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RESEARCH ARTICLE

Sulfamethoxazole and tetracycline induced alterations in biomass, photosynthesis, lipid productivity, and proteomic expression of *Synechocystis* sp. PCC 6803

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Abstract

Since antibiotics show hormesis effects in cyanobacteria at the nanogram per liter concentration level, the possibility for two commonly used antibiotics (sulfamethoxazole and tetracycline) to increase lipid productivity in *Synechocystis* sp. PCC 6803 was assessed in the present study. The two target antibiotics significantly promoted ($p < 0.05$) the biofuel productivity of *Synechocystis* sp. PCC 6803 through the increase of both biomass and lipid content. Sulfamethoxazole and tetracycline significantly stimulated ($p < 0.05$) cyanobacterial growth by upregulating proteins related to cell differentiation, cell division, and gene expression; significantly enhanced ($p < 0.05$) the photosynthetic activity by upregulating photosynthesis-related proteins; and significantly increased ($p < 0.05$) the lipid content in cyanobacterial cells by downregulating carbohydrate catabolic proteins and carbohydrate transport proteins. Due to the altered expression pattern of biosynthesis-related proteins, the two antibiotics increased the proportion of monounsaturated fatty acids, while tetracycline reduced the proportions of saturated and polyunsaturated fatty acids. The changes in fatty acid composition may improve the combustion performance of biofuel. This study provided insights into the application of antibiotics in cyanobacteria-based biofuel production.

Keywords Cyanobacteria · Hormesis · Biofuel · Photosynthetic activity · Proteome · Fatty acid methyl esters

Introduction

Biofuel is considered to be a compatible alternative to fossil fuels and has various advantages including innocuity, sustainability, and good biodegradability (Aslam et al. 2018). Compared with fossil fuels, biofuel is an environmentally friendly fuel that releases less greenhouse gas and generates less air pollution (Živković et al. 2017). Biofuel comes from oil-rich plants, animals, and photosynthetic microorganisms, among which cyanobacteria, as the third-generation biofuel feedstock, present good application future in biofuel production (Han et al. 2016). Due to the high growth rate, cyanobacterial cultivation requires less land occupation than terrestrial plants and shows good adaptability to extreme culture conditions (Han et al. 2016; Wahlen et al. 2011). Establishing a simple and efficient production process of quality biofuel is the major hurdle for the economization of cyanobacteria-based biofuel (Bharte and Desai 2019).

Cyanobacteria are photosynthetic organisms with the ability to fix CO_2 and produce lipids in the form of diacylglycerols and triacylglycerols (Deshmukh et al. 2019a, b), which can be easily transformed to biofuel through transesterification using alcohols (e.g., methanol) and catalysts (base or acid) (Raheem et al. 2018). The reported lipid content in cyanobacteria varies from 4 to 21% (Shuba and Kifle 2018), and increased lipid content is an important issue for the commercial realization of cyanobacteria-based biofuel. The lipid content was reported to be regulated by culture conditions including temperature, light, salinity, and CO_2 concentration (Aslam et al. 2018; Chandra et al. 2019; Dineshbabu et al. 2020). Recent studies also observed an increased lipid content in cyanobacteria exposed to exogenous stresses, such as nitrogen starvation, phosphorous deficiency, ultrasonication, ultraviolet radiation,

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and TiO_2 stresses (Casazza et al. 2015; Cordeiro et al. 2017; Ellison et al. 2019). However, the final biofuel production amount is determined by biomass × lipid content. Reports on the simultaneous stimulation of biomass and lipid content during oil-producing cyanobacterial culture are still limited.

Antibiotics are a group of antibacterial chemicals that are broadly used in human disease treatment, veterinary medicine, and aquaculture (Ben et al. 2019). With a bacteria-like structure, cyanobacteria are sensitive to antibiotics (Maul et al. 2006). Various hormetic responses have been observed in cyanobacteria under exposure to low concentrations of antibiotics, including the promotion of biomass growth, photosynthetic activity, and cellular substance synthesis. For instance, the growth of *Microcystis flos-aquae* was stimulated under exposure to 1–100 ng/L erythromycin (Wan et al. 2015). Exposure to 0.1 mg/L ofloxacin led to increased photosynthetic activity in *Microcystis aeruginosa* (Deng et al. 2015). Previous studies by our group found that 100–600 ng/L amoxicillin promoted the synthesis of proteins and polysaccharides in *M. aeruginosa*, and the proteomic responses suggested a stimulatory effect of amoxicillin, ciprofloxacin, and sulfamethoxazole on carbohydrate synthesis (Liu et al. 2016; Liu et al. 2017). If antibiotic stress could increase biomass growth and lipid content at the same time, a significant increase in lipid productivity of oil-producing cyanobacteria could be achieved. However, information on the influence of antibiotics on oil-producing cyanobacteria was still limited.

In this study, two commonly used antibiotics effective against gram-negative bacteria, sulfamethoxazole (SMZ) and tetracycline (TET), were selected as target chemicals (Yang et al. 2018). A commonly used oil-producing cyanobacterial strain with high lipid content, *Synechocystis* sp. PCC 6803, was selected as the target cyanobacterium (Sivaramakrishnan and Incharoensakdi 2018). The genome of *Synechocystis* sp. PCC 6803 has been fully recorded (Pattanaik et al. 2020), and it is regarded as an attractive model species for oil-producing cyanobacteria. The regulatory effects of target antibiotics on the biomass, lipid content, and fatty acid composition of *Synechocystis* sp. PCC 6803 were investigated, based on which the possibility of using antibiotics in cyanobacterial biofuel production was evaluated. In addition, the regulatory mechanisms of antibiotics in *Synechocystis* sp. PCC 6803 were interpreted according to the responses of photosynthetic activity and the whole proteome.

Materials and methods

Culture of cyanobacterial cells

SMZ and TET with purities of 99.5% and 96.2%, respectively, were purchased from Dr. Ehrenstorfer, Inc. (Germany). The target antibiotics were dissolved in methanol and stored at − 20 °C. *Synechocystis* sp. PCC 6803 was precultivated using sterile BG11 medium at 25 ± 1 °C under fluorescent white light at an intensity of 2000 lx with a 16:8 light:dark cycle. In the antibiotic exposure test, a certain amount of cyanobacterial cells were inoculated into 200 mL of sterile BG11 medium, and the initial optical density value at 730 nm (OD_{730}) was 0.05 ± 0.002. Next, the cyanobacterial cells were aseptically cultured for 18 days under the same conditions as those used for precultivation. At the beginning of the antibiotic exposure test, each antibiotic was spiked into the culture medium at an exposure dose of 100 ng/L. Before the antibiotic exposure test, a preliminary experiment showed that target antibiotics were degraded by 20–30% every day because of hydrolysis and photolysis. Therefore, TET and SMZ were replenished into the culture medium every 24 h to maintain a stable exposure dose. The final concentration of methanol solvent in each antibiotic-treated group was below 0.005% (v/v). A solvent control group containing *Synechocystis* sp. PCC 6803 spiked with 0.005% methanol was prepared. Three independent replicate experiments were conducted for two antibiotic-treated groups and solvent control.

Determination of cellular responses

To assess the growth conditions, 3 mL of cyanobacterial culture was aseptically taken from each flask at a regular time interval of 24 h, and the OD_{730} value was measured through spectrophotometry. The specific growth rate was calculated as Eq. (1):

$$\mu(\text{per day}) = \frac{\ln OD_{t1} - \ln OD_{t0}}{t_1 - t_0} \quad (1)$$

where OD_{t0} and OD_{t1} are the OD_{730} values at t_0 and t_1 of the exponential growth phase, respectively.

Three parameters indicating the photosynthetic activity, namely, the chlorophyll *a* content, the maximum photochemical quantum yield of Photosynthesis system II (F_v/F_m), and the maximum relative electron transport rate ($rETR_{max}$), were analyzed every 3 days. The chlorophyll *a* content was measured according to Bland and Angenent (2016). The F_v/F_m and $rETR_{max}$ values in the cyanobacterial cells were analyzed using a Dual-PAM-100 fluorometer (Walz GmbH, Germany) after dark-adaption for 10 min. The rapid light curve was determined using a series of 20-s light exposures, and $rETR_{max}$ was estimated through curve-fitting calculations (Fu et al. 2012). The (F_v/F_m) was calculated as Eq. (2):

$$\frac{F_v}{F_m} = \frac{F_m - F_0}{F_m} \quad (2)$$

where F_0 is the minimum fluorescence intensity recorded at a low-frequency light pulse and F_m is the maximum

fluorescence intensity recorded at 600-ms blue saturation pulse.

On the last day of exposure, 100 mL of cyanobacterial culture was sampled from each flask and used for the analysis of biofuel production ability. The cyanobacterial cells were collected through centrifugation and freeze-dried under vacuum. The dry cyanobacterial cells were first extracted with a solvent system containing chloroform and methanol (2:1, v/v) and then extracted with 0.9% sodium chloride solution. The final extract was dried under a gentle flow of nitrogen and weighed to assess the lipid content and the lipid productivity. The fatty acids in *Synechocystis* sp. PCC 6803 were methylesterified into fatty acid methyl esters (FAMEs) according to the method developed by Lepage and Roy (1984) and determined through an Agilent (7890A-5975C) GC/MS system according to Anahas and Muralitharan (2015). The extraction of lipid, the calculation of lipid content and lipid productivity, and the transesterification process were described in detail in the Supplementary Material. Student's *t* test was used to determine the differences in the biomass, photosynthetic parameters, total lipid content, and fatty acid composition between each antibiotic-treated group and the solvent control using the SPSS software (version 23.0).

Determination of proteomic responses

Through tandem mass tag (TMT)–based quantitative proteomic analysis, the proteomic profiles of *Synechocystis* sp. PCC 6803 cells were compared between each antibiotic-treated group and the solvent control on the 9th day of antibiotic exposure. Twenty milliliters of culture medium containing cyanobacterial cells was taken from each replicate of each test group. The proteins in the cyanobacterial cells were extracted with SDT lysis buffer, digested through the filter-aided sample preparation (FASP) method (Wiśniewski et al. 2009), and labeled with the TMT 10-plex isobaric label reagent set (Thermo Scientific Corp., USA). Next, the labeled peptides were fractionated and quantitatively analyzed through a UHPLC/MS/MS system. Proteins were identified according to the database of *Synechocystis* sp. PCC 6803 in UniProt (updated on 30/11/2019). Differentially expressed proteins presented a fold change of higher than 1.5 or lower than 0.67 at $p < 0.05$ according to Student's *t* test. The procedures of protein extraction, digestion, labeling, fractionation, quantitative analysis, and protein identification are described in detail in the Supplementary Material. Each differentially expressed protein was annotated according to the biological process defined in Gene Ontology database. The protein-protein interaction (PPI) network of differentially expressed proteins was constructed through the software Cytoscape (version 3.8.0), according to the interaction information obtained from the STRING web-based tool (https://string-db.org). Functional modules composed of highly interconnected proteins ($p < 0.05$) within the PPI network were constructed through the ClusterONE plugin of Cytoscape.

Results and discussion

Influence of antibiotics on cyanobacterial growth

Figure 1 shows the variation in biomass in different test groups during the antibiotic exposure test. There was no significant difference ($p > 0.05$) in the OD_{730} values between the solvent control and each antibiotic-treated group during the first 3 days of exposure. The exponential growth started from the fourth day and lasted until the twelfth day of the culture period. After that point, the cyanobacteria growth gradually entered the stationary phase. Two antibiotics stimulated the growth of *Synechocystis* sp. PCC 6803 and significantly increased ($p < 0.05$) the biomass (represented by OD_{730}) in the exponential phase and the stationary phase. The specific growth rate in the solvent control was 0.186 ± 0.006 per day, while the growth rates in the SMZ-treated group and TET-treated group were 0.234 ± 0.005 and 0.212 ± 0.019 per day, respectively. Compared with that of the solvent control, the growth rate was increased by 25.8% and 14.0% following exposure to SMZ and TET, respectively. Cyanobacterial biomass was increased by 53.1% and 48.1% following exposure to SMZ and TET, respectively.

The proteomic responses provided insights into the mechanism of action of the target antibiotics in *Synechocystis* sp. PCC 6803 for growth stimulation (Table S1 in the Supplementary Material). SMZ induced the upregulation of a protein related to cell differentiation (spoIID) and two proteins related to cell division (minE and sepF) and consequently facilitated cell division. TET also stimulated cyanobacterial growth through the upregulation of spoIID and sepF. In addition, 28 differentially expressed proteins related to gene expression were identified in the SMZ-treated group, among which 25 proteins were upregulated. Similarly, 17 proteins related to gene expression and 3 proteins related to the regulation of gene expression were differentially expressed in the TET-treated group, and 17 of the above 20 proteins were upregulated. The above results suggested that both SMZ and TET showed a tendency to stimulate the gene expression process of *Synechocystis* sp. PCC 6803. Due to the positive correlation between cell division and gene expression (He et al. 2017), SMZ and TET may stimulate cyanobacterial growth through the promotion of gene expression.

In this study, 8 of 9 differentially expressed stress response proteins (rnhB, slr6040, sodB, lexA, slr1694, priA, nth, and sll0729) were upregulated in the SMZ-treated group, and 5 of 6 differentially expressed stress response proteins (sodB, mntB, slr1436, blaOXA-3, and pds) were upregulated in the TET-treated group. The upregulation of stress response

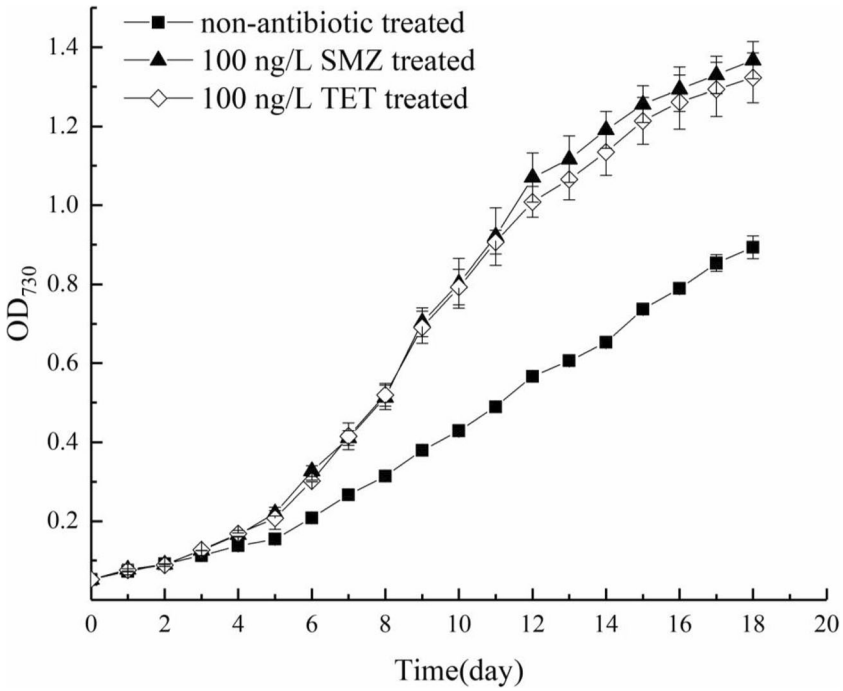

Fig. 1 Growth curves of *Synechocystis* sp. PCC 6803 under exposure to sulfamethoxazole (SMZ) and tetracycline (TET) (mean ± standard deviation of three replicates are shown for each OD value)

proteins also suggested that *Synechocystis* sp. PCC 6803 showed an adaptive stress response to antibiotic exposure. Previous studies suggested that increased growth was also an adaptive response to the stress induced by antibiotics at low exposure doses, which was characterized as hormesis (Liu et al. 2016; Zou et al. 2013). Similar growth stimulation effects were reported in another cyanobacterium (*M. aeruginosa*) exposed to spiramycin and ampicillin at a low exposure dose of 300 ng/L (Wang et al. 2019). Furthermore, Aderemi et al. (2018) reported the stimulatory effects of SMZ, erythromycin, and ciprofloxacin in the oil-producing microalga *Raphidocelis subcapitata* at exposure doses of 2–8 nM. Therefore, low concentrations of antibiotics have the potential to be introduced into the culture of oil-producing cyanobacteria/microalgae to increase biomass accumulation.

Notably, the stimulatory effects of antibiotics in cyanobacteria have an optimum concentration range. For instance, erythromycin was found to stimulate the growth of *Synechocystis* sp. at a test concentration of 10 μg/L while inhibiting the growth of *Synechocystis* sp. at test concentrations of 1, 100, and 1000 μg/L (Pomati et al. 2004). Our group also conducted a preliminary experiment to investigate the effects of SMZ and TET in *Synechocystis* sp. PCC 6803 at different test concentrations, and the most significant stimulatory effects were observed at the test concentration of 100 ng/L. Therefore, only the experimental results at an antibiotic concentration of 100 ng/L are shown in this study. When antibiotics are applied to the oil-producing cyanobacteria/microalgae culture system, the antibiotic concentration is supposed to be the essential operation parameter.

Influence of antibiotics on the photosynthetic activity

The stimulation of photosynthetic activity is a typical hormesis effect of antibiotics in cyanobacteria. For instance, erythromycin and ofloxacin were found to increase the F_v/F_m and $rETR_{max}$ values in cyanobacteria at the ng/L concentration level (Deng et al. 2015; Wan et al. 2015). According to Fig. 2, this study also observed increased photosynthetic activity in antibiotic-treated groups. In each test group, the chlorophyll *a* content increased with increasing culture time and varied with a similar trend as that of the biomass. In the exponential phase and stationary phase, the chlorophyll *a* content was significantly ($p < 0.05$) increased by 70.6–87.1% and 49.7–78.3% under exposure to SMZ and TET, respectively. In each test group, F_v/F_m and $rETR_{max}$ gradually increased in the exponential phase, reached the highest values on the twelfth day, and gradually decreased in the stationary phase. The SMZ and TET treatments significantly ($p < 0.05$) increased the F_v/F_m and $rETR_{max}$ values. During the whole exposure period, the F_v/F_m values were significantly increased by 21.5–51.5% and 24.9–48.8% under exposure to SMZ and TET, respectively. The $rETR_{max}$ values were significantly increased by 28.8–69% and 21.3–59.9% under exposure to SMZ and TET, respectively. These results indicated that both SMZ and TET stimulated photosynthetic activity through the promotion of light energy conversion efficiency (as indicated by F_v/F_m) and photochemical electron transport (as indicated by $rETR_{max}$). Furthermore, this study observed that SMZ and TET upregulated 22 and 23 proteins involved in the photosynthetic process, respectively (Table S1). This result was in consistence with a previous study, in which upregulated

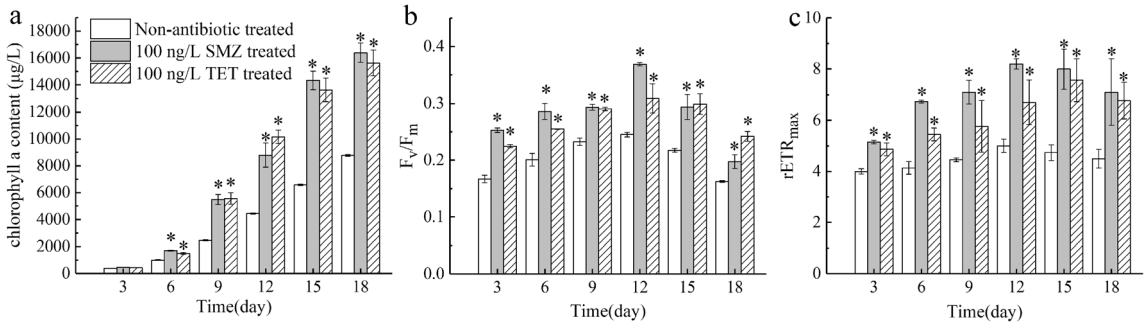

Fig. 2 Effects of sulfamethoxazole (SMZ) and tetracycline (TET) on **a** the chlorophyll a content, **b** the F_v/F_m value, and **c** the $rETR_{max}$ value of *Synechocystis* sp. PCC 6803 (mean ± standard deviation of three replicates are shown for each value; * indicates significant difference between each antibiotic-treated group and the solvent control at $p < 0.05$ according to Student's *t* test)

photosynthesis-related proteins were found to play an important role in tolerance to environmental stresses (Rowland et al. 2010).

Overexpression of photosynthesis-related proteins and increased photosynthetic activity may result in an increased energy supply for cyanobacterial growth and fatty acid biosynthesis (Paul 2013).

Influence of antibiotics on biofuel production ability

Previous studies observed that some environmental stresses could increase the lipid content in cyanobacteria. For instance, UV radiation and TiO_2 stresses were found to increase the lipid content in *Arthrospira* (*Spirulina*) *platensis* without affecting the biomass (Casazza et al. 2015). Ultrasonic treatment was found to increase the lipid content in oil-producing cyanobacteria, but the influence of ultrasonic treatment on biomass remained unclear (Ellison et al. 2019). The present study observed that antibiotic stress could simultaneously increase the biomass growth and lipid content. According to Table 1, antibiotic exposure significantly increased ($p < 0.05$) the dry weight of the cyanobacterial cells. This result was in consistence with the responses of the OD_{730} values. The lipid content (%DW) in the cyanobacterial cells of the solvent control was 14.71%, which was close to the results obtained by Touloupakis et al. (2015). Exposure to SMZ and TET significantly increased ($p < 0.05$) the lipid content to 19.10% and 16.98%, respectively. Furthermore, the lipid productivity in the SMZ-treated group and TET-treated group was 2.49- and 2.19-fold higher than that in the solvent control, respectively.

According to the proteomic responses to target antibiotics (Table S1), 4 proteins involved in energy derivation by oxidation of organic compounds (sdhB, norB, fumC, and malQ), 3 proteins involved in the catabolic process (gloB, gst1, and sll0654), and one protein involved in carbohydrate metabolic process (sll0529) were differentially expressed in the SMZ-treated group. Four proteins related to carbohydrate metabolic process (slr0453, pgm, fbp and nplT), 2 proteins involved in the catabolic process (sll0828 and gst1), and 2 proteins involved in energy derivation by oxidation of organic compounds (sdhB and norB) were differentially expressed in the TET-treated group. All of the above proteins were downregulated by the target antibiotics, suggesting that the two target antibiotics regulated the proteomic expression profile of *Synechocystis* sp. PCC 6803 towards an inhibition of carbohydrate catabolism. Furthermore, SMZ downregulated four carbohydrate transport proteins (slr1841, slr1908, sll1550, and slr0042), and TET downregulated six carbohydrate transport proteins (slr0042, sll1271, slr1908, sll0772, sll1550, and slr1841) in *Synechocystis* sp. PCC 6803. The downregulation of carbohydrate transport proteins may inhibit the transmembrane excretion of carbohydrates. Inhibited carbohydrate catabolism and suppressed carbohydrate excretion might contribute to the accumulation of carbohydrates (including fatty acids) in *Synechocystis* sp. PCC 6803 and consequently led to increased lipid productivity.

Twenty-six and 25 proteins related to the biosynthetic process were differentially expressed in the SMZ-treated group and the TET-treated group, respectively (Table S1). The proteomic responses suggested that exposure to target antibiotics may cause an alteration in the biosynthetic pattern of cellular substances. The altered composition of fatty acids in each antibiotic-treated group compared with the solvent control further verified this hypothesis. The fatty acids in *Synechocystis* sp. PCC 6803 are methyl-esterified into FAMEs and quantified by GC/MS, as shown in Table 1. Eight fatty acids were identified in the solvent control, among which palmitic acid (C16:0) and linoleic acid (C18:2) were major components. In each antibiotic-treated group, one more fatty acid, tetradecanoic acid (C14:0), was identified. The proportion of linolenic acid (C18:3) in the three test groups was lower than 12%, which met the requirements for a qualified biofuel according to the European Standards (EN 2004). Compared with the solvent control, SMZ and TET exposure significantly ($p < 0.05$) increased the proportions of monounsaturated fatty acids (MUFAs), while TET exposure significantly reduced ($p < 0.05$) the proportion of saturated fatty acids (SFAs) and

Table 1 Comparison of total lipid content and FAME composition between the solvent control and each antibiotic-treated group (mean ± standard deviation of three replicates is shown for each value; FAMEs were obtained through the esterification of fatty acids in *Synechocystis* sp. PCC 6803)

Parameters		Test groups		
		Solvent control	100 ng/L SMZ-treated group	100 ng/L TET-treated group
Dry weight (DW) of cyanobacteria (g)		0.0177 ± 0.0194	0.0339 ± 0.0004*	0.0329 ± 0.0022*
Lipid content (%DW)		14.71	19.10*	16.98*
Lipid productivity (mg/L/d)		2.89	7.19*	6.21*
FAME composition (%)	C14:0	ND	0.76	0.62
	C15:0	0.61	2.98	3.83
	C16:0	65.29	62.48	58.89*
	C16:1	5.79	8.43*	9.75
	C17:0	0.94	ND	0.34
	C18:0	1.72	1.24	2.01
	C18:1	1.79	2.21*	3.17*
	C18:2	21.98	19.04	19.01*
	C18:3	1.88	2.87*	2.38*
	SFA[a]	68.57	66.69	65.07*
	UFA[b]	31.43	32.55*	34.31*
	MUFA[c]	7.58	10.63*	12.92*
	PUFA[d]	23.86	21.92	21.39*

*Significant difference between each antibiotic-treated group and the solvent control at $p < 0.05$ according to Student's *t* test

ND; not detected

[a] Saturated fatty acid

[b] Unsaturated fatty acid

[c] Monounsaturated fatty acid

[d] Polyunsaturated fatty acid

polyunsaturated fatty acids (PUFAs). The length and unsaturation degree of fatty acid chains and the composition of individual fatty acids are key factors that determine the oxidative stability and physical characteristics of biofuel (Deshmukh et al. 2019a, b; Islam et al. 2013). The biofuel containing more MUFAs and less SFAs and PUFAs presents better combustion performance (Adu-Mensah et al. 2019). In addition, oleic acid (C18:1) is considered to be an optimal fatty acid with good oxidative stability and cold-flow properties (Singh et al. 2019). The production of oleic acid (C18:1) in the cyanobacterial cells was significantly ($p < 0.05$) stimulated by the two target antibiotics. The above results indicated that both SMZ and TET improved the combustion performance of the biofuel produced by *Synechocystis* sp. PCC 6803. The above results indicated that antibiotics have good prospects for application in cyanobacterial biofuel production.

Interactions of differentially expressed proteins in the antibiotic-treated groups

SMZ upregulated 83 proteins and downregulated 51 proteins in *Synechocystis* sp. PCC 6803, whereas TET upregulated 73 proteins and downregulated 44 proteins. There were more upregulated proteins than downregulated proteins, which further verified the adaptive response to the target antibiotics at the proteomic level. Although the two target antibiotics have different antibacterial mechanisms (González-Pleiter et al. 2013), both of them affected 13 biological processes in *Synechocystis* sp. PCC 6803, namely, biosynthetic process, catabolic process, carbohydrate metabolic process, cell differentiation, cell division, energy derivation by oxidation of organic compounds, nitrogen compound metabolic process, gene expression, photosynthesis, oxidation-reduction process, methylation, transport, and response to stimulus. In addition, 68 differentially expressed proteins were commonly shared by the SMZ-treated group and the TET-treated group. These results suggested that different antibiotics may regulate *Synechocystis* sp. PCC 6803 in similar patterns.

According to the PPI networks shown in Fig. 3, 129 of the 134 differentially expressed proteins participate in 1235 pairs of interactions in the SMZ-treated group, and 113 of the 117 differentially expressed proteins participate in 935 pairs of interactions in the TET-treated group. In the SMZ-treated group, 27 hub proteins with high connection degrees (≥ 30 interacting proteins) were recognized (Table 2), which were regarded as the most essential proteins in the PPI network. The

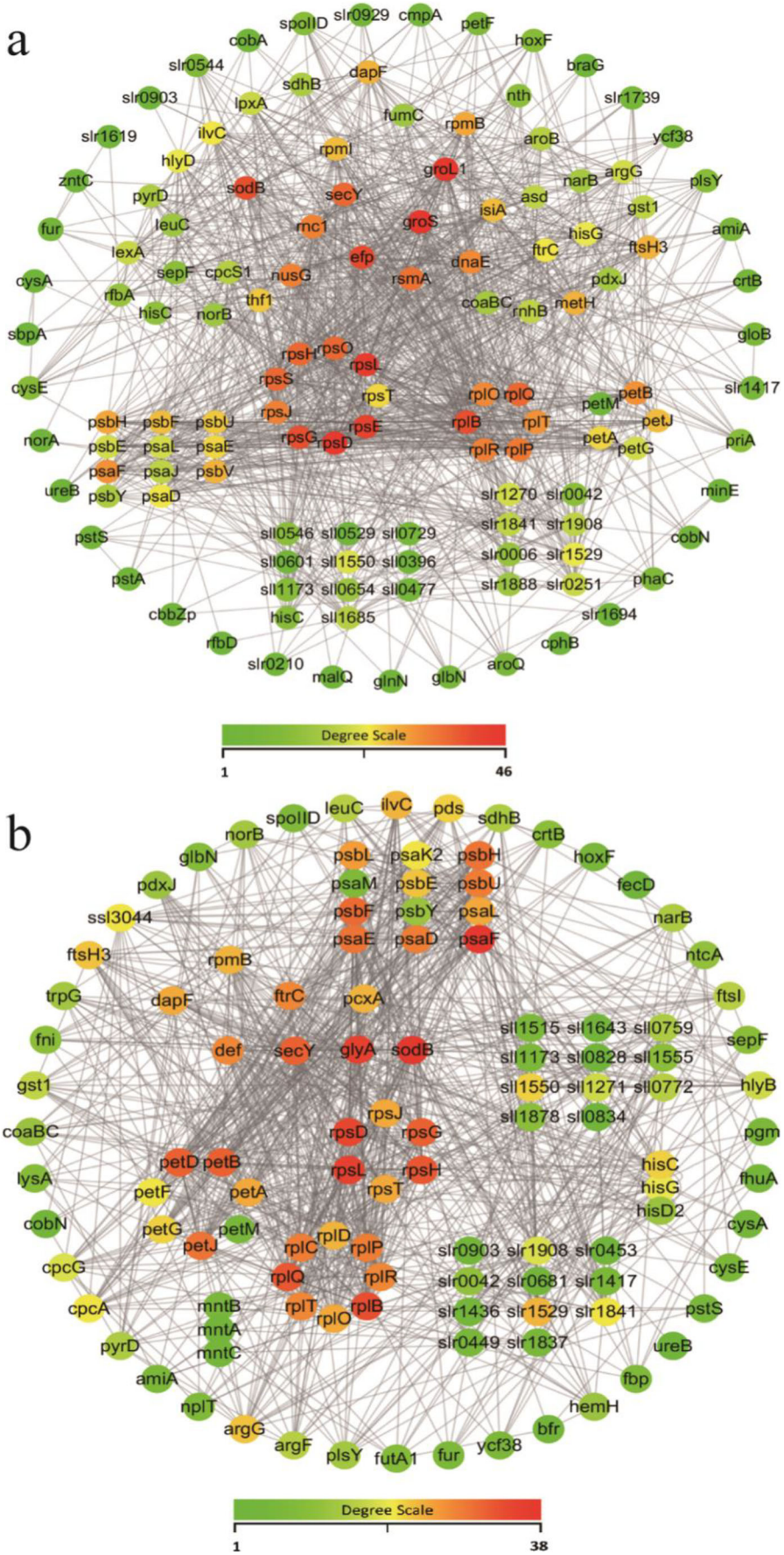

Fig. 3 The interaction networks of the differentially expressed proteins in *Synechocystis* sp. PCC 6803 exposed to 100 ng/L of **a** sulfamethoxazole (SMZ) and **b** tetracycline (TET), constructed according to the STRING database. The protein symbols are shown in Table S1 in the Supplementary Material. The connection degree of each protein is indicated by color

Table 2 Hub proteins in the PPI networks of two antibiotic-treated groups

Test group	Uniprot accession	Protein name	Protein Symbol	Degree
SMZ-treated group	Q05972	60 kDa chaperonin 1	groL1	46
	Q05971	10 kDa chaperonin	groS	46
	P48939	30S ribosomal protein S4	rpsD	45
	P74230	30S ribosomal protein S12	rpsL	44
	P73304	30S ribosomal protein S5	rpsE	42
	Q55119	Elongation factor P	efp	41
	P74229	30S ribosomal protein S7	rpsG	40
	P73317	50S ribosomal protein L2	rplB	40
	P77968	Superoxide dismutase [Fe]	sodB	40
	P73316	30S ribosomal protein S19	rpsS	37
	P73307	30S ribosomal protein S8	rpsH	37
	P77964	Protein translocase subunit SecY	secY	37
	P73296	50S ribosomal protein L17	rplQ	37
	P72866	30S ribosomal protein S15	rpsO	37
	P72666	Ribosomal RNA small subunit methyltransferase A	rsmA	36
	P73313	50S ribosomal protein L16	rplP	35
	P74226	30S ribosomal protein S10	rpsJ	34
	P73305	50S ribosomal protein L18	rplR	34
	P36265	Transcription termination/antitermination protein NusG	nusG	34
	P74368	Ribonuclease 3 1	rnc1	34
	P73303	50S ribosomal protein L15	rplO	33
	P74750	DNA polymerase III subunit alpha	dnaE-N	33
	P29256	Photosystem I reaction center subunit III	psaF	32
	P48957	50S ribosomal protein L20	rplT	32
	Q57038	Cytochrome b6	petB	32
	P72851	50S ribosomal protein L28	rpmB	30
	P14835	Photosystem II reaction center protein H	psbH	30
TET-treated group	P29256	Photosystem I reaction center subunit III	psaF	38
	P77968	Superoxide dismutase [Fe]	sodB	38
	P74230	30S ribosomal protein S12	rpsL	35
	P77962	Serine hydroxymethyltransferase	glyA	35
	P48939	30S ribosomal protein S4	rpsD	34
	P73317	50S ribosomal protein L2	rplB	33
	P73307	30S ribosomal protein S8	rpsH	32
	P73296	50S ribosomal protein L17	rplQ	32
	P74229	30S ribosomal protein S7	rpsG	31
	P77964	Protein translocase subunit SecY	secY	31
	Q57038	Cytochrome b6	petB	31
	P27589	Cytochrome b6-f complex subunit 4	petD	31
	P09191	Cytochrome b559 subunit beta	psbF	30

hub proteins included 20 proteins (74.1%) related to gene expression (rpsD, rpsL, rpsE, efp, rpsG, rplB, rpsS, rpsH, rplQ, rpsO, rsmA, rplP, rpsJ, rplR, nusG, rnc1, rplO, dnaE-N, rplT, and rpmB), 3 proteins (11.1%) involved in stress response (groL1, groS, and sodB), 3 proteins (11.1%) involved in photosynthesis (psaF, petB, and psbH), and one protein (3.7%) involved in transport (secY). Most of the hub proteins (23 of 27) were upregulated. Similar results were also observed in the PPI network of the TET-treated group. Thirteen hub proteins were recognized in the PPI network,

namely, 4 proteins (30.8%) involved in the photosynthesis process (psaF, petB, petD, and psbF), 6 proteins (46.1%) involved in gene expression (rpsL, rpsD, rplB, rpsH, rplQ, and rpsG), one protein (7.7%) involved in biosynthetic process (glyA), one protein (7.7%) involved in response to stimulus (sodB), and one protein (7.7%) involved in transport (secY). Twelve of the 13 hub proteins in the TET-treated group were upregulated. Elevated gene expression and stimulated photosynthesis played essential roles in the response to the stress caused by antibiotic exposure.

A significantly enriched ($p < 0.05$) functional module containing highly interconnected proteins was identified in the PPI network of the SMZ-treated group (Fig. S1 in the Supplementary Material). In this module, 20 upregulated proteins involved in the photosynthesis process (psbH, psbF, psbU, psbE, psaL, psaE, psaF, psaJ, psbV, psbY, psaD, petB, petM, petA, petJ, petG, thf1, isiA, slr1739, and ftrC) were closely correlated with an upregulated protein involved in the biosynthetic process (crtB), a downregulated protein involved in the catabolic process (sll0654), and four downregulated carbohydrate transport proteins (sll1550, slr1908, slr1841, and slr0042). Similar to the SMZ-treated group, in the functional module of the TET-treated group, close correlations among 21 upregulated photosynthesis-related proteins (psbL, psaK2, psbH, psaM, psbE, psbU, psbF, psbY, psaL, psaE, psaD, psaF, petF, petD, petB, petA, petJ, petG, cpcA, cpcG, and ftrC), one upregulated biosynthesis-related protein (hemH), and six downregulated carbohydrate transport proteins (slr0042, sll1271, slr1908, sll1550, sll0772, and slr1841) were observed (Fig. S2 in the Supplementary Material). This result indicated that the stimulation of photosynthesis by antibiotics may further trigger the altered pattern of carbohydrate metabolism and transport. The hub proteins and functional modules in the two PPI networks further verified that the two different antibiotics shared similar regulation mechanisms for the biofuel production of *Synechocystis* sp. PCC 6803 at the proteomic level.

Environmental impact on the utilization of antibiotics in biofuel production

Antibiotics have been widely used in agriculture to promote the growth of plants and animals (Cerqueira et al. 2019; Zhao et al. 2020). However, the discharge of antibiotic residues was proved to induce antibiotic resistance in environmental bacteria (Cerqueira et al. 2019). In this study, SMZ and TET were highly effective in improving the lipid productivity of oil-producing cyanobacteria, which could consequently increase economic efficiency. However, the potential adverse environmental impacts during the utilization of antibiotics should also be considered. Previous studies observed that antibiotic resistance in bacteria correlated with the differential expression of proteins related to carbohydrate metabolism, transport, and biosynthesis (Jones-Dias et al. 2017; Opoku-Temeng et al. 2019; Zuñiga-Navarrete et al. 2019). These findings were in accordance with the proteomic responses of *Synechocystis* sp. PCC 6803 under exposure to antibiotics. In addition, the overexpression of ribosomal proteins plays an important role in bacterial resistance to antibiotics (Vranakis et al. 2014). In this study, ribosomal proteins also accounted for a large proportion of the upregulated proteins in *Synechocystis* sp. PCC 6803 under exposure to antibiotics. The above results suggested that cyanobacteria showed antibiotic resistance mechanisms similar to those of bacteria. Recent studies have suggested that antibiotic resistance in cyanobacteria results in an increased bloom-forming ability, which poses a threat to aquatic ecosystems (Le Page et al. 2019). To eliminate the spread of antibiotic resistance in the environment, antibiotic residues should be completely removed from the culture medium after utilization in the culture of oil-producing cyanobacteria. Various advanced oxidation technologies are effective for the removal of antibiotics, and the elimination of antibiotic residues is easy to accomplish (Eniola et al. 2019; Wu et al. 2020). Furthermore, photolysis and hydrolysis of antibiotics could not be avoided during the cyanobacterial culture process (Biošić et al. 2017), and the influence of antibiotic degradation products on oil-producing cyanobacteria and the environment deserves further investigation.

Conclusions

This study observed beneficial effects of sulfamethoxazole and tetracycline on the biomass growth, lipid content, photosynthetic activity, and fatty acid composition of *Synechocystis* sp. PCC 6803 at the ng/L concentration level. The proteomic responses indicated that upregulation of gene expression-related proteins and photosynthesis-related proteins contributed the most to the stimulatory effects of the target antibiotics. The upregulated photosynthesis-related proteins further triggered the differential expression of proteins related to carbohydrate metabolism and transport, which resulted in increased lipid accumulation and altered fatty acid composition in cyanobacterial cells. The introduction of antibiotic stress into the oil-producing cyanobacterial culture system showed good prospects for increasing biofuel productivity.

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