



# Cylindrospermopsin enhances the conjugative transfer of plasmid-mediated multi-antibiotic resistance genes through glutathione biosynthesis inhibition

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## ABSTRACT

Cylindrospermopsin (CYN), a cyanobacterial toxin, has been detected in the global water environment. However, information concerning the potential environmental risk of CYN is limited, since the majority of previous studies have mainly focused on the adverse health effects of CYN through contaminated drinking water. The present study reported that CYN at environmentally relevant levels (0.1–100 µg/L) can significantly enhance the conjugative transfer of RP4 plasmid in *Escherichia coli* genera, wherein application of 10 µg/L of CYN led to maximum fold change of ~6.5-fold at 16 h of exposure. Meanwhile, evaluation of underlying mechanisms revealed that environmental concentration of CYN exposure could increase oxidative stress in the bacterial cells, resulting in ROS overproduction. In turn, this led to an upregulation of antioxidant enzyme-related genes to avoid ROS attack. Further, inhibition of the synthesis of glutathione (GSH) was also detected, which led to the rapid depletion of GSH in cells and thus triggered the SOS response and promoted the conjugative transfer process. Increase in cell membrane permeability, upregulation of expression of genes related to pilus generation, ATP synthesis, and RP4 gene expression were also observed. These results highlight the potential impact on the spread of antimicrobial resistance in water environments.

## 1. Introduction

Cyanobacteria are essential primary producers in aquatic ecosystems such as lakes and reservoirs (Picossi et al., 2015). The rapid growth of cyanobacteria in eutrophic waters leads to the formation and proliferation of cyanobacterial blooms, which not only severely diminishes water quality but also poses a serious risk to human and ecosystem health (Merel et al., 2013). In addition, cyanobacteria can release high levels of undesirable biologically active secondary metabolites, known as cyanotoxins. These toxins are often classified by the nature of their toxic effects into categories such as hepatotoxins (e.g., microcystins [MCs] and cylindrospermopsin [CYN]), neurotoxins, cardiotoxins, and dermatoxins.

CYN was first detected and isolated from *Cylindrospermopsis raciborskii*, and this compound is a stable tricyclic alkaloid that mainly acts as a hepatotoxin (Ohtani et al., 1992; Puerto et al., 2014). Due to its high

water-soluble nature (Chiswell et al., 1999), CYN has been detected in water environments worldwide, and this toxin occurs commonly in aquatic ecosystems in North America, Oceania, Europe, and Asia (Scarlett et al., 2020). According to some recent reports, CYN concentrations mainly ranged from 0.1 to 100 µg/L in water bodies globally and the highest total CYN level was detected in the water supply of a farm in central Queensland at a concentration of 1050 µg/L (Scarlett et al., 2020; Yang et al., 2021). It is shocking that CYN has even been detected in Antarctic waters, which highlights the widespread distributions of the microalgae that synthesize this cyanotoxin (Kleintech et al., 2014). More recently, CYN has been identified as cardiotoxins, neurotoxins, carcinogenic and potentially genotoxic (Pearson et al., 2010; Scarlett et al., 2020). To protect the public from the deleterious effects of CYN, a guideline value (lifetime) of total CYN of 0.7 µg/L was proposed by the World Health Organization (WHO) in 2022. Nevertheless, the majority of research on CYN has mainly concentrated on evaluating the adverse

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health effects of consuming CYN-contaminated drinking water, little information is available concerning the environmental risk of CYN in aquatic environment.

The spread of antimicrobial resistance (AMR) poses a growing global threat to public health (Allen et al., 2010; Prestinaci et al., 2015). AMR is the capacity of bacteria to withstand the biocidal effect of antimicrobial agents, causing approximately 700,000 deaths per year. The WHO recently estimated that, by 2050, up to 10 million people will die each year due to AMR (Watkins and Bonomo, 2016). To date, a great variety of antibiotic resistance bacteria (ARB) and antibiotic resistance genes (ARGs) have been detected in different water bodies such as rivers, lakes, wetlands, and the sea (Hendriksen et al., 2019; Holmes et al., 2016; Martinez, 2008). ARGs and ARB not only exist in water bodies but also can spread and diffuse among bacteria through several mechanisms in aquatic environments. It is generally accepted that horizontal gene transfer is the most significant driver of the current AMR pandemic, with its mechanisms including conjugation, transformation, and transduction, among which the conjugation transfer is the most prevalent (von Wintersdorff et al., 2016). Further, apart from antibiotics, several studies have recently demonstrated that a number of non-antibiotic environmental contaminants in water ecosystems such as disinfection by-products (He et al., 2022), nanomaterials (Li et al., 2020; Qiu et al., 2012), non-antibiotic antimicrobial agents (Lu et al., 2018), nonnutritive sweeteners (Yu et al., 2021), and heavy metals (Wang et al., 2020a) can accelerate the conjugation transfer of ARGs even in the absence of antibiotic selection pressure.

According to some previous studies, cyanobacterial blooms have been demonstrated to increase the numbers and relative abundances of total ARGs in water environments. Additionally, cyanobacteria can play a critical role as reservoirs and sources of ARGs (Wang et al., 2020b; Zhang et al., 2020). Therefore, cyanotoxins and ARGs are extremely likely to co-occur in various water ecosystems worldwide. However, little information is available concerning the effects of cyanotoxins on the spread of ARGs in water environments. To date, only one recent study has reported that MCs promote the horizontal transfer of ARGs by stimulating the formation of reactive oxygen species (ROS) and increasing cell membrane permeability (Xu et al., 2020). Up to now, no study has addressed the role of CYN in bacteria and the spread of ARGs. In fact, CYN and MCs are two different chemicals, though both of them are classified as cyanotoxins (Díez-Quijada et al., 2020). Further, it has been reported that toxicity mechanisms for CYN and MCs are cyanotoxin-specific, depending on their toxicokinetic and toxicodynamic characteristics and chemical structures. As for MCs, its toxicity mechanisms are related primarily to inhibition of protein phosphatase 1 and 2 A (PP1 & PP2A) and disruption of mitochondrial electron transport chain (Guzman-Guillen et al., 2017). With regard to CYN, its toxicity mechanisms have been indicated to mainly associate with the irreversible inhibition of protein synthesis and induction of oxidative stress (Guzman-Guillen et al., 2017; Runnegar et al., 1995). Therefore, it is reasonable to speculate whether environmentally relevant concentrations of CYN could enhance the plasmid-mediated horizontal transfer through cyanotoxin-specific mechanisms.

In the present laboratory-based study, conjugation model using two different strains of *Escherichia coli* (*E. coli*) was established according to previously published studies in this laboratory (Qiu et al., 2012). The conjugation system was treated with selected CYN concentrations ranging from 0.1 to 100 µg/L, and the mating time was extended up to 24 h to emphasize the presence of CYN in real the water environment. To elucidate the underlying mechanisms of CYN effects on plasmid conjugation, oxidative stress indicators including intracellular ROS levels and antioxidant enzymatic activities were determined. Then, measurements of intracellular glutathione (GSH) levels and GSH biosynthesis were conducted to evaluate potential mechanisms of CYN toxicity. Besides, a series of underlying mechanisms were also clarified by determining changes in cell membrane permeability. Apart from this, genome-wide transcriptomic analysis was further applied to evaluate differentially

expressed genes under CYN exposure. Collectively, these findings suggested that CYN not only poses a serious threat to human and environmental health but also plays a nonnegligible role in the spread of ARGs by stimulating conjugative transfer between bacteria.

## 2. Materials and methods

### 2.1. Bacterial strains, culture conditions, and CYN

The optimized RP4-mediated conjugation model was chosen to assess the effects of CYN on the conjugative transfer of ARGs based on previous studies in this laboratory (He et al., 2022; Qiu et al., 2012). Briefly, *E. coli* HB101 carried RP4 plasmid (a multi-host conjugative plasmid that carries ARGs against ampicillin [Amp], kanamycin [Km], and tetracycline [Tet]). *E. coli* K12, which is resistant to rifampicin (Rif), was used as recipient. The bacterial strains were incubated overnight at 37 °C in Luria-Bertani broth (LB, Becton, Dickinson and Company, USA, pH = 7.4), supplemented with Amp, Km, and Tet for the donor, and with Rif for the recipient. Amp, Km, and Tet were obtained from Sangon Biotech (China), Rif from Solarbio (China), and CYN was purchased from Macklin (China).

### 2.2. Conjugation experiments under CYN exposure

Overnight cultures were centrifuged for 5 min at 6000 rpm, 4 °C, washed 3 times with substrate-free phosphate-buffered saline (PBS), and resuspended in LB to obtain ~2 × 10<sup>8</sup> CFU/ml and mixed at a donor/recipient ratio of 1:1. The mixture was then treated with different concentrations of CYN (0.1, 1, 10, 100 µg/L) at 37 °C for 4, 8, 16, and 24 h without shaking, with Milli-Q water being used as a control. Following incubation, the mixture was mixed thoroughly and inoculated on LB agar selection plates containing Amp, Km, Tet, and Rif to determine the numbers of transconjugants. Total recipients were determined by inoculating diluted cell mixtures onto LB agar plates containing Rif. The conjugative transfer frequency was calculated via the ratio of transconjugants to total recipients (Wang et al., 2019).

Furthermore, to eliminate the possibility of spontaneous mutation, experiments were also conducted as described in the previous study. Growth curve analysis, plasmid gel electrophoresis, and PCR experiments were conducted to exclude any growth inhibition caused by the selected CYN dosages and to corroborate the transfer of the RP4 plasmid from the donor to the transconjugants (He et al., 2022).

Additionally, 100 µM of thiourea was added as ROS scavenger to the above-described conjugation system to determine whether different CYN concentrations promote conjugative transfer by inducing ROS production (Lu et al., 2018). Besides that, the role of GSH in regulating conjugation was confirmed by adding exogenous GSH (100 µM). All experiments were conducted in biological triplicates.

### 2.3. Detection of ROS levels and cell membrane permeability

Intracellular ROS levels and cell membrane permeability were measured using a microplate reader (SpectraMax iD5, Molecular devices, USA) coupled with 2',7'-dichlorofluorescein diacetate cellular ROS detection assay kit (Solarbio, China) and propidium iodide dye (PI, AAT Bioquest, USA), respectively. In brief, the mating cultures were treated with the same CYN doses as in the conjugation experiment, then samples were collected and ROS levels and membrane permeability were measured using the kits according to the manufacturer's instructions (He et al., 2022; Lu et al., 2020). Cell suspensions without treatment were used as a negative control. A 10 µM solution provided by the ROS detection assay kit and a heat-treated group (30 min at 80 °C) were used as positive controls for ROS levels and membrane permeability, respectively.

## 2.4. Measurement of GSH levels

GSH levels in the conjugation system were detected using the Reduced GSH Content Assay Kit (D799614-0100, Sangon Biotech, China) to verify the role of CYN in inhibition of GSH synthesis. Briefly, bacterial suspensions treated with different CYN concentrations (0, 0.1, 1, 10, 100 µg/L; 2 h of exposure) were collected, washed twice with PBS, and then resuspended in lysis buffer from the kit. Then the lysates were fragmented by using an ultrasonic crusher in an ice bath, and the mixture was centrifuged for 10 min at 8000 rpm. The supernatant was collected and analyzed for the GSH levels using the kit as per the manufacturer's instructions (Zhang et al., 2022). All tests were performed in biological triplicates.

## 2.5. Measurement of GSH synthesis related enzyme and antioxidant enzymatic activities

To validate the effects of CYN on GSH synthesis and the role of antioxidant system in CYN-induced conjugative transfer, the activities of enzymes related to GSH biosynthesis and several antioxidant enzymes were determined. These included  $\gamma$ -glutamyl cysteine ligase (GCL), glutathione peroxidase (GPx), superoxide dismutase (SOD), and catalase (CAT). A Bacterial Total Protein Extraction Kit (BBI Life Science, Hong Kong, China) was used for the extraction of bacterial proteins of different CYN doses treated samples (0, 0.1, 1, 10, 100 µg/L; 2 h of exposure) (Ji et al., 2022). Then, activities of GCL, GPx, SOD and CAT in extract were measured using the GCL Activity Assay Kit (BC1215, Solarbio, China), GPx Assay Kit (BC1195, Solarbio, China) (Zhao et al., 2022), Superoxide Dismutase Assay Kit (BC5165, Solarbio, China) (Wang et al., 2021a) and Catalase Assay Kit (BC0205, Solarbio, China) (Wang et al., 2021b), respectively. All procedures were conducted strictly in accordance with the protocols provided by the manufacturers. All tests were performed in biological triplicates.

## 2.6. Measurement of ATP levels

Intracellular ATP levels were quantified employing a microplate reader (SpectraMax iD5, Molecular devices, USA) coupled with ATP assay kit (Beyotime, China). The assay was performed following the manufacturer's protocol. Briefly, following the treatment of mating cultures with identical CYN concentrations (2 h of exposure) as utilized in the conjugation experiments, ATP was extracted from the samples. Following centrifugation for the removal of cellular debris, the supernatant was then introduced into the substrate mixture, followed by the quantification of luminescence to assess ATP levels. All tests were performed in biological triplicates.

## 2.7. mRNA Expression analysis

The transcriptional expression levels of GSH synthesis genes (*gshA*, *gshB*) were quantified by reverse transcription-PCR (RT-PCR). The mRNA transcriptional expression levels were normalized to the level of 16s rRNA. Details about RNA extraction, cDNA construction, the primers and annealing temperature for qPCR can be seen in SI (Text. S2 and Table. S5).

## 2.8. Transmission electron microscopy (TEM)

TEM was used to investigate the effects of CYN on the microstructure and morphology of cells, following the method previously reported by Zhou et al. (Zhou et al., 2023). Briefly, 10 µg/L and 100 µg/L CYN were added to the conjugation mating systems for 16 hours, as described in Method 2.2, with Milli-Q water serving as the control. Subsequently, the samples were observed using a field emission scanning electron microscope equipped with a STEM-Detector (Sigma 300, Zeiss, Germany).

## 2.9. RNA extraction, genome-wide RNA sequencing and transcriptomic analysis

Conjugation mating systems were treated with CYN at 0, 0.1, 10, and 100 µg/L for 2 h at 37 °C. Then, the bacterial cells were centrifuged for 5 min at 6000 rpm and 4 °C and resuspended in PBS. Stabilized bacterial samples ready for genome-wide RNA sequencing were then submitted to Novogene (China), where their total RNA was extracted using a Bacterial Genomic RNA Extraction Kit (Tiangen, China) according to the manufacturer's instructions and sequenced on the Illumina NovaSeq 6000 Platform, followed by global transcriptional analysis. Gene expression level was quantified in terms of fragments per kilobase per million mapped reads (FPKM). Differences in gene transcriptional values between the control (without CYN treatment) and the CYN-treated groups were identified based on their log<sub>2</sub> fold change (LFC) values. All experiments were performed with at least three biological replicates.

## 2.10. Statistical analysis

All data analyses were conducted using SPSS for Windows version 25.0 (SPSS, Chicago, USA) and GraphPad Prism 9.3.1 (California, USA). All phenotypic data were reported as mean  $\pm$  standard deviation (SD). Analysis of variance (ANOVA) and independent sample t-tests with Benjamini-Hochberg correction were used to analyze the results. P-values below 0.05 were indicative of statistical significance and presented as  $P_{adj}$ . Levels of significance were indicated as follows: \*, #,  $P_{adj} < 0.05$ , \*\*, ##,  $P_{adj} < 0.01$ .

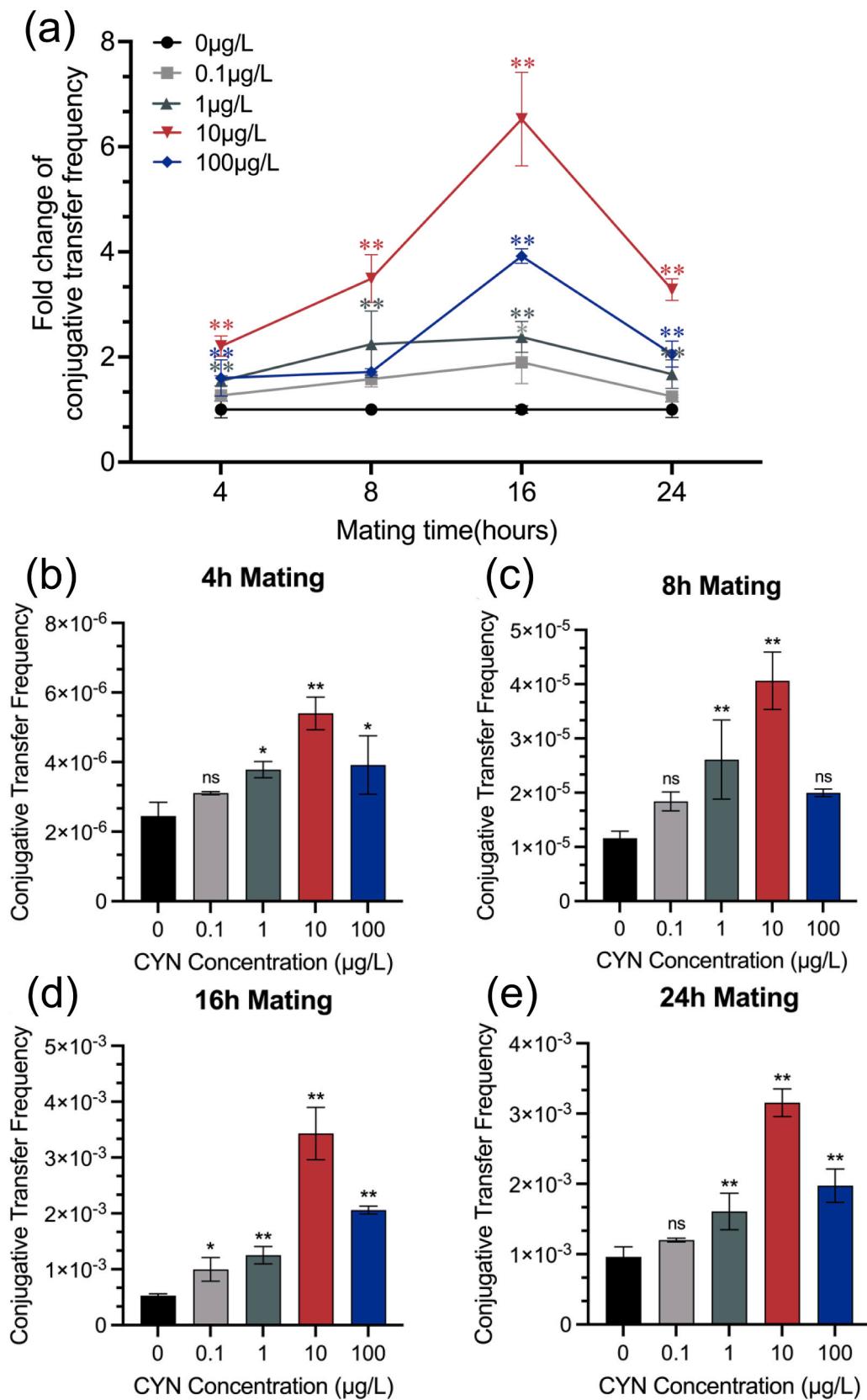
## 3. Results and discussion

### 3.1. CYN significantly promoted conjugative transfer of ARGs

To examine the effects of environmentally relevant levels of CYN on conjugative transfer, intra-genera transfer system under 0 – 100 µg/L CYN exposure was assessed. Notably, these selected CYN concentrations are much lower than the minimum inhibitory concentration (MIC) of CYN tested in the present study (> 50 mg/L). As shown in Fig. 1a, after mating for 4, 8, 16, and 24 h, the RP4 plasmid-mediated average spontaneous conjugative transfer frequencies were calculated as  $2.45 \times 10^{-6}$ ,  $1.16 \times 10^{-5}$ ,  $5.26 \times 10^{-4}$ , and  $9.61 \times 10^{-4}$ , respectively. Particularly, the strongest enhancement of conjugative transfer frequencies occurred at a CYN dose range of 10 – 100 µg/L ( $P_{adj} < 0.01$ ). For instance, there was a 2.2-fold increase in the conjugative transfer frequencies in 10 µg/L CYN-treated group compared to the control after 4 h of exposure ( $P_{adj} < 0.01$ ). During continuous exposure times of up to 24 h, the highest promoted conjugation frequencies were monitored after 16 h exposure, reaching up to 1.90–, 2.38–, 6.53–, and 3.92–fold increases compared with that of control, respectively. Since CYN concentrations in global water bodies have been reported to be 0.1 – 100 µg/L, this study therefore concluded that environmentally relevant CYN levels may contribute greatly to the spread of ARGs in aquatic environments.

Additionally, several experiments were conducted to validate the transfer of the RP4 plasmid. The MICs of the antibiotics Amp, Km, Tet, and Rif were tested. As expected, the tested transconjugants exhibited multiple resistance and high MICs against Rif (> 1000 mg/L), indicating that the RP4 plasmid was successfully transferred (Table S1). Plasmid RP4 was analyzed in randomly selected donor, recipient, and transconjugant strains. PCR analysis further confirmed that the transconjugants were indeed recipient strains containing the RP4 plasmid from the donor (Fig. S2).

It is worth noting that transconjugants can also propagate in LB broth during the 24 h mating process and this effect has been minimized by comparing the fold changes of conjugative transfer frequency in each group. Using this approach, if CYN exposure did not significantly affect conjugation transfer, the conjugative transfer frequencies in CYN-



**Fig. 1.** (a) Fold changes of conjugative transfer frequencies. (b–e) Effects of 0.1 – 100 µg/L CYN on conjugation at selected time points. Significant differences between the CYN-treated and the control groups. (ANOVA, \*  $P_{adj} < 0.05$ ; \*\*  $P_{adj} < 0.01$ ).

treated groups would be similar to those of the control group. Further, growth curve analyses of transconjugants were conducted to verify the effects of different CYN levels on transconjugants. As illustrated in Fig. S1, the selected CYN concentrations did not affect the growth of transconjugants significantly. Therefore, CYN exposure can promote conjugative transfer frequency by increasing the number of transconjugants in the mating mixture (Fig. 1b–e).

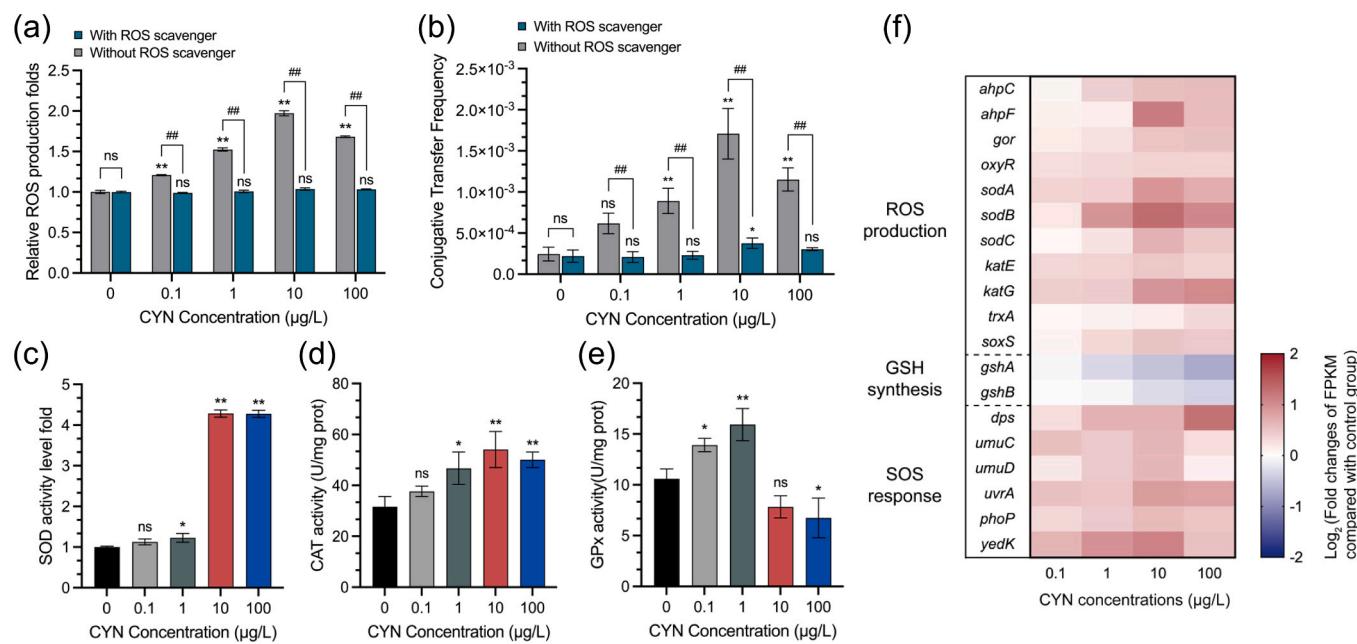
Interestingly, compared to 16 h of mating time, all CYN dosages showed decreased fold changes in conjugative transfer frequencies at 24 h. This decrease might be attributed to the physiological state of bacteria, which could be impacted by prolonged exposure to CYN. A similar phenomenon has been reported in previous studies (He et al., 2022), and confirmed by several other research groups. For instance, Lu et al. (2018) demonstrated that the conjugative transfer frequencies of AGRs peaked with a 2 µg/L triclosan dose (ranging from 0 to 2000 µg/L). To account for this phenomenon, detection of bacterial vitality in mating system was investigated (Text. S1). As the mating time reached 24 h, lower bacterial vitality was observed in all tested groups compared to 16 h mating time (Fig. S3), presumably explaining the decreased promotional effects at 24 h.

### 3.2. CYN induced ROS overproduction and SOS response

Induction of modest oxidative stress has been demonstrated to fulfil a significant role in facilitating conjugative transfer (Lu et al., 2020, 2018; Qiu et al., 2012). In the present study, increased ROS levels in the conjugative transfer system upon treatment with all the selected CYN concentrations were shown in Fig. 2a ( $P_{adj} < 0.01$ ). Particularly, ROS induction in the 10 µg/L CYN-treated group was nearly 2-fold compared with that of the control group. This is in agreement with some previous studies that identified CYN induce ROS overproduction (Hinojosa et al., 2023; Zhang et al., 2021). Further, to verify the effect of ROS overproduction on conjugation, comparison of conjugative transfer frequencies between with and without the aforementioned ROS scavenger was also conducted. As exhibited in Fig. 2a, after treated with

thiourea, ROS levels in the CYN-exposure groups were similar to those of the controls regardless of CYN dose ( $P_{adj} > 0.05$ ). Furthermore, the addition of thiourea also reversed increased conjugation frequencies induced by all CYN doses (Fig. 2b). It should be noticed that the addition of exogenous ROS scavenger did not significantly affect the conjugative transfer frequency of 0 µg/L CYN-treated group ( $2.19 \times 10^{-4}$ ), compared to the control group ( $2.44 \times 10^{-4}$ ) ( $P_{adj} > 0.05$ ). As shown in Fig. 2b, a remarkable decline in transfer frequencies was observed in all treated samples ( $P_{adj} < 0.05$ ). Specifically, the conjugative transfer frequencies decreased from  $6.47 \times 10^{-4} - 2.08 \times 10^{-3}$  to  $2.09 \times 10^{-4} - 3.77 \times 10^{-4}$  after 16 h of exposure. It is worth noting that increased conjugative transfer frequencies induced by CYN were not completely inhibited by ROS scavenger (Fig. 2b). For instance, after adding ROS scavenger, 10 µg/L CYN-treated group still exhibited significantly higher conjugation frequencies compared with that of 0 µg/L CYN-treated group and the control group. These findings suggest that the overproduction of ROS plays a significant role in CYN-enhanced conjugative transfer but is not the only underlying mechanism.

Redox balance has also been reported to play a crucial role in maintenance of normal cell function (Bae et al., 2011). To further evaluate the relationship between antioxidant system and CYN-induced increased conjugative transfer frequencies, activities of SOD, CAT and GPx were determined in the present study. As shown in Fig. 2c to d, both SOD and CAT activities in the conjugation system increased with increasing CYN doses, exhibiting maximum activity in samples treated with 10–100 µg/L of CYN, broadly consistent with the trend observed in the conjugative transfer experiment above. However, it is worth noting that GPx activities tended to increase and then decrease with increasing CYN concentrations in the current study (Fig. 2e). For instance, GPx activity showed the maximum activity upon 0.1–1 µg/L CYN exposure, and then a decrease trend was detected in 10–100 µg/L CYN groups. The increase of GPx activity could be due to bacteria attempting to activate antioxidant function to combat the increased ROS, whereas the decrease might be attributed to the decreasing GSH levels, in which GSH is a reduced substrate of GPx, indicating GPx activity is GSH-dependent



**Fig. 2.** Effects of CYN on ROS generation. (a) Relative ROS production levels after CYN exposure and effects of the exogenous addition of the ROS scavenger thiourea (2 h exposure,  $N = 3$ ). (b) Effects of thiourea on the CYN-induced conjugative transfer of RP4 plasmid (16 h exposure,  $N = 3$ ). Significant differences between the individual thiourea-treated and thiourea-free groups were indicated as follows: # ( $P_{adj} < 0.05$ ), ## ( $P_{adj} < 0.01$ ), independent sample t-test. Significant differences between individual CYN-treated and control groups (0 µg/L CYN), \* ( $P_{adj} < 0.05$ ) and \*\* ( $P_{adj} < 0.01$ ), one way ANOVA. Effects of CYN on (c) SOD activity fold changes (d) CAT activity changes (e) GPx activity changes after 2 h of exposure ( $N = 3$ ). Significant differences between the CYN-treated groups and the control were marked with \* ( $P_{adj} < 0.05$ ) and \*\* ( $P_{adj} < 0.01$ ), ANOVA. (f) Fold changes in the expression of stress related genes.

(Forman et al., 2009).

These above results were also strongly substantiated by transcriptomic analyses (Fig. 2f and Table. S3). Particularly, gene *oxyR* which involved in oxidative stress defense regulation (Pomposiello and Demple, 2001) was significantly upregulated in samples after 2 h mating time. The gene *oxyR*, encoding a transcription factor OxyR, plays a key role in the oxidative stress response. OxyR is known to respond to H<sub>2</sub>O<sub>2</sub> stress and regulates the expression of antioxidant-related genes to protect against oxidative damage (Ran et al., 2022). Moreover, CYN exposure significantly upregulated the expression of *ahpC* and *ahpF*, which are the alkyl hydroperoxide reductase genes (Bae et al., 2011), and the expression of the superoxide radical degradation genes *katE* and *katG* (He et al., 2022). The gene *ahpC* and *ahpF* can be modulated directly by OxyR, and the gene *katG* is a direct target gene of OxyR, the expression of these genes is regulated by OxyR (Jo et al., 2015). The superoxide removal transcriptional activator gene *soxS*, is similarly a ROS sensor as *oxyR*, primarily responds to oxidative stress caused by superoxide radicals (Gou et al., 2014). As a transcriptional regulator, SoxS is an essential component of SoxRS redox stress response system. The SoxRS system influences numerous genes related to oxidative stress response including *sodA*, which encodes superoxide dismutase, responsible for converting superoxide radicals into oxygen and H<sub>2</sub>O<sub>2</sub> (Dong et al., 2015). In the present study, a significant upregulation of genes *soxS* and *sodA* was observed under CYN exposure (Fig. 2f and Table. S3), suggesting that CYN can induce ROS and oxidative stress. Simultaneously, the GSH oxidoreductase gene, *gor*, was also significantly upregulated under CYN exposure (Wang et al., 2019). Further, activation of SOS response was induced (Beaber et al., 2004; Ubeda et al., 2005). Particularly, significant increase in the expression of *dps* was observed, which has been previously shown to encode a protein that binds to DNA to prevent DNA damage (Lackraj et al., 2020). DNA repair genes (*umuC*, *uvrA*) (Sargentini and Smith, 1986) and other SOS response-related genes (*phoP*, *yedK*) were also significantly increased. It is also found that the two GSH synthesis related genes *gshA* and *gshB* (Copley and Dhillon, 2002), exhibited 0.6– to 0.8– fold and 0.7– to 0.8– fold downregulation, respectively (Fig. 2f and Table. S3).

Collectively, based on the observed phenotypic and genetic outcomes, it is evident that CYN enhances the ARGs conjugation process, which can be attributed in part to elevated oxidative stress and the activation of the SOS response within bacterial cells. This is consistent with findings from other studies, which report that oxidative stress increases the frequency of bacterial conjugative transfer (He et al., 2022; Jiang et al., 2022; Qiu et al., 2012; Wang et al., 2019; Yu et al., 2021).

### 3.3. CYN exacerbated oxidative stress through the inhibition of GSH biosynthesis

GPx, which can mediate the reduction of GSH to glutathione disulfide (GSSG), is involved in balancing the H<sub>2</sub>O<sub>2</sub> homeostasis in signaling cascades (Brigelius-Flohe and Flohe, 2020). As mentioned above, decreased GPx activities were detected under 10–100 µg/L CYN exposure and the proper functioning of GPx necessitates the presence of GSH (Brigelius-Flohe and Maiorino, 2013). Further, GSH is one of the most important endogenous antioxidants that maintains redox balance in cells, which directly involved in neutralizing free radicals and ROS (Griffith, 1999). It has been documented that CYN could exert its toxicity via protein and GSH synthesis inhibition (Humpage et al., 2005; Runnegar et al., 1995; Yang et al., 2021). Recent studies have similarly confirmed that exposure to CYN leads to a reduction in total GSH levels, consistent with our observations (Gutierrez-Praena et al., 2011; Hinojosa et al., 2023). Hence, it is reasonable to speculate that the promoted conjugative transfer might be closely associated with CYN-induced GSH synthesis inhibition.

To confirm this speculation, total intracellular GSH levels were determined. As shown in Fig. 3a, intracellular GSH levels experienced a dose-dependent reduction in mating system after 2 h CYN exposure ( $P_{adj}$

< 0.01), except for 0.1 µg/L of CYN. Lower intracellular GSH levels would deactivate the GSH-dependent GPx (Brigelius-Flohe and Maiorino, 2013), which is consistent with the decreasing trend of GPx activities in 10–100 µg/L CYN-treated groups. Endogenous GSH biosynthesis pathway is regulated by two rate limiting enzymes, γ-glutamate-cysteine ligase (GCL) and glutathione synthetase (GS), encoded by the genes *gshA* and *gshB*, respectively (Copley and Dhillon, 2002; Zhang and Forman, 2012). As revealed in Fig. 3b, significant decreases in GCL activities were observed after 2 h of exposure to the 10–100 µg/L CYN ( $P_{adj}$  < 0.01), which exhibited a similar tendency as the reduced intracellular GSH content. These findings are consistent with studies of CYN in mammalian tissue cells and fish embryos (Gutierrez-Praena et al., 2011; Runnegar et al., 1995).

Furthermore, the transcriptional expression levels of GSH synthesis-related genes *gshA* and *gshB* were quantified by qRT-PCR. As showcased in Fig. 3c-d, the expression levels of *gshA* and *gshB* were detected as significant down-regulates under 10–100 µg/L CYN exposure. Therefore, it can be inferred that the decrease in cellular GSH could be due to the inhibition of GSH synthesis. This inference was further validated by the majority reversal of CYN-induced increased conjugation frequencies and ROS accumulation resulting from exogenous GSH treatment. For instance, the exogenous GSH supplement significantly decreased the intracellular ROS levels induced by exposure of CYN, and partially reversed corresponding CYN-induced conjugative transfer frequencies 70.90–72.03 % of the exogenous GSH-free groups (Fig. 3e-f). These results suggested that disruption of the GSH pools caused by CYN-induced GSH synthesis inhibition perturbed the equilibrium of intracellular redox state. Bacterial cells tried to degrade excessive ROS, which led to a compensatory increase in SOD and CAT activities.

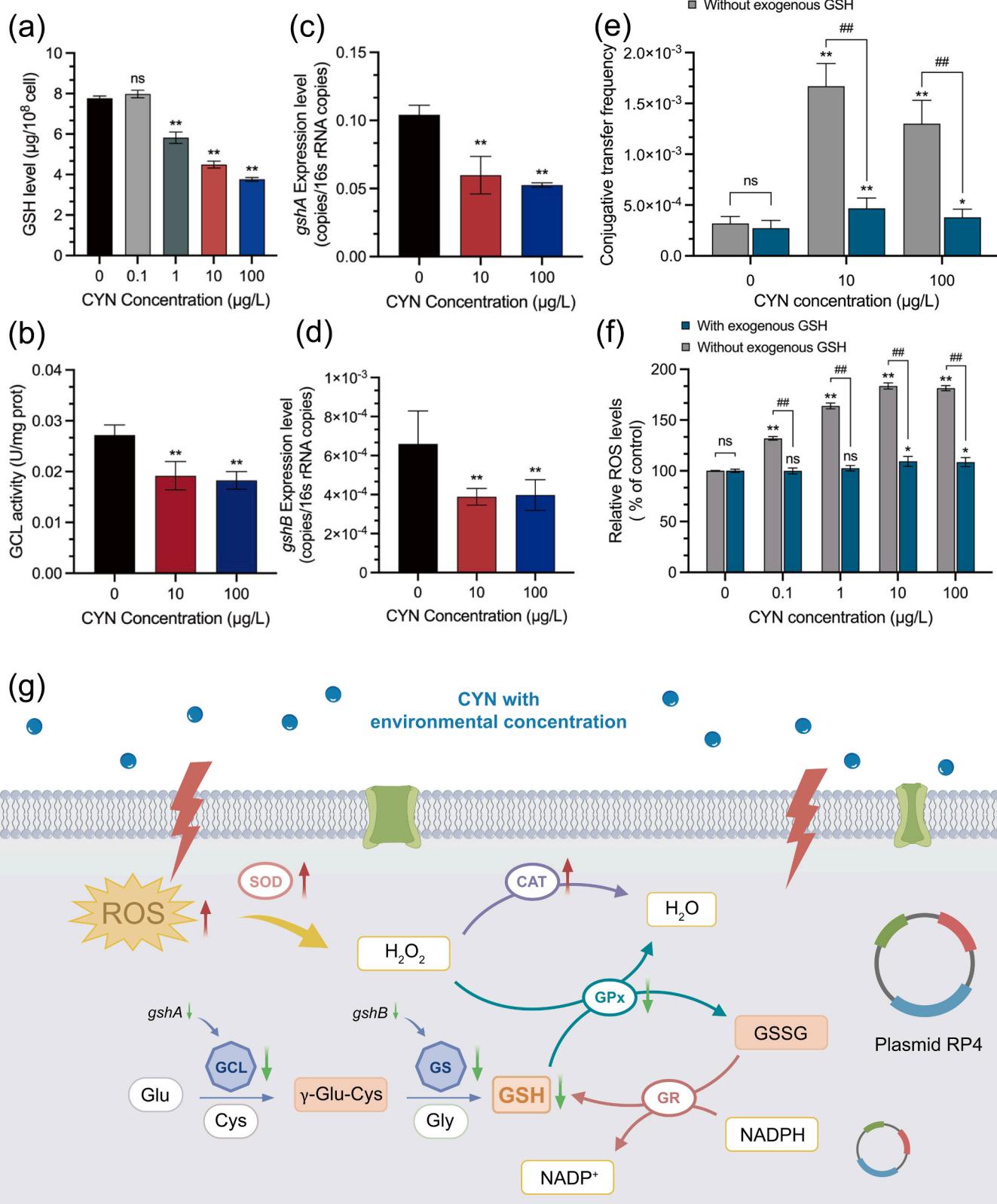
Taken together, our results indicate that environmental concentration of CYN exposure could increase oxidative stress in the bacterial cells, resulting in ROS overproduction. In turn, this led to an upregulation of antioxidant enzyme-related genes to avoid ROS attack. Furthermore, inhibition of the synthesis of GSH was also detected, leading to the rapid depletion of GSH in cells and thus triggered the SOS response and promoted the conjugative transfer of ARGs (Fig. 3g).

### 3.4. CYN increased cell membrane permeability

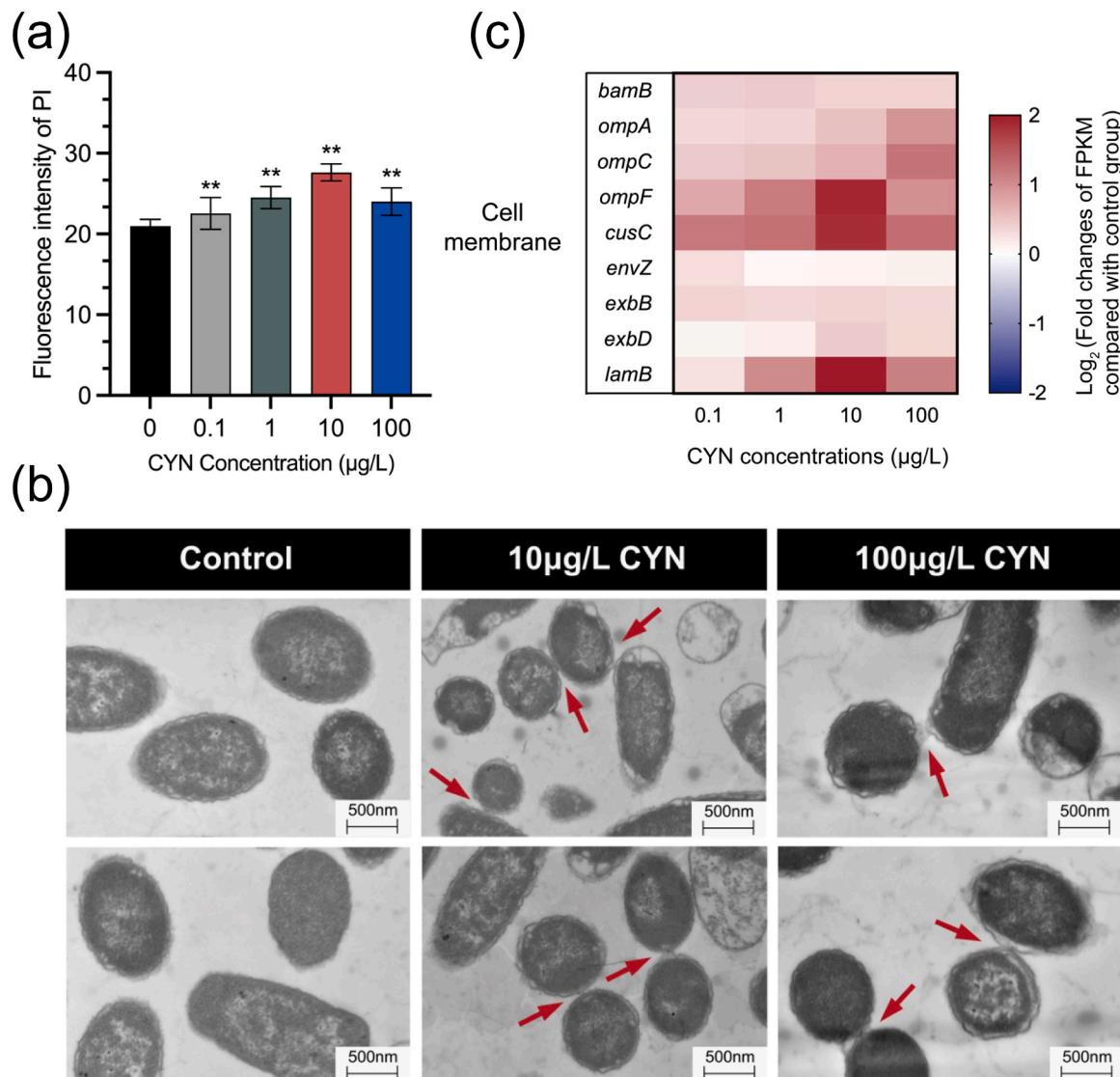
According to some previous studies, excess levels of ROS could initiate an assault on the polyunsaturated fatty acid present in the cellular membrane, thereby leading to lipid peroxidation of the membrane and an elevation in its permeability (Jaime et al., 2012), which could benefit horizontal transfer of plasmid (Wang et al., 2019).

As shown in Fig. 4a, significant changes were observed in the cell membrane permeability in conjugation system after CYN exposure, exhibiting an approximately 1.2– to 1.3– fold change compared to the control at 2 h ( $P_{adj}$  < 0.01). This was also confirmed by TEM images, which showed that cells were dispersed with limited physical contact. In untreated groups, the surface of the cell membrane was distinct and smooth after 16 h of mating. Upon exposure to 10 and 100 µg/L CYN, was observed, along with crumpled and damaged cell membranes (Fig. 4b).

The transcriptional levels of genes associated with changes in cell membrane permeability were also analyzed to support the above-described results (Fig. 4c and Table S3). For instance, exposure to CYN increased the expression of the major outer membrane protein family regulator genes *ompA*, *ompC*, and *ompF* by 1.2– to 1.9– fold, 1.4– to 2.3– fold, and 1.7– to nearly 2.3– fold, respectively. The gene *cusC*, which encodes an outer membrane transport protein (Koebnik et al., 2000; Munson et al., 2000), was found to be highly upregulated by all CYN doses tested herein, reaching 2.3– to 3.6– fold increases. Moreover, CYN also significantly increased the expression of the *exbB* and *exbD* genes, which were previously reported to modulate membrane stability and are related to the energization of outer membrane transport (Bishop et al., 1998; Pramanik et al., 2010).



**Fig. 3.** Effects of CYN on (a) GSH levels (b) GCL activity after 2 h of exposure ( $N = 3$ ). Effects of CYN on the expression of GSH biosynthetic pathway related genes (c) *gshA* and (d) *gshB* ( $N = 3$ ). Significant differences between the CYN-treated groups and the control were marked with \* ( $P_{\text{adj}} < 0.05$ ) and \*\* ( $P_{\text{adj}} < 0.01$ ), ANOVA. Effects of exogenous GSH (100  $\mu\text{M}$ ) supplement on (e) ROS generation and (f) conjugation frequencies of plasmid RP4 (16 h exposure,  $N = 3$ ). Significant differences between the CYN-treated and the control group were marked with \* ( $P_{\text{adj}} < 0.05$ ) and \*\*, (#) ( $P_{\text{adj}} < 0.01$ ), ANOVA. Significant differences between the individual GSH-treated groups and GSH-free groups were indicated as follows: # ( $P_{\text{adj}} < 0.05$ ), ## ( $P_{\text{adj}} < 0.01$ ), independent sample t-test. (g) The underlying mechanisms related to CYN-induced inhibition of GSH biosynthesis. Abbreviations are as in Table S6.



**Fig. 4.** (a) Effects of CYN exposure on cell membrane permeability. Significant differences between the individual CYN-treated and control groups were marked with \* ( $P_{\text{adj}} < 0.05$ ) and \*\* ( $P_{\text{adj}} < 0.01$ ). (b) TEM images of the control, 10  $\mu\text{g/L}$  CYN, and 100  $\mu\text{g/L}$  CYN-treated groups. (c) Fold changes in the expression of core genes related to bacterial cell membrane integrity.

### 3.5. CYN enhanced expression of conjugation-related genes on plasmid RP4

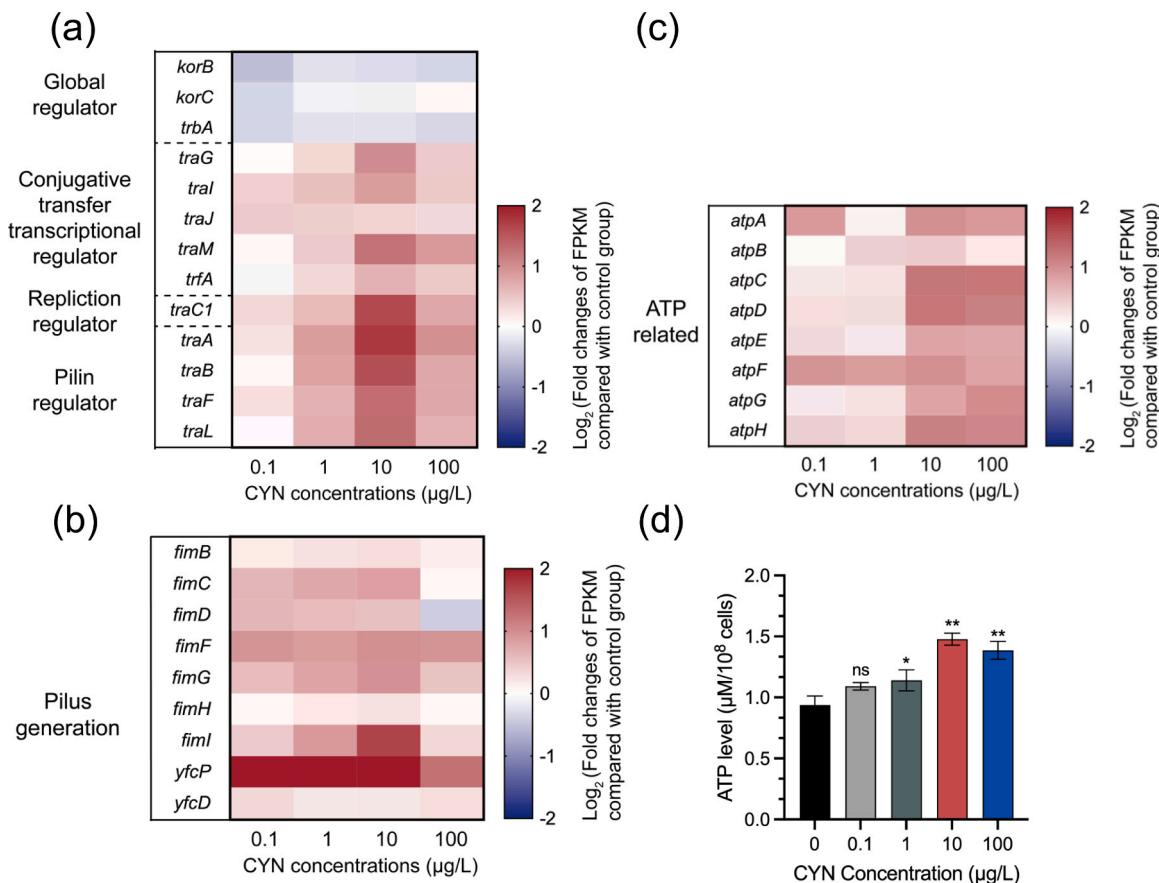
Plasmid conjugation is regulated by the DNA transfer and replication system (Dtr), and operates together with the MpF system for conjugation (Samuels et al., 2000). In this study, CYN exposure significantly decreased the expression of core global regulatory genes *korB* and *korC* (Fig. 5a). In turn, this downregulation of global regulatory genes was associated with an increase in the expression of the plasmid replication gene *trfA*, as well as the Dtr (*traC*) and MpF system genes (*traA*, *traB*, *traF*, *traL*).

CYN exposure also upregulated the expression of adhesion-relevant genes (*fim* and *yfc*) in the present study. CYN exposure significantly upregulated fimbrium biogenesis genes such as *fimC*, *fimD*, *fimG*, and *fimH* (Fig. 5b and Table S3). In addition, the expression of sex pili biogenesis genes on the RP4 plasmid, including *traA*, *traB*, *traF*, and *traL*, was notably upregulated in the 10  $\mu\text{g/L}$  CYN groups (Fig. 5a and Table S4). This is consistent with some previous studies that reported adhesive pilus generation can increase the physical contact between the donor and recipient and therefore facilitate conjugative transfer (Ochman et al., 2000). Moreover, both regulation of piliation and DNA

transfer are processes that require energy (Berzhinskene Ia et al., 1980), whereas increased energy provision could enhance conjugation frequency (Chen et al., 2005). Exposure to the selected dosages of CYN increased the expression of genes involved in ATP synthesis. Specifically, as shown in Fig. 5c and Table S3, the ATP regulator family genes *atpA* – *atpH* exhibited a 1.1– to 2.1– fold increase, especially upon exposure to 10–100  $\mu\text{g/L}$  CYN. Measurements of intracellular ATP levels within the mating system treated with CYN were also conducted. As shown in Fig. 5d, ATP levels showed a significant increase after exposure to 1–100  $\mu\text{g/L}$  CYN ( $P_{\text{adj}} < 0.05$ ), indicated a similar trend to that of the conjugation experiments (Fig. 1). However, there was no significant differences ( $P_{\text{adj}} > 0.05$ ) in intracellular ATP levels under 0.1  $\mu\text{g/L}$  CYN exposure. In this study, CYN exposure increased intracellular ATP levels (Fig. 5d) and the mRNA expression of ATP synthesis genes (Fig. 5c), suggesting that the dysregulation of energy metabolism was another underlying mechanism of CYN-induced promotional conjugative transfer.

### 4. Conclusions

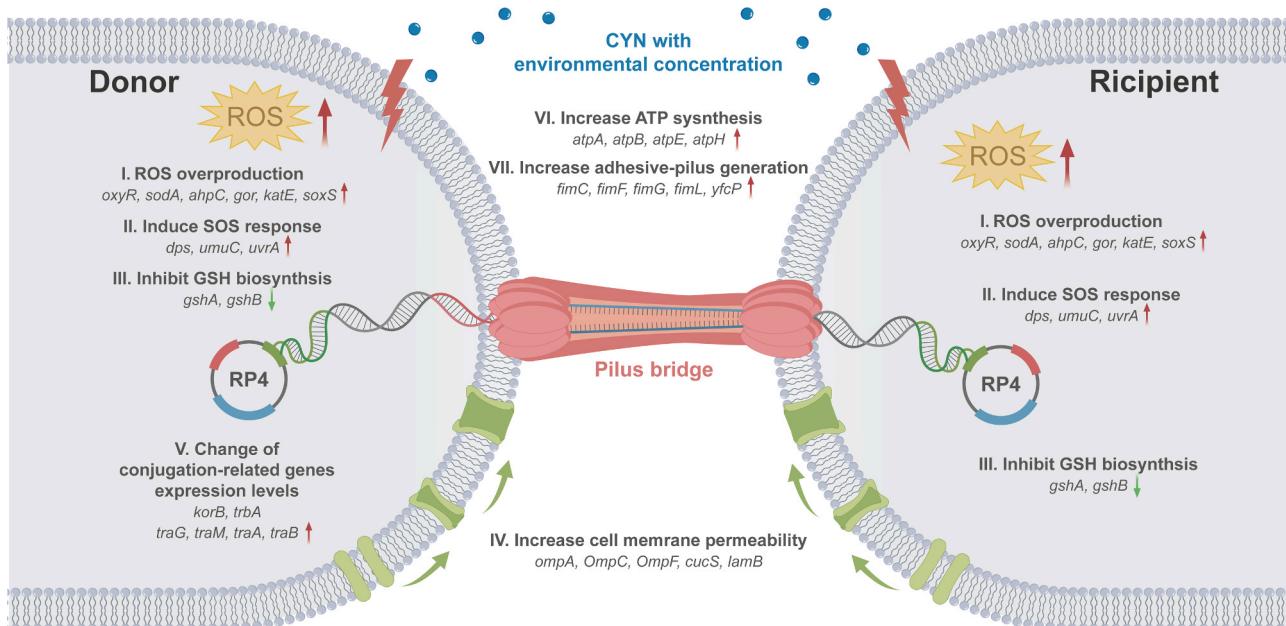
In the present study, effects and mechanisms of CYN at



**Fig. 5.** Fold changes in the expression of (a) conjugation-related genes in the RP4 plasmid, (b) pilus generation-related genes, and (c) ATP-related genes. (d) Effects of CYN exposure on intracellular ATP levels. Significant differences between the individual CYN-treated and control groups were marked with \* (P<sub>adj</sub> < 0.05) and \*\* (P<sub>adj</sub> < 0.01).

environmentally relevant concentrations (0.1–100 µg/L) on the conjugative transfer of ARGs during long-term exposure were investigated. Considering the widespread detection of CYN in various aquatic environments worldwide, the promotion of conjugative transfer induced by

CYN could be significant for the transmission and diffusion of AMR in natural aquatic environments. Moreover, these effects could be further exacerbated by the intensification of global warming caused by human activities in recent years, as this increases the frequency of



**Fig. 6.** Mechanisms of CYN-enhanced conjugative transfer of ARGs.

cyanobacterial blooms. This paper also highlights that the CYN-specific promotion mechanisms on conjugation frequency were mainly attributed to ROS overproduction, exacerbated oxidative stress due to the decreased cellular GSH pool, which was the result of CYN-induced GSH biosynthesis (Fig. 6). Moreover, this promotion effect is also implicated in increased membrane permeability, and enhanced expression of ATP-regulated genes and conjugative apparatus-related genes on the plasmid (Fig. 6). Notably, our findings of significant increases in ARG transfer enhanced by environmentally relevant CYN concentrations were still detected under laboratory conditions. Nevertheless, the influence of CYN on the diffusion of ARGs is likely to be more complex in real aquatic environments due to the influence of microbial communities, ARB abundance, algae, and other pollutants in natural ecosystems. Therefore, the potential role of CYN in promoting the conjugative transfer of ARGs in natural aquatic environments cannot be ignored, as this could pose a serious threat to public health. More studies are needed to estimate the impact of CYN on the spread of ARGs within the microbial community under complex environmental conditions.

#### CRediT authorship contribution statement

**Xiaobo Yang:** Validation, Data curation. **Shang Wang:** Validation, Data curation. **Chenyu Li:** Validation, Data curation. **Xi Zhang:** Methodology, Investigation. **Chen Zhao:** Methodology, Investigation. **Jinrui Cao:** Writing – original draft, Investigation, Conceptualization. **Zhi-qiang Shen:** Investigation, Conceptualization. **Shuran Yang:** Writing – original draft, Investigation, Conceptualization. **Bin Xue:** Investigation, Data curation, Conceptualization. **Jingfeng Wang:** Writing – review & editing. **Chao Li:** Methodology, Investigation. **Zhigang Qiu:** Writing – review & editing.

#### Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

#### 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.2024.116288](https://doi.org/10.1016/j.ecoenv.2024.116288).

#### References

- Allen, H.K., Donato, J., Wang, H.H., Cloud-Hansen, K.A., Davies, J., Handelsman, J., 2010. Call of the wild: antibiotic resistance genes in natural environments. *Nat. Rev. Microbiol.* 8 (4), 251–259.
- Bae, Y.S., Oh, H., Rhee, S.G., Yoo, Y.D., 2011. Regulation of reactive oxygen species generation in cell signaling. *Mol. Cells* 32 (6), 491–509.
- Beaber, J.W., Hochhut, B., Waldor, M.K., 2004. SOS response promotes horizontal dissemination of antibiotic resistance genes. *Nature* 427 (6969), 72–74.
- Berzhinskene Ia, A., Zizaitė, L., Baronaite, Z.A., Grinius, L.L., 1980. Energy supply for transport of plasmid R 100-1 during conjugation of *Escherichia coli* cells. *Biokhimiia* 45 (6), 1103–1112.
- Bishop, R.E., Leskiw, B.K., Hodges, R.S., Kay, C.M., Weiner, J.H., 1998. The entericidin locus of *Escherichia coli* and its implications for programmed bacterial cell death. *J. Mol. Biol.* 280 (4), 583–596.
- Brigelius-Flohe, R., Flohé, L., 2020. Regulatory phenomena in the glutathione peroxidase superfamily. *Antioxid. Redox Signal* 33 (7), 498–516.
- Brigelius-Flohe, R., Maiorino, M., 2013. Glutathione peroxidases. *Biochim Biophys. Acta* 1830 (5), 3289–3303.
- Chen, I., Christie, P.J., Dubnau, D., 2005. The ins and outs of DNA transfer in bacteria. *Science* 310 (5753), 1456–1460.
- Chiswell, R.K., Shaw, G.R., Eaglesham, G., Smith, M.J., Norris, R.L., Seawright, A.A., Moore, M.R., 1999. Stability of cylindrospermopsin, the toxin from the cyanobacterium, *Cylindrospermopsis raciborskii*: effect of pH, temperature, and sunlight on decomposition. *Environ. Toxicol.* 14 (1), 155–161.
- Copley, S.D., Dhillon, J.K., 2002. Lateral gene transfer and parallel evolution in the history of glutathione biosynthesis genes. *Genome Biol.* 3 (5) research0025.
- Díez-Quijada, L., Medrano-Padial, C., Llana-Ruiz-Cabello, M., Cătunescu, G.M., Moyano, R., Risalde, M.A., Cameán, A.M., Jos, Á., 2020. Cylindrospermopsin-microcystin-LR combinations may induce genotoxic and histopathological damage in rats. *Toxins* 12 (6).
- Dong, T.G., Dong, S., Catalano, C., Moore, R., Liang, X., Mekalanos, J.J., 2015. Generation of reactive oxygen species by lethal attacks from competing microbes. *Proc. Natl. Acad. Sci. USA* 112 (7), 2181–2186.
- Forman, H.J., Zhang, H., Rinna, A., 2009. Glutathione: overview of its protective roles, measurement, and biosynthesis. *Mol. Asp. Med.* 30 (1–2), 1–12.
- Gou, N., Yuan, S., Lan, J., Gao, C., Alshawabkeh, A.N., Gu, A.Z., 2014. A quantitative toxicogenomics assay reveals the evolution and nature of toxicity during the transformation of environmental pollutants. *Environ. Sci. Technol.* 48 (15), 8855–8863.
- Griffith, O.W., 1999. Biologic and pharmacologic regulation of mammalian glutathione synthesis. *Free Radic. Biol. Med.* 27 (9–10), 922–935.
- Gutiérrez-Praena, D., Pichardo, S., Jos, A., María Cameán, A., 2011. Toxicity and glutathione implication in the effects observed by exposure of the liver fish cell line PLHC-1 to pure cylindrospermopsin. *Ecotoxicol. Environ. Saf.* 74 (6), 1567–1572.
- Guzman-Guillen, R., Puerto, M., Gutierrez-Praena, D., Prieto, A.I., Pichardo, S., Jos, A., Campos, A., Vasconcelos, V., Cameán, A.M., 2017. Potential Use of Chemoprotectants against the Toxic Effects of Cyanotoxins: a Review. *Toxins* 9 (6).
- He, K., Xue, B., Yang, X., Wang, S., Li, C., Zhang, X., Zhao, C., Wang, X., Qiu, Z., Shen, Z., Wang, J., 2022. Low-concentration of trichloromethane and dichloroacetonitrile promote the plasmid-mediated horizontal transfer of antibiotic resistance genes. *J. Hazard. Mater.* 425, 128030.
- Hendriksen, R.S., Munk, P., Njage, P., van Bunnik, B., McNally, L., Lukjancenko, O., Roder, T., Nieuwenhuijse, D., Pedersen, S.K., Kjeldgaard, J., Kaas, R.S., Clausen, P., Vogt, J.K., Leekitcharoenphon, P., van de Schans, M.G.M., Zuidema, T., de Roda Husman, A.M., Rasmussen, S., Petersen, B., Global Sewage Surveillance project, C., Amid, C., Cochrane, G., Sicheritz-Ponten, T., Schmitt, H., Alvarez, J.R.M., Aidara-Kane, A., Pamp, S.J., Lund, O., Hald, T., Woolhouse, M., Koopmans, M.P., Vigre, H., Petersen, T.N., Aarestrup, F.M., 2019. Global monitoring of antimicrobial resistance based on metagenomic analyses of urban sewage. *Nat. Commun.* 10 (1), 1124.
- Hinojosa, M.G., Cascajosa-Lira, A., Prieto, A.I., Gutierrez-Praena, D., Vasconcelos, V., Jos, A., Cameán, A.M., 2023. Cytotoxic effects and oxidative stress produced by a cyanobacterial cylindrospermopsin producer extract versus a cylindrospermopsin non-producing extract on the neuroblastoma SH-SY5Y Cell Line. *Toxins* 15 (5).
- Holmes, A.H., Moore, L.S., Sundsfjord, A., Steinbakk, M., Regmi, S., Karkey, A., Guerin, P.J., Piddock, L.J., 2016. Understanding the mechanisms and drivers of antimicrobial resistance. *Lancet* 387 (10014), 176–187.
- Humpage, A.R., Fontaine, F., Froscio, S., Burcham, P., Falconer, I.R., 2005. Cylindrospermopsin genotoxicity and cytotoxicity: role of cytochrome P-450 and oxidative stress. *J. Toxicol. Environ. Health Part A* 68 (9), 739–753.
- Jaime, M.D., Lopez-Llorca, L.V., Conesa, A., Lee, A.Y., Proctor, M., Heisler, L.E., Gebbia, M., Giaevers, G., Westwood, J.T., Nislow, C., 2012. Identification of yeast genes that confer resistance to chitosan oligosaccharide (COS) using chemogenomics. *BMC Genom.* 13, 267.
- Ji, H., Cai, Y., Wang, Z., Li, G., An, T., 2022. Sub-lethal photocatalysis promotes horizontal transfer of antibiotic resistance genes by conjugation and transformability. *Water Res.* 221, 118808.
- Jiang, Q., Feng, M., Ye, C., Yu, X., 2022. Effects and relevant mechanisms of non-antibiotic factors on the horizontal transfer of antibiotic resistance genes in water environments: a review. *Sci. Total Environ.* 806 (Pt 3), 150568.
- Jo, I., Chung, I.Y., Bae, H.W., Kim, J.S., Song, S., Cho, Y.H., Ha, N.C., 2015. Structural details of the OxyR peroxide-sensing mechanism. *Proc. Natl. Acad. Sci. USA* 112 (20), 6443–6448.
- Kleintech, J., Hildebrand, F., Wood, S.A., Ciřs, S., Agha, R., Quesada, A., Pearce, D.A., Convey, P., Kpper, F.C., Dietrich, M.R., 2014. Diversity of toxin and non-toxin containing cyanobacterial mats of meltwater ponds on the Antarctic Peninsula: a pyrosequencing approach. *Antarct. Sci.* 26 (5), 521–532.
- Koebnik, R., Locher, K.P., Van Gelder, P., 2000. Structure and function of bacterial outer membrane proteins: barrels in a nutshell. *Mol. Microbiol.* 37 (2), 239–253.
- Lackraj, T., Birstones, S., Kacori, M., Barnett Foster, D., 2020. Dps protects enterohemorrhagic *Escherichia coli* against acid-induced antimicrobial peptide killing. *J. Bacteriol.* 202 (11).
- Li, G., Chen, X., Yin, H., Wang, W., Wong, P.K., An, T., 2020. Natural sphalerite nanoparticles can accelerate horizontal transfer of plasmid-mediated antibiotic-resistance genes. *Environ. Int.* 136, 105497.
- Lu, J., Wang, Y., Li, J., Mao, L., Nguyen, S.H., Duarte, T., Coin, L., Bond, P., Yuan, Z., Guo, J., 2018. Triclosan at environmentally relevant concentrations promotes horizontal transfer of multidrug resistance genes within and across bacterial genera. *Environ. Int.* 121 (Pt 2), 1217–1226.
- Lu, J., Wang, Y., Jin, M., Yuan, Z., Bond, P., Guo, J., 2020. Both silver ions and silver nanoparticles facilitate the horizontal transfer of plasmid-mediated antibiotic resistance genes. *Water Res.* 169, 115229.
- Martinez, J.L., 2008. Antibiotics and antibiotic resistance genes in natural environments. *Science* 321 (5887), 365–367.

- Merel, S., Walker, D., Chicana, R., Snyder, S., Baurès, E., Thomas, O., 2013. State of knowledge and concerns on cyanobacterial blooms and cyanotoxins. *Environ. Int.* 59, 303–327.
- Munson, G.P., Lam, D.L., Outten, F.W., O'Halloran, T.V., 2000. Identification of a copper-responsive two-component system on the chromosome of *Escherichia coli* K-12. *J. Bacteriol.* 182 (20), 5864–5871.
- Ochman, H., Lawrence, J.G., Groisman, E.A., 2000. Lateral gene transfer and the nature of bacterial innovation. *Nature* 405 (6784), 299–304.
- Ohtani, I., Moore, R.E., Runnegar, M.T.C., 1992. Cylindrospermopsin: a potent hepatotoxin from the blue-green alga *Cylindrospermopsis raciborskii*. *J. Am. Chem. Soc.* 114 (20), 7941–7942.
- Pearson, L., Mihali, T., Moffitt, M., Kellmann, R., Neilan, B., 2010. On the chemistry, toxicology and genetics of the cyanobacterial toxins, microcystin, nodularin, saxitoxin and cylindrospermopsin. *Mar. Drugs* 8 (5), 1650–1680.
- Picossi, S., Flores, E., Herrero, A., 2015. The LysR-type transcription factor PacR is a global regulator of photosynthetic carbon assimilation in *Anabaena*. *Environ. Microbiol.* 17 (9), 3341–3351.
- Pomposiello, P.J., Demple, B., 2001. Redox-operated genetic switches: the SoxR and OxyR transcription factors. *Trends Biotechnol.* 19 (3), 109–114.
- Pramanik, A., Zhang, F., Schwarz, H., Schreiber, F., Braun, V., 2010. ExbB protein in the cytoplasmic membrane of *Escherichia coli* forms a stable oligomer. *Biochemistry* 49 (40), 8721–8728.
- Prestinaci, F., Pezzotti, P., Pantosti, A., 2015. Antimicrobial resistance: a global multifaceted phenomenon. *Pathog. Glob. Health* 109 (7), 309–318.
- Puerto, M., Jos, A., Richardo, S., Moyano, R., Blanco, A., Cameán, A.M., 2014. Acute exposure to pure cylindrospermopsin results in oxidative stress and pathological alterations in tilapia (*Oreochromis niloticus*). *Environ. Toxicol.* 29 (4), 371–385.
- Qiu, Z., Yu, Y., Chen, Z., Jin, M., Yang, D., Zhao, Z., Wang, J., Shen, Z., Wang, X., Qian, D., Huang, A., Zhang, B., Li, J.W., 2012. Nanoalumina promotes the horizontal transfer of multi-resistance genes mediated by plasmids across genera. *Proc. Natl. Acad. Sci. USA* 109 (13), 4944–4949.
- Ran, M., Li, Q., Xin, Y., Ma, S., Zhao, R., Wang, M., Xun, L., Xia, Y., 2022. Rhodaneses minimize the accumulation of cellular sulfane sulfur to avoid disulfide stress during sulfide oxidation in bacteria. *Redox Biol.* 53, 102345.
- Runnegar, M.T., Kong, S.M., Zhong, Y.Z., Lu, S.C., 1995. Inhibition of reduced glutathione synthesis by cyanobacterial alkaloid cylindrospermopsin in cultured rat hepatocytes. *Biochem. Pharmacol.* 49 (2), 219–225.
- Samuels, A.L., Lanka, E., Davies, J.E., 2000. Conjugative junctions in RP4-mediated mating of *Escherichia coli*. *J. Bacteriol.* 182 (10), 2709–2715.
- Sargentini, N.J., Smith, K.C., 1986. Quantitation of the involvement of the recA, recB, recC, recF, recJ, recN, lexA, radA, radB, uvrD, and umuC genes in the repair of X-ray-induced DNA double-strand breaks in *Escherichia coli*. *Radiat. Res.* 107 (1), 58–72.
- Scarlett, K.R., Kim, S., Lovin, L.M., Chatterjee, S., Scott, J.T., Brooks, B.W., 2020. Global scanning of cylindrospermopsin: critical review and analysis of aquatic occurrence, bioaccumulation, toxicity and health hazards. *Sci. Total Environ.* 738, 139807.
- Ubeda, C., Maiques, E., Knecht, E., Lasa, I., Novick, R.P., Penades, J.R., 2005. Antibiotic-induced SOS response promotes horizontal dissemination of pathogenicity island-encoded virulence factors in staphylococci. *Mol. Microbiol* 56 (3), 836–844.
- Wang, K., Han, L., Hong, H., Pan, J., Liu, H., Luo, Y., 2021a. Purification and identification of novel antioxidant peptides from silver carp muscle hydrolysate after simulated gastrointestinal digestion and transepithelial transport. *Food Chem.* 342, 128275.
- Wang, Q., Liu, L., Hou, Z., Wang, L., Ma, D., Yang, G., Guo, S., Luo, J., Qi, L., Luo, Y., 2020a. Heavy metal copper accelerates the conjugative transfer of antibiotic resistance genes in freshwater microcosms. *Sci. Total Environ.* 717, 137055.
- Wang, Y., Lu, J., Mao, L., Li, J., Yuan, Z., Bond, P.L., Guo, J., 2019. Antiepileptic drug carbamazepine promotes horizontal transfer of plasmid-borne multi-antibiotic resistance genes within and across bacterial genera. *ISME J.* 13 (2), 509–522.
- Wang, Y., Zhao, H., Liu, Y., Li, J., Nie, X., Huang, P., Xing, M., 2021b. Environmentally relevant concentration of sulfamethoxazole-induced oxidative stress-cascaded damages in the intestine of grass carp and the therapeutic application of exogenous lycopene. *Environ. Pollut.* 274, 116597.
- Wang, Z., Chen, Q., Zhang, J., Guan, T., Chen, Y., Shi, W., 2020b. Critical roles of cyanobacteria as reservoir and source for antibiotic resistance genes. *Environ. Int.* 144, 106034.
- Watkins, R.R., Bonomo, R.A., 2016. Overview: Global and Local Impact of Antibiotic Resistance. *Infect. Dis. Clin. N. Am.* 30 (2), 313–322.
- von Wintersdorff, C.J., Penders, J., van Niekerk, J.M., Mills, N.D., Majumder, S., van Alphen, L.B., Savelkoul, P.H., Wolfs, P.F., 2016. Dissemination of antimicrobial resistance in microbial ecosystems through horizontal gene transfer. *Front. Microbiol.* 7, 173.
- Xu, L., Zhou, Z., Zhu, L., Han, Y., Lin, Z., Feng, W., Liu, Y., Shuai, X., Chen, H., 2020. Antibiotic resistance genes and microcysts in a drinking water treatment plant. *Environ. Pollut.* 258, 113718.
- Yang, Y., Yu, G., Chen, Y., Jia, N., Li, R., 2021. Four decades of progress in cylindrospermopsin research: the ins and outs of a potent cyanotoxin. *J. Hazard. Mater.* 406, 124653.
- Yu, Z., Wang, Y., Lu, J., Bond, P.L., Guo, J., 2021. Nonnutritive sweeteners can promote the dissemination of antibiotic resistance through conjugative gene transfer. *ISME J.* 15 (7), 2117–2130.
- Zhang, H., Forman, H.J., 2012. Glutathione synthesis and its role in redox signaling. *Semin. Cell Dev. Biol.* 23 (7), 722–728.
- Zhang, Q., Zhang, Z., Lu, T., Peijnenburg, W., Gillings, M., Yang, X., Chen, J., Penuelas, J., Zhu, Y.G., Zhou, N.Y., Su, J., Qian, H., 2020. Cyanobacterial blooms contribute to the diversity of antibiotic-resistance genes in aquatic ecosystems. *Commun. Biol.* 3 (1), 737.
- Zhang, Q., Wang, L., Chen, G., Wang, M., Hu, T., 2021. Cylindrospermopsin impairs vascular smooth muscle cells by P53-mediated apoptosis due to ROS overproduction. *Toxicol. Lett.* 353, 83–92.
- Zhang, X., Zheng, Q., Yue, X., Yuan, Z., Ling, J., Yuan, Y., Liang, Y., Sun, A., Liu, Y., Li, H., Xu, K., He, F., Wang, J., Wu, J., Zhao, C., Tian, C., 2022. ZNF498 promotes hepatocellular carcinogenesis by suppressing p53-mediated apoptosis and ferroptosis via the attenuation of p53 Ser46 phosphorylation. *J. Exp. Clin. Cancer Res.* 41 (1), 79.
- Zhao, Z., Yang, S., Deng, Y., Wang, L., Zhang, Y., Feng, Z., Li, H., Chi, Z., Xie, Y., Dong, D., 2022. Naringin Interferes Doxorubicin-Induced Myocardial Injury by Promoting the Expression of ECHS1. *Front. Pharmacol.* 13, 859755.
- Zhou, H., Yang, X., Yang, Y., Niu, Y., Li, J., Fu, X., Wang, S., Xue, B., Li, C., Zhao, C., Zhang, X., Shen, Z., Wang, J., Qiu, Z., 2023. Docosahexaenoic acid inhibits pheromone-responsive-plasmid-mediated conjugative transfer of antibiotic resistance genes in *Enterococcus faecalis*. *J. Hazard. Mater.* 444 (Pt A), 130390.