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Original Research

Photosynthesis Inhibition of Pyrogallol Against the Bloom-Forming Cyanobacterium *Microcystis aeruginosa* TY001

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Abstract

Severe eutrophication and harmful cyanobacterial blooms of freshwater ecosystems has been a persistent environmental topic in recent decades. Allelochemical inhibition has received great attention in aquatic ecology and quality management. This study investigates the growth and full photosynthesis performance of pyrogallol on *Microcystis aeruginosa* TY001. The growth and pigment contents of *M. aeruginosa* were seriously inhibited by pyrogallol. The relative expression levels of the *nblA* gene were up-regulated under pyrogallol treatments. Unexpectedly, the relative transcript abundance of the *psaB* and *psbA* genes significantly increased compared with the control, but the chlorophyll fluorescence parameters of *M. aeruginosa* TY001 decreased significantly, except at 1 mg L^{-1} pyrogallol. In conclusion, the target sites of pyrogallol's toxic effect on the PSII of *M. aeruginosa* TY001 were mainly on the active reaction centers and the electron transport at the acceptor side.

Keywords: pyrogallol, allelopathic, *Microcystis aeruginosa*, gene expression, photosystem II

Introduction

Harmful algae blooms (HABs) have become a widespread concern in recent decades due to their frequent occurrence in eutrophic freshwater ecosystems [1-2]. Cyanobacterial blooms, especially of *Microcystis aeruginosa*, can cause serious water-quality problems in aquatic environments such as issues of oxygen depletion, aquaculture, drinking water supply, and recreational use [3-5]. Moreover, toxic secondary metabolites (cyanotoxins) produced by some bloom-forming cyanobacterial species are lethal to aquatic organisms and highly poisonous to people and livestock [6-7]. The Taiyuan region of the Fenhe River is a large park with a theme of "people, city, ecosystem, and culture," which promotes relaxation, vacationing, and sightseeing [8]. In August 2011, the section of Fenhe River in Taiyuan experienced unprecedented HABs dominated by *M. aeruginosa* TY001 over a large area. From then on, cyanobacterial blooms occurred every year in the river. Therefore, controlling the excessive growth of planktonic phototrophs in aquatic ecosystems has been a hot topic in HAB management.

Biological control agents have recently received considerable attention worldwide [9]. Allelochemicals have been confirmed to have obvious inhibitory effects on algae proliferation [10]. To date, at least 37 allelochemicals have been demonstrated to be highly effective on phytoplankton [11, 12]. Among them, pyrogallol (polyphenol) was confirmed to exhibit one of

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the most intensive inhibitory effects on *M. aeruginosa* [13-14]. Liu et al. [15] demonstrated that the cell density of *M. aeruginosa* only decreased by 1.6% after a two-day incubation with 4.5 mg L^{-1} pyrogallol in BG_{11} medium. Lu et al. [16] showed that the biomass of *M. aeruginosa* had a slight decrease after 10-day exposure to pyrogallol at a concentration of 1 mg L^{-1}. However, the effects of high concentrations of pyrogallol on phytoplankton – especially in the photosynthetic processes – has not been described.

Photosynthesis is one of the most important physiological process of algae cells and is sensitive to allelochemicals [17-18]. In recent years, gene expression analysis has been widely used to evaluate the impacts of allelochemicals on algae in aquatic systems [19-20]. Shao et al. [21] discovered that *M. aeruginosa* PCC7806 treated with a low concentration of pyrogallol caused a significant increase in the transcription of the *psbA* gene, which encodes the D1 protein and is the key to photosystem II (PS II) [19, 22]. But the photosynthesis of cyanobacteria could not be predicted based on only one gene. Moreover, chlorophyll fluorescence measurements have been proved to be a rapid and reliable means to exhibit changes in photosynthesis of algae under environment stress [23-24]. The effects of chlorophyll fluorescence parameters of *M. aeruginosa* treated with pyrogallol have not been adequately studied, despite the fact that these effects can reliably indicate changes in photosynthetic efficiency under environmental stress [25]. Additionally, more genes must be researched to investigate the photosynthesis inhibition effect of pyrogallol on *M. aeruginosa*.

This research was performed to explore the potential targets for toxicity from pyrogallol on the photosynthetic systems of *M. aeruginosa* TY001. We probed the chlorophyll fluorescence parameters to evaluate photosynthetic performance and the photosystem II defense mechanism in *M. aeruginosa* TY001. Furthermore, two photosynthesis-related genes (*psbA* and *psaB*) and a phycobilisome-related gene (*nblA*) were selected as target markers to participate in stress responses to pyrogallol.

Materials and Methods

Algal Strain and Cultivation

M. aeruginosa TY001 was originally isolated by the authors during HAB events from the Taiyuan region of the Fenhe River, Shanxi province, China (37.87°N latitude, 112.55°E longitude). The cyanobacteria cells were inoculated into sterile flasks with MA liquid medium (pH 8.2) [26]. The Erlenmeyer flasks were cultured in a homeothermic incubator at 25±1ºC under the light at a 12:12 light/dark cycle and an intensity of 40 μM photons m^{-2} s^{-1} (cool-white fluorescent tube). The flasks were shaken manually twice daily and rearranged randomly to prevent cells adhering to the side wall and uneven growing caused by minor differences in photon irradiance.

Exposure to Pyrogallol

Pyrogallol was purchased from Tianjin Guangfu Fine Chemical Research Institute (Tianjin, China). The stock solutions of pyrogallol were compounded in water prior to use. The algal cells were cultured in 250-mL Erlenmeyer flasks containing 95 mL of sterile MA liquid medium at an initial OD_{680} of 0.110, then 5 mL pyrogallol was added into the flasks to make the final concentrations of 0 (control), 1, 5, 10, 15, and 20 mg L^{-1}, respectively. Each treatment was performed in triplicate and cultivated as above for 24 h.

Cell Integrity Test

Cyanobacterial cell viability was assayed using fluorescein diacetate (FDA, Sigma-Aldrich, USA) and propidium iodide (PI, Sigma-Aldrich, USA). Thirty μL *M. aeruginosa* TY001 were resuspended in 50 μL fresh phosphate buffer sodium (PBS, 50 mM, pH 7.4). For all staining, PI at a final concentration of 30 μM L^{-1} and FDA (2.4 μM L^{-1}) for 30 min at 25°C and in darkness. PI can intercalate and stain DNA when the cell membrane is damaged or a cell dies, so PI was a good indicator for the dead or dying cells [27]. Cells with a damaged cell membrane can emit red fluorescence upon excitation stained by PI, whereas cells with an intact cell membrane can emit green fluorescence upon excitation when stained by FDA [28]. Microscopic images were obtained using laser confocal microscopy (Zeiss LSM 880, Germany). Cell membrane integrity was analyzed by flow cytometer (Accuri C6; BD Accuri Cytometers, Ann Arbor, MI, USA). The detailed procedures were described in Xiao et al. [29]. Data were expressed as the percentage of PI-stained cells.

Determination of Cell Density and Chlorophyll a

Cell density and OD_{680} are highly correlated [30], and a strong linear correlation (R^2>0.99) between optical density (OD_{680}) and algal cell density has been confirmed [31]. Thus, the cell density of *M. aeruginosa* TY001 was determined at OD_{680} using a spectrophotometer (UV1800, Shimadzu, Japan).

The chlorophyll a contents of *M. aeruginosa* TY001 were determined according to the ethanol extracting method [32]. In brief, 5 mL of homogenized algal cells were centrifuged at 4,500 rpm for 10 min, and the supernatant was discarded. The cell pellets were then washed with phosphate buffer sodium (PBS, 50 mM, pH 7.4). The pigment was extracted from the suspended solid residue using 5 mL ethanol (95%) at 4ºC without light for 24 h. The chlorophyll a contents were then determined at 665-nm and 649-nm wavelengths using a spectrophotometer (UV1800, Shimadzu, Japan) with a blank of ethanol (95%). The chlorophyll a content was calculated as follows [33]: $C_{Chl\text{-}a}$ (mg L^{-1}) = (13.95 × OD_{665}) – (6.88 × OD_{649}).

RNA Extraction, Reverse Transcription, and Quantitative Real-Time PCR

After exposure to pyrogallol, the algal cells were harvested by centrifugation at 8,000 rpm for 5 min. Pelleted cells were resuspended in RNAiso Plus reagent, which were quickly frozen in liquid nitrogen for 1 h. Total RNA was extracted with RNAiso Plus (TaKaRa, China) according to the reagent manual and dissolved in RNase-free water. RNA was quantified by a NanoDrop 2000 UV-Vis Spectrophotometer (Thermo, Waltham, MA, USA). The total RNA was transcribed reversely into cDNA using the PrimeScript RT reagent Kit with gDNA Eraser (TaKaRa, China), following the manufacturer's protocol. To measure the expression levels of mRNA, qRT-PCR was performed with 10 μL SYBR Premix Ex Taq (TaKaRa, China), 0.8 μL (10 μM) forward and reverse primer, 2 μL of 20-fold diluted cDNA, and 6.4 μL distilled water using the Applied Biosystems 7300 Real-time PCR System (Applied Biosystems, USA). The cycle parameters consisted of one cycle at 95°C for 3 min and then 40 cycles of 95°C for 15 s and 60°C for 40 s. The relevant primers used in the study and three target genes are presented in Table 1. The *16S rRNA* was used as a normalized gene. Transcript levels of the target genes from the qRT-PCR were analyzed by comparing the cycle threshold value (Ct) to the reference gene [34]. This formula was determined as follows: $\Delta\Delta Ct = (Ct_{\text{target gene}} - Ct_{16S\ rrn})_{\text{stress}} - (Ct_{\text{target gene}} - Ct_{16S\ rrn})_{\text{control}}$. Three replicates were used for all experiments, and each experiment included two technical replicates. Amplification specificity was tested using the dissociation curve for all samples.

Determination of Polyphasic Fast Fluorescence Induction and JIP-Test Analysis

To determine polyphasic fast fluorescence induction, chlorophyll fluorescence was measured with a double-modulation fluorometer FL3500 (PSI, Inc., Brno, Czech Republic). Thylakoid membranes were dark-adapted for 20 min before each test. The chlorophyll fluorescence measurements were recorded according to Wang et al. [25]. Moreover, the JIP-test was used to identify polyphasic fluorescence transient, and the terms of the O-J-I-P dynamics curves and the parameters calculated from the fluorescence transient are presented in Table S1 [35].

Table 1. Sequences of primer pairs used in real-time PCR.

Target gene	Primer sequence (5′- 3′)	Ref.
16S rrn	F: GGACGGGTGAGTAACGCGTA R: CCCATTGCGGAAAATTCCCC	[18]
psbA	F: GGTCAAGARGAAGAAACCTACAAT R: GTTG AAACCGTTGAGGTTGAA	[18]
psaB	F: CGGTGACTGGGGTGTGTATG R: ACTCGGTTTGGGGATGGA	[20]
nblA	F: TTTTCTCTGACCATCATTTGTTCG R: CAGTTCAACATTCGTTCTTTTCAG	[42]

Statistical Analysis

The data were obtained in triplicate for every treatment. Significant differences were performed using SPSS 13.0. Parametric one-way analysis of variance (ANOVA) was employed to calculate significant differences ($P<0.05$) among different treatments.

Results

Effects of Pyrogallol on Cell Viability

Damaged cell membranes can emit red fluorescence upon excitation stained by PI, whereas cells with an intact cell membrane can emits green fluorescence upon excitation stained by FDA. In Fig. 1b, the proportion of damaged *M. aeruginosa* TY001 cells was 5.6% under 5 mg L^{-1} pyrogallol stress after 24 h. But in Fig. 1c, the proportions were increased 7.8% under 15 mg L^{-1} pyrogallol stress compared with 5 mg L^{-1}. There were nearly no dead cells in the control group (Fig. 1a). As shown in Fig. 1d, all the *M. aeruginosa* TY001 cells emit green fluorescence upon excitation. But in Fig. 1e, the cells were treated with pyrogallol for 24 h and they emit green and red fluorescence upon excitation.

Effects of Pyrogallol on Cell Density, Chlorophyll a

The effects of pyrogallol on the cell density of *M. aeruginosa* TY001 are presented in Fig. 2a. Low concentrations (1 mg L^{-1}) of pyrogallol had almost no inhibitory effect during the exposure period, but a significant decrease was found in a concentration-dependent manner from 5 mg L^{-1} to 20 mg L^{-1} of pyrogallol ($p<0.05$). The chlorophyll a content exhibited a similar trend (Fig. 2b).

Effects of Pyrogallol on the Gene Expression of *M. aeruginosa* TY001

The results of the expression the of *psbA* (A), *psaB* (B), and *nblA* (C) genes in *M. aeruginosa* TY001 under pyrogallol stress after 24 h are summarized in Fig. 3. The effects of the allelochemical on the relative expression level of photosynthesis-related genes of exposure are presented in Figs 3a and 3b. The influence of pyrogallol on the expression of *psbA* and *psaB* were increased significantly ($p<0.05$), except at 1 mg L^{-1}. After 24 h of exposure, the relative transcript abundance of the *psbA* gene was 1.20, 1.47, 1.36, and 1.67 times that of the control at the 5, 10, 15, and 20 mg L^{-1} concentrations, respectively. The relative expression level of *psbA* was not significantly affected by pyrogallol at the 1 mg L^{-1} concentration, and

Table S1. Explanation of the terms of the O-J-I-P curves and the selected JIP-test parameters.

Technical fluorescence parameters and formula	Illustrations
F_o	Initial Chl fluorescence (50 μs) obtained in dark-adapted samples
F_J	Fluorescence intensity at the J-step (at 2 ms)
F_I	Fluorescence intensity at the I-step (at 30 ms)
Fm	Maximal Chl fluorescence measured under saturating illumination
$F_{300\ \mu s}$	Calculation of the initial slope (Mo) of the relative variable fluorescence kinetics (at 300 μs)
Fv = Fm - F_o	Variable fluorescence intensity
Fv/ Fm = (Fm - F_o) / Fm	Potential maximum photosynthetic capacity
V_J = ($F_{2\ ms}$ - F_o) / (Fm- F_o)	Relative variable fluorescence intensity at the J-step, reflecting the open state of reaction centers
Mo=4($F_{300\ \mu s}$- F_o) / (Fm - F_o)	Approximated initial slope of the fluorescence transient, reflecting the maximum speed of Q_A^- reduction
Quantum efficiencies or flux ratios	
$\psi_o = ET_o/ TR_o = 1 - V_J$	Probability that a trapped excition moves an electron into the electron transport chain beyond Q_A^-(at t = 0)
φ_{Eo} = [1 - (F_o/ Fm)] ψ_o	Quantum yield for electron transport (at t = 0)
φ_{Po} = TR_o/ ABS = Fv/ Fm	Maximum quantum yield for primary photochemistry (at t = 0)
φ_{Do} = 1-φ_{Po}	Quantum yield for dissipation (at t = 0)
Specific fluxes or specific activities	
ABS / RC = Mo(1 / V_J)(1/φ_{Po})	Absorption flux per reaction center
TR_o / RC = Mo(1 / V_J)	Trapped energy flux per reaction center (at t = 0)
ET_o / RC = Mo(1 / V_J)ψ_o	Electron transport flux per reaction center (at t = 0)
DI_o / RC = ABS / RC - TR_o / RC	Dissipated energy flux per reaction center (at t = 0)
$RC / ABS = (1 / Mo).\varphi_{Po}.V_J$	Density of RCs based on absorbed energy
Phenomenological energy fluxes(per excited cross section)	
ABS / $CS_o \approx F_o$	Absorption flux per cross section (CS) (at t = 0)
Density of reaction centers	
RC/ CS_o=φ_{Po}(V_J/Mo)(ABS/ CS_o)	Number of RCs per CS, reflecting density of RCs
Performance indexes	
PI_{ABS}= (RC/ABS) [φ_{Po}/(1−φ_{Po})][ψ_o/(1−ψ_o)]	Performance index on absorption basis
PI_{CS}=(RC/ CS_o) [φ_{Po}/(1−φ_{Po})][ψ_o/(1−ψ_o)]	Performance index on cross-section basis (at t = 0)

the transcript abundance was close to the control. The relative expression level of *psaB* in *M. aeruginosa* TY001 varied significantly in a manner similar to that of the *psbA* gene. The *psaB* relative transcript abundance was 6.51, 7.10, 4.36, and 19.95 times that of the control at the 5, 10, 15, and 20 mg L^{-1} concentrations, respectively. The *psaB* gene was not significantly affected (p>0.05) by 1 mg L^{-1} of pyrogallol. The relative transcript abundance of the *nblA* gene was 11.28, 9.64, 16.20, and 27.81 times that of the control at the 5, 10, 15, and 20 mg L^{-1} concentrations, respectively (Fig. 3c). The expression of *nblA* was not significantly affected (p>0.05) by 1 mg L^{-1} of pyrogallol at 24 h of exposure, as the relative transcript abundance exhibited only a slight increase compared with the control.

Effect of Pyrogallol on the Fast Fluorescence Rise Transient

The fast fluorescence kinetic induction curves of *M. aeruginosa* TY001 treated with various concentrations of pyrogallol for 24 h are presented in Fig. 4, and the fluorescence transient was concentration-dependent except at 1 mg L^{-1}. F_J and F_m dropped clearly and the J-P phase decreased as the pyrogallol concentration increased,

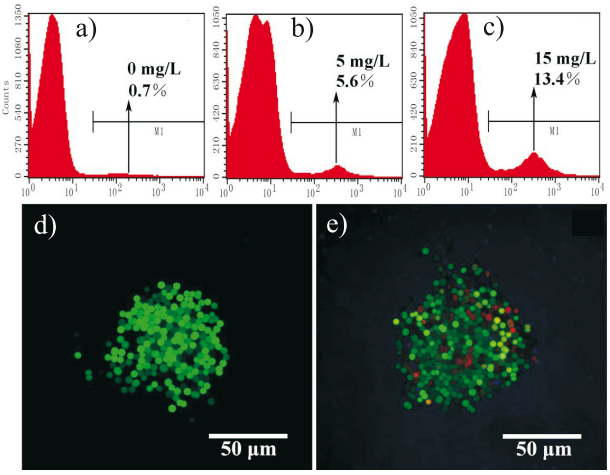

Fig. 1. Flow cytometric histogram of *M. aureginosa* TY001 stained with PI (M1: the proportion of damaged cells). The proportion of healthy and damaged cells treated with various concentrations of pyrogallol for 24 h are shown, (a) control group, (b) 5 mg L^{-1} pyrogallol-treated, and (c) 15 mg L^{-1} pyrogallol-treated. Fluorescence visualization of viability for *M. aeruginosa* TY001 treated with various concentrations of pyrogallol for 24 h, (d) control group, and (e) treatment group.

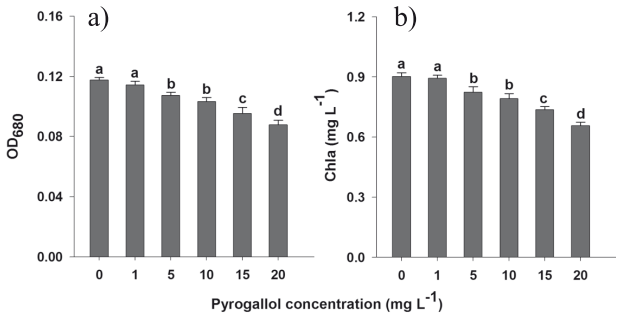

Fig. 2. Cell density (OD_{680}) (a) and chlorophyll-a (b) contents of *M. aeruginosa* TY001 treated with various concentrations of pyrogallol for 24 h. Mean values and standard deviation of three replicates are shown. Bars with different alphabet letters indicate significant difference in means among groups with different concentrations (p<0.05).

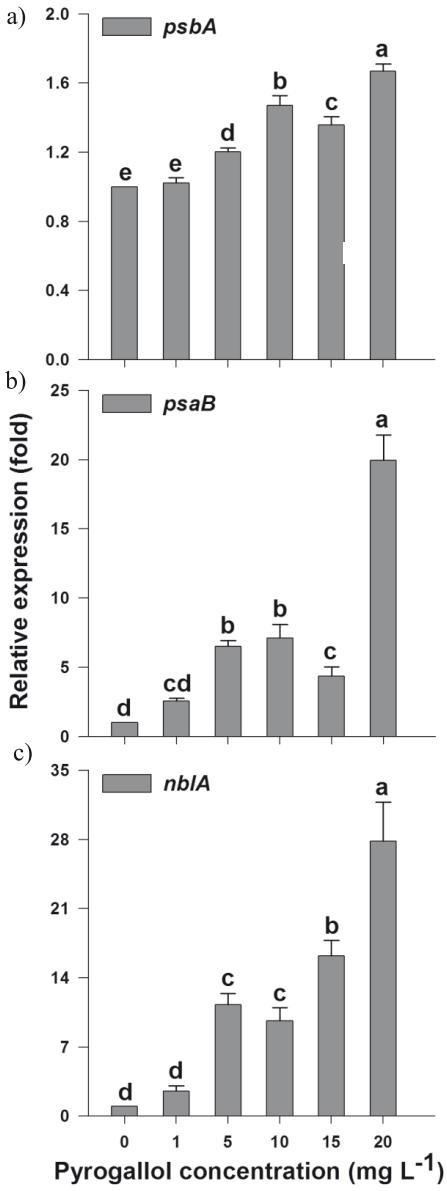

Fig. 3. Effects of pyrogallol on *psbA* (a), *psaB* (b), and *nblA* (c) genes of *M. aeruginosa* TY001. Mean values and standard deviation of three replicates are shown. Bars with different alphabet letters indicate significant difference in means among groups with different concentrations (p<0.05).

especially at the 15 and 20 mg L^{-1} concentrations. Compared with controls, the treated *M. aeruginosa* TY001 exhibited markedly lower F_o values at concentrations of 5, 10, 15, and 20 mg L^{-1}, but the treated *M. aeruginosa* TY001 exhibited a higher F_o value at concentrations of 1 mg L^{-1}.

More photochemical information from the JIP-test parameters is presented in Fig. 5. The maximum quantum yield of primary photochemistry (Fv/Fm), ψ_o, the quantum yield of electron transport (φE_o), and the number of RCs per CS (RC/CS_o), PI_{CS}, and PI_{ABS} were significantly decreased by pyrogallol at concentrations of 5, 10, 15, and 20 mg L^{-1}, but no significant change was observed following treatment with 1 mg L^{-1}. Following incubation with 5, 10, 15, and 20 mg L^{-1} pyrogallol, ABS/RC and DI_o/RC exhibited remarkable increases compared with the control, but TR_o/RC and ET_o/RC decreased drastically. Compared with the control, the M_o were significantly increased following treatment with 5, 10, 15, and 20 mg L^{-1} pyrogallol, and no significant difference was observed at concentrations of 1 mg L^{-1}.

Discussion of Results

Cyanobacterial blooms cause serious water-quality problems and are becoming a widespread concern in freshwater ecosystems. One current potential and effective

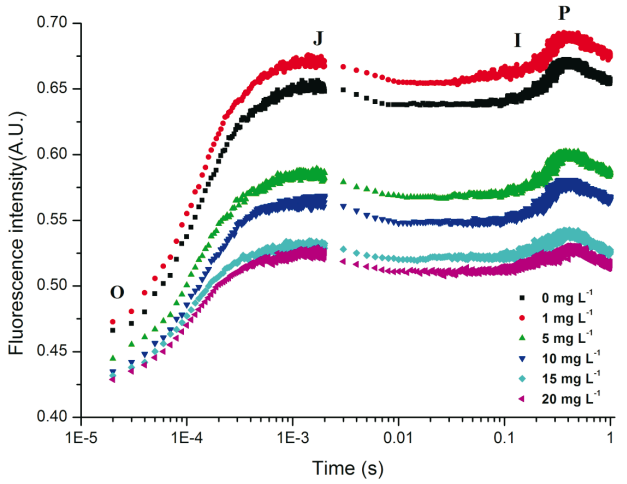

Fig. 4. The fast fluorescence transient of *M. aeruginosa* TY001 treated with various concentrations of pyrogallol for 24 h. Each value represents the mean of three replicates.

method to inhibit the growth of unpleasant algae is the use of allelochemicals released from aquatic macrophytes [36]. Allelochemicals extracted from various submerged and emergent plants have been reported to possess certain inhibitory effects on target cyanobacteria [37]. Among all chemicals, pyrogallol has been confirmed to exhibit one of the most intensive inhibitory effects on *M. aeruginosa* [13, 38]. In the present study, the growth of cyanobacteria cells exhibited a positive correlation with the test concentrations of pyrogallol after 24 h of exposure. This may be because stress exceeded the tolerance limit of *M. aeruginosa* TY001, causing cell integrity, membrane structure, and photosynthetic capacity to fail. Chlorophylls are the primary algal photosynthetic pigment and play a significant role in light harvesting, light energy transfer,

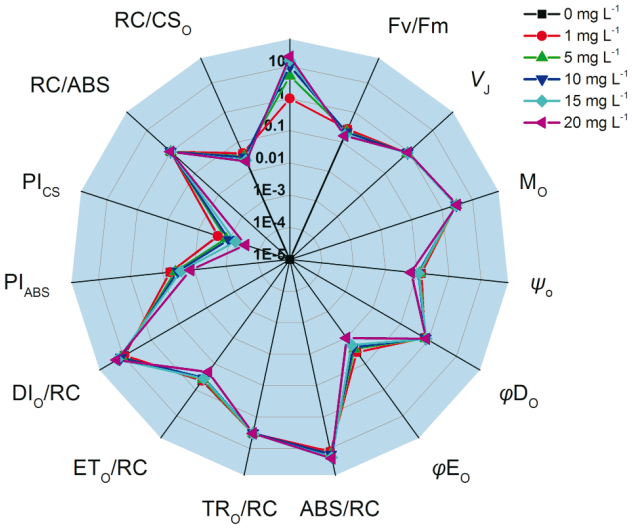

Fig. 5. Radar plot of the JIP-test parameters calculated from the fluorescence transient. Each value represents the mean of three replicates.

and conversion during the photosynthesis process [24]. The chlorophyll a content decreased markedly after exposure to pyrogallol, which may be because the stress of the allelochemical decreased the photo-oxidation capacity of *M. aeruginosa* TY001.

Photosynthesis is one of the most significant metabolic activities of cyanobacterial cells. Photosynthesis converts light energy to chemical energy by means of two large protein complexes, photosystems I (PS I) and II (PS II), located in the thylakoid membranes [39, 40]. The gene *psaB* encodes the P700 chlorophyll *a* A2 apoproteins, which is one of the essential component of PS I, whereas *psbA* encodes the D1 protein that forms the key reaction center subunits of photosystem II (PS II) [19, 22]. Shao et al. [21] reported that pyrogallol increased *psbA* gene expression in *M. aeruginosa* PCC7806. In the present study, the relative expression level of *psaB* in the PS I and *psbA* in the PS II reaction centers significantly increased compared with the control after exposure to pyrogallol for 24 h. The results indicated that the photosynthetic system of *M. aeruginosa* TY001 had already sensed the environmental stress and needed to generate enough new P700 chlorophyll A2 apoproteins and mature D1 protein to replace the damaged ones, likely explaining the reason for the significant increase of *psaB* and *psbA* gene expression. The *nblA* is a gene encoding the low-molecular-weight phycobilisome degradation protein NblA (non-bleaching A), which is involved in the proteolytic degradation of the key cyanobacteria light-harvesting complex, the phycobilisomes (PBSs), under the stress of nitrogen starvation [41]. Lu et al. [42] demonstrated that medium concentrations of allelochemical Epigallocatechin-3-gallate (EGCG) caused a significant increase in the transcription of the *nblA* gene of *M. aeruginosa* PCC7806. In the present work, the relative transcript abundance of the *nblA* gene was shown to significantly increase compared with the control, which may induce an added PBS degradation protein and further reduce the efficiency of photosynthesis. Consequently, we deduced that more degradation of PBSs may be another explanation for the photoinhibition of pyrogallol-treated *M. aeruginosa* TY001 cells.

To confirm this speculation, the chlorophyll fluorescence parameters and fluorescence transients in *M. aeruginosa* TY001 were measured. It is well known that the chlorophyll fluorescence parameters can exhibit changes in photosynthesis under environment stress, such as the presence of allelochemicals [18], antibiotics [23], and heavy metals [43]. Measurement of fluorescence transience permits the evaluation of the fluxes of photons, electrons, excitons, and energy in PSII [43]. The fast fluorescence kinetic induction curves of *M. aeruginosa* TY001 treated with various concentrations of pyrogallol for 24 h is presented in Fig. 4, and the fluorescence transient was concentration-dependent except at 1 mg L^{-1}. The results demonstrated that pyrogallol possibly causes hormesis of cyanobacteria. F_J and F_m significantly decreased, and the J-P phase decreased as the pyrogallol concentration increased, illustrating that the electron

transfer at the donor side of PSII was injured, resulting in the accumulation of $P680^+$ [44]. The decrease of *F*o with increasing pyrogallol concentration indicates that more fluorescence-quenching centers were present and that the structure of antenna pigments were changed [45-46]. To obtain more important information about the absorption, distribution, and utilization of energy in the photosynthesis of *M. aeruginosa* TY001 after exposure to pyrogallol for 24 h, the JIP-test of the chlorophyll fluorescence transience was performed. In this work, the significant decreases in the φEo and Ψo indicate that the electron transport chain was more vulnerable to the stress of pyrogallol. Moreover, the increase of V_J suggested that the proportion of closed PSII RCs and reduced QA at J step were enhanced. In this study, the ABS/RC was increased significantly, which caused drastic increases in the DIo/RC and φDo. These findings suggest that the number of light-harvesting complexes (LHCs) per RC or the inactivation of some RCs may have been increased [47]. Furthermore, the density of RC/CSo was clearly decreased, which further suggests that inactivation of the reaction centers (RCs) resulted in the increase of ABS/RC, indicating that pyrogallol damaged the RCs in *M. aeruginosa* TY001. Although pyrogallol induced an increase in the φDo, non-photochemical de-excitation of excited energy can produce ROS [18]. This finding illustrates that the oxidative stress of *M. aeruginosa* TY001 was induced not only from the photo-oxidation of pyrogallol but also from the non-photochemical de-excitation of excited energy in the photosystem itself.

These results, together with data on the chlorophyll fluorescence parameters and fluorescence transients, indicate that membrane disruption leads to photoinhibition and eventually results in the increasing proportion of dead cells as treated with higher concentrations of pyrogallol.

Conclusions

Our results indicate that pyrogallol, like other allelochemicals, has a significant impact on photosynthesis in *M. aeruginosa* TY001. First, the expression of the *nblA* gene was up-regulated, which may induce an added PBS degradation protein and further reduce the efficiency of photosynthesis. Second, the chlorophyll fluorescence parameters (*F*v/*F*m, PI_{ABS}, PI_{CS}) of *M. aeruginosa* TY001 decreased significantly, except at 1 mg L^{-1} pyrogallol, which indicated that the full photosynthesis performance of cyanobacteria was markedly inhibited. These data suggest that photosynthesis inhibition may be an important mechanism by which pyrogallol acts on *M. aeruginosa* TY001. In summary, the high concentration of pyrogallol inhibited *M. aeruginosa* TY001 to the zero or negative growth state after 24 h of exposure. Further investigation is warranted to determine more inhibitory mechanisms of algae in greater detail to mitigate or eliminate harmful algal blooms in the Taiyuan region of the Fenhe River.

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