown to inhibit the expression of numerous functional genes, including those related to photosynthesis and *mcy* genes (Kim et al. 2021). Therefore, the alleviation of oxidative stress would result in the up-regulation of these genes,

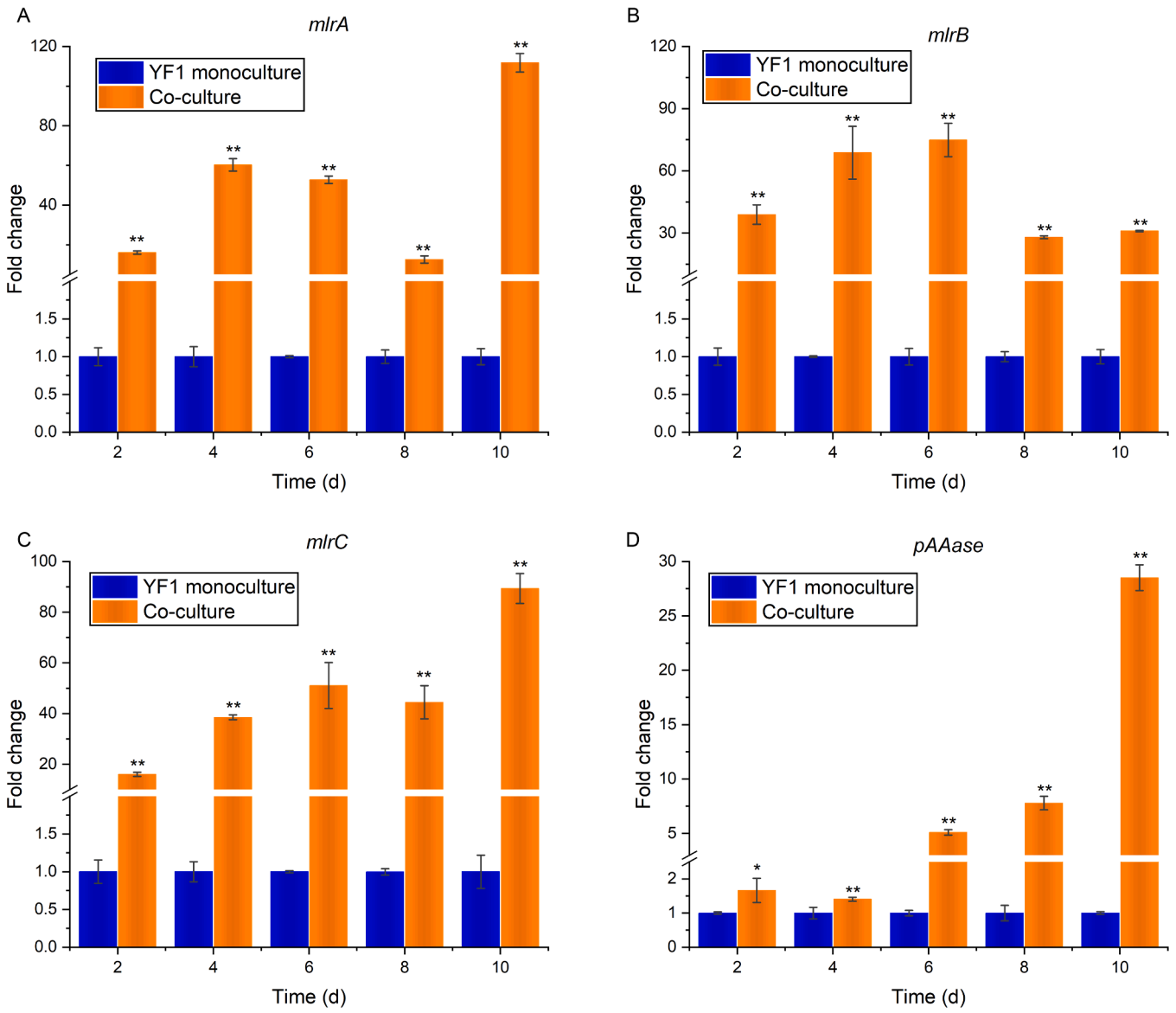

Fig. 6. Expression of MC-LR degradation-associated genes in the monoculture of *Sphingopyxis* sp. YF1 and the co-culture. A, *mlrA*, B, *mlrB*, C, *mlrC*, and D, *pAAase*. $p < 0.05$, **, $p < 0.01$.

as observed in the present study (Figs. 5 and 7). We also found a significant increase in the SOD activity in the co-culture on day 6 (Fig. 4B),

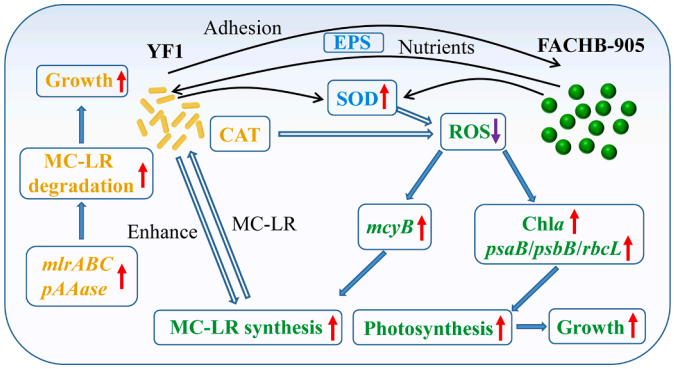

Fig. 7. Proposed bacterial-cyanobacterial interaction mechanisms in this study. Green characteristics are those of FACHB-905, yellow ones belong to YF1, and the blue ones are produced by both.

possibly due to the rapid increase in MDA content after day 4 (Fig. 4A) necessitating the synthesis of more SOD. Therefore, it is speculated that SOD also plays a role in reducing ROS (Fig. 7). The SOD activity in the co-culture was lower than that in the monoculture after day 6, which may be attributed to the involvement of other ROS-scavenging enzymes, such as peroxidases and glutathione, which can act as major antioxidants during these stages (Kim et al. 2021; Rezayian et al. 2019).

In turn, cyanobacteria can influence the growth of associated bacteria. Some cyanobacteria have been shown to negatively affect bacterial growth. Zhang et al. (2019) found that the growth of *M. aeruginosa* increased while the growth of some bacterial species was suppressed in a co-culture system. It was speculated that the cyanotoxins, including MCs, can inhibit the energy metabolism of these associated microorganisms. In the present study, *M. aeruginosa* FACHB-905 significantly promoted the growth of strain YF1 (Fig. 1C). Unlike those species, due to the strong MC-LR metabolic capability, MC-LR acts as carbon and nitrogen sources rather than a metabolism inhibitor for strain YF1. Meanwhile, strain YF1 may also use cyanobacterial exudates such as EPS as its nutrients such as N and P (refer to the discussion on EPS below). Previous research has shown that the exudates from 12 cyanobacterial

species can support the growth of *Pseudomonas* sp. P1, *Ancylobacter aquaticus* CN13, and *Ralstonia eutropha* JMP134, and the maximum cell densities of strain CN13 and JMP134 were positively correlated with the content of total organic carbon of cyanobacterial exudates (Kirkwood et al. 2006).

The production of MCs in the presence of epiphytic bacteria has been reported previously. Briand et al. (2016) reported that the bacterial community isolated from the mucilage of *M. aeruginosa* 7806 did not significantly influence the intracellular MC-LR and desmethyl MC-LR (Des-MC-LR) concentrations (normalized by the dry weight). To date, the underlying mechanism by which MC-degrading bacteria affect MC production remains poorly understood. MCs have various functions such as the modulation of proteins, involvement in photosynthesis, and acting as a self-protective mechanism (Dziallas and Grossart 2012; Wang et al. 2018). In the present study, we observed that the production of MC-LR by *M. aeruginosa* FACHB-905 in the presence of *Sphingopyxis* sp. YF1 was promoted, especially on days 8 and 10 (Fig. 2B). This might be attributed to the degradation of MC-LR by strain YF1, which stimulated *M. aeruginosa* FACHB-905 to increase MC-LR production to maintain an adequate level of MC-LR for its functions.

As a MC-LR degrader, strain YF1 significantly decreased ExMC-LR concentration, especially on day 10 (Fig. 2C). On day 10, more cells lysed and released MC-LR, resulting in much higher ExMC-LR concentrations (which is inferred from the rapid increase in ExMC-LR concentration in the monoculture). The InMC-LR concentration in the co-culture is ~ 2 folds that in the monoculture, it can be inferred that the actual MC-LR concentration released by strain FACHB-905 in the co-culture may be significantly higher than that the ExMC-LR concentration in the FACHB-905 monoculture (4.58 μg/L). Therefore, strain YF1 played a significant role in the degradation of MC-LR. However, because MC-LR has not been completely released, the role of strain YF1 in degrading MC-LR has not been fully realized. This study reveals two sides of MCs-degrading bacteria in the dynamic of MCs. It is the first study that suggests the dual roles of a MCs-degrading bacterium in promoting cyanotoxins synthesis and degrading cyanotoxins (Fig. 7).

The present study showed that cells of YF1 and FACHB-905 were in close contact (Fig. 3), which is conducive to their interactions. Moreover, considerable amounts of exudates were produced (Fig. 3). The elements of the exudates suggest the presence of extracellular polymeric substances (EPS), which are complex mixtures of biomolecules surrounding microbial cells (Le et al. 2022; Peng et al. 2022; Seviour et al. 2019). EPS may facilitate the attachment of bacterial cells to cyanobacterial cells. It has been found that EPS ingredients such as polysaccharides can contribute the cell adhesion (Shi et al. 2022). EPS are rich in nutrients, and one of the functions of EPS is to store and transport nutrients (e.g., N and P) between different species (Liu et al. 2018; Tang et al. 2021). Previous research has shown that EPS from *Microcystis* spp. can be degraded by the bacterial community obtained from the bloom zone (Li et al. 2009). It is inferred that strain YF1 may use EPS as its carbon source. The findings in this study suggest that EPS may play a role in the interactions of *Sphingopyxis* sp. YF1 and *M. aeruginosa* FACHB-905 (Fig. 7).

The frequent outbreaks of HCBs and the resulting harms have become a global environmental and public health issue (Li et al. 2016). Various factors, including climate change and eutrophication, influence the occurrence of HCBs (Liu et al. 2020; Smucker et al. 2021). However, the biological factors that affect this process are often overlooked. Although it is recognized that associated bacteria play an essential role in the formation of HCBs (Pound et al. 2021), the underlying mechanisms remain unclear. As a special bacterial group, the cyanotoxin-degrading bacteria exhibit a relatively high abundance in the cyanobacteria-related microbial community (Gao et al. 2022; Shao et al. 2014), highlighting their importance in the cyanobacterial phycosphere. Our study reveals the complexity of interactions between cyanotoxin-degrading bacteria and bloom-forming cyanobacteria. Firstly, the results show that a MCs-degrading bacterium promotes the growth of a toxic cyanobacterium specifically during the early and middle stages, which suggests cyanotoxin-degrading bacteria may drive the initial or mid-term development of HCBs but may not significantly affect the eventual outcome of HCBs. Moreover, our findings show that *Sphingopyxis* sp. YF1 can significantly reduce the ExMC-LR concentration, suggesting an important role of bacteria in cyanotoxin degradation in aquatic environments. Meanwhile, the present study shows that cyanotoxin-degrading bacteria also affect the production of cyanotoxins. The present study emphasizes the importance of interactions between MCs-degrading bacteria and bloom-forming cyanobacteria in the progression of HCBs and the environmental fate of cyanotoxins. This study also has implications for the control of HCBs and cyanotoxins detoxification. Liu et al. (2020) have revealed that microcystinase A can simultaneously inhibit the growth of *Microcystis aeruginosa* and degrade MC-LR. As cyanotoxin-degrading bacteria may promote cyanobacterial growth and toxins synthesis, the use of degrading enzymes instead of bacterial cells for HCB control and cyanotoxin removal could be a preferable approach.

The present study described the interactions between a MCs-degrading bacterium and toxic *Microcystis aeruginosa.* However, in natural environment, cyanobacteria can occur in the unicellular or colonial forms, and bacteria always exist as communities rather than single species. Moreover, bacteria can occur as free-living communities or they can attach to cyanobacterial cells. These factors make the interactions between bacteria and cyanobacteria in the natural environment much more complex. This study also did not explore the entire process from the lag phase to the decline phase of cyanobacterial growth. Therefore, future studies should simulate microbial communities in the real environment, focusing on the interactions between free-living and/or attached bacterial communities and cyanobacterial cells on a longer time scale.

5. Conclusion

This study investigated the interactions between a typical microcystin-degrading bacterium and a toxic bloom-forming cyanobacterium and the underlying mechanisms. The bacterium and the cyanobacterium mutually affected each other's growth. The bacterium increased the intracellular MC-LR content (cellular quota), especially on the later period, and significantly decreased the extracellular MC-LR concentration, which was consistent with the up-regulation of critical functional genes involved in MC-LR synthesis and biodegradation. The alleviation of oxidative stress may be a key mechanism to enhance cyanobacterial growth, and EPS may play a role in the bacterium-cyanobacterium interactions. These findings will further enhance our understanding of the role that biological factors play in influencing the occurrence and development of HCBs.

CRediT authorship contribution statement

Tangjian Peng: Writing – review & editing, Writing – original draft, Visualization, Supervision, Project administration. **Yanqing Tang:** Visualization, Software, Investigation, Data curation. **Danping Cai:** Visualization, Methodology, Investigation, Data curation, Conceptualization. **Yuqing Gu:** Visualization, Methodology, Investigation. **Jia Wei:** Writing – review & editing, Methodology. **Jiajia Zhang:** Methodology, Investigation, Data curation. **Juan Ni:** Methodology, Investigation. **Jun Liu:** Writing – review & editing, Supervision. **Xiaoya Ren:** Writing – review & editing, Supervision. **Jiafeng Pan:** Writing – review & editing, Supervision. **Xizi Long:** Writing – review & editing, Methodology. **Hui Wang:** Writing – review & editing, Conceptualization. **Fei Yang:** Writing – review & editing, Supervision, Funding acquisition, Conceptualization.

Declaration of competing interest

The authors declare no conflict of interest.

Data availability

Data will be made available on request.

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Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.watres.2024.122241.

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