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Development of a Potentially New Algaecide for Controlling Harmful Cyanobacteria Blooms Which is Ecologically Safe and Selective

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ABSTRACT: Harmful cyanobacterial blooms (HCBs) caused by *Microcystis aeruginosa* are of great concern as they negatively affect the aquatic environment and human health. Chemical methods could rapidly eradicate HCBs and have been used for many decades. However, many chemical reagents are not recommended to eliminate HCBs in the long term, given the possible destructive and toxic effects of the chemicals employed on non-target aquatic organisms. We developed a new algaecide, 2-((1,3,4-thiadiazol-2-yl)thio)-*N*-(4-chlorophenyl) acetamide (Q2), to control harmful cyanobacteria while being environmentally friendly and selective. In our study, Q2 effectively inhibited cyanobacterial growth, especially of *M. aeruginosa*, but did not affect eukaryotic algae in test concentrations. A critical mechanism was revealed by transcriptome and metagenomic results showing that Q2 affects multiple cellular targets of cyanobacteria for HCB control, including the destruction of organelles, damage in the photosynthesis center, as well as inhibition of gas vesicle growth, and these changes can be highly relevant to the decrease of quorum-sensing functional KEGG pathways. Furthermore, Q2 did not affect the microbial composition and could recover the disrupted aquatic functional pathways in a short period. This is different from the impact on ecosystem functioning of the traditionally used harmful algaecide diuron. All these results verified that Q2 could be friendly to the aquatic environment, providing a new directional choice in managing HCBs in the future.

KEYWORDS: *microcystis-dominated blooms, eco-friendly algaecide, selective inhibition mechanism, transcriptional profile, metagenome*

INTRODUCTION

Cyanobacteria are known as ancient oxygen-evolving prokaryotes capable of energy collection via photosynthesis. Cyanobacteria, therefore, serve a fundamentally critical part in the supply of oxygen and carbon dioxide sequestration.[1−3] However, because of the overnutrition from the growth of the human population and urbanization, as well as from the expansion of agriculture and industry, some bloom-forming cyanobacteria multiply to reach high biomass, thus forming harmful cyanobacterial algal blooms (HCBs) and producing hazardous toxins.[4−6] This trend is being intensified by the increase in global warming.[7] A recent study predicted that the average number of days for HCBs would increase from about 7 days per water body per year now to 16−23 days in 2050 and 18−39 days in 2090. This prediction was made based on climate alteration projections from five global circulation models coupling water quantity and quality.[8] HCBs not only result in severe damage to the aquatic environment but also endanger the safety of drinking water and even cause the death of animals and humans. It is, therefore, a paramount imperative for performing all possible measures to suppress and control HCBs.

Several approaches have been applied to restrain HCBs, including (1) physical measures, such as nutrient control through supply of phosphorus-binding clays and photosynthetic activity reduction via high-frequency sonication;[9,10] (2) biological measures, such as the introduction of natural enemies (viruses, pathogenic bacteria, or fungi), and change of the whole food web by removing planktonic fish and benthivorous fish or by introducing piscivorous fish;[11−13] and (3) chemical measures, such as herbicides (e.g., diuron), hydrogen peroxide (H_2O_2), and copper-based additives (e.g., $CuSO_4$).[14−16] However, although the chemical reagents currently in use can rapidly eradicate cyanobacterial blooms, they are not recommended for long-term use since these chemicals destroy aquatic ecological systems without selectivity.[17] It is necessary and urgent to develop a chemical material that is highly selective for HCB elimination and for which no traces remain in the environment in the long term.

Q2, a member of the thioacetamide compound, was selected as a new type of algaecide to eliminate HCBs in a friendly manner.[18,19] In our study, we compared the impact of Q2 on the development of cyanobacteria and eukaryotic algae to reveal whether Q2 exerts selective inhibition on cyanobacteria. Transcriptomic and metagenomic approaches

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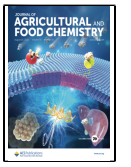

were performed to uncover the mechanisms of inhibition of cyanobacterial growth by Q2. Furthermore, to evaluate its aquatic environmental safety, the effects of Q2 on the community structures of prokaryotic and eukaryotic microorganisms were investigated through high-throughput 16S/18S rRNA sequencing. Our findings provide an unambiguous potential for ecologically controlling cyanobacterial blooms in a sustainable manner.

MATERIALS AND METHODS

Material Preparation and Microalgal Monoculture. Q2 was kindly provided by Professor L.L. Feng (Central China Normal University). Diuron and other reagents were purchased from Aladdin (Shanghai, China). The description of the development of Q2 was added in the Supporting Information (Section S1). The strains of the cyanobacteria *Microcystis aeruginosa* (FACHB-905), *Anabaena*, and *Pseudanabaena* sp. (FACHB-2209), as well as of the eukaryotic algae *Chlorella vulgaris* (FACHB-24), *Monoraphidium* sp. (FACHB-1853), and *Chlorella pyrenoidosa* (FACHB-9), were used in the experiment. All strains were purchased from the Institute of Hydrobiology at the Chinese Academy of Sciences (Wuhan, China). The strains were cultivated in a sterile BG-11 medium under the conditions as follows: 25 ± 0.5 °C under a fluorescent light of 46 μmol m^{-2} s^{-1}, with a 12 h: 12 h light/dark cycle.

Exposure Experiment of Q2 and Diuron. To determine the inhibitory selectivity of Q2 on algae, cyanobacterial algae of *M. aeruginosa*, *Anabaena*, and *Pseudanabaena* sp. and eukaryotic algae of *C. vulgaris*, *C. pyrenoidosa*, and *Monoraphidium* sp. were exposed to Q2 and diuron (set at a series of concentrations of 0.1, 0.5, 1, 2, 5, and 10 mg/L), with four replicates for each group. Nitrogen and phosphorus were added at 6 and 0.3 mg/L concentrations to supply the nutrients for microorganism growth for 2 weeks, respectively.[20] To determine the algal growth performance, a spectrophotometer was used to measure the cell density at 680 nm every 24 h. The inhibition of algal growth was calculated as described by Zhou et al. (2020).[20] To ensure uniform algal distribution, all cultures were manually agitated thrice daily during the experiment.

RNA Extraction and Transcriptional Sequencing. Since Q2 at a concentration of 0.5 mg/L exhibited a good selectively inhibitive performance on *M. aeruginosa*, this concentration of Q2 was used for transcriptomic analysis after 2 days of exposure. For RNA extraction, *M. aeruginosa* and *C. vulgaris* were collected by gentle filtration on a 0.2 μm filter. Total RNA was purified using TRIzol (Invitrogen, Carlsbad, CA, USA) and a RNeasy Mini reagent. The quantification and characterization of the extracted RNA were performed using a Nanodrop2000 Bioanalyzer and agarose electrophoresis. High-quality RNA samples (prokaryote RNA: $RNA_{\text{total biomass}} \geq 2.0$ μg, OD260/280 = 1.8∼2.2 and $RNA_{\text{concentration}} \geq 100$ ng/μL; eukaryote RNA: $RNA_{\text{total biomass}} \geq 1.0$ μg, $RNA_{\text{concentration}} \geq 35$ ng/Ml, OD260/230 ≥ 1 and OD260/280 = 1.8∼2.2) were used to construct the sequencing library. Then, the fragmentation buffer was utilized to randomly screen and break the prokaryote and eukaryote RNA into tiny fragments of approximately 200 and 300 bp, respectively. mRNA served as a template for the first-strand cDNA synthesis under the action of reverse transcriptase. When a second-stranded synthesis was performed, dUTP was substituted for dTTP in the dNTP reagent to guarantee that the base in the second strand of cDNA included A/U/C/G. The End Repair Mix was applied for making the cDNA structure of the double chain have a flat end, followed by an A base at the 3′ end, which is used to connect the *Y*-connector before PCR amplification. The UNG enzyme was applied for the digestion of the second strand of cDNA, which guarantees that only the first chain of cDNA is included in the library before Illumina HiSeq sequencing.

Transcriptome Data Analysis. High-quality clean data were obtained after removing the inferior quality reads (<Q20) and reads containing adapters or poly-N (<10%) from the raw data and then mapped to the *M. aeruginosa* and *C. vulgaris* genomes. The read maps of each gene were counted, and the transcripts per million reads of each transcript were calculated. The differential expression genes (DEGs) between samples based on $p < 0.05$ and log2 fold-change >1 were analyzed using DESeq2. Finally, GOATOOLS software was applied for the Gene Ontology (GO) enrichment analyses of DEGs.

Quantitative Real-Time PCR Validation. Quantitative real-time PCR (qRT-PCR) was used to confirm the transcriptional level of target genes revealed from transcriptome sequence data. Primers for RT-PCR were designed based on the known sequences of *M. aeruginosa* in the National Center for Biotechnology Information. As described by Gan et al., 2022,[21] we performed the qRT-PCR with an Eppendorf MasterCycler ep RealPlex thermal cycler (Wesseling-Berzdorf, Germany) using the PCR protocol.

Aquatic Microcosm Setup and Q2 Exposure. We acquired the freshwater samples used in our study from an urban river (30°18′25″N, 120°12′38″E) in July 2019. The river is located in Hangzhou, China. Water samples were filtered through eight layers of gauze to remove impurities and plant residues. In the Q2 and diuron toxicity experiments, 0.5−2 mg/L of Q2 showed good selective toxicity on cyanobacteria and the eukaryotic algae, but diuron indistinguishably inhibited both the growth of the cyanobacteria and the eukaryotic algae. We selected 0.5 mg/L as the exposure concentration of Q2 in four replicates for the microcosm experiment. Diuron was set up as the positive control at the same exposure concentration.

DNA Extraction and 16/18S rRNA Gene Sequencing for the Microcosm Experiment. We used a 0.45 μm pore diameter membrane to filter the water sample (200 mL) to obtain microorganisms upon exposure of 0, 7, and 14 days. A FastDNA SPIN kit (MP Biomedicals, OH, USA) was used to extract the obtained total nucleic acid following the manufacturer's protocol. The established primers 338F/806R and SSU0817F/1196R were used for PCR amplification of the prokaryotic 16S rRNA genes spanning the V3−V4 hypervariable region and the eukaryotic 18S rRNA genes in the V5−V7 regions, respectively. The sequence of primers is provided in Table S1. An Illumina MiSeq platform (Illumina, San Diego, USA) was used to sequence the purified amplicons.

Zebrafish Embryos and Larva Exposure to Q2 for Evaluation of Its Toxic Effects on Aquatic Species. We performed the zebrafish toxicity test according to the OECD test guidelines (OECD, 1998). Briefly, we pipetted the embryos in 24-well plates (Corning, NY, USA), along with one embryo per well containing 2 mL of 0.5 mg/L Q2, jointly with the negative control group. The exposure test was carried out in triplicate (3 microplates, 10 embryos in each group, and a total of 30 embryos per experimental condition). The incubation was performed at a constant temperature of 26 ± 1 °C with a photoperiod of 16:8 h light: dark. During the testing period, the hatching and survival rates were recorded twice daily using a stereoscopic microscope, and dead embryos were removed as soon as possible. At a 120 h exposure period (hpf), the larvae's body length and swimming behavior were measured. The effects of Q2 on zebrafish behavior were evaluated by altering the light conditions as follows: after initial acclimatization for 20 min, the distance and time of free-swimming under continuous light were recorded every 60 s during the second 20 min, followed by two rounds of 10 min light:10 min dark. Zebralab Video-Track (ViewPoint Life Sciences, France) was utilized to record and analyze the swimming behavior.

Microcosm Metagenomic Sequence and Analysis. To collect aquatic microorganisms, a water sample of approximately 200 mL was filtered through a 0.45 μm pore diameter membrane at 0, 7, and 14 days after Q2 exposure. We set three biological replicates for each treatment. A Maxwell16 DNA extraction system (Promega) and LEV DNA kit (AS1290, Promega, Madison, WI, USA) were utilized to extract the total genomic DNA. Then, the quality of the genomic DNA was determined using 1% agar gel electrophoresis. Sequence libraries were established using the NEXTFLEX Rapid DNA-Seq Kit following the manufacturer's instructions. Electrophoresis (Agilent 4200, Agilent, Santa Clara, CA) was performed to evaluate the library quality using a fluorometer (Qubit 3.0 fluorometer, Life Tech-

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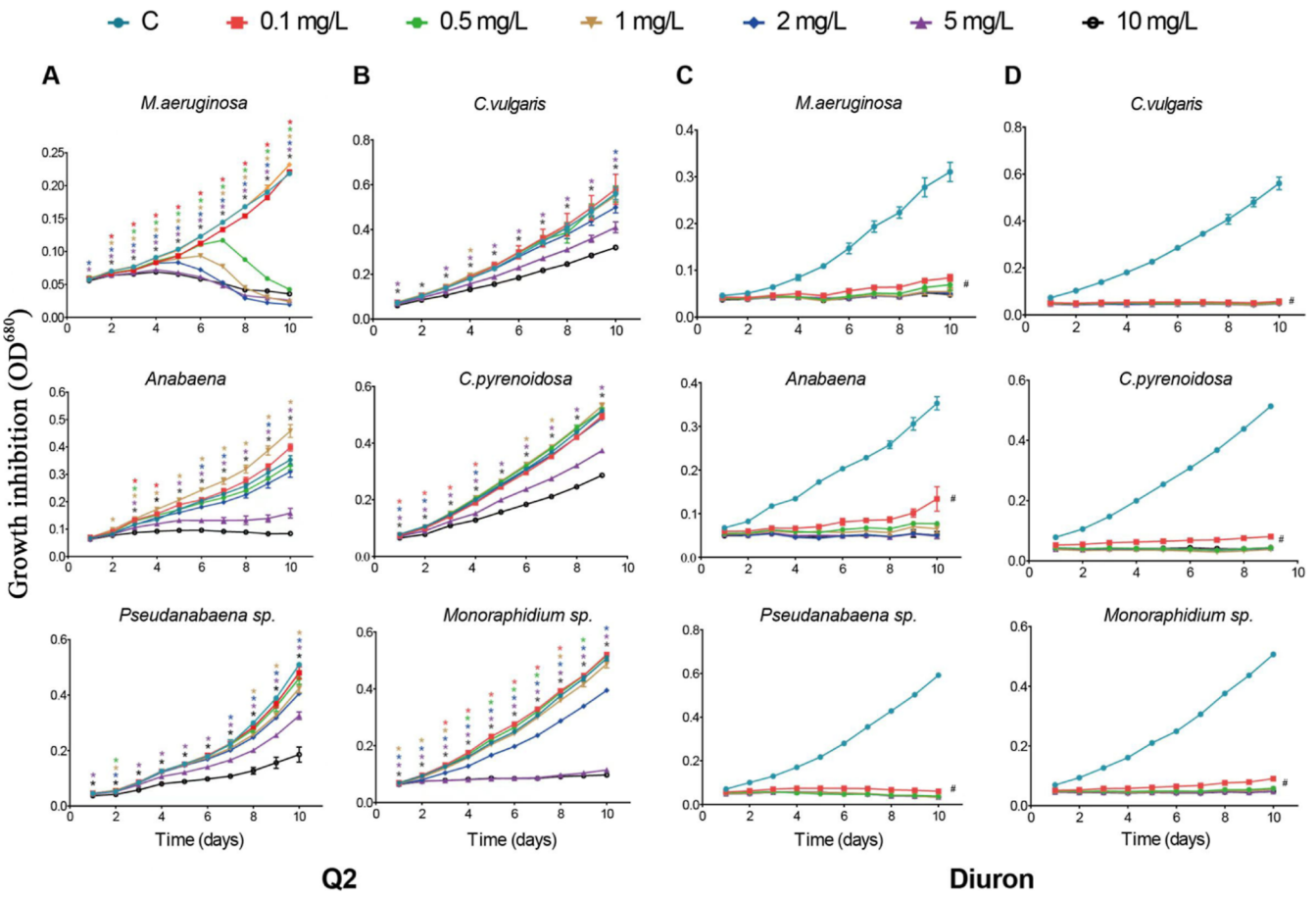

Figure 1. Effects of Q2 (A,B) and diuron (C,D) within a 0.1−10 mg/L concentration range on the growth of *M. aeruginosa*, *Anabaena*, and *Pseudanabaena* sp. as well as on the eukaryotic algae of *C. vulgaris*, *C. pyrenoidosa*, and *Monoraphidium* sp. The notable differences ($p < 0.05$) between the Q2 exposure groups and control groups were presented as "*", and the colors indicated different exposure concentrations. "#" of the diuron group indicated a significant difference ($p < 0.05$) after exposure to all concentrations of diuron at all exposure time points.

nologies, Grand Island, NY). The bridge PCR and sequencing were conducted using NovaSeq Reagent Kits/HiSeq X Reagent Kits.

Microcosm Metagenome Analysis. Raw reads containing low-quality bases, adapters, and ploy-*N* were deleted in each metagenome data set using fastp software (https://github.com/OpenGene/fastp) to acquire clean data for subsequent analysis. MEGAHIT (https://github.com/voutcn/megahit) was utilized to assemble the sequences of different sequencing depths.[22] We use MetaGene (http://metagene.cb.k.u-tokyo.ac.jp/) to predict the open reading frames (ORFs) of each assembled contig (>100 bp),[23] with default parameters. CD-HIT (http://www.bioinformatics.org/cd-hit/)[24] was applied for removing redundant ORFs and obtaining unique initial gene (Unigene) clusters (90% identity with 90% coverage). We searched Unigenes using the basic local alignment search tool (BLAST) for taxonomic and functional annotations against the Kyoto Encyclopedia of Genes and Genomes (KEGG) (http://www.kegg.jp/kegg/) databases, which defaults to using DIAMOND software.[25]

Data Analysis and Statistics. The vegan R package was utilized to qualify the diversity and richness of the aquatic microbial communities (prokaryotic and eukaryotic) at the genus level.[26] We conducted the principal coordinate analysis (PCoA), as well as permutational analysis of variance (ANOVA), for microbial community composition at the genus level based on Bray−Curtis dissimilarity. The functional potential (KO level) was performed using the vegan package to determine the discrepancy between groups. ggplot2 was used to visualize the statistical data. Mean ± standard deviation was shown for all experimental data. The statistically significant differences between the control and treatments were validated using a one-way ANOVA (Statview 5.0), followed by the Kolmogorov−Smirnov one-sample test and Levene's test. Differences were considered statistically significant in the case of $p < 0.05$.[27]

RESULTS

Inhibitory Activity and Selectivity of Q2 on the Development of Cyanobacteria and Eukaryotic Algae. There is a significant discrepancy in the growth impacts on both cyanobacteria and eukaryotic algae induced by Q2 across different concentrations, except at high concentrations of 10 mg/L, which indistinguishably suppressed the growth of both cyanobacteria and eukaryotic algae ($p < 0.05$, Figure 1A,B). Q2 at lower concentrations, including 0.5, 1, and 2 mg/L, exhibited significant inhibition on the growth of *M. aeruginosa*, *Anabaena*, and *Pseudanabaena* sp after 7 d of exposure, but they did not affect the growth of the eukaryotic algae *C. vulgaris*, *C. pyrenoidosa*, and *Monoraphidium* sp. (Figure 1A,B). Notably, Q2 showed the most significant growth inhibition on *M. aeruginosa* compared with other cyanobacteria at IC50 values of 0.5 mg/L. However, diuron exerted significant inhibition on cyanobacteria and eukaryotic algae at any concentration. Different from diuron, Q2 had a certain selectivity to suppress the growth of some cyanobacteria but not eukaryotic algae within a certain range of exposure concentrations (Figure 1C,D).

Global Transcriptional Changes of *M. aeruginosa* and *C. vulgaris* in Response to Q2 Treatment. We

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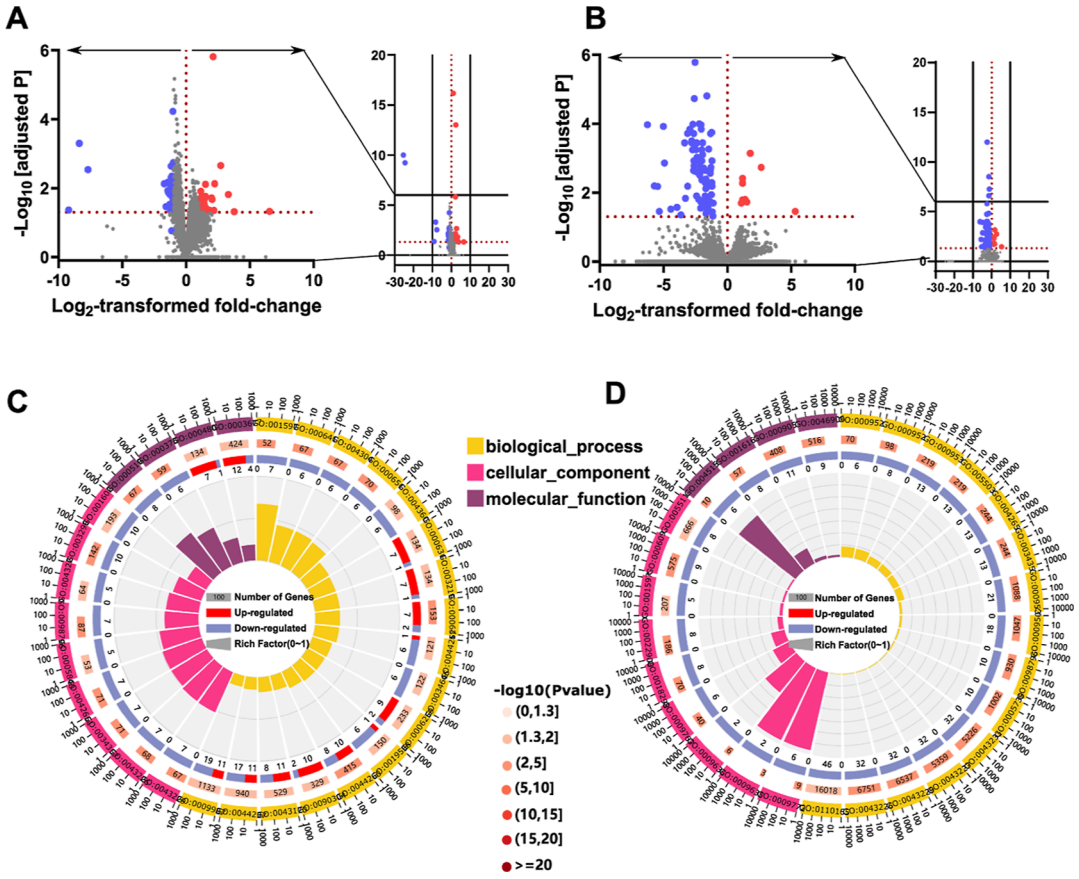

Figure 2. Transcriptome analysis of *M. aeruginosa* (A,C) and *C. vulgaris* (B,D) in response to 0.5 mg/L of Q2. Volcano plots show the enriched gene signatures of *M. aeruginosa* (A) and *C. vulgaris* (B) (two-tailed Wilcoxon rank-sum test with FDR correction), comparing Q2-treated samples and control samples. Enrichment circle diagram of GO category analysis in *M. aeruginosa* (C) and *C. vulgaris* (D). The first lap indicates the sort of GO enrichment; outside the circle are the coordinates of the number of genes. Here, both the whole data set and a magnified version are shown. Different GO categories are presented as different colors; the second lap indicates the number and *P* value for the category of the background gene; the third lap indicates the radio of up-and down-regulated foreground genes and numbers; the down-regulated gene ratio is indicated by light purple, and the up-regulated gene ratio is indicated by a red color; the fourth lap indicates the enrichment factor of each GO term.

investigated the global transcriptional effects of Q2 on *M. aeruginosa* and *C. vulgaris* to reveal the mechanism by which Q2 inhibited the cyanobacteria. Using RNA-seq data, a total of 125 differentially expressed genes (DEGs) were identified in *M. aeruginosa* and 97 DEGs in *C. vulgaris* relative to the control, according to the cutoff of the adjusted *p*-value (based on Benjamini-Hochberg correction) ≤ 0.05 and fold-change ≥ 2 (Figure 2A,B). In the *M. aeruginosa* group, we identified 65 up-regulated genes and 60 down-regulated genes after Q2 treatment. In the *C. vulgaris* group, 88 down-regulated genes and 9 up-regulated genes were identified (Figure 2B). To assign functional information, GO enrichment pathway analyses were performed to identify enriched biological processes from the coral DEGs and the algal transcriptome. The DEGs in the *M. aeruginosa* group were involved in 27 significant up-regulated and 64 down-regulated pathways. These pathways were distributed over three GO categories: molecular functions, biological processes, and cellular components. However, all DEGs in *C. vulgaris* were down-regulated pathways distributed over molecular functions and cellular components (Figure 2C,D).

To further reveal the inhibition targets of Q2 on *M. aeruginosa*, the top 20 KO items with the most significant differential (up- or down-regulated) pathways were analyzed (Figure 3A−C). Most elevated genes were involved in DNA repair pathways, including DNA metabolic process, DNA binding, DNA reconstruction, DNA-mediated, and nucleic acid metabolic pathways (Figure 3A). Among the down-regulated GO items, the transcript abundance of DEGs ($p < 0.05$) involved in the photosynthesis pathway (thylakoid membrane, photosystem I/II reaction center, and photosynthetic membrane) decreased significantly after exposure of Q2. Q2 significantly decreased the core protein of both PS-I and PS-II to half of the control, including *psa*K, *psa*A, *psa*B, *psa*E, *psb*K, *psb*I, *psb*N, and *psb*D. Three ribosome synthesis genes (*rpl*F, *rpl*Q, and *rpm*B) were reduced by approximately 0.4-fold of the control after Q2 treatment. Notably, the pathway of the gas vesicle (GV) shell had the highest enrichment factor, where the first genes of the two contiguous operons (*gvp*A1 and *gvr*A2) in the GV gene locus transcription exerted a significant reduction after Q2 exposure (Figure 3B,C). These alterations in the transcriptional level of the above genes were consistent with the relative transcriptional abundance performed by qRT-PCR (Figure S2). Besides, all genes related to microcystin (MC) synthesis were not changed after exposure to Q2 (Figure S3).

Changes in the Prokaryotic and Eukaryotic Microorganism Community Structure and Diversity after Q2

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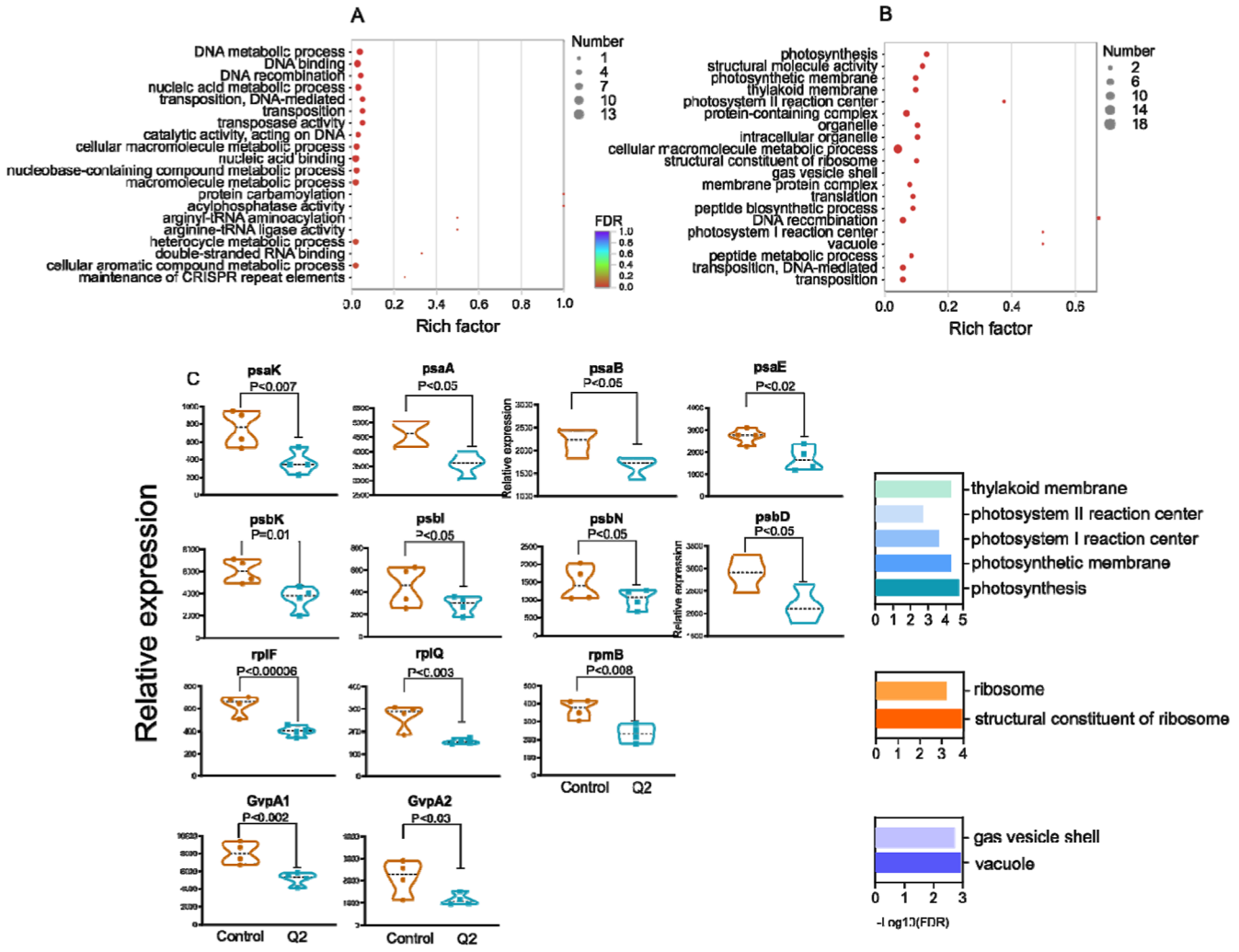

Figure 3. Bubble chart showing the significant alterations [(A): up-regulation and (B) down-regulation] of the GO pathway in *M. aeruginosa*, comparing Q2-treated and control samples. (C) Relative expression levels of down-regulated genes related to photosynthesis, organelles (especially ribosomes), thylakoid membranes, and GVs.

and Diuron Treatment. We utilized a laboratory aquatic microcosm to evaluate the impact of Q2 and diuron on the prokaryotic and eukaryotic community structure and diversity through high-throughput sequencing of 16S/18S rRNA genes. Q2, as well as diuron, exerted no impact on the alpha- (Shannon, ACE, and Chao indices) diversity of the aquatic prokaryotic and eukaryotic communities ($p > 0.05$, two-tailed Welch's *t*-test) after treatment with Q2 for 7 and 14 days (Figures 4A and S4A). By PCoA and PERMANOVA analysis, it was found that the prokaryotic and eukaryotic communities in the diuron treatment showed a markable separation with the control group along with PC1 after 7 days and 14 days of exposure, while the control and the Q2 treatment clustered together (Figures 4B and S4A). The Venn diagram revealed that most prokaryotic and eukaryotic taxa were shared in all treatments, but a more significant number of unique prokaryotic and eukaryotic taxa were found in the diuron than in the Q2 treatment (Figures 4C and S4A). At the genus level of prokaryotes, the abundance of 77 and 53 bacteria was differentially altered after diuron treatment for 7 and 14 days, respectively. In contrast, only 21 and 13 bacteria changed significantly after Q2 exposure. Among the genera mentioned above, some potential pathogenic microbes in Q2, including *Acinetobacter*, *Zoogloea*, *Thauera*, *Dechloromonas*, and *Limnothrix*, decreased significantly or did not alter as compared with the control group. However, these bacteria increased dramatically from 0.4- to fivefold after diuron exposure. Notably, the beneficial bacteria (*Mesorhizobium*) showed a higher abundance in the Q2 treatment than in the control, but this bacterium had disappeared in the diuron group (Figure 4D).

Zebrafish Embryos and Larva Exposure to Q2 for Evaluation of Its Toxic Effects on Aquatic Species. The survival rate [0−120 h exposure period (hpf)] and hatching rate (48−96 hpf) of zebrafish embryos, as well as the body weight and swimming behavior of the larvae (120 hpf), are shown in Figure S5. No significant alteration in the hatching and survival rates relative to the control treatments was observed for Q2-exposed embryos. Q2 did not affect the development of larvae since no significant difference occurred between the control and Q2-treated groups. Besides, Q2 exhibited almost no considerable effect on zebrafish larval swimming, although it reduced swim speeds between 60 and 70 min.

Effects of Q2 on the Microbial Community Function Potential. To more deeply understand the potential

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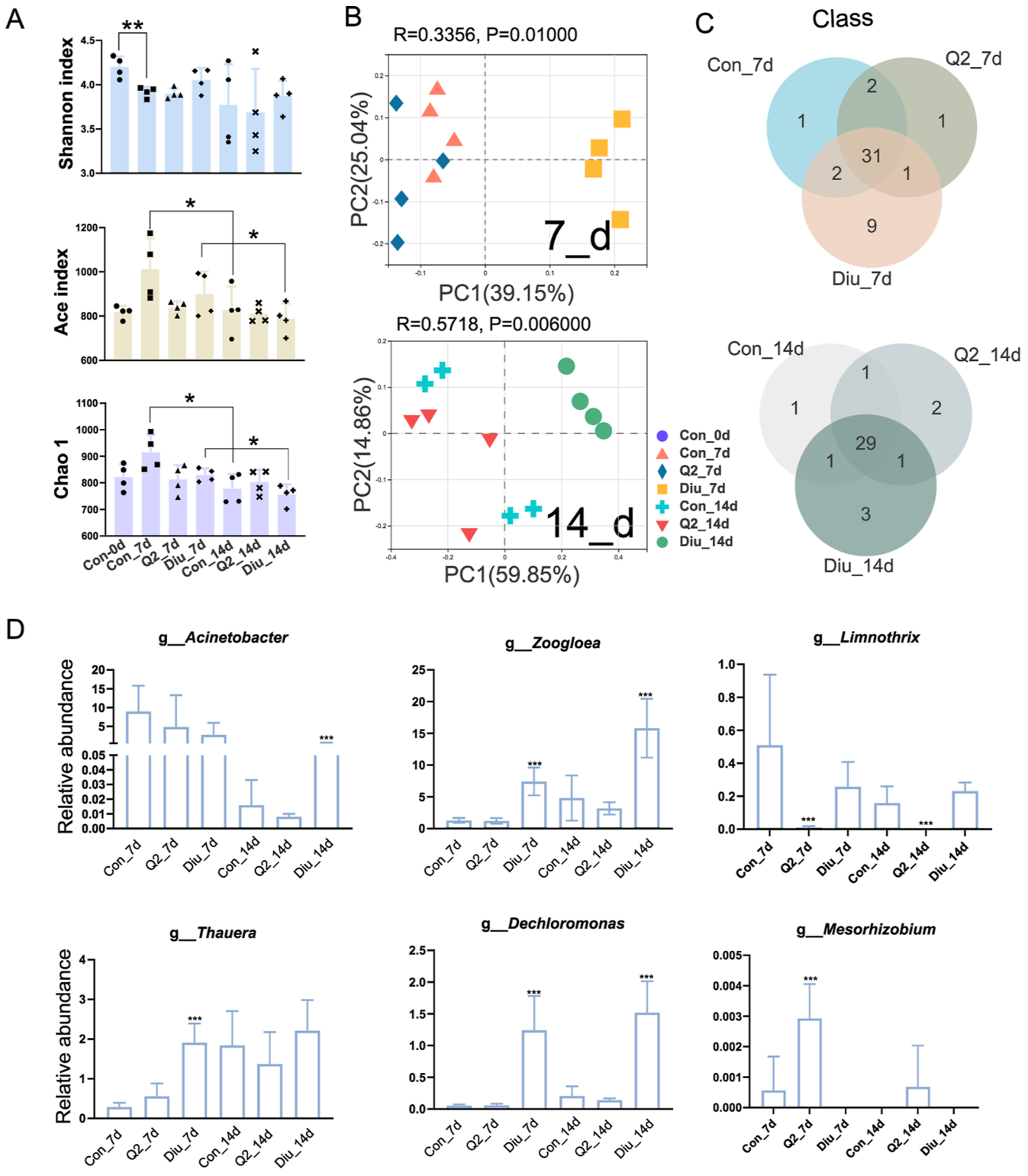

Figure 4. Overview of the impact of Q2 on the diversity and structure of prokaryotic microbial communities. (A) Diversity index (Shannon, ACE, and Chao 1). The statistically significant differences ($p < 0.05$ from a one-way ANOVA) between treatments are shown in "*". Sample profile. (B) PCoA score plots of prokaryote community profiles in different treatments after 7 days and 14 days of Q2 exposure. (C) Venn diagram of shared and unique genus numbers at the class level observed in different treatments. (D) Relative abundance of differential genera in different groups.

regulatory mechanisms and the environmental impacts of Q2 on *M. aeruginosa*, we investigated the functional attributes of the aquatic microbiome with metagenomic analyses. From the top abundance of 15 pathways, Q2 caused a significant decrease in a lower abundance of genes significantly involved in carbohydrate metabolism, amino acid metabolism, global and overview map, xenobiotic biodegradation, and metabolism (Figure 5A). However, the pathways mentioned above did not

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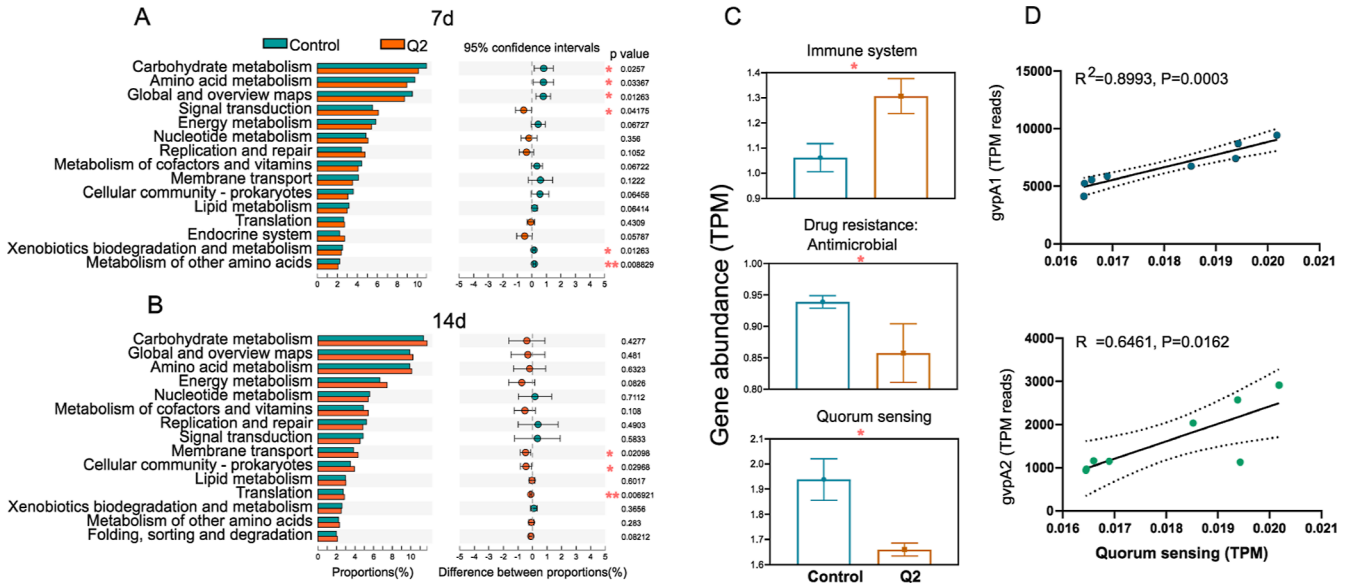

Figure 5. KEGG metabolic pathways of two levels of *M. aeruginosa* in Q2 and the control group after 7 (A) and 14 days (B) of exposure. (C) Gene abundance of some pathways (immune system, drug resistance: antimicrobial, quorum sensing) after exposure of 7 days. (D) Ordinary least squares linear regression between the functional abundance of quorum sensing and the transcriptional abundance of genes related to GVs. The notable differences ($p < 0.05$) between Q2 and the control group are presented by "*".

change after exposure to Q2 for 14 days (Figure 5B). The pathway of the immune system was significantly increased to 1.23-folds of the control, while the antimicrobial pathway for drug resistance was markedly reduced to 0.9-folds of the control after 7 days of exposure to Q2 (Figure 5C). Furthermore, the quorum sensing pathway was significantly down-regulated by 14.3% of the control in the Q2 groups (Figure 5C), which also positively correlated with the transcription gene cluster related to the GV (Figure 5D).

DISCUSSION

Over the past few decades, freshwater cyanobacterial blooms have constituted an ever-increasing challenge to the global aquatic environment because they pose a risk to public health and aquatic ecological systems.[28,29] Currently, *M. aeruginosa* seem to be the most deleterious algae present in eutrophic freshwater, secreting toxic MC to endanger the aquatic environment.[30,31] For example, *M. aeruginosa* dominated the phytoplankton community and caused a recent drinking water crisis.[32] Therefore, much attention has been paid to developing new algaecides with low toxicity, high selectivity, and eco-friendly performance.[33] In this study, Q2 displayed significant selective algicidal effects against *M. aeruginosa* at a low concentration (0.5 mg/L), while the commercial chemical diuron suppressed the growth of *M. aeruginosa* and *C. vulgaris* without selectivity. This indicates that Q2 has the potential of being a promising algaecide for the alleviation of *M. aeruginosa*-dependent blooms.

We utilized transcriptional and metagenomic technology to reveal the selective inhibition mechanism of Q2 to *M. aeruginosa*. Cyanobacteria harbor several traits, providing them with an obvious competitive advantage over eukaryotic phytoplanktons, guaranteeing their dominance and enabling the progress of dense cyanobacterial blooms such as harmful metabolite production,[34] GVs providing buoyancy,[35] nitrogen fixation,[36] and so on. MCs, a hepatotoxic product produced by *M. aeruginosa*, are transcribed as two polycistronic transcripts (*mcy*ABC and *mcy*DEFGHIJ). However, in our study, the transcriptional level of genes related to MC synthesis was not affected by Q2, suggesting that inhibiting the synthesis of MCs was not involved. Photosynthesis, as a critical source of energy for cyanobacteria, utilizes two large protein complexes—photosystems I (PS−I) and II (PS-II)—to convert solar energy into chemical energy in the thylakoid membranes.[37] Therefore, in our study, reductions in the expressional level of *psa*K, *psa*A, *psa*B, and *psa*E in PS-I and the expressional level of *psb*K, *psb*I, *psb*N, *and psb*D in PS-II suggest that PS-I and PS-II in algal cells were impaired by Q2. These results indicate that Q2 causes inhibition of growth of *M. aeruginosa* by blocking the electron transport chain and disturbing the photosynthesis process.[38] We also observed that the ribosome pathway with the ability to link tRNA-delivered amino acids to form proteins was decreased by Q2 exposure. This indicates that the N cycle was disturbed.[39] GV is a hollow protein structure filled with gas and can help bloom-forming cyanobacteria float upward by providing buoyancy.[35,40] The reduction of gvpA1 and gvrA transcription by Q2 treatment indicated GV deficiency in *M. aeruginosa*, which could cause the failure to form cyanobacterial eruptions.[35] Notably, quorum sensing (QS) can be a direct effector of photosynthesis, a regulator of the cell cycle, and can act as a morphogen, initiating GV morphogenesis, as evidenced by the positive regulation between QS and GV production revealed by genetic screens.[41,42] The metagenome results exhibited a significant decrease in the KEGG pathway of QS that exerted a positive linkage with the abundance of genes related to GVs. Therefore, it is likely that Q2 destroyed the QS functional pathways to affect photosynthesis and inhibit the growth of the GVs, which may be a critical molecular mechanism.

It is challenging for traditional algal killers to protect the ecological safety of water bodies while effectively controlling water blooms. Copper sulfate, for instance, negatively affects the aquatic microbiomes with the capability of degrading organic substances and other microbial ecosystem services,

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although it can rapidly control cyanobacterial blooms.[14] Diuron decreased the resilience of microbial communities and delayed the recovery of efficient microbial food webs.[43] This was similar to our findings of diuron exhibiting a greater disruption on both the prokaryotic and the eukaryotic community, lasting up to 14 days.[44,45] Q2 exerted almost no fluctuation on both the prokaryotic and the eukaryotic community (including diversity and composition) in our study. Especially, Q2 treatment significantly increased the abundance of the beneficial bacterium *Mesorhizobium*, which can produce cobalamin (vitamin B12). Cobalamin, a complex metabolite, serves as an indispensable cofactor for many branches of aquatic organisms.[46,47] Q2 totally eradicated *Limnothrix*, a toxin-producing cyanobacterium.[48] Besides, some pathogenic prokaryotes, the latent hosts for genes related to antibiotic resistance genes or heavy metal resistance, did not change after Q2 exposure while they significantly increased after diuron exposure,[48−50] which may enhance resistance genes' dissemination risk. The metagenome results revealed a significant decrease in the pathways related to drug resistance to an antimicrobial and a significant increase in the pathways of the immune system. These results indicated that Q2 has a lower ability to damage the environment than diuron. Moreover, our metagenomic results demonstrated that Q2 has a good recovery capacity of aquatic ecological functioning after 14 days of exposure compared to 7 days. Nevertheless, we found that exposure to Q2 had no impact on the growth of zebrafish through effects on the survival rate and hatching rate of the embryos as well as the body weight and swimming mobility of the larvae. These results thus confirm the environmentally friendly effects of Q2 on aquatic organisms and imply that Q2 is a promising algaecide to alleviate *M. aeruginosa* blooms.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acs.jafc.2c02489.

> Development of Q2; chemical structure of Q2; relative transcriptional abundance of genes related to photosynthesis, organelles (especially ribosomes), thylakoid membranes, and GVs in *M. aeruginosa;* relative expression levels of down-regulated genes related to MC synthesis; overview of the effects of the Q2 on microbial communities' diversity and structure eukaryote; diversity index (Shannon, ACE, and Chao 1); survival rate (A), hatching rate (B), body length (C), and swimming speed (D); sequence of established primers for the prokaryotic 16S rRNA genes and the eukaryotic 18S rRNA genes; and effects of HQ7 and Q2 on Cy-FBP/SBPase and *Microcystis aeruginosa* (FACHB-905) (PDF)

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Notes

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