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Mechanistic insights into hormesis induced by erythromycin in the marine alga *Thalassiosira weissflogii*

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**A R T I C L E   I N F O**

*Hormetic effects*  
*Antibiotic agents*  
*Marine diatoms*  
*Systems biology*  
*Ribosome*  
*Total soluble protein*

**A B S T R A C T**

Erythromycin (ERY) is a typical macrolide antibiotic with large production and extensive use on a global scale. Detection of ERY in both freshwaters and coaster seawaters, as well as relatively high ecotoxicity of ERY have been documented. Notably, hormesis has been reported on several freshwater algae under ERY stress, where growth was promoted at relatively lower exposures but inhibited at higher treatment levels. On the contrary, there is limited information of ERY toxicity in marine algae, hampering the risk assessment on ERY in the coaster waters. The presence of hormesis may challenge the current concept of dose-response adopted in chemical risk assessment. Whether and how exposure to ERY can induce dose-dependent toxicity in marine algae remain virtually unknown, especially at environmentally relevant concentrations. The present study used a model marine diatom *Thalassiosira weissflogii* (*T. weissflogii*) to reveal its toxicological responses to ERY at different biological levels and decipher the underlying mechanisms. Assessment of multiple apical endpoints shows an evident growth promotion following ERY exposure at an environmentally relevant concentration (1 µg/L), associated with increased contents reactive oxygen species (ROS) and chlorophyll-a (Chl-a), activated signaling pathways related to ribosome biosynthesis and translation, and production of total soluble protein. By contrast, growth inhibition in the 750 and 2500 µg/L treatments was attributed to reduced viability, increased ROS formation, reduced content of total soluble protein, inhibited photosynthesis, and perturbed signaling pathways involved in xenobiotic metabolism, ribosome, metabolism of amino acid, and nitrogen metabolism. Measurements of multiple apical endpoints coupled with de novo transcriptomics analysis applied in the present study, a systems biology approach, can generate detailed mechanistic information of chemical toxicity including dose-response and species sensitivity difference used in environmental risk assessment.

1. Introduction

Erythromycin (ERY), a typical macrolide antibiotic, is commonly used to treat bacterial infections since the 1950s (Berthet et al., 2010). Following the administration of ERY in humans or animals, most of the parent compound and its metabolites are excreted via feces and urine, before being received by sewage treatment plants (Schafhauser et al., 2018); however, the removal rates of ERY were approximately 30%, and it was not uncommon that the detected levels of ERY in effluents exceeded that in influents (Leung et al., 2012). Effluents from hospitals and aquaculture pounds can be another point sources of ERY (Schafhauser et al., 2018). ERY has been detected in freshwater and marine systems at the maximum concentration of 75.5 and 1.9 µg/L, respectively (Lin and Tsai, 2009; Minh et al., 2009). Moreover, exposure to ERY can introduce undesired impacts on bacteria, algae, invertebrates, fish, etc. (Ji et al., 2012; Schafhauser et al., 2018), and thus ERY is recognized as one of the prioritized pollutants in aquatic environments (see reviews in Mo et al., 2022).

Algae are more vulnerable to the exposure and hazards imposed by antibiotics in aquatic systems such as ERY. Several studies have been
conducted on freshwater algal species (e.g., *R. subcapitata*, *M. aeruginosa*, *M. flos-aquae*) to evaluate the toxicity of ERY (Campa-Cordova et al., 2006; Guo et al., 2021; Wang et al., 2019). Indeed, cyanobacteria appear to be highly sensitive to ERY toxicity, and the 6-day EC50 for *M. aeruginosa*, *M. wesenbergii*, *S. leopoldiana*, *A. cylindrica*, *Nostoc* sp., *Synechococcus* sp., *A. flos-aquae*, and *A. variabilis* was 23, 23, 35, 160, 200, 230, 270, 430 µg/L, respectively (Ando et al., 2007). Besides, for green algae, *R. subcapitata* (4-day EC50: 44 – 160 µg/L) is generally more sensitive to ERY toxicity than *D. tertiolecta* (4-day EC50 = 5750 µg/L) and *C. vulgaris* (4-day EC50: 26330 – 84100 µg/L) (Adremi et al., 2021; Ma et al., 2021; Machado and Soares, 2019b; Zhang et al., 2021). These studies showed that exposure to higher levels (from sub-µg/L to mg/L) of ERY reduced growth performance, which is associated with modulation of structure and activity of photosystems (e.g., inhibition of chlorophyll biosynthesis, pigment production, photosynthesis), alterations in energy metabolism (e.g., hyperpolarization of mitochondrial membrane, reduction in electron transport, etc.), induction of oxidative stress (e.g., excessive ROS production, elevated lipid peroxidation, and altered enzymatic or non-enzymatic antioxidants), alterations in cell size, and/or decrease in cell viability (Deng et al., 2014; González-Pleiter et al., 2013; Liu et al., 2011; Machado and Soares, 2019a, 2019b; Nie et al., 2013; Wang et al., 2021; Wu et al., 2020). However, these studies only looked at apical endpoints at the population, cellular and/or biochemical levels, but neither the global gene expression patterns nor the molecular mechanisms were revealed, especially at environmentally relevant exposure concentrations.

It is of particular concern that ERY can impose hormetic effects in cyanobacteria including *M. aeruginosa* and *M. flos-aquae*, where the growth performance was enhanced at relatively low ERY exposure levels but reduced at higher ERY exposure levels (Wan et al., 2015; Zhang et al., 2020). This may have ecological and human health consequences such as the low-dose enhancement of harmful microorganisms, including bacteria, fungi, and algae, degrading the water quality and facilitating the development and spread of diseases (Agathokleous et al., 2018, 2020). Importantly, the presence of hormesis challenges the current concept of environmental risk assessment, as chemical dose–response models dominantly adopted in environmental risk assessment by regulatory agencies are either threshold models or linear non-threshold models using high exposures, both of which refute the possibility of promoted actions at low exposure levels as in hormesis (Calabrese and Baldwin, 2002; Calabrese, 2013; Agathokleous et al., 2020).

Hormesis, a biphasic dose response phenomenon, is qualitatively characterized by stimulation under low-dose treatments and inhibition under high-dose treatments. The dose response curves of hormesis are typically inverted U-shape or J-shape depending on the endpoints measured (Calabrese, 2013). Additionally, the quantitative features of hormesis can be characterized by the maximum stimulation (~130–160% compared to the control) (Calabrese and Baldwin, 2002). Antibiotics have been reported to induce hormesis in algae and high plants, but most of these studies only measured endpoints at the individual (cellular) and/or physiological (biochemical) levels, but rarely at the molecular level (Agathokleous et al., 2018). Thus, molecular mechanisms underpinning hormesis revealed by omics technologies are particularly lacking and warranted. Notably, Guo et al. (2021) recently found that ERY exposure induced hormesis in *R. subcapitata*, in which the growth promotion was linked to the modification of xenobiotic metabolism, DNA replication, and metabolism of porphyrin and chlorophyll at the transcriptional level. Metabolomics analysis showed that ABC transporters, metabolism of nucleosides, and energy metabolism were responsible for the observed hormesis (Mo et al., 2023). However, physiological (biochemical) apical endpoints, such as cell viability, photosynthesis, ROS production, soluble protein content, etc., were not determined in these studies, and thus the links between physiological responses and global gene expression patterns remain undereexplored.

Unlike their freshwater counterparts, although ERY has been detected in coastal seawater, limited ERY toxicity information is available for marine algae, hampering the risk assessment and environmental surveillance of ERY in the marine coaster waters. For instance, exposure of *P. yezoensis* to high levels of ERY (e.g., 7.5 g/L) reduced Chl-a fluorescence and photochemical efficiency of PSII (Fv/Fm) while increasing the non-photochemical quenching (Oh et al., 2005). Marine diatoms (3-day EC50 < 1000 µg/L for *C. closterium*, *C. gracilis*, and *P. tricornutum*) showed relatively less sensitive to ERY toxicity, and inhibited growth, reduced Fv/Fm, and elevated ROS formation were observed in a marine diatom *P. tricornutum* exposed to high levels of ERY (Sendra et al., 2018). However, whether and how exposure to ERY can induce dose-dependent toxicity in marine algal species remain obscure, especially at environmentally relevant concentrations.

Diatoms are a predominant group of microalgae in marine ecosystems, in which they contribute approximately 40% of the global carbon fixation and serve as food for zooplankton (Armbrust et al., 2004). Particularly, *Thalassiosira weisflogii* (*T. weisflogii*) is a commonly used model marine diatom for ecotoxicological studies and biomonitoring due to its global distribution, ecological importance, rapid growth, good sensitivity, and easy cultivation (Araújo and Souza-Santos, 2013; US EPA, 1987).

In this study, using *T. weisflogii* as a model marine diatom, it is hypothesized that ERY exposure causes hormetic effects in *T. weisflogii* through perturbing key signaling pathways and biochemical processes involved in exobiocet metabolism, photosynthesis, energy metabolism, and DNA replication-coupled repair. A holistic assessment on ERY to *T. weisflogii* was performed to assess the toxicological responses at the population (growth performance), physiological (photosynthesis, ROS production, and metabolomic activity), and transcriptional (*de novo* transcriptome) levels.

2. Materials and methods

2.1. Chemical preparation and algae cultivation

A marine diatom *T. weisflogii* (CCMP 1587) was obtained from Provasoli-Guillard National Centre for Culture of Marine Phytoplankton, Bigelow. *T. weisflogii* was cultured in the f/2 medium made with artificial seawater (30 ppt salinity; filtered with a 0.45 µm membrane) under 22 ± 1 °C and 80 µmol photons m⁻² s⁻¹ with a 12 h light: 12 h dark cycle. To keep the algae in suspension and facilitate the transfer of CO₂, algal cultures were manually shaken thrice daily. Algal stocks were sub-cultured each week. *T. weisflogii* in the logarithmic growth phase was used for the subsequent experiments. A stock solution of ERY (CAS# 114–07–8, HPLC ≥ 98%; Merck, Germany) at concentration of 10 µg/mL was freshly prepared and used in the exposure experiments (Nie et al., 2013; Yoshinobu et al., 1983).

2.2. Growth inhibition test

Freshwater algae are usually exposed to a chemical for 3 or 4 days in the acute toxicity tests (OECD, 2011; US EPA, 2012). In the present study, the marine diatom (*T. weisflogii*) was exposed to ERY for 7 days to evaluate the chronic toxicity. Moreover, it allowed sufficient algal biomass to be harvested for the transcriptomic analysis. Briefly, *T. weisflogii* (1 × 10⁶ cells/mL) was seeded in a 250 mL conical flask filled with 150 mL of f/2 medium. A calculated proportion of ERY stock solution was added into the f/2 medium within a conical flask. The ERY concentrations in the exposure medium were measured at day 0 and day 7 using HPLC-MS/MS as in our previous works (Guo et al., 2021; Ma et al., 2021). At day 7, the remaining ERY were 56.2% ± 11.1%, 70.7% ± 8.0%, 76.4% ± 8.8% of their initial exposure concentrations for the 1, 750 and 2500 µg/L ERY treatment groups, respectively, while ERY was undetectable in the control group. Cell density was then estimated by counting the algal cells with a hemacytometer under a microscope (Nikon Eclipse 96i, Japan). At day 7, collected algae were either used to measure physiological parameters or transcriptomic analysis.
3. Results

3.1. ERY exposure altered growth and viability of *T. weissflogii*

A clear hormesis was observed in *T. weissflogii* under ERY stress, in which the cell densities increased by 24.1% ± 15.1%, 48.2% ± 4.0%, and 36.3% ± 24.4% in the 1, 10 and 50 µg/L ERY exposure groups, respectively, but decreased by 23.2% ± 2.2%, 52.3% ± 1.7%, and 93.4% ± 1.5% in the 750, 2500, and 5000 µg/L ERY exposure groups (Fig. 1A). The calculated EC50 of ERY to *T. weissflogii* is 2398 µg/L. Accordingly, ERY exposure concentration at 1, 750, and 2500 µg/L used in this study is considered as an environmentally relevant concentration, EC100 and EC50 value, respectively (Figs. 1A and S1). In terms of toxicity sensitivity of aquatic plants to ERY toxicity, cyanobacteria > *R. subcapitata* > *D. tertiolecta* > marine diatoms (including *T. weissflogii*) > *C. vulgaris* > *L. minor* (Aderemi et al., 2021; Ando et al., 2007; Eguchi et al., 2004; Isidori et al., 2005; Ma et al., 2021; Machado and Soares, 2019b; Pomati et al., 2004; Sendra et al., 2018; Zhang et al., 2021). In addition, algal cell viability was slightly (p > 0.05) elevated in the 1 and 10 µg/L ERY treatments, but it was significantly (p < 0.05) reduced by 64.1% ± 6.2% and 65.4% ± 2.4% in *T. weissflogii* exposed to 750 and 2500 µg/L ERY (Fig. 2).

2.3. Measurement of physiochemical parameters

The viability of *T. weissflogii* was evaluated by measuring the cellular esterase activity, Hydroxyl radical (HO•), and singlet oxygen (1O2) using fluorescein diacetate (FDA) (Brookes et al., 2000), 3′-(p-hydroxyphenyl) fluorescein (PHF) (Brochmann et al., 2014), and Singlet Oxygen Sensor Green (SOSG) (Prasad et al., 2018), respectively. *T. weissflogii* were collected through centrifugation (3000g, 10 min), rinsed with PBS twice, and stained with 25 µmol/L FDA, 25 µmol/L HPF, or 50 µmol/L SOSG for 30 min at room temperature in the dark. Finally, the stained algal samples were analyzed using a flow cytometer (FACSCanto II, Becton Dickinson, USA). A setting of excitation 488 nm/emission 530 nm, excitation 492 nm/emission 515 nm, and excitation 504 nm/emission 525 nm was used for fluorescence measurement of FDA, PHF, and SOSG, respectively.

2.4. Determination of total soluble protein, chlorophyll-a and chlorophyll fluorescence

Algae were counted and collected through centrifugation at 3000g for 10 min. Phosphate buffer solution (pH = 7.3) supplemented with a protease inhibitor cocktail (Beyotime, Shanghai, China) was added to resuspend the algae, followed by sonication (HN-650Y, Shanghai, China) in an ice-water bath. The resulting homogenates were centrifuged (12000g) at 4 °C for 10 min. The content of total soluble protein was determined using a BCA assay kit with bovine serum albumin as a standard (Beyotime, Shanghai, China).

Chl-a of collected microalgae were extracted using 90% acetone at 4 °C in the dark for 24 h. Following the extraction, centrifugation at 5000g for 10 min was performed. The absorbances of the algal extracts were recorded using a spectrophotometer (UV-2700, Shimadzu, Japan). The content of Chl-a was calculated based on the Eq. (1) (Ritchie, 2006).

\[
\text{Chl-a} = 11.47 \times A_{664} - 0.45 \times A_{430}
\]

where, A664 and A430 is the absorbance measured at 664 and 630 nm, respectively.

The collected algae were transferred to a 24-well plate. As described in Gao et al. (2018), after dark adaptation for 15 min, the minimum fluorescence yield (F0) and the maximum fluorescence yield (Fm) were measured using an Imaging-PAM fluorometer (Maxi version; Heinz Walz GmbH, Germany). The maximum quantum yield of PSII was calculated as Fv/Fm = (Fm – F0)/Fm.

2.5. Transcriptomic analysis

Based on the data of growth inhibition test, algae from the control and the 1 (an environmentally realistic concentration), 750 (EC100), and 2500 µg/L (EC50) ERY treatments were collected at day 7 for transcriptomic analysis. Total RNA of *T. weissflogii* was extracted using Trizol method. RNA quality was checked using Agilent 2100 Bioanalyzer (Agilent Technologies, USA), and algal samples (RIN > 8) were used for the subsequent library preparation. In total, 12 cDNA libraries were prepared and sequenced using an Illumina NovaSeq platform. Reads of paired-end with a 150 bp read-length were generated. The resulting raw reads were cleared up, and high-quality reads (> Q20) were then de novo assembled using Trinity. The transcript with the longest sequence was considered as a unigene. All the assembled unigenes were compared with the databases including NR, Swiss-Prot, GO, KEGG, and eggNOG, and Pfam using NCBI BLASTX program. Based on matches in these databases, an E-value cutoff < 1 × 10^-5 was applied to assign annotations to these unigenes. Transcript abundance was then calculated by mapping clean reads to unigene sequences using RSEM, followed by normalization using the FPKM method. Differential expression of genes was analyzed using DESeq2 package in R, and the differentially expressed genes (DEGs) were selected using an adj-*p* value < 0.05 and foldchange > 1.5. The identified DEGs were finally subjective to the enrichment analysis of GO and KEGG pathway, with a p < 0.05 considered statistically significant.

2.6. Statistical analysis

Statistical analysis was conducted using Graph Pad Prism 9 (San Diego, USA). Normality test was performed on collected data including growth, content of total soluble protein, Chl-a content and Chl-a fluorescence and ROS formation. A one-way analysis of variance (ANOVA) followed by Tukey’s posthoc test was applied, and a p < 0.05 was deemed significant (*).

3. Results
3.2. ERY exposure altered soluble protein content and photosynthesis

The content of total soluble protein significantly \((p < 0.05)\) increased from \(1.36 \pm 0.04\) pg/cell (control) to \(1.92 \pm 0.04\) and \(2.05 \pm 0.03\) pg/cell in the 1 and 10 \(\mu\)g/L ERY exposure groups, respectively, compared to the control (Fig. 3A). On the contrary, it was reduced significantly \((p < 0.05)\) to \(0.36 \pm 0.02\) and \(0.29 \pm 0.02\) pg/cell in algae exposed to 750 and 2500 \(\mu\)g/L ERY, respectively. Similarly, the alterations in the content of Chl-a are also in good agreement with algal growth performance under ERY stress. Specifically, it was elevated significantly \((p < 0.05)\) from \(4.63 \pm 0.24\) pg/cell (control) to \(6.63 \pm 0.92\) and \(6.44 \pm 0.80\) pg/cell in \textit{T. weissflogii} exposed to 1 and 10 \(\mu\)g/L ERY, respectively, but was reduced in the 750 and 2500 \(\mu\)g/L ERY exposure groups to \(2.98 \pm 0.65\) and \(2.15 \pm 0.39\) pg/cell, respectively (Fig. 3B). Additionally, Fv/Fm slightly \((p > 0.05)\) increased in the 10 \(\mu\)g/L ERY treatment, but it declined significantly \((p < 0.05)\) from \(0.64 \pm 0.01\) (control) to \(0.58 \pm 0.01, 0.55 \pm 0.02, 0.42 \pm 0.01,\) and \(0.39 \pm 0.01\) in \textit{T. weissflogii} exposed to ERY at concentration of 500, 750, 2500 and 5000 \(\mu\)g/L, respectively, compared with the control (Fig. 3C).

3.3. ERY exposure altered ROS formation

ROS formation in \textit{T. weissflogii} exposed to low levels of ERY slightly but non-significantly \((p > 0.05)\) increased in the 1 \(\mu\)g/L ERY treatment, but it was markedly elevated following exposure to higher levels of ERY (Fig. 4A-D). Notably, the production of \(\text{HO}^*\) was significantly \((p < 0.05)\) increased by \(109.8\% \pm 9.8\%\) and \(141.9\% \pm 65.9\%\) in \textit{T. weissflogii} exposed to 750 and 2500 \(\mu\)g/L ERY, respectively (Fig. 4B), while the production of \(1\text{O}_2\) was significantly \((p < 0.05)\) augmented by \(43.6\% \pm 15.0\%\) in the 2500 \(\mu\)g/L ERY treatment (Fig. 4D).
3.4. ERY exposure altered transcriptomic profiles

3.4.1. Transcriptomic sequencing and de novo assembly

Execution of transcriptomic sequencing on the algal samples yielded an average of 50,440,295 (7616,484,495), 45,546,545 (6877,528,345), 50,232,128 (7585,051,328), and 45,567,447 (6878,662,929) high-quality raw reads (base reads) for the control, 1, 750, and 2500 µg/L ERY exposure groups, respectively. In all samples, reads of > Q20 range from 97.95% to 98.26%. After trimming and filtration, the resulting quality raw reads (base reads) for the control, 1, 750, and 2500 µg/L ERY exposure groups, respectively. In all samples, reads of > Q20 range from 97.95% to 98.26%. After trimming and filtration, the resultingreads of high quality from each sample were inputted for de novo assembly, and the generated contigs were assembled into 48447 transcripts (N50 = 6146 bp) and 25785 unigenes (N50 = 3009 bp). The maximum length and average length of the assembled unigenes was 32583 bp and 1483 bp, respectively.

3.4.2. Functional annotation and differential expression analysis

The proportion of genes matched and annotated successfully is 46.89%, 24.47%, 32.01% and 46.96%, 47.00%, 43.09%, respectively. In the NR species distribution (Fig. S2A), sequencing data of T. weissflogii (20.31%) and Thalassiosira Pesudonana (16.4%) were enriched into 35 KEGG pathways (Fig. S4) and 26 eggNOG categories including phases I, II and III (Coleman et al., 1997). Redox reactions in biochemical processes involved in exobiotic metabolism, energy metabolism, and photosynthesis. Notably, signaling pathways related to hormonal response, Chl-a content, Fv/Fm, and soluble protein content in T. weissflogii exposed to 2500 µg/L ERY (Table 1 and Fig. S8C). In contrast, KEGG pathways, such as ribosome, arginine biosynthesis, histidine metabolism, nitrogen metabolism, beta-alanine metabolism, photosynthesis-antenna proteins, porphyrin metabolism, were highlighted in T. weissflogii exposed to 2500 µg/L ERY (Table 1 and Fig. S8C).

3.4.3. Gene enrichment analysis

Significant (p < 0.05) GO terms including MF, BP, and CC are shown in Fig. S7A-C. There are 4, 8 and 15 KEGG pathways significantly (p < 0.05) enriched in the 1, 750, and 2500 µg/L ERY exposure groups, respectively (Table 1). Specifically, pentose phosphate pathway, ribosome biogenesis in eukaryotes, glutathione metabolism, arginine and proline metabolism were significantly enriched in the 1 µg/L ERY treatment (Table 1 and Fig. S8A). In the 750 µg/L ERY exposure group, KEGG pathways including nitrogen metabolism, pyrimidine metabolism, purine metabolism, arginine biosynthesis, and pentose phosphate pathway were identified (Table 1 and Fig. S8B). In contrast, KEGG pathways, such as ribosome, arginine biosynthesis, histidine metabolism, nitrogen metabolism, beta-alanine metabolism, photosynthesis-antenna proteins, porphyrin metabolism, were highlighted in T. weissflogii exposed to 2500 µg/L ERY (Table 1 and Fig. S8C).

4. Discussion

Using T. weissflogii as a model marine diatom, in accordance with the hypothesis, the present study revealed that ERY exposure induced hormesis through the modification of signaling pathways and biochemical processes involved in exobiotic metabolism, energy metabolism, and photosynthesis. Notably, signaling pathways related to ribosome and protein translation, but not DNA replication and repair as in R. subcapitata (Guo et al., 2021), were shown to be highly involved in the ERY-induced hermetic effects in T. weissflogii. Novel mechanistic information was generated, in which the transcriptional alterations were linked to the physiological responses including cell viability, ROS production, Chl-a content, Fr/Fm, and soluble protein content in ERY-induced hormesis.

4.1. Genes of exobiotic metabolism

Xenobiotics in plants are metabolized in the detoxification system including phases I, II and III (Coleman et al., 1997). Redox reactions in phase I are catalyzed by cytochrome P450 monooxygenases, and through which hydrophobic compounds are typically converted into less polar metabolites which are then transferred to other detoxification systems for further biotransformation.
hydrophobic metabolites (Ma et al., 2015). Notably, hydrolases and/or laccases may also play a critical role in phase I detoxification reactions in plants including algae. Subsequently, metabolites resulting from the phase I reactions are conjugated with hydrophilic moieties, including glutathione, glucuronic acid, and sulfate, catalyzed by glutathione S-transferases (GSTs), glycosyltransferases, and sulfotransferases, respectively, to further improve their solubility (Zhang and Yang, 2021). Metabolites generated from reactions of phases I and II are finally transported into vacuoles intracellularly and/or exported into extracellular matrix by ATP – binding cassette (ABC) – type transporters in phase III processes (Coleman et al., 1997).

In the present study, no genes of detoxification systems were differentially expressed in T. weissflogii exposed to 1 µg/L ERY, nor DEGs of phrase I detoxification was identified in the 750 and 2500 µg/L ERY exposure group; however, several genes related to exobiotic metabolism and transportation were highlighted in T. weissflogii exposed to 750 and 2500 µg/L ERY. Specifically, gst, large (encodes glycosyltransferase-like protein LARGE), and abcg2 were downregulated, while abca3 and abch1
were upregulated in the 750 µg/L ERY treatment. By contrast, downregulation of genes including *gst*, *gpx* (encodes glutathione peroxidase), *abcg2*, and *abcf1*, and upregulation of ABC transporter genes (i.e., *abcb1*, *abcf2*, and *abcf3*) were observed in the 2500 µg/L ERY exposure group. Downregulation of phase II genes suggest that the detoxification capacity of algae may be hampered following ERY treatment at concentrations above 750 µg/L, resulting in prolonged presence of ERY within the algal cells and ultimately reduced algal growth performance. While ABCG subfamily (encoded by *abcg*) are linked to antibiotic resistance, ABCF subfamily (encoded by *abcf*) participate in the}

**Fig. 5.** Ribosome biogenesis pathway altered in *T. weissflogii* following exposure to 1 µg/L erythromycin (ERY) for 7 days. Up-regulated genes are highlighted in red font. UTP21: U3 small nucleolar RNA-associated protein 21; Bms1: ribosome biogenesis protein BMS1; KRE33: N-acetyltransferase 10; Rio2: RIO kinase 2.
processes of ribosome recycling and translational control (Kang et al., 2010; Pisarev et al., 2010). The altered expression of ABC transporter genes may contribute to disturbed molecular signaling and biochemical processes of ribosome and translation (discussed in Section 4.2) and reduced resistance to ERY stress, amplified as growth inhibition in the 750 and 2500 μg/L ERY exposure groups. These findings are partially in agreement with ERY-induced growth inhibition in R. subcapitata, in which genes of exobiotic metabolism (encodes CYP450, hydrolases, and amine oxidases) and ABC transporter genes (encodes ABCA, ABCB, ABCI, ABCG) were exclusively downregulated following exposure to 80 or 120 μg/L of ERY (Guo et al., 2021). These molecular findings supported the hypothesis that plant defense mechanisms could be activated by exposure of antibiotics, leading to stimulation in the framework of xenohormesis to enhance tolerance to expected adverse environmental conditions (Agathokleous et al., 2018; Howitz and Sinclair, 2008).

4.2. Pathways related to ribosome and translation

Ribosomes are the cellular organelles where proteins are produced through translation (Thomson et al., 2013). In eukaryotic cells, ribosome biogenesis involves correct generation and dedicate assembly of 4 rRNAs (i.e., 18 S, 5.8 S, 25 S, and 5 S rRNA) and 79 ribosomal proteins (i.e., 46 large subunits and 33 small subunits), with the assistance of numerous factors (e.g., ATPases, GTPases, helicases, kinases, and nucleases) in multiple steps (Thomson et al., 2013).

Ribosome biogenesis starts with co-transcription of the 18 S, 5.8 S and 25 S rRNA, followed by their assembly into the 90 S pre-ribosomes with other components (e.g., UTP-C complex, t-UTP complex, UTP-B complex, and MMP10 complex) in the nucleolus (Piazzì et al., 2019; Thomson et al., 2013). The co-transcript of rRNAs is then cleaved to produce two particles, pre-40S and pre-60S. Subsequently, both pre-40S and pre-60S are matured in the nucleoplasm with multiple assembly factors (e.g., the A3 factors, dynein-like Rea1). The resulting matured pre-40S and pre-60S particles are processed into mature 40 S and mature 60 S in the cytoplasm (Fig. 5). Ribosome synthesis is a highly energy-demanding process, and all these components and associated factors are essential for ribosome biogenesis (Piazzì et al., 2019). The mature 40 S and mature 60 S are finally assembled into ribosomes functioning as protein production machines (Thomson et al., 2013).

In this study, genes enriched in signaling pathway of ribosome biogenesis in eukaryotes, such as utp21, nat10, bms1, and riok2, were all upregulated in the 1 μg/L ERY exposure group (Table 1 and Fig. 5). Notably, ribosome biogenesis protein BMS1 (encoded by bms1) and N-acetyltransferase 10 (encoded by nat10) serve as a molecular switch for the maturation of pre-40S and pre-60 particle in the nucleolus, respectively. U3 small nuclear RNA-associated protein 21 (encoded by utp21) is a critical subunit of UTP-B complex in the 90 S pre-ribosome, and RIO kinase 2 (encoded by riok2) is required for the maturation of 40 S particle in the cytoplasm (Ni and Buszczak, 2022). The consistent upregulation of these genes may suggest an increase in ribosome biogenesis and translation in algae treated with 1 μg/L ERY, resulting in increased content of total soluble protein and ultimately promoted growth of T. weissflogii. By contrast, signaling pathway of ribosome was altered in the 2500 μg/L ERY exposure group, the expression of multiple ribosomal protein genes either encoding large subunits (e.g., rpl8e, rpl1, rpl2, rps11e, rps12e, rpl24, rpl39e, rpl4, rpl40e, rpl44e, rpl7) or ribosomal...
protein small subunits (e.g., rpS15e, rpS16e, rpS17e, rpS19e, rpS20e, rpS15, rpS23e, rpS27ae0, rpS29, rpS30e) were dysregulated (Table 1 and Fig. 6), suggesting the biogenesis of ribosomes was perturbed and more energies were demanded for the corrections (Shore and Albert, 2022). This may lead to less energy investment to cell growth and division, supported by reduced content of total soluble protein and growth inhibition of 
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**4.4. Photosynthesis system**

Oxygentic photosynthesis in plants including algae requires Chl to absorb and transmit light energy in the light harvesting processes (Wang and Grimm, 2021). Chl bind to antenna proteins to form the core components of photosynthesis systems I and II. Biosynthesis of Chl starts with L-glutamate to produce 5-aminolevulinate through three enzymatic reactions generating two intermediates (L-glutamyl-RNA (Glu) and L-glutamate 1-semialdehyde) catalyzed by glutamyl-RNA synthetase (encoded by *ears*), glutamyl-RNA reductase, and glutamate-1-semialdehyde in each step, respectively (Bollivar, 2006). Subsequently, from 5-aminolevulinate, it takes six more enzymatic reactions to generate protoporphyrin IX. These reactions and the enzyme catalyzes result in hydroxymethylbilane synthase (encoded by *hemC*), uroporphyrinogen decarboxylase (encoded by *hemE*), coproporphyrinogen oxidative decarboxylase (encoded by *hemF*), etc. (Bollivar, 2006; Wang and Grimm, 2021). Down-regulation of genes including *ears*, *hemC*, *hemE*, and *hemF* were observed in algae treated with 2500 µg/L ERY, suggesting the biosynthesis of protoporphyrin IX was inhibited (Table 1).

Following the generation of protoporphyrin IX, another eight enzymatic reactions are required to finally produce Chl-a, in which eight enzymes are responsible for the catalysis (Bollivar, 2006; Wang and Grimm, 2021). Of which, magnesium chelatase (encoded by *chld*, *chlB*, and *chlD*) catalyzes the insertion of magnesium into protoporphyrin IX to produce magnesium protoporphyrin. Another important intermediate, divinyl-protoporphyrin, is converted into divinyl chlorophyllide a by protochlorophyllide reductase (encoded by *por*), followed by the generation of divinylchlorophyll a catalyzed by divinyl chlorophyllide a 8-vinyl-reductase (encoded by *dvr*) in the next step (Bollivar, 2006). Downregulation of *chlB*, *por*, and *dvr* was evident in the 2500 µg/L ERY treatment (Table 1), suggesting that biosynthesis of Chl-a was inhibited. Indeed, these results agree with physiological measurement data, where the contents of Chl-a declined in the 750 and 2500 µg/L ERY treatments but increased in T. weissfogeli exposed to 1 µg/L ERY. Notably, protoporphyrin IX can also be converted to heme and then cytochrome c, an essential component of the mitochondrial respiratory chain, with the catalysis of protoporphyrin ferrochelatase and cytochrome c heme-lyase (encoded by *hccs*), respectively (Heinemann et al., 2008). The downregulation of *hccs* suggests that the production of cytochrome c may be reduced, causing structural and functional impairments to mitochondria in algae exposed to high levels of ERY (Nie et al., 2013; Machado and Soares, 2019a).

Pigments are assembled into antenna proteins to form the cores of light-harvesting complex I and II (LHCl and LHClII), serving as essential components embedded in the chloroplastic thylakoid membrane for the light harvesting in photosynthesis (Wang and Grimm, 2021). In this study, expression of *lha01* and *lha04* (encodes light-harvesting complex I Chl-a/b binding protein 1 and 4) were downregulated, suggesting the structure and function of LHCl may be impaired following exposure to high levels of ERY, associated with the reduced Fv/Fm and excessive production of ROS (e.g., HO2 and O2). These findings are generally in agreement with hormesis induced in *M. flos-aquae* where a slight increase in ROS production and Fv/Fm, accompanied by significantly elevated Chl-a content and rETR in the low-dose stimulation. By contrast, ROS production, enzymatic activities of superoxide dismutase (SOD) and catalase (CAT), and malonaldehyde (MDA) significantly increased, while Chl-a content, Fv/Fm, rETR were significantly reduced in high-dose inhibition (Wan et al., 2015). Taken together, a slight but non-significant elevation of ROS production is likely to serve as an initial incentive for the low-dose growth stimulation in ERY-induced hormesis (Agathokleous et al., 2020), followed possibly by a slight increase in ribosome resemble and protein translation, photosynthesis, and cell viability. Exposure to high dose of ERY, on the other hand, continued to increase the ROS production, causing structural and functional impairments to T. weissfogeli (e.g., ribosomes, protein translation, photosynthesis, and cell viability) and ultimately resulting in growth inhibition.
5. Conclusions
In ERY-induced hormesis in T. weissflogii, promoted growth in the 1 μg/L ERY exposure group was possibly attributed to a light increase in ROS production, activation of ribosome biogenesis, and increase in production of total soluble protein. By contrast, inhibited algal growth caused by treatment with 750 and 2500 μg/L ERY was attributed to suppressed signaling related to xenobiotic metabolism and transport, ribosome, amino acid metabolism, nitrogen metabolism, and photosynthesis system. Pollution of macroalgal antibiotics at low levels (ng/L to sub-μg/L) is likely to serve as a critical factor for the promotion of phytoplankton blooms, potentially changing the structures and functions of the aquatic food chains (Tang et al., 2021; Zhang et al., 2020). The stability and bioavailability of ERY in the natural environment can affect the toxic responses of nontargeted organisms like algae to ERY. Therefore, the contribution/interference of environmental factors (e.g., light, temperature, salinity, pH, nutrients, pollutants, etc.) to the ERY toxicity shall be considered in subsequent studies. Assessment of multiple apical endpoints at population, physiological, and transcriptional levels in the present study, a systems biology approach, can generate detailed mechanistic information for the chemical toxicity including dose-response and species sensitivity difference used in environmental risk assessment.

CRediT authorship contribution statement
Jiezhang Mo: Conceptualization, Methodology, Data curation, Writing – original draft, Funding acquisition, Supervision. Runnn Lv, Xian Qin, Xintong Wu, Haibo Chen: Investigation, Methodology, Data curation. Neng Yan, Jingchun Shi, Yinglin Wu, Wenhua Liu, Jiahua Guo: Writing – review & editing. Richard Y.C. Kong: Writing – review & editing, Funding acquisition.

Declaration of Competing Interest
The authors reported no declarations of competing interests.

Data availability
Data will be made available on request.

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Appendix A. Supporting information
Supplementary data associated with this article can be found in the online version at doi:10.1016/j.ecoenv.2023.115242.

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