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Effect of Pyrogallol on the Growth and Pigment Content of Cyanobacteria-Blooming Toxic and Nontoxic *Microcystis Aeruginosa*

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M. aeruginosa is a commonly occurring cyanobacterium and has been responsible for some of the most serious pernicious algal blooms in China (Song et al., 1999). Although some methods have been proposed to control harmful algal growth, only a few of them are applicable due to high cost, secondary pollution, or impracticability (Anderson, 1997). The effects of aquatic plants on harmful algae bloom were proposed as a measure to control undesired algal growth in aquatic ecosystems recently (Nakai et al., 1999). Several compounds with algicidal activity against cultured algae and natural phytoplankton assemblages have been isolated and identified from aquatic plants (Mulderij et al., 2005; Li and Hu, 2005). Nakai et al. (2000) showed that *M. spicatum* tissue released pyrogallol and produced an inhibitory effect on the growth of blue–green algae *Microcystis aeruginosa*. Although the inhibitory effect of pyrogallol on the growth of *Microcystis aeruginosa* has been demonstrated, only a few studies on the effect of pyrogallol on the different strains of *Microcystis aeruginosa*, including photosynthetic pigment contents and growth at different pH values, have been reported. The main objectives of present study were: (1) to investigate and compare the influence of pyrogallol on the growth and pigment absorption spectrum of toxic *M. aeruginosa* and nontoxic *M. aeruginosa*, and (2) to evaluate the effect of pyrogallol on the inhibition rate of toxic *Microcystis aeruginosa* at different pH mediums.

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Materials and Methods

Stock cultures of the toxic *M. aeruginosa* (FACHB-942) and nontoxic *M. aeruginosa* (FACHB-469) were obtained from the Institute of Hydrobiology, Chinese Academy of Sciences (Wuhan, China). The axenic cultures were grown in a sterilized BG11 liquid medium under a 12:12 LD cycle with a light intensity of 2200 lux provided by daylight fluorescent tubes at 25 ± 1°C. Pyrogallol (AR) was supplied by Sinopharm Group Chemical Reagent Ltd (Shanghai, China). Prior to use, a stock solution of pyrogallol was freshly made up in ultrapure water (Millipore, USA) including methanol. The final solvent concentration in the algae medium never exceeded 0.01%. An algae medium containing an equivalent solvent concentration in the absence of pyrogallol was used as the control. Pyrogallol solution was added to the mediums to give final pyrogallol concentrations of 4.5, 6.5, 7.5 and 8.5 mg/L to toxic *Microcystis aeruginosa*, or 4.5, 6.5 and 7.5 mg/L to nontoxic *Microcystis aeruginosa*. The initial algae concentration was 3×10^6 cell/ml. Each treatment was replicated three times. The cell density in each culture was determined at intervals of 48 h for eight days by using a hemocytometer.

For determination of chlorophyll a (Chl a) and carotenoid contents, samples of algae suspensions were centrifuged and the pellets were extracted with 90% acetone for 24 hours at 4°C. The supernatants obtained from each extraction were used for spectrophotometric pigment determination. Chl a and carotenoid contents were determined with the method described by Li (2000).

For determination of the absorption spectrum of photosynthetic pigments, photosynthetic pigments extracted as described above were scanned in a UV–VIS recording

spectrophotometer (Shimadzu UV-1601, Japan) and the absorption spectrum were recorded from 350 nm to 750 nm. The inhibition rate (IR) was calculated by the usual formula: IR% = (1 - N_t/N_0)*100 where N_t is the cell density and N_0 is the cell density of control at time t after pyrogallol exposure.

The values were statistically analyzed by an overall one-way analysis of variance (ANOVA) inserted in the graphic program Origin. *Significant at $P < 0.05$ and ** significant at $P < 0.01$.

Results and Discussion

There were clear dose relations for the inhibitory effect of pyrogallol on the toxic and nontoxic *M. aeruginosa* (Fig. 1, Fig. 2). After two days of pyrogallol treatment, the cell densities of toxic *M. aeruginosa* above 4.5 mg/L were significantly inhibited (ANOVA, $P < 0.05$, $n = 3$) and the cell densities of 4.5, 6.5, 7.5 and 8.5 mg/L were 98.4, 86.9, 68.4 and 35.7% in contrast to the control. Nontoxic *M. aeruginosa* was more susceptive to pyrogallol than toxic *M. aeruginosa* (ANOVA, $P < 0.01$, $n = 3$) and the cell densities of 4.5, 6.5 and 7.5 mg/L were 53.2, 44.1 and 35.8% in comparison with the control, respectively. Table 1 showed that the Chl a and carotenoid contents of toxic and nontoxic *M. aeruginosa* were reduced with increasing pyrogallol concentrations for 72h exposure.

The Chl a and carotenoid concentrations of toxic *M. aeruginosa* were changed significantly above 4.5 mg/L. The Chl a concentrations of 4.5, 6.5, 7.5 and 8.5 mg/L were 75.93, 65.12, 45.06 and 35.19%, and the carotenoid concentrations were 83.25, 65.4, 44.5 and 10.99%, respectively. The Chl a and carotenoid concentrations of nontoxic *M. aeruginosa* decreased more quickly than that of toxic *M. aeruginosa* after 72 h treatment. The lower pigment contents suggested that the collecting solar energy was decreased.

The pigment absorption spectrums of the toxic and nontoxic *M. aeruginosa* exposed to pyrogallol for 48 hours were shown in Figs. 3 and 4. The absorption spectrum indicated that absorption of pigments gradually decreased

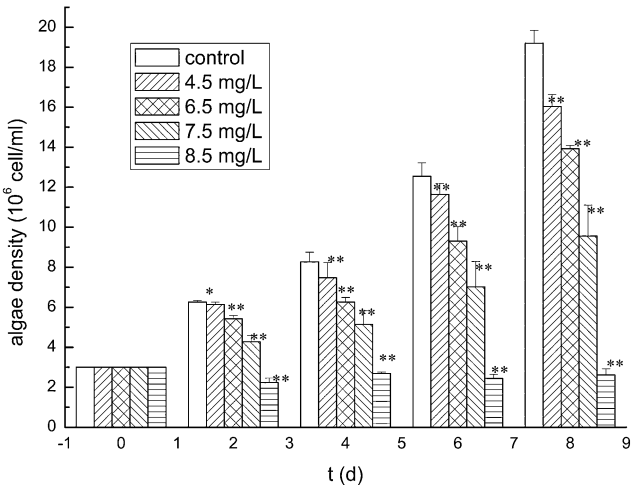

Fig. 1 Effects of pyrogallol on the biomass of toxic *M. aeruginosa* (means ± SE, $n = 3$)

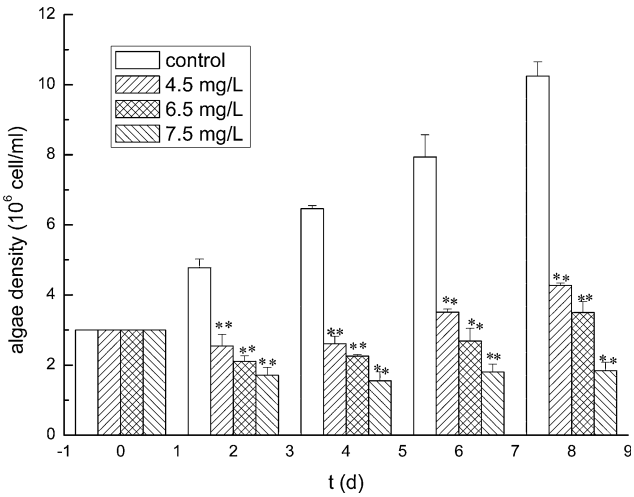

Fig. 2 Effects of pyrogallol on the biomass of nontoxic *M. aeruginosa* (means ± SE, $n = 3$)

Table 1 Chlorophyll a and carotenoid contents of *M. aeruginosa* exposed to pyrogallol for 72 h (means ± SE, $n = 3$)

	Toxic *M. aeruginosa*		Nontoxic *M. aeruginosa*	
	Chl a (μg/ml)	Carotenoid (μg/ml)	Chl a (μg/ml)	Carotenoid (μg/ml)
Control	1.62 ± 0.01	0.382 ± 0.005	1.49 ± 0.07	0.313 ± 0.025
4.5 mg/L	1.23 ± 0.05**	0.318 ± 0.012*	0.925 ± 0.01**	0.184 ± 0.006**
6.5 mg/L	1.08 ± 0.02**	0.25 ± 0.02**	0.747 ± 0.01**	0.123 ± 0.002**
7.5 mg/L	0.85 ± 0.06**	0.162 ± 0.004**	0.541 ± 0.05**	0.08 ± 0.008**
8.5 mg/L	0.603 ± 0.07**	0.049 ± 0.011**	NT	NT

NT: no tested

at 440 nm and 665 nm. Absorption of nontoxic *M. aeruginosa* at 440 nm and 665 nm decreased more quickly than that of toxic *M. aeruginosa*. The results were consistent with pigment changes of toxic and nontoxic *M. aeruginosa* (Table 1). Interesting results were the changes of absorption in the vicinity of 410 nm, which revealed that not only was pigment concentration decreased but also the property of pigments was changed.

Figure 5 showed that the control biomass of toxic *M. aeruginosa* grew well at all pH values tested and the algae biomass was not significantly different at the pH 8.0, 8.5 and 9.0. It was appropriate that pH values (8.0, 8.5 and 9.0) were chosen to investigate pH effects on pyrogallol.

The inhibitory effect of pyrogallol at different pH values on the toxic *M. aeruginosa* is displayed in Fig. 6. The results indicated that the algae growth at 6.5 mg/L pyrogallol mediums was clearly dependent on pH values. The results supported that the growth inhibition of *M. aeruginosa* could be related to pyrogallol property (Nakai et al., 2000). Pyrogallol was known to auto-oxidise in alkaline solutions and at the same time the radicals were produced (Bors et al., 1997). These radical species which could cleave DNA and peroxidize low-density lipoproteins and lipids would be very toxic to cells (Halliwell et al., 1987). There were some reports that in the presence of the copper(II) ion and aerobic conditions, catechin was able to induce DNA cleavage and accelerate the peroxidation of unsaturated fatty acids (Hayakawa et al., 1997). Nevertheless, pyrogallol generated far more OH• than did catechol for all pH values studied (Do Céu Silva et al., 2003).

The results from Fig. 7 showed that the pH values had different effects on the inhibition rate of toxic *M. aeru-*

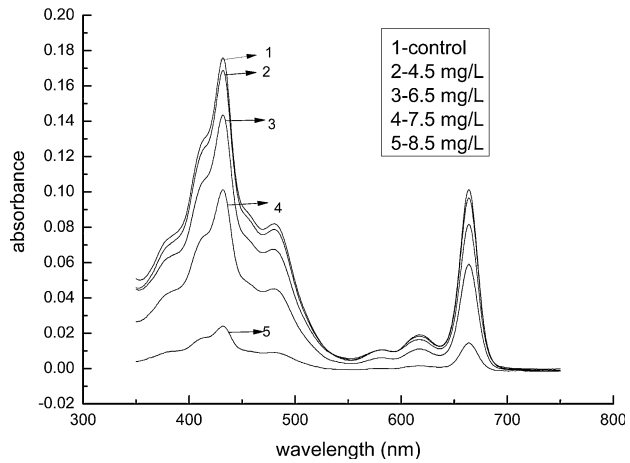

Fig. 3 Absorption spectrum of toxic *M. aeruginosa* exposed to pyrogallol for 48 h

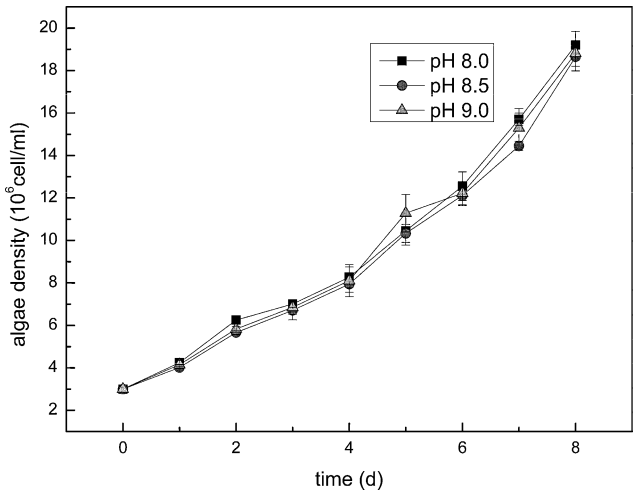

Fig. 5 Effects of different pH on the growth of the toxic *M. aeruginosa* control

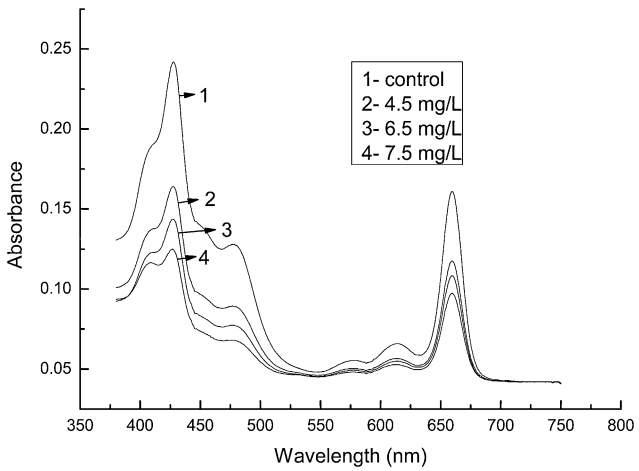

Fig. 4 Absorption spectrum of nontoxic *M. aeruginosa* exposed to pyrogallol for 48 h

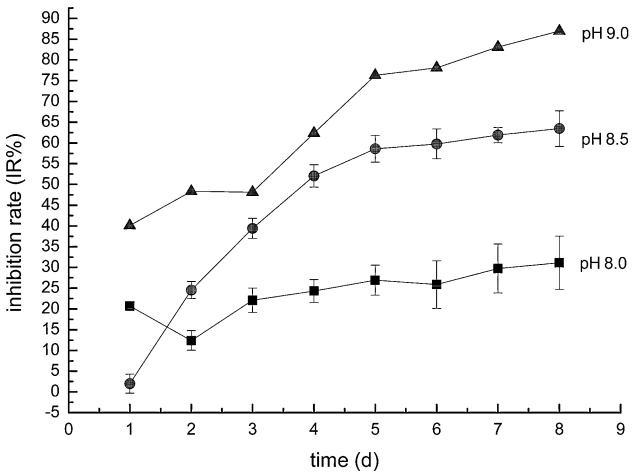

Fig. 6 Inhibition rate of toxic *M. aeruginosa* exposed to 6.5 mg/L pyrogallol mediums at pH values from 8.0–9.0 (means ± SE, $n = 3$)

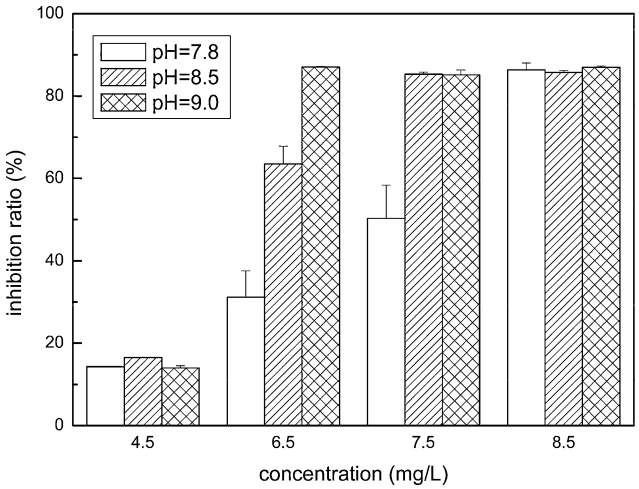

Fig. 7 Maximum inhibition rate (%) of toxic *M. aeruginosa* treated with pyrogallol at different pH values (means ± SE, $n = 3$)

ginosa in different concentrations of pyrogallol. At a lower concentration of pyrogallol (4.5 mg/L) and the highest concentration of pyrogallol (8.5 mg/L), the effects of pH on the inhibition rate were insignificant. However, the maximum inhibition rate of algae exposed to 6.5 mg/L and 7.5 mg/L pyrogallol medium increased with increasing pH. The phenomenon demonstrated that the rate of pyrogallol autooxidation strongly depended on pH values as well as pyrogallol concentration (Gao et al., 1998). At a higher pH and higher concentration medium, the inhibition effects on toxic *M. aeruginosa* were stronger.

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