Interspecific competition between *Microcystis aeruginosa* and *Chlamydomonas microsphaera* stressed by tetracyclines

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Abstract

The extensive tetracyclines in human and veterinary medicines cause contamination in the environment that could contribute to the antibiotic-resistant bacteria or the species competition in phytoplankton. In this study, *Microcystis aeruginosa* (bloom-forming cyanobacteria) and *Chlamydomonas microsphaera* (common green alga) were selected to test the effect of different concentrations of tetracyclines (tetracycline and oxytetracycline) in mono-culture and co-culture. The results showed that compared with mono-culture, the cell growth of *C. microsphaera* decreased significantly in co-culture treated with different concentrations of tetracycline and oxytetracycline. With the expose of 0.1, 2, 10 mg L^{-1} of tetracycline, the inhibition ratios of *M. aeruginosa* varied between 17.7% and 31.37% in co-culture compared to mono-culture, while the cell growth of *M. aeruginosa* was promoted treated with 0.1, 2, 7.25 mg L^{-1} of oxytetracycline in co-culture. However, the cell growth of *C. microsphaera* was significantly inhibited with all treatments in co-culture. With the treatment of tetracycline, the specific growth rate of *M. aeruginosa* was 0.36 to 0.31 day^{-1} in mono-culture and co-culture, while that of *C. microsphaera* was ranged from 0.38 to 0.26 day^{-1} in mono-culture, and it decreased from 0.25 day^{-1} (0 mg L^{-1}) to 0.08 day^{-1} (10 mg L^{-1}) in co-culture. With the treatment of oxytetracycline, the specific growth rate of *M. aeruginosa* was stimulated in co-culture while that of *C. microsphaera* showed an extremely significant inhibition in co-culture compared to mono-culture. Therefore, although *M. aeruginosa* outcompeted *C. microsphaera* in co-culture with the tetracyclines-free treatment, the competitive advantage of *M. aeruginosa* expanded upon addition of low or high concentrations of tetracyclines.

1. Introduction

Phytoplankton, including microalgae and cyanobacteria, are primary producers and form the base of the food web in aquatic ecosystems (Seymour et al. 2017). Phytoplankton with small cell types have a good capacity to uptake nutrients and convert light energy to chemical energy via photosynthesis (Häder et al. 2015). Since high sensitivities of some species in phytoplankton community to environmental disturbance and pollution, some algal species were used as environmental indicators for ecological assessments in terms of species composition, biomass, metabolism and chemical byproducts (Pannard et al. 2009; Stevenson 2014; Bi et al. 2018).

Microcystis, the most common bloom-forming cyanobacteria, is widespread in eutrophic lakes and reservoirs, which cause environmental and ecological problems (Carmichael 1995). The release metabolites (2-methylisoborneol and geosmin) by cyanobacteria can cause odors problems (Sugimoto et al. 2016). It is also well-known that *M. aeruginosa* produces microcystin (a hepatotoxin) that deteriorates water quality and threat livestock and human health (Watanabe et al. 1988; Hee Jin et al. 2005; Zhai et al. 2013). *C. microsphaera* is also a common and nontoxic single-celled green alga in the freshwater ecosystem (Song et al. 2007; Zhang et al. 2012). *M. aeruginosa* and *C. microsphaera* normally coexist in the same aquatic system and dominate respectively along with spatial and temporal variations (Zhang et al. 2013). The phytoplankton community can be affected by abiotic and biotic factors include pH, light,

nutrients, temperature, and allelopathic interactions (Zhang et al. 2012; Sugimoto et al. 2016; Tan et al. 2019b, 2020). Many studies have mentioned that the external contaminants could also change the community structure. Low concentration of pentachlorophenol (1 μg L^{-1}) that closed to the concentration in surface water stimulated the growth of *M. aeruginosa*, a shift dominance from *M. aeruginosa* to *Chlorella vulgaris* was observed when pentachlorophenol was over 0.25 mg L^{-1} (de Morais et al. 2014). *M. aeruginosa* outcompeted *Scenedesmus obliquus* in co-culture without linear alkylbenzene sulfonate (LAS) or 1 mg L^{-1} of LAS, while *S. obliquus* dominated with above 20 mg L^{-1} of LAS (Zhu et al. 2016). Similarly, the effect of phenol on competition between *M. aeruginosa* and *Chlorella pyrenoidosa* in co-culture were investigated (Tan et al. 2019a), described that phenolic pollution overturned the competition between both species.

Tetracyclines are one of the most extensively used in agricultural activities, livestock, and aquaculture as broad-spectrum antibiotics because of their cost-efficient, better antimicrobial activity, and low toxicity. Tetracyclines found in China river or lake basins are ranged from 67 to 25538 ng L^{-1} for tetracycline, 170 to 361107 ng L^{-1} for oxytetracycline, and 267 to 25538 ng L^{-1} for chlortetracycline (Jiang et al. 2014; Wang et al. 2017). However, the high concentrations (100 to 500 mg L^{-1}) of antibiotics were found in pharmaceutical manufacturing wastewater (Jing et al. 2014), which require further treatment. The solution for waste antibiotics is wildly searching and the wastewater treatment for tetracyclines can be categorized as biochemical and physicochemical technologies. Biochemical processes are mainly biodegradation by activated sludge, while physicochemical technologies include adsorption, oxidation, membrane processes, photocatalytic, and electrochemical methods (Jing et al. 2014; Scaria et al. 2021). However, due to the toxicity of antibiotics for activated sludge and technique application problems, the incomplete removal of tetracyclines was found, in which the tetracyclines (up to 32 mg L^{-1}) were detected in the effluent of a pharmaceutical wastewater treatment plant (Hou et al. 2016).

The effect of oxytetracycline and sulfamethoxazole on the physiological characteristics of *M. aeruginosa* and *C. microsphaera* was individually investigated (Zhou et al. 2021). In this study, we investigated the effect of tetracyclines on the interspecific competition between the toxic *Microcystis* strain (*M. aeruginosa*) and the nontoxic *Chlamydomonas* strain (*C. microsphaera*), which was analyzed by the cell growth in mono-culture and co-culture, and by the established competitive parameters. This work aims to evaluate the dominant species on the phytoplankton community under tetracyclines-free and tetracyclines exposure.

2. Materials And Methods

2.1 Organisms and reagents

M. aeruginosa (FACHB-1343) and *C. microsphaera* (FACHB-52) were purchased from the Freshwater Algae Culture Collection at the Institute of Hydrobiology, Chinese Academy of Sciences (Wuhan, China).

Tetracycline and oxytetracycline were selected as test pharmaceuticals (purchased from Aladdin, Shanghai, China).

The medium, included WC (Guillard and Lorenzen 1972), BG11, SE and SP, were pre-selected before the study of the effect of antibiotics on algae competition. The composition of mediums is shown in supplementary information Table S1 and S2. Two algae were cultured under different mediums, and the medium (BG-11) with the best growth rate was selected as the algae culture. Before the experiment, *M. aeruginosa* and *C. microsphaera* were cultured in BG11 medium for one week separately.

2.2 Experimental design

The mono-culture and co-culture were designed to identify the interspecific competition between *M. aeruginosa* and *C. microsphaera*. The amount of inoculation was based on the total volume ratio of *M. aeruginosa* and *C. microsphaera* at 1:1, thus, the initially inoculated algae cell concentration of *M. aeruginosa* and *C. microsphaera* were 10^6 and 10^4 cells mL^{-1}, respectively, in mono-culture and co-culture. Two species were cultured under the axenic condition in 250 mL Erlenmeyer flasks containing 150 mL of the medium at 25 ± 1°C with a light: dark cycle of 12:12 h under illumination by a cool-white fluorescent light at 50 μmol photons m^{-2} s^{-1}. Mono-culture and co-culture were treated with four treatment groups of tetracycline (0, 0.1, 2, and 10 mg L^{-1}) and oxytetracycline (0, 0.1, 2, and 7.25 mg L^{-1}). The concentration range of tetracycline was according to 96 h half-maximal inhibitory concentration (IC_{50}) on both species (as shown in supplementary information Table S3) and the concentration range of oxytetracycline was set based on Zhou et al. (2021). Triplicate was performed for each treatment, the flasks were shaken twice every day and randomly placed.

2.3 Determination of phytoplankton biomass

The cell densities of both organisms were quantified in mono-culture using a spectrophotometer (UV-1600PC, Mapada, China), and counted with a hemocytometer and a microscope (XSP-2CA, Shanghai Optical Instrument Factory, China). The linear regression between the cell density and optical density at 675 nm had a high correlation index ($R^2 > 0.99$), as shown in supplementary information (Fig. S1). The cell densities of both species were counted directly in co-culture with the hemocytometer and the microscope. Cell counts were performed on days 1, 3, 5, 7, 9, 11, 13, and 15.

2.4 Data analysis

The parameters of interspecific competition determined for the significant differences between mono-culture and co-culture were analyzed by Student t-test. The significant differences in the means of the variables between different concentrations of tetracyclines were analyzed by one-way analysis of variances (ANOVA) using the LSD test ($P < 0.05$). All of the results were expressed as the mean ± standard deviation. IC_{50} values were analyzed by SPSS 23.0.

The effects of co-culture on the growth of algae can be described by the cell inhibition ratio, described as follows:

$$IR=(C_{mono}-C_{co})/C_{mono}\times 100$$

1

Where IR is the cell inhibition ratio. C_{mono} is cell density in mono-culture, and C_{co} is cell density in co-culture.

The specific growth rate of algae is calculated as follows (Wang et al. 2010):

$$\mu=(\ln C_t-\ln C_0)/t$$

2

Where μ is the specific growth rate, C_0 and C_t is the cell biomass at the initial time and day t of the exponential phase.

The formula for fitting the logistic growth model of *M. aeruginosa* and *C. microsphaera* is as follows:

$$N=K/(1+e^{a-r*t})$$

3

N represents cell density, K represents the maximum cell growth density, r represents the intrinsic cell growth rate in mono-culture, a represents the coefficient indicating the intercept of the growth curves, and t represents the culture time.

The competitive relationship between the two algae in mixture culture used the Lotka-Volterra competition model (Volterra 1926), and formulas are as follows:

$$\frac{N_{C+1}-N_C}{t_{n+1}-t_n}=r_C N_C\left(1-\frac{N_C+\alpha N_M}{K_C}\right)$$

4

$$\frac{N_{M+1}-N_M}{t_{n+1}-t_n}=r_M N_M\left(1-\frac{N_M+\beta N_C}{K_M}\right)$$

5

Where N_{C+1} (N_C) and N_{M+1} (N_M) represent the cell density of *C. microsphaera* and *M. aeruginosa* under co-culture at day t_{n+1} (t_n). K_C and K_M represent the maximum cell density of *C. microsphaera* and *M. aeruginosa*, respectively, in mono-culture. r_C and r_M represent the intrinsic cell growth rate of *C. microsphaera* and *M. aeruginosa*. α (the effect of *M. aeruginosa* on *C. microsphaera*), and β (the effect of *C. microsphaera* on *M. aeruginosa*) are the competition coefficient under co-culture.

3. Results

3.1 Growth of *M. aeruginosa* and *C. microsphaera* treated with tetracyclines or tetracyclines-free in mono-culture and co-culture

The growth curves of *M. aeruginosa* and *C. microsphaera* in mono-culture and co-culture treated with different concentrations of tetracycline for 15 days are shown in Fig. 1. In mono-culture, compared with tetracycline-free treatment, the same growth trend of *M. aeruginosa* was observed under the expose of low concentrations of tetracycline (0.1 and 2 mg L^{-1}), and the cell concentration of *M. aeruginosa* was promoted during the culture time at 0.1 and 2 mg L^{-1} of tetracycline. However, the growth of *M. aeruginosa* was inhibited with 10 mg L^{-1} of tetracycline. The cell growth of *C. microsphaera* was simulated at 0.1 mg L^{-1} of tetracycline, but it was inhibited significantly at 2 and 10 mg L^{-1} of tetracycline in mono-culture.

In co-culture, compared with tetracycline-free treatment, the cell growth of both species was accelerated at 0.1 mg L^{-1} of tetracycline but was inhibited by 2 and 10 mg L^{-1} of tetracycline. Compared with mono-culture, the biomass of *C. microsphaera* decreased significantly in co-culture treated with different concentrations of tetracycline. In the absence of tetracycline, after 15 days of culture, the cell concentration in mono-culture of *C. microsphaera* was 13.1 times higher than in co-culture.

Figure 2 shows the cell growth of *M. aeruginosa* and *C. microsphaera* treated with different concentrations of oxytetracycline for 15 days. In mono-culture, the cell growth of *M. aeruginosa* and *C. microsphaera* increased similarly between the treatment of low concentration of oxytetracycline (0.1 mg L^{-1}) and oxytetracycline-free treatment. Compared with the oxytetracycline-free treatment, the inhibition degree of the biomass of *C. microsphaera* (52.9%) was higher than that of *M. aeruginosa* (13.7%) under the treatment of 2 mg L^{-1} of oxytetracycline. Both species were inhibited significantly with 7.25 mg L^{-1} of oxytetracycline.

In co-culture, compared with oxytetracycline-free treatment, the cell growth of *M. aeruginosa* was promoted at 0.1 and 2 mg L^{-1} of oxytetracycline, and after 15 days of culture, the biomass of *M. aeruginosa* treated with oxytetracycline at 0.1 and 2 mg L^{-1} increased by 68.5% and 24.2%, respectively. The inhibition degree of the biomass of *M. aeruginosa* treated with 7.25 mg $L^{-1}$of oxytetracycline in co-culture was lower than that in mono-culture. In contrast to mono-culture, the cell growth of *C. microsphaera* treated with different concentrations of oxytetracycline was markedly inhibited in co-culture.

3.2 Growth parameters

The growth kinetic parameters of *M. aeruginosa* and *C. microsphaera* in both cultures treated with tetracycline and oxytetracycline were calculated by Eq. (3) and are listed in Tables 1 and 2, respectively. High coefficient of regression (R^2) indicates the cell growth of *M. aeruginosa* and *C. microsphaera* in

Fig. 1 and Fig. 2 well-fitted by logistic growth model. The maximum cell density (K) of *M. aeruginosa* and *C. microsphaera* treated with tetracycline in mono-culture were higher than that in co-culture (Table 1), which indicates the cell growth of *M. aeruginosa* and *C. microsphaera* was limited in co-culture compared to mono-culture. In mono-culture, comparing different concentrations of tetracycline treatment, it was found that the maximum cell density of *M. aeruginosa* was higher at 0.1 and 2 mg L^{-1} of tetracycline than that of tetracycline-free treatment, while in co-culture, the maximum cell density of *M. aeruginosa* was the highest in tetracycline-free group. The maximum cell density of *C. microsphaera* was the highest at 0.1 mg L^{-1} of tetracycline treatment in both mono-culture and co-culture.

Table 1
The growth kinetic parameter of *M. aeruginosa* and *C. microsphaera* in mono-culture and co-culture treated with tetracycline

Species	Culture type	Tetracycline (mg L^{-1})	K (10^6 cells mL^{-1})	a	r	R^2
M. aeruginosa	Mono-culture	0	497.8	5.04	0.32	0.998
		0.1	565.0	5.17	0.31	0.998
		2	456.6	4.39	0.28	0.998
		10	185.2	4.82	0.38	0.983
	Co-culture	0	346.2	3.78	0.27	0.984
		0.1	220.4	3.78	0.35	0.979
		2	185.6	3.51	0.32	0.971
		10	145.0	3.99	0.34	0.983
C. microsphaera	Mono-culture	0	4.58	4.09	0.35	0.995
		0.1	4.69	3.88	0.35	0.996
		2	3.11	4.13	0.33	0.998
		10	N.A.	11.61	0.30	0.963
	Co-culture	0	0.28	2.59	0.41	0.956
		0.1	0.38	2.60	0.34	0.987
		2	0.22	2.28	0.33	0.960
		10	0.04	0.87	0.77	0.559

Note: N.A. means data was not available.

The maximum cell density (K) of *M. aeruginosa* and *C. microsphaera* treated with oxytetracycline in mono-culture was also higher than that in co-culture (Table 2). The highest of the maximum cell density

of *M. aeruginosa* was found with 0.1 mg L^{-1} of tetracycline treatment in mono-culture and with tetracycline-free treatment in co-culture. However, in mono-culture, under 0, 0.1, and 2 mg L^{-1} of oxytetracycline treatment, the calculated maximum cell density of *C. microsphaera* was too high, which indicates that the cell was in the exponential growth phase for 15 days. This suggests to extend the culture time to calculate the maximum cell density.

Table 2
The growth kinetic parameter of *M. aeruginosa* and *C. microsphaera* in mono-culture and co-culture treated with oxytetracycline

Species	Culture type	Oxytetracycline (mg L^{-1})	K (10^6 cells mL^{-1})	a	r	R^2
M. aeruginosa	Mono-culture	0	339.5	3.99	0.34	0.996
		0.1	367.1	4.37	0.35	0.996
		2	322.1	4.44	0.35	0.998
		7.25	243.1	4.67	0.09	0.496
	Co-culture	0	346.2	3.78	0.27	0.984
		0.1	343.8	4.21	0.45	0.976
		2	327.6	3.82	0.33	0.986
		7.25	130.5	6.13	0.56	0.995
C. microsphaera	Mono-culture	0	1957.5	10.59	0.28	0.989
		0.1	2096.3	10.59	0.27	0.986
		2	1054.6	11.31	0.32	0.993
		7.25	0.73	4.15	0.25	0.990
	Co-culture	0	0.55	3.38	0.60	0.995
		0.1	0.50	3.57	0.65	0.970
		2	0.34	3.08	0.46	0.963
		7.25	0.16	2.32	0.40	0.928

Figure 3 describes the effect of co-culture on the cell inhibition ratios of *M. aeruginosa* and *C. microsphaera* treated with different concentrations of tetracyclines at day 15. *M. aeruginosa* and *C. microsphaera* were inhibited in co-culture treated with different concentrations of tetracycline, in which the inhibition ratios of *M. aeruginosa* varied between 8.2% and 31.4%, while the inhibition ratios of *C. microsphaera* were around 90.2–93.5%. In terms of feeding oxytetracycline, the inhibition ratios of *M.*

aeruginosa were 22.2% in oxytetracycline-free group and all negative values in oxytetracycline-treated groups, which indicated the cell of *M. aeruginosa* treated with oxytetracycline was promoted. The inhibition ratios of *C. microsphaera* decreased from 88.0% (0.1 mg L^{-1} of oxytetracycline) to 25.6% (7.25 mg L^{-1} of oxytetracycline).

The specific growth rates (μ) of *M. aeruginosa* and *C. microsphaera* treated with tetracycline are shown in Fig. 4. The specific growth rates of *M. aeruginosa* were from 0.36 to 0.31 day^{-1} in mono-culture and co-culture following exposure to tetracycline. In mono-culture, the specific growth rate of *M. aeruginosa* was significantly decreased at 10 mg L^{-1} of tetracycline, while that rates of *M. aeruginosa* at 2 and 10 mg L^{-1} of tetracycline were significantly different from the rates at 0 and 0.1 mg L^{-1} of tetracycline in co-culture. Significant differences were found between mono-culture and co-culture in the specific growth rate of *M. aeruginosa* following exposure to all concentrations of tetracycline. The specific growth rates of *C. microsphaera* decreased from 0.38 day^{-1} at 0.1 mg L^{-1} of tetracycline to 0.26 day^{-1} at 10 mg L^{-1} of tetracycline in mono-culture, while the rates decreased from 0.25 day^{-1} (0 mg L^{-1}) to 0.08 day^{-1} (10 mg L^{-1}) in co-culture. Highly significant differences ($P < 0.01$) were identified between mono-culture and co-culture in tetracycline-free and tetracycline-treated groups.

In mono-culture, the specific growth rate of *M. aeruginosa* was significantly decreased at 10 mg L^{-1} of oxytetracycline (Fig. 5). In co-culture, the highest specific growth rate of *M. aeruginosa* was 0.39 day^{-1} at 0.1 mg L^{-1} of oxytetracycline. The specific growth rates of *M. aeruginosa* in co-culture were higher than that in mono-culture, which significant differences between two types of culture were observed at 0.1 and 7.25 mg L^{-1} of oxytetracycline. In terms of *C. microsphaera*, the specific growth rates decreased from 0.37 day^{-1} in oxytetracycline-free treatment to 0.21 day^{-1} at 10 mg L^{-1} of oxytetracycline in mono-culture, while the rates decreased from 0.25 day^{-1} (0 mg L^{-1}) to 0.18 day^{-1} (10 mg L^{-1}) in co-culture, and all treatments under co-culture were significantly different from mono-culture treatments.

Table 3 is listed the competition parameters α (the effect of *M. aeruginosa* on *C. microsphaera*) and β (the effect of *C. microsphaera* on *M. aeruginosa*).α was lower than 0.1 treated with 0, 0.1, and 2 mg L^{-1} of tetracycline, while α increased to 27.8 with 10 mg L^{-1} of tetracycline. It suggested that *M. aeruginosa* hardly affected the cell growth of *C. microsphaera* when tetracycline was less than 2 mg L^{-1}, but *M. aeruginosa* inhibited the growth of *C. microsphaera* at 10 mg L^{-1} of tetracycline. All negative values of β in all treatments of tetracycline suggested that *C. microsphaera* promoted the growth of *M. aeruginosa*. When oxytetracycline was added, α decreased with the increase of oxytetracycline concentration. This indicated that *M. aeruginosa* inhibited the growth of *C. microsphaera*, and the degree of inhibition decreased with the increasing oxytetracycline concentration. The value of β was also negative in all treatments with oxytetracycline, indicating that *C. microsphaera* promoted the growth of *M. aeruginosa*.

Table 3
The competitive coefficient of *M. aeruginosa* and *C. microsphaera* treated with tetracycline and oxytetracycline.

Tetracyclines	mg L^{-1}	α	β
Tetracycline	0	0.04 ± 0.02	-1181 ± 22
	0.1	0.04 ± 0.02	-1156 ± 1657
	2	0.03 ± 0.02	-578 ± 433
	10	27.80 ± 5.82	-569 ± 293
Oxytetracycline	0	17.03 ± 2.71	-282 ± 55
	0.1	9.53 ± 3.67	-552 ± 325
	2	6.86 ± 2.77	-230 ± 19
	7.25	0.01 ± 0.02	-16607 ± 4795

4. Discussion

4.1 Interspecific competition in tetracyclines-free treatment

Through the effect of co-culture on the cell growth of both species, it can be found that in the absence of tetracyclines, the inhibition ratio of cell growth of *C. microsphaera* (around 90%) was higher than that of *M. aeruginosa* (less than 23%). Moreover, compared to mono-culture, the specific growth rate of *M. aeruginosa* was not affected in co-culture, but that rate of *C. microsphaera* was significantly decreased in co-culture. According to the competitive coefficient of Lotka-Volterra model, the effect of *M. aeruginosa* on *C. microsphaera* (α) with tetracyclines-free treatment was positive, indicating that *M. aeruginosa* inhibited the growth of *C. microsphaera*. The negative values of β pointed out that *C. microsphaera* promoted the growth of *M. aeruginosa*. Thus, these parameters confirmed the dominance of *M. aeruginosa* in co-culture. The same phenomenon has been previous described. *M. aeruginosa* became the dominant species when competing with *Scenedesmus* in co-culture (Zhu et al. 2016).

The dominance of *M. aeruginosa* may be due to the size of the cells. *Microcystis* is 6 to 12 times smaller than *Scenedesmus* in cell volume (Zhu et al. 2016). *C. microsphaera* is about 100 times larger than *M. aeruginosa*, identified in this study. Small bacterial cells require higher nutrients than larger size organisms in the phytoplankton in terms of mass-to-mass basis (Karl 2000), while the smaller size and large surface-to-volume ratio allow a high competitive capacity of nutrients absorption (Azam et al. 1983). This explains the competitive advantage of *M. aeruginosa* for nutrients. Furthermore, the production of *M. aeruginosa* may affect the dominance species. Microcystins (such as Microcystin-LR) and anatoxin-a, productions of *M. aeruginosa*, influenced the growth and physiology of eukaryotic and prokaryotic phytoplankton (Chia et al. 2019). Microcystins not only reduce survival and growth rate of fish by interfering with embryonic hatching, but also trigger histopathological effects (Malbrouck and

Kestemont 2006). It is interesting to note that *M. aeruginosa* was stimulated to increase its microcystins production in the presence of green algae (Bittencourt-Oliveira et al. 2015). Although the total biomass of zooplankton was not affected by the exposure of microcystin, microcystin had a positive effect at a population-level (Paes et al. 2016). Besides, extracellular allelopathic compounds (like D-limonene and 1-chlorine heptacosane) produced by *M. aeruginosa* may contribute to its dominance (Zhai et al. 2013).

4.2 Interspecific competition under different concentration of tetracyclines

Growth hormesis of *M. aeruginosa* was observed with the exposure of a low concentration of tetracycline and oxytracycline. The cell growth of *M. aeruginosa* was promoted with low concentrations of tetracyclines (0.1 or 2 mg L^{-1}) in mono-culture or co-culture and inhibited with high concentrations of tetracyclines (Fig. 1 and Fig. 2). Hormesis is a dose-response relationship characterized by low-dose stimulation and high-dose inhibition (Stebbing 1982; Axelrod et al. 2004). Hormesis of pollutants has been reported previously. Tan et al. (2019) found that phenol stimulated the growth of *M. aeruginosa* at low concentrations (2 mg L^{-1}). The growth of *Skeletonema costatum* was accelerated when florfenicol concentration below 2 mg L^{-1}, and inhibited significantly over 4 mg L^{-1} (Liu et al. 2012).

The cell response of hormesis could simulate the change of community structure. The inhibition ratio of *M. aeruginosa* was significantly lower than that of *C. microsphaera* with the exposure of tetracycline, whereas the negative inhibition ratio of *M. aeruginosa* means that the growth of *M. aeruginosa* was promoted in co-culture with the treatment of oxytetracycline. This indicates that the addition of tetracyclines significantly inhibited the growth of *C. microsphaera*, less inhibited or even stimulated the growth of *M. aeruginosa*. According to the competitive coefficient of Lotka-Volterra model, the effect of *M. aeruginosa* on *C. microsphaera* (α) was higher in treatment with 10 mg L^{-1} tetracycline than other concentrations of tetracycline. The negative values of β (the effect of *C. microsphaera* on *M. aeruginosa*) in tetracyclines-treatments, especially in the exposure of 7.25 mg L^{-1} oxytetracycline, pointed out that *C. microsphaera* promoted the growth of *M. aeruginosa*. These parameters confirmed the dominance of *M. aeruginosa* in the presence of tetracyclines.

In mono-culture, the specific growth rate of *M. aeruginosa* was not affected at 0.1 and 2 mg L^{-1} of tetracycline treatment, and even was stimulated under oxytetracycline treatment. While the specific growth rate of *C. microsphaera* was unaffected at 0.1 mg L^{-1} of tetracycline, but significantly decreased at 2 mg L^{-1} of tetracyclines. Under the exposure of high concentrations of tetracyclines, the specific growth rate of *M. aeruginosa* was inhibited to a lower degree than that of *C. microsphaera*. Under co-culture, this situation was exacerbated, that is, under the effect of low concentrations of tetracyclines, the specific growth rate of *C. microsphaera* was significantly inhibited, while under the exposure of high concentration of tetracyclines the inhibition degree of the specific growth rate of *C. microsphaera* was higher than that in mono-culture. This phenomenon was caused by the different responses of different species to pollutants. *M. aeruginosa* and *C. microsphaera* were tested for acute toxicity of tetracyclines and identified by 96 h IC_{50}. Table S3 shows that IC_{50} of tetracycline for *M. aeruginosa* and *C.*

microsphaera was 10.39 and 2.04 mg L^{-1}, respectively, whereas IC_{50} of oxytetracycline were 7.25 and 4.20 mg L^{-1}, respectively (Zhou et al. 2021). It indicated that *M. aeruginosa* has stronger antibiotic resistance than *C. microsphaera*. Thus, low concentrations of tetracyclines stimulated growth of *M. aeruginosa* and inhibited the growth of *C. microsphaera*, while high concentrations of tetracyclines on the growth inhibition rate of *C. microsphaera* was higher than that of *M. aeruginosa*, which promoted the dominance of *M. aeruginosa* in the presence of tetracyclines.

Moreover, some studies have mentioned that the pollutants can overturn the species community structure. Zhu et al. (2016) described dominant species changed from *M. aeruginosa* to *S. obliquus* when LAS was over 20 mg L^{-1} in co-culture. *M. aeruginosa* dominated with the expose of 0, 2, and 20 mg L^{-1} of phenol in co-culture, while *C. pyrenoidosa* outcompeted with 200 mg L^{-1} of phenol (Tan et al. 2019a). However, in this study, the dominance of *M. aeruginosa* with all treatment of tetracyclines differed from the previously mentioned studies. It can be explained by the toxicity resistance. The inhibitory concentration (IC_{50}) of LAS for *M. aeruginosa* and *S. obliquus* were 10 and 100 mg L^{-1}, respectively. In terms of phenol treatments, IC_{50} for *M. aeruginosa* and *C. pyrenoidosa* were 80.8 and 631.4 mg L^{-1}. *M. aeruginosa* were more sensitive than *S. obliquus* and *C. pyrenoidosa*. When the concentration of pollutants exceeds the tolerance range of the cells, the cell growth are affected, which leads to changes in community structure. In our work, *M. aeruginosa* was more tolerant than *C. microsphaera*, thus under the effect of high concentrations of tetracyclines, *M. aeruginosa* dominated in all treatments. Therefore, it could be concluded that the community structure of organisms during co-culture is also affected by the toxicity of the pollutants on different concentrations.

Collectively, in the presence of tetracyclines, *M. aeruginosa* dominated co-cultured with *C. microsphaera*. *Microcystis* has been reported to be more competitive in the co-culture under eutrophic conditions, compared to *Scenedesmus obliquus* (Zhu et al. 2016), *C. pyrenoidosa* (green algae) (Tan et al. 2019a), *Monoraphidium convolutum* (green algae) (Bittencourt-Oliveira et al. 2015). However, *Cylindrospermopsis raciborskii* (Cyanobacteria) outcompeted over *M. aeruginosa* with a high concentration of phosphorus resource. The interspecific competition is dependent on a combination of factors relating to the species or strains community or variable environments, such as light, temperature, or nutrients (Sugimoto et al. 2016; Xiao et al. 2017). Moreover, according to the different responses of species to the pollutants, the community structure could be influenced. Although the tetracyclines do not reach the level of mg L^{-1} in the natural river and lake basins, the stimulation of low concentration of antibiotics (such as oxytetracycline) also needs to be considered in the interspecific competition.

5. Conclusion

This study investigated the interspecific competition between cyanobacteria (*M. aeruginosa*) and green algae (*C. microsphaera*) in the absence and presence of tetracycline and oxytetracycline. The results of our study demonstrate that *M. aeruginosa* is a superior competitor to *C. microsphaera* in the co-culture system. In the tetracyclines-free treatment, *M. aeruginosa* outcompeted *C. microsphaera* in co-culture. *C.*

microsphaera was more sensitive than to tetracyclines compared to *M. aeruginosa*. In co-culture, low concentrations of tetracyclines unaffected or stimulated the growth of *M. aeruginosa*, but unaffected or inhibited the growth of *C. microsphaera*. High concentrations of tetracyclines on the growth inhibition rate of *C. microsphaera* was higher than that of *M. aeruginosa*, which enhanced the competitive advantage of *M. aeruginosa* and intensified the dominance of *M. aeruginosa* in the presence of tetracyclines.

Declarations

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Competing Interests

The authors have no relevant financial or non-financial interests to disclose.

Author Contributions

All authors contributed to the study conception and design. Material preparation, data collection and analysis were performed by Xudong ZHOU, and Xincheng JIANG. The first draft of the manuscript was written by Xudong ZHOU and reviewed by Jibao CHEN and Pengcheng GAO. All authors commented on previous versions of the manuscript. All authors read and approved the final manuscript.

Ethical Approval

Not applicable

Consent to Participate

Not applicable

Consent to Publish

Not applicable

Availability of data and materials

The datasets generated during and/or analyzed during the current study are available from the corresponding author on reasonable request.

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Figures

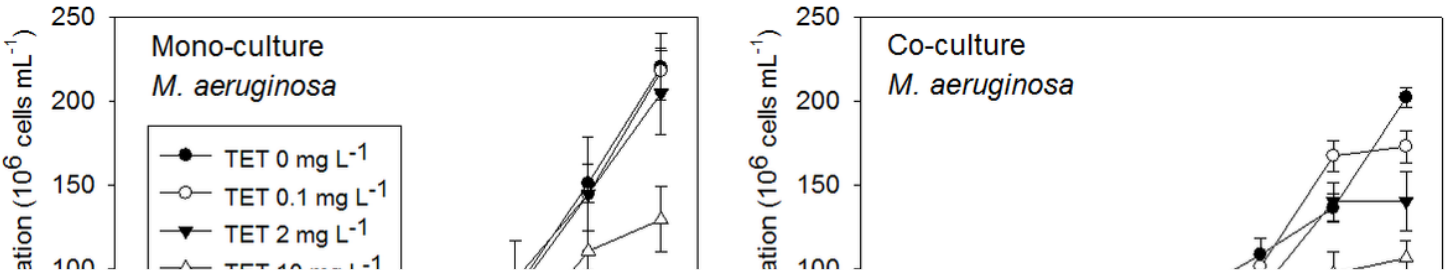

Figure 1

The growth curve of *M. aeruginosa* and *C. microsphaera* in mono-culture and co-culture treated with different concentrations of tetracycline (TET).

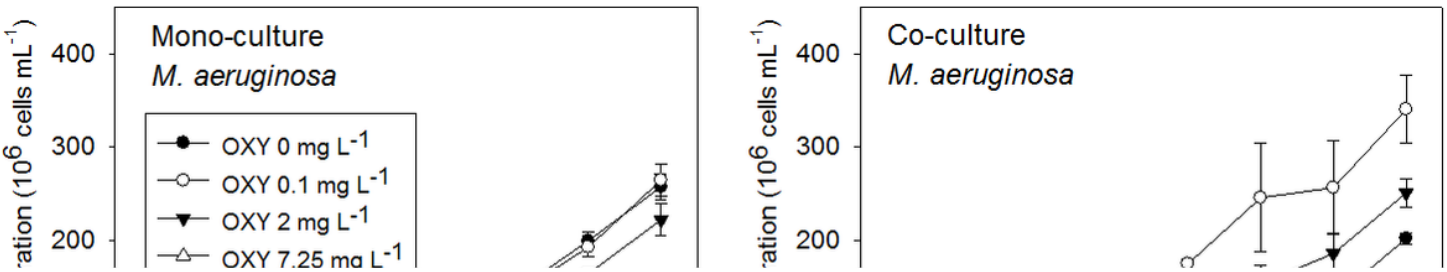

Figure 2

The growth curve of *M. aeruginosa* and *C. microsphaera* in mono-culture and co-culture treated with different concentrations of oxytetracycline (OXY).

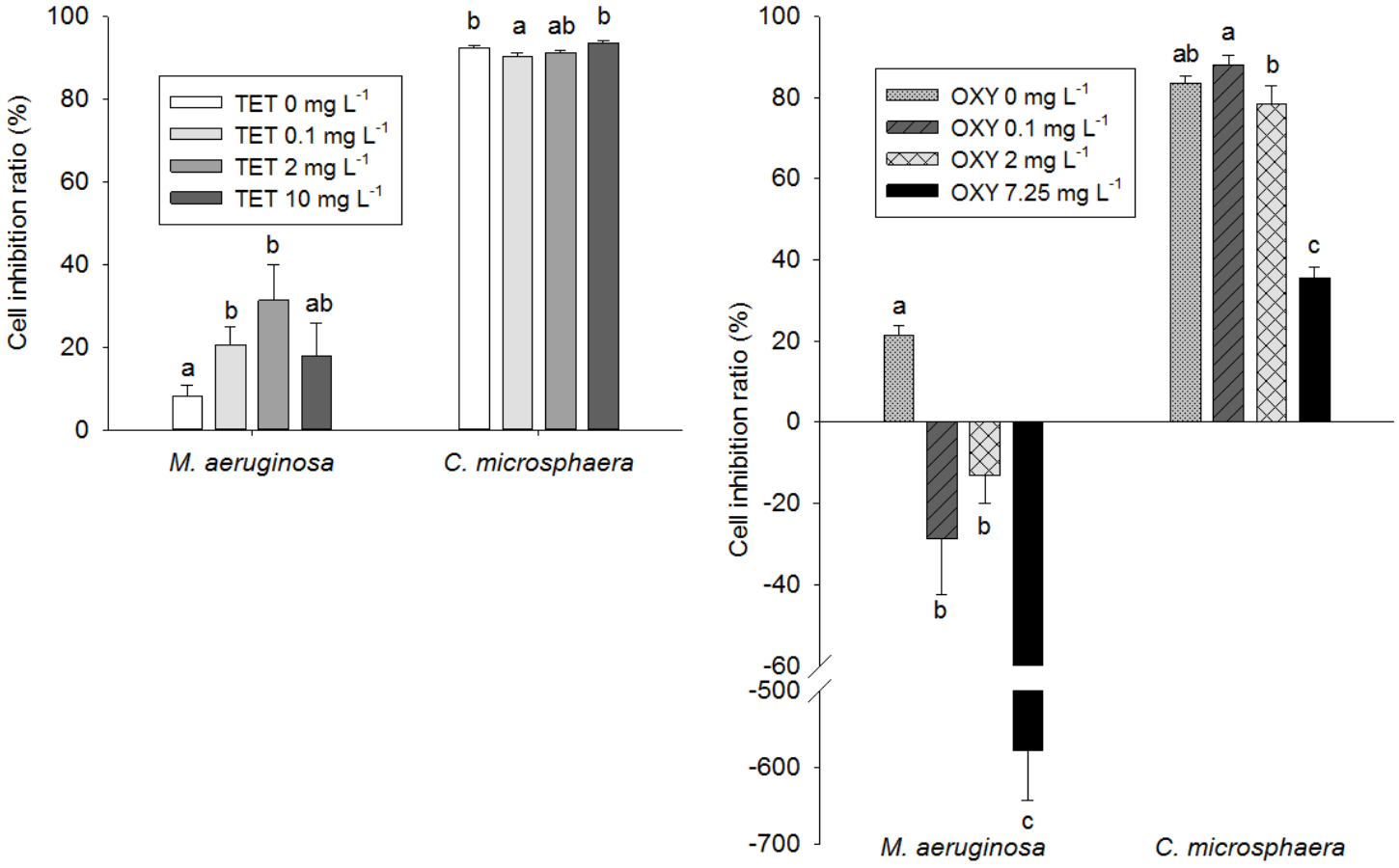

Figure 3

The effect of co-culture on the cell inhibition ratio of *M. aeruginosa* and *C. microsphaera* treated with different concentrations of tetracycline (TET) and oxytetracycline (OXY). Different letters above (below) columns represent significant differences (P<0.05) between different concentrations of tetracyclines.

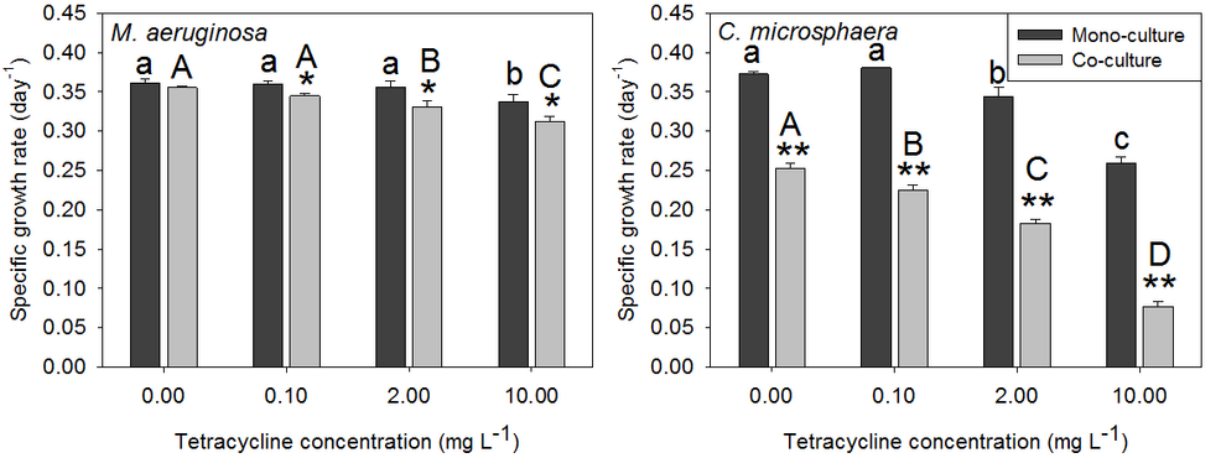

Figure 4

The specific growth rate of *M. aeruginosa* and *C. microsphaera* in mono-culture and co-culture treated with tetracycline. Single asterisk and double asterisk represent the significant differences between mono-culture and co-culture at $P < 0.05$ and $P < 0.01$, respectively. Lowercase letter (Uppercase letter) above columns represent significant differences in mono-culture (co-culture) between different concentrations of tetracycline at $P < 0.05$.

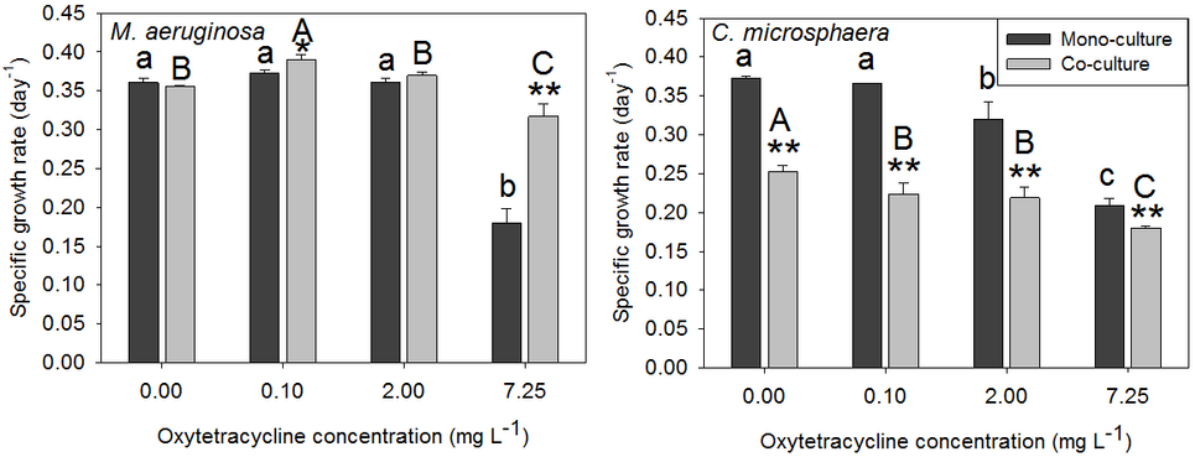

Figure 5

The specific growth rate of *M. aeruginosa* and *C. microsphaera* in mono-culture and co-culture treated with oxytetracycline. Single asterisk and double asterisk represent the significant differences between mono-culture and co-culture at $P < 0.05$ and $P < 0.01$, respectively. Lowercase letter (Uppercase letter) above columns represent significant differences in mono-culture (co-culture) between different concentrations of oxytetracycline at $P < 0.05$.

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