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Antibiotic-resistance gene transfer in antibiotic-resistance bacteria under different light irradiation: Implications from oxidative stress and gene expression

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ABSTRACT

Due to the significant public health risks, there is substantial scientific interest in the increasing abundance of antibiotic-resistance bacteria (ARB) and the spread of antibiotic-resistance genes (ARGs) in aquatic environments. To clearly understand the mechanism of ARG transfer, this study examined the conjugative transfer of genes encoding resistance to cephalosporin (bla_{CTX}) and polymyxin (*mcr-1*) from two antibiotic-resistant donor strains, namely *E. coli* DH5α (CTX) and *E. coli* DH5α (MCR), and to a streptomycin-resistant receptor strain (*E. coli* C600 (Sm)). Conjugative transfer was specifically studied under different light irradiation conditions including visible light (VL), simulated sunlight (SS) and ultraviolet light (UV_{254nm}). Results show that the conjugative transfer frequency was not affected by VL irradiation, while it was slightly improved (2–10 fold) by SS irradiation and extremely accelerated (up to 100 fold) by UV irradiation. Furthermore, this study also explored the link between ARG transfer and stress conditions. This was done by studying physiological and biochemical changes; oxidative stress response; and functional gene expression of co-cultured AR-*E. coli* strains under stress conditions. When correlated with the transfer frequency results, we found that VL irradiation did not affect the physiological and biochemical characteristics of the bacteria, or induce oxidative stress and gene expression. For SS irradiation, oxidative stress occurred slowly, with a slight increase in the expression of target genes in the bacterial cells. In contrast, UV irradiation, rapidly inactivated the bacteria, the degree of oxidative stress was very severe and the expression of the target genes was markedly up-regulated. Our study could provide new insight into the underlying mechanisms and links between accelerated conjugative transfer and oxidative stress, as well as the altered expression of genes relevant to conjugation and other stress responses in bacterial cells.

1. Introduction

In recent decades, the increasing abundance of antibiotic resistance in the environment and the spread of antibiotic-resistance genes (ARGs) have drawn increased attention due to their significant public health risks (Bouki et al., 2013; Taylor et al., 2011; Walsh et al., 2011). Antibiotic-resistance bacteria (ARB) and ARGs have been found in various environments, especially in aquatic systems (Di Cesare et al., 2016; Jia et al., 2017), which act as reservoirs and conduits for the spread of both ARB and ARGs (Munir et al., 2011; Schwartz et al., 2003). Antibiotic overuse has been established as a factor associated with the outbreak of antibiotic resistance (Christou et al., 2017; Zhang et al., 2017).

Horizontal gene transfer (HGT), which is usually necessary for competitive bacteria to evolve in the environment, is one of the major drivers for the dissemination of ARGs (Erickson, 2001; Huddleston, 2014; Kruse and Sørum, 1994). There are three main mechanisms of HGT: transformation, transduction and conjugation

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(Heuer and Smalla, 2007). The latter is thought to be the most frequent and efficient mechanism for gene transfer in the environment, including ARGs (Bellanger et al., 2014; Dang et al., 2017; Erickson, 2001; Sørensen et al., 2005) thus contributing to the spread of ARGs among bacteria (Zhang et al., 2017).

Recent studies have demonstrated bacterial ARG transfer can be enhanced by different environmental and chemical stressors, including chlorination (Guo et al., 2015), nano-materials (Qiu et al., 2012), antibiotics (Zhang et al., 2017) and organic contaminants (Jiao et al., 2017). This genetic transfer among the bacterial cells is usually induced by stressors, such as antibiotics (Beaber et al., 2004; Jutkina et al., 2016), metals and biocides (Seier-Petersen et al., 2014). Further evidence indicates that the horizontal transfer of ARGs between bacteria is induced by antibiotics and occurs via broadly conserved cellular response pathways, such as those involved in reactive oxygen species (ROSs) response systems (Beaber et al., 2004) and the SOS response (Andersson and Hughes, 2014; Beaber et al., 2004). The involvement of these cellular pathways and mechanisms raise important questions about the possible role of other environmental factors in ARG transmission.

Due to the issues described above, there is increasing interest in studying the effects of environmental factors on the transfer of ARGs. In the natural environment, bacteria encounter a myriad of stressors, among which light could be one of the most important factors affecting the survival of enteric bacteria in the aquatic environment during the daytime (Clerc and Simonet, 1996). Although there are many studies focused on the spread of ARGs by conjugative transfer in environmental matrices (Bellanger et al., 2014; Dröge et al., 1999), it is still unclear how light irradiation may affect their conjugation transfer frequency and the mechanism by which it triggers oxidative stress responses or alters gene expression. Furthermore, the possible correlations between them are unknown.

Therefore, the main objective of this study was to investigate the mechanisms of ARG conjugative transfer under different types of light irradiation. A comprehensive study was conducted by exposing ARB and antibiotic-susceptible bacteria (ASB) to different light irradiation. This was done by (1) studying the physiological and biochemical changes in the ARB and ASB; (2) quantifying the expression of different genes, including those involved in oxidative stress regulation, cell repair, DNA repair, conjugation and antibiotic resistance; (3) analyzing the bacterial oxidative stress response (including the ROS content and activity of antioxidant enzymes) in both bacterial strains; and (4) studying the change in frequency of ARG transfer. The objective of this study was to reveal the link between the ARG conjugative transfer efficiencies and bacterial stress conditions in the aquatic environment by investigating the effects of different light irradiations on conjugative transfer.

2. Materials and methods

2.1. Bacterial strains and culture conditions

All the strains used in this study are listed in Table S1. The two donor strains were *E. coli* DH5α (CTX), which harbors a plasmid containing the bla_{CTX} gene that confers resistance to cephalosporins, and *E. coli* DH5α (MCR), which harbors a plasmid containing the *mcr-1* gene that confers resistance to polymyxin B. These two plasmids belong to the transferable plasmid of IncI2. Both strains were cultured in Luria-Bertani (LB) medium supplemented with cefotaxime (16 μg mL^{-1}) and polymyxin B (8 μg mL^{-1}), respectively. The recipient strain (*E. coli* C600 (Sm)) was grown in LB supplemented with 3000 μg mL^{-1} of streptomycin (Sm). In this case, resistance to streptomycin was encoded within the bacterial genome. Prior to the ARG transfer experiments, the susceptibility of the recipient strain was validated based on its inability to grow on plates containing cefotaxime (16 μg mL^{-1}) and polymyxin B (8 μg mL^{-1}).

Bacteria were cultured by inoculating 1 mL of frozen stock culture (−80 °C) into 100 mL of sterile LB broth. Cultures were grown at 37 °C for 18 h under continuous agitation in a rotating shaker until growth approached the logarithmic phase, which was defined as an optical density at 600 nm (OD_{600}) between 1.8 and 2.0, as measured by microplate readers (Varioskan LUX; Thermo Fisher Scientific, USA). The logarithmic phase bacteria were then centrifuged at 8000 rpm for 2 min. The supernatant was discarded and the cell pellet was rinsed twice with a sterilized natural saline solution (0.9% NaCl, pH = 7.2). The pellet was then resuspended in natural saline solution to an OD_{600} of 0.1, which represented approximately 10^8 colony-forming units (CFU) mL^{-1}. A stability curve was created for each of the bacteria in natural saline to assess the viability and potential growth throughout the experimental process (An et al., 2017).

2.2. Light sources

Three different light sources were used in this study, namely visible light (VL), simulated sunlight (SS) and ultraviolet light (UV_{254nm}). An LED light (PerfectLight, Inc., Beijing, China) was used as the VL source, while a 300 W xenon lamp (PerfectLight, Inc.) coupled with a sunlight simulated filter was used as the SS source. For the VL and SS, the irradiance spectra were measured using a spectrometer (USB 2000+, Ocean Optics Inc., USA) and the fluence rate (irradiance) was 60 mW cm^{-2}. A low voltage mercury lamp (single-wavelength germicidal, peak wavelength 254 nm; Trojan Technologies Inc., Canada) was used as the UV source. The fluence rate was determined using a UVX Radiometer with a UVX-25 (UV_{254}) sensor (UVP, LLC), which was calibrated by the manufacturer to the National Institute of Standards and Technology specifications. The fluence rate was set at approximately 4 μW cm^{-2}. The samples were exposed to a range of UV doses (0-2.88 × 10^4 μW s cm^{-2}) by varying the exposure time from 0 to 120 min. The spectra of the three light sources are shown in Fig. S1. Pictures of the three reactors are shown in Figs. S2, S3 and S4.

2.3. Scanning electron microscopy (SEM) and detection of reactive oxygen species

Preparation of the SEM samples is outlined in the SI and was similar to the procedure described in our previous work (Sun et al., 2014).

To explore whether light irradiation affects ARB at a molecular level, intracellular ROSs were quantified using the fluorogenic dye 2′,7′-dichlorofluorescein diacetate (DCFH-DA) (Invitrogen, Carlsbad, USA). This method has been used for similar purposes in other studies (Sun et al., 2014, 2016). Briefly, bacterial suspensions (approximately 10^8 CFU mL^{-1}) were stained with DCFH-DA (final concentration 1 mM) for 20 min at 37 °C in the dark. The suspensions were gently shaken before and after photo-irradiation, as described above. The bacterial pellets were washed twice with 1 × phosphate buffer solution (PBS) and then re-suspended in a sterilized natural saline solution. The treated samples were pipetted into a microplate to measure the fluorescence at 525 nm with the excitation wavelength set at 488 nm. The ROS production level for each light irradiation treatment was normalized to that of the control samples at the initial time (0 min). All experiments were conducted at least in triplicate. The relative ROS levels were expressed in terms of fluorescence intensity. The detailed protocol of the fluorescent assay is provided in the SI.

2.4. Enzymatic activity assays

Enzymatic assays were conducted to determine catalase (CAT), superoxide dismutase (SOD) and glutathione peroxidase (GSH-Px) activities. Cells, harvested from 1.5 mL of bacterial suspension, were lysed using 100 μL of B-PER® Bacterial Protein Extraction Reagent (Pierce Biotechnology, USA). The lysate was centrifuged at 12000 rpm for 2 min. The supernatant was then used to measure CAT, SOD and GSH-Px activity with the Catalase Assay Kit (Beyotime Institute of Biotechnology, China), Superoxide Dismutase Assay Kit (Cayman Chemical, USA) and Glutathione Peroxidase Assay Kit (Nanjing Jiancheng Bioengineering Institute, China), respectively, following manufacturer's instructions. Details of the assay procedures are summarized in the SI.

2.5. Evaluation of the mRNA gene expression

Total RNA was extracted from the bacterial samples at different light irradiation intervals using RNAiso Plus (TaKaRa, Dalian, China). The RNA was then transcribed into cDNA using the PrimeScript™ Reverse Transcriptase kit (TaKaRa) following manufacturer's instructions. Real-time polymerase chain reaction (RT-PCR) was used to quantify the expression of genes involved in oxidative stress regulation (*oxyR, rpoS, soxR, soxS, marA, ompR, osmC, osmY*), cell repair (*basS, cusC, mdtB, motA, yiaD*), DNA repair (*mukB, radA, recF, recJ, recA, rpoD, rpoH, ruvB, lexA, rcsC*) and conjugation (*tesB, ftsY, gspE*), which respectively represents lipid synthesis, cell differentiation and signal transduction genes, while the differential expression of bacterial transcription indirectly reflects the changes in ARG transfer and ARGs (bla_{CTX}, *mcr-1*). The 16S rRNA gene was used as an internal control for data normalization. RT-PCR was performed with SYBR Green I (TaKaRa) in a RT-PCR thermal cycler (CFX 96; Bio-Rad, Hercules, CA, USA).

The primers used in this study are listed in Table S2. The RT-PCR mixtures (25 μL) consisted of 12.5 μL of 2 × SYBR Premix Ex Taq (TaKaRa), 0.75 μL of each primer (10 μM final concentration), 1 μL of cDNA template and 10 μL of distilled H_2O. The thermal cycler amplification profile was 95 °C for 30 s, followed by 40 cycles of 95 °C for 45 s, 60 °C for 45 s, a melting curve analysis at 95 °C for 15 s, and, finally, annealing at 60 °C for 1 min. Each experiment was conducted at least in duplicate. The mRNA expression levels of conjugation related-genes were calculated using the $2^{-\triangle\triangle CT}$ method as follows (Lin et al., 2016):

$$\triangle Ct = Ct_{(\text{Functional genes})} - Ct_{(\text{16S rRNA gene})}$$

$$\triangle\triangle Ct = \triangle Ct_{(\text{Irradiation})} - \triangle Ct_{(\text{Control})}$$

$$FC(\text{fold change}) = 2^{-\triangle\triangle Ct}$$

where the Ct value is the cycle threshold and the functional genes are those mentioned above. No irradiation was added for the control group.

2.6. Establishment of conjugative transfer models

A conjugative transfer model was established to evaluate the conjugative transfer of ARGs under different light irradiations. First, the donor strains *E. coli* DH5α (CTX) or *E. coli* DH5α (MCR) were re-suspended in separate sterile natural saline solutions, and one of the two strains was exposed to different light irradiation. For UV irradiation, donor cells were mixed with recipient cells (*E. coli* C600 (Sm)) at a 1:1 ratio (1 mL each) at different time points (0–120 min). All steps were carried out in the dark to prevent photoreactivation. The mixtures were cultured at 37 °C for 16 h with continuous shaking (150 rpm). For SS and VL irradiation, donor cells re-suspended in sterile natural saline were exposed to SS or VL irradiation at different time points between 0 and 480 min. Re-suspended donor and recipient cells were mixed at a 1:1 ratio (1 mL each) and then cultured under the same conditions as described for UV-irradiated cultures.

To optimize the conjugative transfer models, single-factor experiments were conducted to optimize the cell concentration (10^4, 10^5, 10^6, 10^7, 10^8, 10^9 and 10^{10} CFU mL^{-1}), donor/recipient ratios (1:1, 1:3, 1:5 and 1:7) and mating times (4, 6, 16, 18 and 24 h) (Fig. S5). Next, bacterial growth curves were prepared to optimize mating time (Fig. S6). All donor and recipient bacteria were incubated at 37 °C under shaking at 200 rpm for 18 h.

2.7. Transconjugant identification

After mating for 16 h, separate 2 mL subsamples were collected from donor and recipient sample mixtures. The cultures were serially diluted and spread on LB medium plates containing 10 g L^{-1} agar and different antibiotics. The transconjugants of the donor strain (*E. coli* DH5α (CTX)) were selected on LB plates supplemented with 16 μg mL^{-1} CTX and 3000 μg mL^{-1} Sm. The transconjugants of the donor strain (*E. coli* DH5α (MCR)) were selected on LB plates containing 8 μg mL^{-1} PB and 3000 μg mL^{-1} Sm.

The data represents the average colony counts (CFU mL^{-1}) from triplicate plates and all figures include error bars. Differences in colony counts across replicate experiments never exceeded 10%. The limit of detection for the plating assay was 10 CFU mL^{-1}. All bacterial activity experiments were performed in duplicate. The frequency of conjugative transfer is expressed as the number of transconjugant cells per recipient cells. This frequency was calculated using the following equation:

$$\text{Frequency of conjugative transfer} = \frac{\text{Number of transconjugants}}{\text{Number of recipents}}$$

where the unit of bacterial cells is CFU mL^{-1}.

2.8. Statistical analysis

One-way analysis of variance (ANOVA) was used to determine significant differences in the frequency of conjugative transfer under different light irradiation at a significance level of $P = 0.05$. A result was considered significant if $P < 0.05$, and highly significant when $P < 0.01$, according to previous work (Zhang et al., 2017). The activation and relative ROS generation concentrations, as well as anti-enzyme activities of antibiotic-resistance strains, were all replicated three times, and results are expressed as the mean ± standard deviation.

3. Results

3.1. Inactivation effects under different light irradiation

To evaluate the bactericidal activity of different light sources, time-killing curves were plotted for ARB (*E. coli* DH5α (CTX) and *E. coli* DH5α (MCR)) and ASB (*E. coli* DH5α) irradiated with the three different light sources. Under VL irradiation, there was no significant change in cell concentrations for the three strains (Fig. 1a), suggesting that VL does not have an inactivation effect on ARB and ASB. This result was further confirmed using SEM (Fig. S7). During VL irradiation, the cells had a solid rod shape and maintained a full and smooth surface, without exhibiting significant surface and

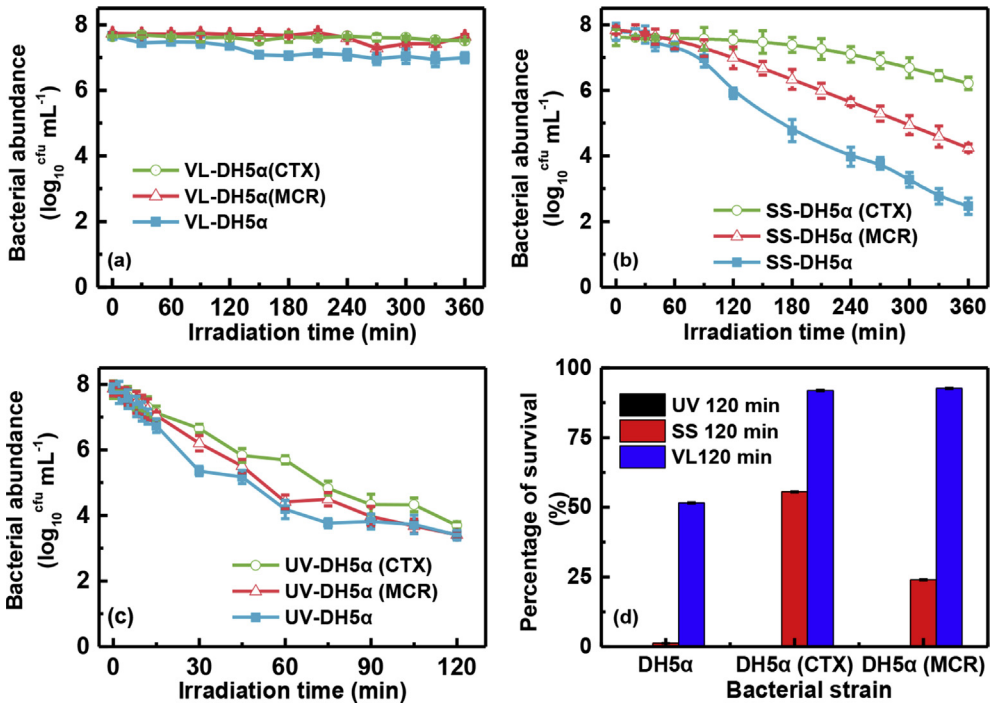

Fig. 1. Culturable bacterial abundance in logarithm of *E. coli* DH5α (MCR), *E. coli* DH5α (CTX) and *E. coli* DH5α during (a) visible light (VL), (b) simulated sunlight (SS), and (c) UV stimulation processes, (d) Effect of three stimulation processes on the bactericidal efficiencies of *E. coli* DH5α (MCR), *E. coli E. coli* DH5α (CTX) and *E. coli* DH5α at 120 min.

microstructure changes. This outcome was identical to that observed for the untreated bacterial cells (Fig. S7), indicating that VL stimulation did not appear to damage the bacteria.

Under SS irradiation, the cell concentrations of the tested bacterial strains initially remained steady during the early period of SS stimulation (60 min of shoulder length), which is similar to VL irradiation (Fig. 1b). In comparison, ASB was inactivated more quickly than the two ARBs, suggesting that the bacteria carrying ARGs are more resistant to external stress than the tested ASB (*E. coli* DH5α). Moreover, when the irradiation time was extended to 360 min, the ASB showed an even more significant reduction in cell concentrations (5-log). Furthermore, SEM images of the bacterial cells irradiated under SS show that long-term SS irradiation could lead to significant damage to the bacterial cells, with deeper sinking and shriveled cell walls observed for all tested bacterial strains (Fig. S7).

UV irradiation exhibited superior inactivation when compared to VL and SS irradiation (Fig. 1c). All bacterial strains were quickly inactivated by UV radiation after the initial 10 min exposure (Fig. 1c). After 120 min of UV irradiation, the cell concentration was reduced up to 5-log for all tested strains. Compared with SS irradiation, the bacteria were more strongly affected under UV irradiation, and their shoulder period (an initial smooth decay period at the beginning period of the reaction, bacteria remained alive with activity intact) (Nie et al., 2014) (10 min of shoulder length) were much shorter. It is likely that the mechanisms causing bacterial damage are quite different between SS and UV irradiation, in which the latter induces DNA damage. Furthermore, SEM images show that UV irradiation caused time-dependent damage to cells of all tested strains (Fig. S7). Some cells exhibited a slight collapsed appearance within 60 min of irradiation, while cell ends were deeply dented after 120 min irradiation. When UV irradiation was prolonged to 180 min, cells experienced a more severe damage. Thereafter, intact cell walls began to shrink and the cells collapsed.

Furthermore, the bactericidal effects of UV irradiation on the three bacterial strains were compared to VL and SS irradiation within a 120 min treatment period. The inactivation efficiencies varied among the *E. coli* strains (Fig. 1d). In particular, the reduction of ARB and ASB concentrations reached 99.9% under UV irradiation within 120 min, while none of the tested bacterial strains were easily inactivated by VL irradiation. Furthermore, ARB, especial *E. coli* DH5α (CTX), were more resistant to all forms of tested light irradiation.

3.2. Stress response under different light irradiation

Generally, intracellular ROSs are considered to be responsible for oxidative stress, which leads to oxidative damage of DNA, enzymes and other cell components (Sun et al., 2014). Furthermore, the formation of intracellular ROSs subsequently induces the generation of various antioxidant enzymes that can catalyze the conversion and detoxification of $^{\bullet}O_2^-$ and H_2O_2 (Cabiscol et al., 2000; Farr and Kogoma, 1991; Fraud and Poole, 2011) to relieve oxidative stress. Among these enzymes, catalase (CAT), a type of bacterial antioxidant enzyme, breaks down H_2O_2 to H_2O and O_2 (Farr and Kogoma, 1991), while superoxide dismutase (SOD), converts superoxide into hydrogen peroxide and other hydrogen peroxides (Das et al., 2012; Holovska et al., 2002). Glutathione peroxidase (GSH-Px) is mainly used to remove intracellular ROSs and works together with SOD and CAT to reduce and prevent the oxidative effects of ROSs (Cabiscol et al., 2000). Therefore, the effect of light irradiation on the level of intracellular ROS formation was monitored using the fluorescent probe DCFH-DA. Results show that intracellular ROS levels remained steady throughout the 420 min of VL irradiation (Fig. S8a).

During the SS irradiation process, an initial increase and then a subsequent decrease in the relative levels of intracellular ROS were observed for three tested bacterial strains. Similar trends in ROS levels were observed for both ARB and ASB (Fig. 2a). These results indicate that oxidative stress increased gradually until it reached a

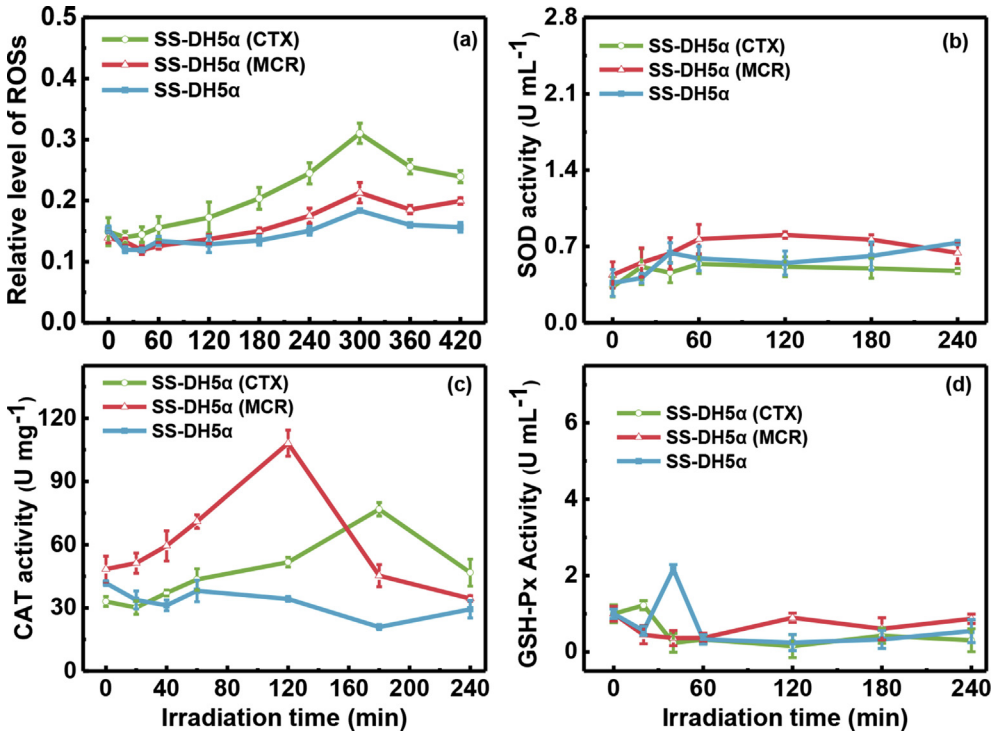

Fig. 2. The effects of SS irradiation on (a) the levels of intracellular ROSs shown by fluorescent intensity of probe DCFH-DA; (b) the activity of SOD; (c) the activity of CAT; (d) the activity of GSH-Px.

maximum after 300 min of SS irradiation. The cells were then damaged and subsequently ruptured due to the ROS produced in response to continuous SS irradiation. These outcomes were clearly demonstrated by the decrease in intracellular ROS when some bacterial cells were inactivated within 300 min. A similar result was obtained in our previous study (Sun et al., 2014). Furthermore, the intracellular ROS levels in *E. coli* DH5α (CTX) cells were higher than those in *E. coli* DH5α (MCR) cells, which were both higher than the levels in the ASB *E. coli* DH5α cells.

During the UV irradiation process, the intracellular ROS levels continuously increased in both ARB throughout the 120 min UV irradiation process (Fig. 3a), thereby demonstrating that UV continuously stimulates the production of ROS in these bacterial cells. In contrast, unlike the ARB, there was no significant change found in the ASB cells. It may be that UV induced more significant and direct DNA damage in the ASB than the inactivation mediated by the generated ROS.

In addition, the induced ROSs, such as H_2O_2, $^{\bullet}O_2^-$ and $^{\bullet}OH$, in the bacterial cells might elicit production of antioxidant enzymes, including SOD, CAT and GSH-Px. To further determine if oxidative stress occurred in the bacterial cells during VL stimulation, the activities of CAT, SOD and GSH-Px were tested. No changes were observed for CAT (Fig. S8b), SOD (Fig. S8c) and GSH-Px (Fig. S8d) activities under VL irradiation, which is consistent with the findings of the bactericidal ability and intracellular ROS generation analysis.

In contrast to VL irradiation, the SOD activities in the three tested strains slightly increased with increasing SS irradiation time (Fig. 2b). Conversely, the activity of CAT continuously increased during the initial SS irradiation period (120 min for *E. coli* DH5α (MCR) and 180 min for *E. coli* DH5α (CTX)), and then decreased thereafter (Fig. 2c). At longer irradiation times, a decrease in CAT activity and a rapid increase of photo-inactivation efficiency was observed. Furthermore, the time required to achieve maximal CAT activity was substantially shorter for *E. coli* DH5α (MCR) than for *E. coli* DH5α (CTX), indicating that *E. coli* DH5α (MCR) is less resistant to H_2O_2. These results agree with those of the bactericidal activity (Fig. 1b). Moreover, the activity of GSH-Px in *E. coli* DH5α cells only increased during the first 30 min, whereas there were no changes in activity for the two ARB cells (Fig. 2d).

Under UV irradiation (Fig. 3b), increased SOD activity in *E. coli* DH5α (CTX), *E. coli* DH5α (MCR) and *E. coli* DH5α was observed for the initial 10 min of light stimulation and then the activity continuously decreased thereafter. Similarly, CAT activity for *E. coli* DH5α (CTX) and *E. coli* DH5α (MCR) cells quickly increased during the initial 10 min and then decreased over a prolonged irradiation time (Fig. 3c). In contrast, the CAT concentration in the *E. coli* DH5α cells did not change during the initial stage; however, it did gradually decrease as the reaction time lengthened. Furthermore, the GSH-Px activity in the three bacterial strains tended to increase at different levels within different time periods (Fig. 3d).

3.3. Gene expression under different light irradiation

Generally, under an antibiotic regime (Andersson and Hughes, 2014) or UV disinfection (Lin et al., 2016), normal bacterial function during aerobic respiration can be directly or indirectly disrupted through over-generation of ROS (Zhang et al., 2018). This subsequently induces DNA damage or alters other cellular components, thereby compromising DNA and cellular membrane integrity. Thus, to articulate the underlying mechanisms used by the three bacterial strains to counteract the inactivating effects of light irradiation, we examined gene expression related to stress response, cell repair, DNA repair and conjugative transfer. The expression of all tested genes is illustrated as a heat map (Fig. 4). The results show that there was a downregulation of gene expression when the bacteria were exposed to VL irradiation as compared with no light irradiation.

Under SS irradiation (Fig. 4), most of the tested genes were

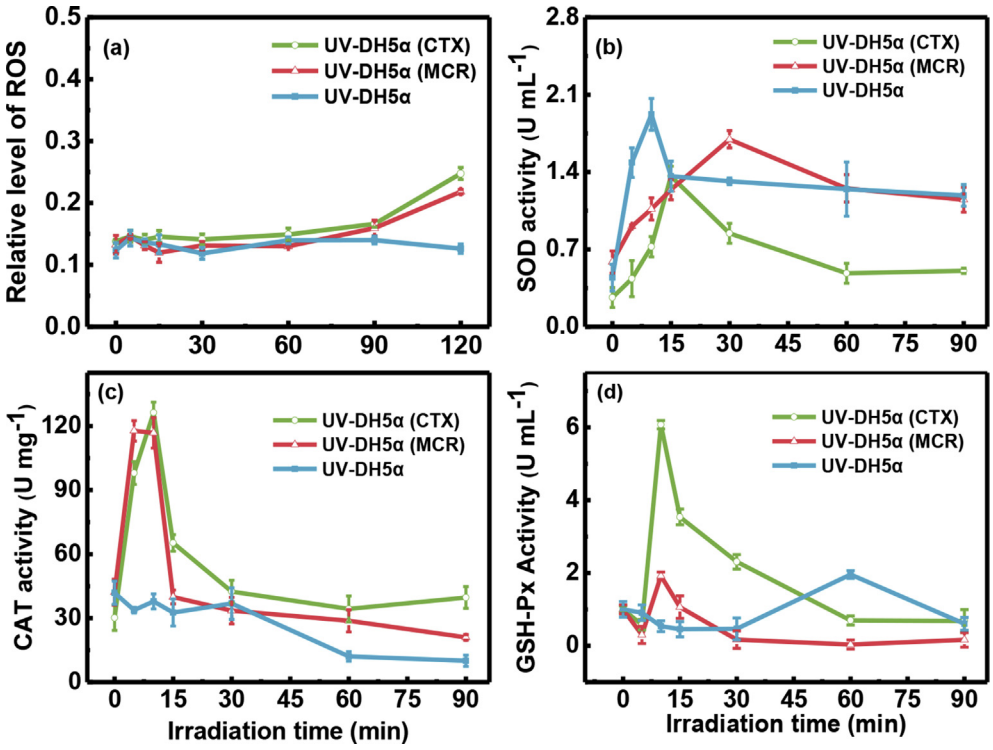

Fig. 3. The effects of UV irradiation on (a) the levels of intracellular ROSs shown by fluorescent intensity of probe DCFH-DA; (b) the activity of SOD; (c) the activity of CAT; (d) the activity of GSH-Px.

upregulated in *E. coli* DH5α within the initial 240 min and then they were subsequently downregulated. In comparison, within the first 240 min, most of the tested genes, including the stress response related genes and cell repair genes, were upregulated 2–5-fold in *E. coli* DH5α (CTX) and slightly upregulated in *E. coli* DH5α (MCR).

Under UV irradiation (Fig. 4), almost all tested genes in *E. coli* DH5α were downregulated during 120 min treatment. In contrast, most of the tested genes in *E. coli* DH5α (CTX) and *E. coli* DH5α (MCR) were upregulated 2–10-fold following different UV irradiation times. The first difference was in the shoulder period between the two ARB when exposed to UV irradiation (Fig. 1c). The second difference was due to differing resistance mechanisms (Harris, 1992), as the antioxidative enzymes have different abilities to respond to oxidative stress (Fig. 3b, c and 3d).

3.4. ARG transfer under different light irradiation

Under VL irradiation, bacterial inactivation did not occur within 480 min exposure (Fig. S9a). This might ensure the number of ARGs up to the threshold for its successful conjugation, since the number of the potential ARG donors and recipients are directly related to the variation in conjugation transfer efficiency (Davies and Davies, 2010; Erickson, 2001; Sørensen et al., 2005). Prior to the conjugative-based cellular experiments conducted in this study, the optimized conditions (mating time, 16 h; donor/recipient ratio, 1:1; and bacterial concentration, 10^8–10^9 CFU mL^{-1}) (Fig. S5) were attained and then used for all subsequent conjugative experiments. The frequency of conjugative transfer (FCT) increased slightly during the early period of VL stimulation, while there was no obviously accelerated effect on the efficiency of conjugative transfer when the overall trend was taken into consideration (Fig. 5a).

Under SS irradiation, gene expression analysis confirmed that all bacterial strains elicited a clear stress response and a high expression of stress response-related genes. To verify the effect of SS irradiation on ARG transfer, we evaluated the conjugative transfer between ARB. The FCT from *E. coli* DH5α (CTX) to *E. coli* C600 (Sm) decreased during the early period (60–120 min) of SS stimulation and then increased by 2- to 10-fold after 180 min, when compared to 0 min of SS irradiation (Fig. 5b). The decreased FCT during 60–120 min might be explained in that the bacteria suffered from a sudden initial stimulation and elicited general stress responses to survive, where the phenotypic traits could be blocked caused by persistence of growth impairment (Mitchell et al., 2009). In contrast, there was a slight change in the efficiency of conjugative transfer from *E. coli* DH5α (MCR) to *E. coli* C600 (Sm). The data indicates that SS irradiation enhances conjugative transfer but operates differently depending on the plasmid involved.

To further reveal the relationship between ARG conjugative transfer and the stress response, we evaluated ARG transfer following exposure to UV irradiation. The FCT from *E. coli* DH5α (CTX) to *E. coli* C600 (Sm) significantly increased by approximately 10- to 100-fold when compared with the control (Fig. 5c). The FCT from *E. coli* DH5α (MCR) to *E. coli* C600 (Sm) increased by 10- to 60-fold, when compared with the levels observed with SS irradiation. This data indicates that UV irradiation can dramatically promote ARG conjugative transfer.

4. Discussion

Recent studies have found that horizontal gene transfer of ARGs could be induced through the conserved ROS pathway of the bacterial intracellular and SOS response (Andersson and Hughes, 2014; Beaber et al., 2004). We thus hypothesized that the generation of intracellular ROS, which is also related to bacterial oxidative stress as well as other stress genes, may facilitate the transfer of ARGs via conjugation. To test the hypothesis that light irradiation affects horizontal gene transfer by altering cellular response pathways, such as regulating changes in gene expression induced by ROS, we

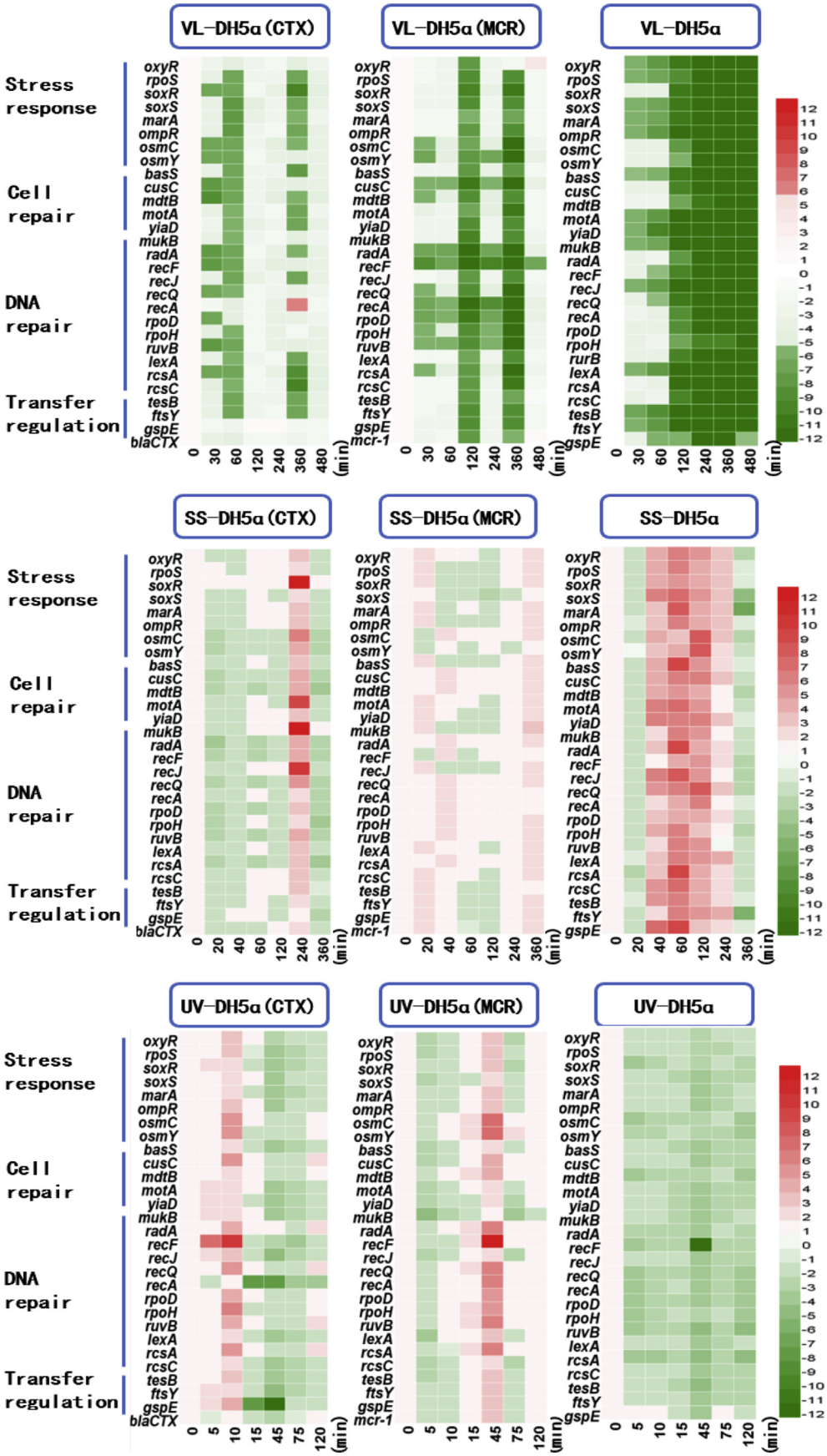

Fig. 4. Gene expression profiles of target genes involved in stress response, cell repair, DNA repair and transfer regulation upon exposure to VL, SS and UV in *E. coli* DH5α (CTX), *E. coli* DH5α (MCR) and *E. coli* DH5α. X-axis: the monitoring time in minutes; Y-axis left: clusters of target genes and list of genes tested, Y-axis right: the figure legend bar (depicted as a green-red color scale. Red spectrum color indicates up-regulated expression; green spectrum color indicated down-regulated expression). (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

evaluated the inactivation effect of ARB and ASB; the generation of intracellular ROS; stress response-related gene expression; cell repair; DNA repair; and conjugative transfer under different light irradiation.

The inactivation effect of ARB and ASB under different light irradiations was evaluated first. It was found that bacterial inactivation only occurred within the UV portion of the spectrum. Since UV can directly penetrate the intact bacterial cell wall, it can then be absorbed by nucleobases, such as DNA and RNA (Connerkerr et al., 1998; Guo et al., 2015), thereby causing significant damage to the DNA and interfering with its function. Thus, it is reasonable that VL irradiation, which is not within the UV spectrum, cannot cause damage to either ARB or ASB. Conversely, SS, which contains a portion of light in the UV spectrum, could cause higher bactericidal efficiencies than VL. Comparatively, pure UV irradiation exhibited the highest inactivation activity and caused more severe damage to the bacterial cells, as shown by the SEM images. Further research found that the ARB were more resistant to VL irradiation than the ASB, possibly because ARGs could confer some advantages to the bacteria that are not only relevant to antibiotic resistance but also to bacterial stress responses or adaptive effects (Hershberg, 2017). Based on the substantial inactivation effect elicited by light irradiation, especially UV irradiation, it can be inferred that bacteria experience extreme stress during the entire light irradiation process.

In this study, during the VL irradiation process, no changes in intracellular ROS concentrations were observed, indicating that the bacteria maintained their normal state and no oxidative stress occurred. Unlike the VL irradiation process, SS irradiation induced an initial increase and then a subsequent decrease in the relative levels of intracellular ROS. Furthermore, the intracellular ROS levels of the ARB were higher than those of the ASB, which is consistent with the results of the inactivation effect. This may be because the ARB are more resistant to oxidative damage caused by the induced ROSs than ASB. In addition, a similar phenomenon to VL irradiation was observed for the intracellular ROS levels of ARB and ASB under UV irradiation, although the trends were different. Dunlop et al. reported similar results, in that antibiotic-resistance *E. coli* could withstand the initial concentration of ROS that was generated by a photocatalysis to a greater extent than antibiotic-sensitive *E. coli* (Dunlop et al., 2015). Nevertheless, the reasons for the differences between ARB and ASB are unclear and need further investigation.

The evolution of antioxidant enzyme activities was also investigated under different light irradiation. No changes on CAT, SOD and GSH-Px activities were observed during the VL irradiation process. In contrast, the activities of the three enzymes increased in all three bacterial strains during the SS irradiation process, although their trends were different. The slight increase in SOD activities may be due to the limited amount of generated $^{\bullet}O_2^-$ in the bacterial cells, and $^{\bullet}O_2^-$ was not the main ROSs involved in killing bacterial cells during SS irradiation. The initial increase and subsequent decrease of CAT activities suggests that H_2O_2 was the main ROS involved in the inactivation of bacteria and that H_2O_2 generated in the system quickly exceeded the CAT protection capacity in the bacterial cell. There was no change in GSH-Px activity for both tested ARB; however, a slight increase was observed for the ASB during the initial SS irradiation. These results indicate that the damage to ASB caused by SS irradiation-induced ROS was mainly limited by GSH-Px; whereas, the weakening effect in ARB might be mainly caused by SOD and CAT, which play a major role in the oxidative stress induced by ROSs.

Under UV irradiation, the SOD activities in three tested strains first increased and then decreased thereafter. The trends in CAT activity for both ARB cells was slightly similar to the trends observed for the SOD activities. This indicates that the activated

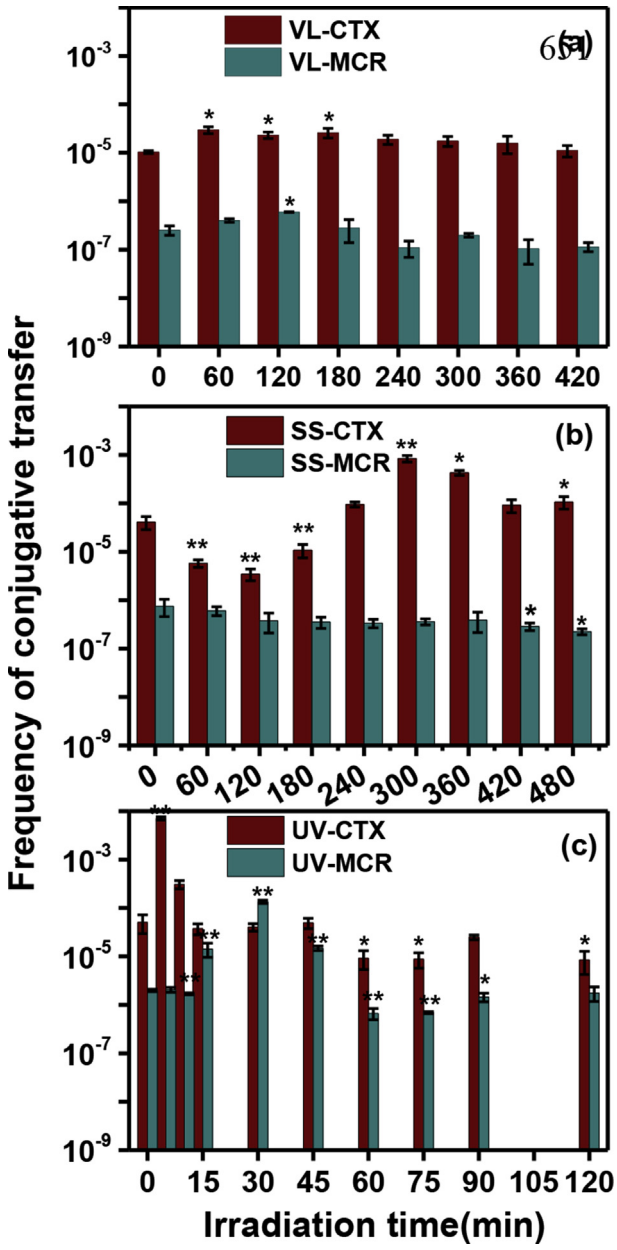

Fig. 5. Effects of (a) VL, (b) SS and (c) UV irradiation on the frequency of conjugative transfer. The donor (*E. coli* DH5α (CTX), *E. coli* DH5α (MCR)) and recipient (*E. coli* C600 (Sm)) bacteria. Light irradiation had a significant effect on the frequency of conjugative transfer (ANOVA, $P < 0.05$); significant differences between light-treated groups and the control group without light irradiation were tested with independent sample *t*-test and shown with * ($P < 0.05$), and ** ($P < 0.01$).

CAT in the ARB cells started to protect the cell from the oxidative stress caused by large amount of H_2O_2 that was produced during the initial period. No significant change in the CAT activity of *E. coli* DH5α suggests that this ASB had a limited capacity to resist UV irradiation. This further confirms the conclusion that this strain is easily inactivated by UV. The different trend in CAT activity across the three strains shows that bacteria with ARGs are more resistant to oxidative damage from H_2O_2. The different trends in GSH-Px activity for the three tested bacterial strains indicates that GSH-Px is involved in protection against oxidative damage of these bacteria during UV irradiation. Overall, these results indicate that light irradiation has an oxidative effect on bacterial strains, which is

demonstrated by the gradual formation of ROSs and the significant changes in antioxidant enzyme activities.

The above results indicate that bacterial cells could be damaged after long-term light stimulation, causing an oxidative stress response. Hence, this study also explored the genetic changes in response to different light irradiation by determining the expression of genes related to stress responses in the three bacterial strains. Under VL irradiation, the down-regulation in the expression of almost all tested genes was observed in all three strains, agreeing to light-induced formation of ROSs and their stimulation of stress response. These results also suggest that the stress response-induced genes were down-regulated when the bacterial cells were exposed to a non-stressing light source (VL) (Luca et al., 2013). In clear contrast, SS irradiation led to the upregulation of most tested genes of the three bacterial strains within 240 min; however, the trends in expression were different for the different strains. Thus, the bacteria that confer differing resistance exhibited different stress responses to SS irradiation. Moreover, the stress responses induced in ASB were more intensive than that in ARB. This could be because the ROS-mediated inactivation efficiency of ASB was higher than that of ARB.

Under UV irradiation, ARB exhibited great changes in gene expression. Moreover, when ARB persisted in the shoulder period (during the shoulder period, the bacteria had high activity and no obvious damage), the transcription and translation levels changed to a large extent. That is, the gene expression associated with UV stimulation changed. In contrast, almost all the tested genes in the ASB were downregulated during the 120 min UV irradiation. This may indicate that the ARB were more likely to produce positive resistance to external stressors than the ASB under UV irradiation. The upregulation in gene expression also suggests that UV irradiation triggered stress-response mechanisms that help cells to cope to external damage. Light irradiation induces bacterial damage by generating ROSs, which invokes stress responses. These responses included the oxidative response and the SOS response that has also been reported by other researchers (Cabiscol et al., 2000; Das et al., 2012; Farr and Kogoma, 1991; Fraud and Poole, 2011).

Our study showed that UV and SS irradiation can effectively increase the frequency of conjugative transfer. In contrast, the studies by Guo et al. and Lin et al. indicate that UV cannot influence or decrease the transfer of ARGs (Guo et al., 2015; Lin et al., 2016). This is because the initial light intensity of the UV irradiation used in those experiments was much higher than that of the UV irradiation used in this study, which could slowly stimulate changes in ARB. The dose required to achieve complete suppression of ARG transfer would be impractical with a high concentration of ARGs. Persistent ARGs during UV irradiation can be captured and transferred to other microorganisms, which presents a new challenge. The development of UV-based disinfection methods, such as UV-Cl_2, UV-TiO_2 and UV-O_3, might also facilitate low-level transfer of ARGs similar to UV alone. Enhanced transfer of ARGs has been observed in the combination of UV and TiO_2 (Dunlop et al., 2015). Our study revealed an over-expression of stress response genes in ARB, along with upregulation and enhanced activities of defense repair genes under light irradiation. Furthermore, this study showed that these bacterial strains are in a very active state when the degree of stress response exceeds the capacity of the cell defense systems. The upregulated gene expression is a genetic response that allows the bacteria to better resist the damaging effects of toxic agents, when first pre-exposed to UV light stimulus.

Furthermore, we evaluated conjugative transfer under different light irradiation. As expected, VL irradiation did not increase the frequency of bacterial conjugative transfer. As mentioned above, during the VL irradiation process, there were no changes in bacterial damage, oxidative stress and gene expression, which were associated with the lack of change in the frequency of conjugative transfer. In comparison, SS irradiation caused a slight increase in the frequency of conjugative transfer; this corresponded to slight damage to bacteria related to oxidative stress and gene expression. UV irradiation significantly promoted ARG conjugative transfer, and correspondingly, showed great effects on the inactivation and surface morphology of the bacteria. The activities of the antioxidant enzymes in the bacteria were also notably enhanced. Furthermore, the oxidative stress and other stress-related genes were overexpressed during UV irradiation. This suggests that there may be a link between bacterial stress conditions and conjugative transfer. This finding is consistent with other studies, which presented evidence that stressors, such as antibiotics and other disinfectants, can enhance conjugative transfer through broad ROS response systems (Zhang et al., 2018) or the SOS response (Andersson and Hughes, 2014; Beaber et al., 2004).

5. Conclusion

This study demonstrates that SS and UV irradiation promotes the spread of ARGs mediated by conjugative transfer of plasmids between *E. coli* strains. UV irradiation causes more intense stimulation, leading to more significant oxidative stress, higher gene expression and stronger acceleration of ARG conjugative transfer. These results reveal a possible connection between conjugative transfer and the bacterial stress response. There is a significant correlation between the conjugative efficiency and the degree of stress response; in that a stronger stress response indicates a higher likelihood that horizontal transfer of ARGs will occur.

Declaration of interests

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.

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.watres.2018.11.019.

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