Influence of tris(2,3-dibromopropyl) isocyanurate on the expression of photosynthesis genes of *Nannochloropsis* sp.

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ABSTRACT

The proteins encoded by *psaA* and *psaB* form a heterodimer, an essential compound of photosystem; while the protein encoded by *psbC* binds with chlorophyll *a* in photosystem II, serving as antennae in photosystem. Here we report that a heterocyclic brominated flame retardant, tris(2,3-dibromopropyl) isocyanurate (TBC), inhibited the expression of *psaA* and *psbC*, then leads to the decrease of *Nannochloropsis* sp.'s growth biomass. TBC exposure inhibited the expression of *psaA* and *psbC* at 10, 100 ng/mL slightly and 1000 ng/mL significantly. In addition, TBC was found to slow down the growth of *Nannochloropsis* sp. at concentrations ranging from 10 to 1000 ng/mL. These results indicated that TBC influenced both photosynthesis and growth performance of *Nannochloropsis* sp.

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1. Introduction

Tris(2,3-dibromopropyl) isocyanurate (TBC) has a wide range of applications, for example, in polyolefin, polyphenyl alkene, unsaturated polyester and synthetic rubber and fibers (Xiong, 1999). In China, TBC has been widely utilized in polymer products since 1980s. Its annual production of TBC in China was >500 metric tons in 1990s (Cao, 1996; Lv et al., 1999). TBC is a heterocyclic hexabrominate with high octanol water partition (K_{ow}) and octanol air partition (K_{oa}) coefficient. The high K_{ow} ($\log K_{ow} = 7.37$) indicates that TBC is easily accumulative in both sediment and organism's body. The exact statistics of the world production of TBC is not available at present; however, its residue in environment must be vigilantly attended. Actually, TBC is a BFR (brominated flame retardant) with a high thermostability, a low viscosity and a tendency to discoloration and corrosiveness. It has been found in many environments (Johnson et al., 2013; Ruan et al., 2009), and its residue is continuously increasing recently (Cristale et al., 2013; Wang et al., 2013).

TBC exposure disrupts the development and endocrine of zebrafish embryo, damages their mitochondria and interferes in their gas bladder

inflation (Li et al., 2011; Zhang et al., 2011). It has been speculated to be toxic to neuronal cells (Qu et al., 2011). Algae are widely distributed in lakes and seas, which are sensitive to contaminants. Algae have been chosen as the most important indicators in aquatic environmental assessment (Chen et al., 2011; Sidharthan et al., 2002; Wu et al., 2009). As an intensively studied microalga, *Nannochloropsis* sp. is abundant in aquatic environment and easy to culture. In order to investigate the relationship of TBC concentration with growth and photosynthesis of *Nannochloropsis* sp., this work had studied the influence of TBC exposure concentration to two important photogenes of *Nannochloropsis* sp.

The proteins encoded by *psaA* and *psaB* form a heterodimer, an essential compound of photosystem I (Hall and Rao, 1999); while the protein encoded by *psbC* binds with chlorophyll *a* in photosystem II, serving as antennae in photosystem II (Bricker, 1990). In this study, the expression of two *Nannochloropsis* sp. photosynthesis essential genes, *psaA* and *psbC*, were analyzed in order to assess the damaging risk of waterborne TBC to aquatic life.

2. Materials and methods

2.1. Chemicals and reagents

Tris(2,3-dibromopropyl) isocyanurate (TBC, purity 97%) was purchased from Sigma-Aldrich (USA). Dimethylsulphoxide (DMSO) was purchased from Merck KGaA (Germany). Stock TBC (100 µg/mL) was prepared by dissolving TBC monomer in DMSO; while working TBC was diluted from the stock solution with DMSO. No observed effect

Abbreviations: *psaA*, photosystem I P700 chlorophyll *a* apoprotein A1; *psaB*, photosystem I P700 chlorophyll *a* apoprotein A2; *psbC*, photosystem II CP43 chlorophyll apoprotein; bp, base pair(s); EC₅₀, 50% effective concentration; cDNA, DNA complementary to RNA.

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concentration (NOEC) of DMSO was determined ahead of TBC treatment (Aldenberg and Slob, 1993). In order to study the effect of TBC to *Nannochloropsis* sp. more accurately, the NOEC of DMSO had been added each experiment. Working TBC concentrations were fixed based on the preliminary test using the TBC with the ranges from 5 to 5000 ng/mL. In the present work, the selected TBC concentrations included 10, 100 and 1000 ng/mL.

2.2. TBC treatment

Nannochloropsis sp. was obtained from The First Institute of Oceanography of State Oceanic Administration of China. The 8000 mL alga that used in experiment was cultured in two containers (Erlenmeyer flasks, 5000 mL in volume) with 4000 mL f/2 medium (Guillard and Ryther, 1962), respectively, under cool-white fluorescent lamps (4000 lx) following a rhythm of 14 h light and 10 h dark and at 24 ± 1 °C in order to obtain the algal biomass for TBC exposure. The medium was prepared with seawater, which was filtrated through a membrane of 0.45 µm pore size and autoclaved at 121 °C for 20 min ahead of use. The alga at the middle of exponential growth phase was equally divided into 8 Erlenmeyer flasks (2000 mL in volume) and mixed with DMSO (final concentration 0.63%, the NOEC). One flask was used as the control; while the remaining 3 were mixed with 10, 100 and 1000 ng/mL TBC, respectively. Two parallel samples for every flask were set in this experiment. The alga in all the 8 flasks (2000 mL in volume) was cultured under the condition set for obtaining the algal biomass for TBC exposure. The number of *Nannochloropsis* sp. cells was counted every another day. About 60 mL of algal culture was sampled at 0, 12, 24, 48 and 72 h, respectively, with cells precipitated by centrifuging at 4 °C and 5000 rpm for 15 min and stored at -80 °C.

2.3. Expression analysis

Total RNA was extracted with Trizol reagent (Beijing CoWin Bioscience Co., Ltd., China) according to the manufacturer's instructions, and stored at -80 °C. The first-strand cDNA was synthesized with RT-PCR kit (Transgen, Beijing, China) on a TP 600 Thermal Cycler Dice PCR (Takara Bio. Inc., Japan) following the manufacturer's instructions and stored at -20 °C. Primers were designed with Primer Premier 5.0 (<http://blast.ncbi.nlm.nih.gov/Blast.cgi>) and synthesized by Shanghai Sunny Biotechnology Co., Ltd. Primers (Table 1) were used to quantify the transcript of *psaA* and *psbC*. In order to qualify the primers, the core region of *psaA* and *psbC* was amplified by predenaturing at 94 °C for 5 min, followed by 30 cycles of denaturing at 94 °C for 45 s, annealing at 55 °C for 45 s, and extending at 72 °C for 45 s, and an extra extension at 72 °C for 5 min. The abundance of *psaA* (108 bp) and *psbC* (187 bp) transcript was determined with real-time quantitative PCR (RT-PCR) and TransStart™ Top Green qPCR SuperMix Kit (Transgen, China). The abundance of β -actin gene transcript was used as the internal control.

2.4. Statistical analysis

The abundance of gene transcripts and the number of cells were expressed as mean $\pm$ SD ($n = 3$). Analysis of variance (ANOVA) and

Table 1
Primers used in this study.

Primer	Sequences (5' → 3')	Tm (°C)
F1	TCCAATCTATGGCGTGCTGAA	59.90
R1	AATGAAACCAACCTGCGAACA	60.50
F2	CCATTCTTTGTTATGACTGGCGT	64.40
R2	CGGCTGCATTAACCGTAGGACTT	67.70
β -Actin F	ACGGCGTCTCACACTG	65.66
β -Actin R	GCTTGACCATCCAATCGG	63.80

Fig. 1. The influence of TBC on the growth of *Nannochloropsis* sp.

Student's t test were conducted with GraphPad software5.0 (GraphPad Prism).

3. Results and discussion

3.1. Growth performance of *Nannochloropsis* sp.

The growth rate of *Nannochloropsis* sp. was significantly inhibited by TBC (Fig. 1) at concentrations ranging from 10 to 1000 ng/mL in a concentration dependent manner, the influence became significant with the concentration of TBC increasing. The EC₅₀ of TBC has been determined (data not shown); however, such a concentration protruded what we may meet in environment. Therefore, the influence of TBC on the growth of *Nannochloropsis* sp. was determined in a concentration range as were selected. Compared with the control (0 ng/mL), TBC treatment (10, 100 and 1000 ng/mL) inhibited the growth of *Nannochloropsis* sp. obviously.

3.2. Expression analysis of *psaA*

The results were presented in Fig. 2. The expression level of *psaA* in control (0 ng/mL) increased during 0 to 48 h, but slightly decreased at 72 h. Contrarily, that of *psaA* decreased significantly when the alga was treated with TBC (10, 100 and 1000 ng/mL) ($P < 0.05$) from 12 to 72 h, reaching the minimum at 48 h. The reduction of the expression in TBC treated groups reached 71% (10 ng/mL), 91% (100 ng/mL) and 94% (1000 ng/mL), respectively. TBC downregulated the transcription of *psaA* gene, the higher the concentration the more obvious the regulation. Although the expression level of *psaA* increased slightly at 72 h, it was still lower 50.5% (10 ng/mL), 68.8% (100 ng/mL), and 82.8% (1000 ng/mL), respectively, than that of control.

The first response of plants to stresses is to protect the cells from possible abiotic damage by weakening the photosynthesis (Bilgin et al., 2010). The photosynthetic activity of plants was reported to be significantly reduced under diverse stresses including temperature, light, heavy metals, toxic substances and among others (Dawson and Dennison, 1996; Han et al., 2003; Li et al., 2005). Besides, some

Fig. 2. The abundance of *psaA* gene transcript in *Nannochloropsis* sp. treated with different concentrations of TBC. The abundance was mean $\pm$ SD ($n = 3$).

Fig. 3. The abundance of *psbC* gene transcript in *Nannochloropsis* sp. treated with different concentrations of TBC. The abundance was mean $\pm$ SD ($n = 3$).

mutations caused by diverse mutagens including TBC may also reduce the photosynthetic activity (Rochaix, 1996). *PsaA* is one of the key gene encoding proteins in reaction center PS I. Therefore, it is understandable that the expression level of *psaA* was inhibited when *Nannochloropsis* sp. was exposed to TBC.

3.3. Expression analysis of *psbC*

From 0 to 24 h, the abundance of *psbC* gene transcript of the control increased continually, and then decreased slightly after 24 h (see in Fig. 3). The abundance of *psbC* gene transcript in treatment groups (10, 100 and 1000 ng/mL) decreased significantly ($P < 0.05$) from 0 to 48 h, but increased at 72 h, however, such abundance was always lower 81.2% (10 ng/mL), 84.4% (100 ng/mL), and 80.2% (1000 ng/mL), respectively, than that of control. Once treated with TBC, the minimum abundance of *psbC* gene transcript appeared at 48 h. The reduction of the expression level of *psbC* reached 65% (10 ng/mL), 75% (100 ng/mL) and 85% (1000 ng/mL) of the control, respectively. Therefore, the transcription of *psbC* gene was downregulated by TBC. When TBC was 1000 ng/mL, such inhibition reached the maximum.

The protein encoded by *psbC* binds with chlorophyll *a* in photosystem II, transporting excitation energy there. According to the experiment results, these photosynthesis genes (*psbC*, *psaA*) had the similar mode of expression in *Nannochloropsis* sp. when exposed to TBC. So the scenarios used to explain for *psaA* may aid to our understanding that TBC reduces the abundance of *psbC* gene transcript in *Nannochloropsis* sp. once being exposed to TBC.

4. Conclusion

The influence of TBC on the growth performance of *Nannochloropsis* sp. and the expression of *psaA* and *psbC* were determined in this study. The growth of *Nannochloropsis* sp. was inhibited by TBC in a concentration dependent manner. TBC also inhibited the expression of *psaA* and *psbC*. TBC had an impact on the growth and photosynthetic gene (*psaA*, *psbC*) expression of *Nannochloropsis* sp.

Conflict of interest

None.

Acknowledgments

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