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Response mechanisms of different antibiotic-resistant bacteria with different resistance action targets to the stress from photocatalytic oxidation

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ABSTRACT

The stress response of antibiotic-resistant bacteria (ARB) and the spread of antibiotic resistance genes (ARGs) pose a serious threat to the aquatic environment and human beings. This study mainly explored the effect of the heterogeneous photocatalytic oxidation (UVA-TiO_2 system) on the stress response mechanism of ARB with different antibiotic resistance action targets, including the cell wall, proteins, DNA, RNA, folate and the cell membrane. Results indicate that the stress response mechanism of tetracycline- and sulfamethoxazole-resistant *E. coli* DH5α, which targets the synthesis of protein and folate, could rapidly induce global regulators by the overexpression of relative antibiotic resistance action target genes. Different stress response systems were mediated via cross-protection mechanism, causing stronger tolerance to an adverse environment than other ARB. Moreover, the photocatalytic inactivation mechanism of bacterial cells and a graded response of cellular stress mechanism caused differences in the intensity of the stress mechanism of antibiotic resistance action targets. *E. coli* DH5α resistant to cefotaxime and polymyxin, targeting synthesis of the cell wall and cell membrane, respectively, could confer greater advantages to bacterial survival and higher conjugative transfer frequency than *E. coli* DH5α resistant to nalidixic acid and rifampicin, which target the synthesis of DNA and RNA, respectively. This new perspective provides detailed information on the practical application of photocatalytic oxidation for inactivating ARB and hampering the spreading of ARGs in the aquatic environment.

1. Introduction

Antibiotics are one of the major means to control the growth of bacteria, especially those with clinical significance. However, the abuse of antibiotics has led to the emergence of many types of antibiotic-resistant bacteria (ARB). Recent studies show that the rapid increase of ARB is closely related to the continuous dissemination of antibiotic resistance genes (ARGs) in the environment (Ben et al., 2019; Parnanen et al., 2019; Pazda et al., 2019). It has been reported that horizontal transfer of ARGs promotes the emergence of multi-antibiotic resistant bacteria (Guo et al., 2017). An important reason for this is that most of the commonly used antibiotics inhibit synthesis of their respective antibiotic action targets, and then inactivated ARB release the corresponding ARGs into the environment. The released ARGs can be transferred to a new host, leading to the emergence of new ARB. This problem can only be eliminated or reduced if the antibiotics used can completely destroy the DNA in the bacteria, including the ARGs (Peter et al., 2017; Pornsukarom and Thakur, 2017).

Unfortunately, most antibiotics currently in use do not have a strong DNA-damaging ability to stop the spread of ARGs. There are two major reasons for the increased spread of ARGs. First, after sub-lethal disinfection, some ARB can survive, and their ARGs can be further transferred between microorganisms. Second, even after inactivation of all ARB, released ARGs can be further transfected to other bacteria via horizontal gene transfer (HGT) (Cai et al., 2021; Li et al., 2020; Yin et al., 2021b). For the second route, there are two important factors that affect the

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spreading frequency of the remaining intact ARGs: 1) the ability and resident time of disinfection to completely destroy DNA molecules; and 2) the effect of disinfection treatment on the HGT frequency of bacterial ARGs (Aminov, 2011; Lerminiaux and Cameron, 2019; von Wintersdorff et al., 2016; Yin et al., 2020).

In response to the increasing spread of ARB/ARGs, it is important to conduct proper disinfection of environmental samples to stop or minimize the spread of ARB and ARGs. Many studies have reported the use of effective water disinfection methods, such as UV (Chen et al., 2019; Templeton et al., 2009), chlorination (Jia et al., 2015; Shi et al., 2013; Yuan et al., 2015) and ozonization (Czekalski et al., 2016), to eliminate ARGs and ARB. However, there is evidence that these disinfection methods do not completely purify the ARGs and ARB but rather promote the spread of ARGs in aquatic environments to some extent (Rizzo et al., 2013; Zhang et al., 2017).

In recent years, UVA-TiO_2 heterogenous photocatalysis has drawn tremendous attention in the area of water treatment (Karaolia et al., 2018; Xie et al., 2021). The fundamental mechanisms of UVA-TiO_2 heterogeneous photocatalysis for inactivating ARB and ARGs are based on the reactions produced by extracellular reactive oxygen species (ROS) (Ganguly et al., 2018). Under UVA light irradiation, the photocatalysts produce holes and electrons, which subsequently initiate oxidation and reduction reactions. Holes and electrons can transform into hydroxyl radicals (Cheng and Xu, 2019). The inactivation efficiency of UVA-TiO_2 heterogenous photocatalysis is much more efficient than UV and does not produce disinfection by-products relative to chlorinated disinfection and ozone disinfection; thus, it has been widely studied and used (Ahmed et al., 2020; Hwangbo et al., 2019; Li et al., 2015; Sun et al., 2014). If the ARB can withstand photocatalytic oxidation, then other weak disinfection methods will undeniably produce similar stress response mechanisms (Yin et al., 2021a). Some studies have shown that ARB can cause a stronger stress response than antibiotic-susceptible bacteria (ASB) during the heterogeneous photocatalytic oxidation process, which accelerates the spread of ARGs and provides survival advantages (Chen et al., 2019; Huang et al., 2017; Yin et al., 2021a). However, the reasons and underlying mechanisms for differences in the stress response of ARB with different antibiotic resistance action targets under exposure to photocatalytic oxidation remains unclear. Furthermore, whether this mechanism affects the HGT frequency of ARGs is unknown.

In order to systematically study the effect of photocatalysis on the destruction of ARB with different antibiotic resistance action targets (i.e., the cell wall, cell membrane integrity, protein synthesis, folate, DNA and RNA), as well as its ARG-damaging ability, a typical heterogeneous photocatalytic oxidation (UVA-TiO_2) system was employed. The selected antibiotics covered the complete action target spectrum of commonly used antibiotics and are very frequently found, along their corresponding ARGs, in environmental waters (Bengtsson-Palme and Larsson, 2015; Cutugno et al., 2020; Milic et al., 2013; Zhu et al., 2020). In addition, antibiotic resistance, cell morphology, conjugational transfer, intracellular ROS, antioxidant enzymes and cellular proteins were analyzed to explore the effect of photocatalytic oxidation on the stress response of different ARB from the perspective of ARGs and antibiotic resistance action targets. The gene expression of ARGs and antibiotic resistance action targets were determined to further dissect the stress response mechanisms of ARB with different antibiotic resistance action targets. These results provide detailed information and a new perspective on the practical application of photocatalytic oxidation for the inactivating ARB and hampering the spread of ARGs in the aquatic environment.

2. Materials and methods

2.1. Bacterial culture and determination of the minimal inhibition concentration

E. coli DH5α used as ASB was obtained from the China General Microbiological Culture Collection Center. The ARB were constructed by introducing a plasmid into *E. coli* DH5α containing the respective ARGs against cefotaxime (CTX), polymyxin (PB), tetracycline (TET), sulfamethoxazole (SMZ), nalidixic acid (NAL), and rifampicin (RA), which were named as *E. coli* DH5α (CTX), *E. coli* DH5α (PB), *E. coli* DH5α (TET), *E. coli* DH5α (SMZ), *E. coli* DH5α (NAL), and *E. coli* DH5α (RA), repspectively. These plasmids were Incl2 transferable plasmids. Details on the ARB are summarized in Table S1. The bacterial strains were cultured overnight in Luria-Bertani (LB) nutrient broth (Sangon, China) with addition of their respective antibiotic (128 mg L^{-1} CTX, 64 mg L^{-1} PB, 4 mg L^{-1} TET, 8 mg L^{-1} SMZ, 256 mg L^{-1} NAL or 256 mg L^{-1} RA) at 37 °C with shaking according to the references (Chen et al., 2018; Jiang et al., 2017; Perreten and Boerlin, 2003; Yin et al., 2019).

The minimal inhibition concentration (MIC) of the ARB was evaluated to assay the evolution of antibiotic resistance under photocatalytic bacterial inactivation. Further details can be found in the Supporting Information (SI).

2.2. Photocatalytic experiments and observation of bacterial morphology by scanning electron microscopy

The bacterial samples and 1.5 g L^{-1} TiO_2 nanoparticles (Aladdin, China) were mixed in 50 mL quartz reaction flasks (Beijing Perfectlight, Beijing, China). The mixtures were then placed in a multi-channel photochemical reaction system with ten identical 150 mW cm^{-2} UVA lamps (Beijing Perfectlight, Beijing, China) for photocatalytic oxidation. The bacterial samples were exposed to photocatalytic oxidation with exposure times from 0 to 50 min. The pictures of the reactors are provided in Fig. S1 and detailed information about photocatalytic experiments can be found in the SI.

Scanning electron microscopy (SEM) was performed to observe the damage process of ARB with different antibiotic resistance action targets under photocatalysis to assess their stress response ability. The sample preparation for SEM characterization followed an earlier study (Li et al., 2015) and the details can be found in the SI.

2.3. Detection of stress response on antibiotic resistance action targets

To evaluate the degree of stress response by ARB under exposure to photocatalytic oxidation, the intracellular ROS and the activities of the respective antioxidant enzymes were determined. The level of intracellular ROS was assayed using the fluorogenic dye 2,7-dichlorodihydrofluorescein diacetate (DCFH-DA) (Invitrogen, Carlsbad, USA) according to the reference (Sun et al., 2016). The antioxidant enzymatic activities of superoxide dismutase (SOD) and catalase (CAT) were quantified. Details of these assays are provided in the SI.

The photocatalytic oxidation effects on antibiotic resistance action targets were explored at a molecular level in different ARB. The effect of stress response was studied by determining the β-lactam content, cell membrane permeability (lactate dehydrogenase, cell membrane potential detection and live/dead cell stanning) and guanosine triphosphate (GTP) content of ARB. The detailed experimental procedures can be found in the SI.

2.4. Quantitative analysis and conjugant identification of antibiotic resistance genes

In order to calculate the inactivation efficiency of photocatalytic oxidation on different ARGs and explore whether HGT will occur, ARGs were detected by quantitative polymerase chain reaction (qPCR). After

determining the reaction time through preliminary experiments, the ARGs were exposed to photocatalytic oxidation at varying exposure times from 0 to 24 h. The qPCR assays, which used ARG-specific primers, standard curve construction and detection limit determination were performed according to a previously reported method (Ren et al., 2018). According to the standard curve, the gene copy numbers were calculated. The detailed experimental procedures can be found in the SI.

To evaluate the conjugative transfer of ARGs and explore the connection between the transfer frequency and antibiotic resistance action targets after exposure to the UVA-TiO_2 system, the best transfer model was selected for the test according to the reference (Chen et al., 2019). Briefly, six ARB, which acted as donors, were independently re-suspended in a reactor solution with TiO_2 and then subjected to photocatalytic oxidization. At different exposure times, donor cells were mixed with streptomycin (SM)-resistant recipient cells (*E. coli* C600 (SM)) at a 1:1 ratio (2 mL in total). After mating, donor-recipient transconjugants were screened on LB agar plates containing SM (3000 mg L^{-1}) and the respective antibiotic concentration.

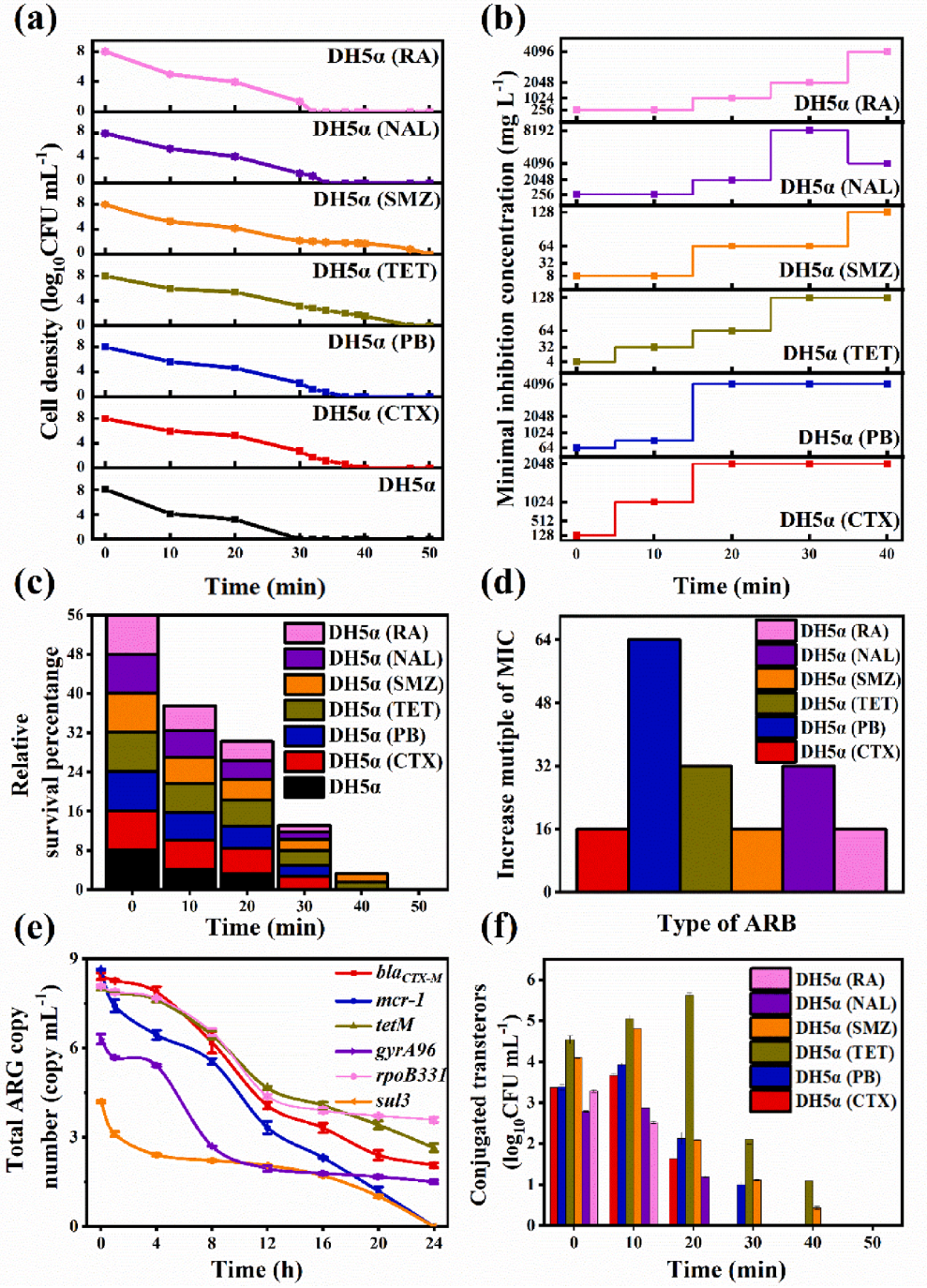

Fig. 1. (a) Time-inactivation curves of antibiotic-susceptible bacteria (ASB) and antibiotic-resistant bacteria (ARB); (b) Minimal inhibition concentration (MIC) curves of ARB; (c) Bacteria abundance curves of ASB and ARB; (d) The increase multiple of MIC; (e) The copy number of total antibiotic resistance genes (ARGs); (f) The conjugative transfer frequency of various ARB, under the exposure to photocatalytic oxidation.

2.5. Determination of stress response mechanism via gene expression assays

In order to further measure the effects of photocatalytic oxidation on the stress response mechanisms of different ARB, the expression of ARGs and antibiotic resistance action target genes were evaluated by qPCR analysis. The *16S rRNA* gene was used as an internal control (Yin et al., 2020). Primer sequences are provided in Table S1 and the detailed procedures are shown in the SI.

3. Results and discussion

3.1. Comparison of inactivation efficiencies and gene transfer of different ARB

In order to evaluate the effects of photocatalytic oxidation on different kinds of ARB, the inactivation efficiency and antibiotic MICs of individual ARB as compared with the ASB were determined for different processes (Fig. 1). Correspondingly, quantification of ARGs and determination of conjugative transfer were also performed to evaluate the effects of photocatalytic oxidation on the bacterial genes (Fig. 1e and 1f). A significant inactivation of bacteria, as determined by the reduction of cell density, was found for all bacterial strains. Furthermore, the survival time of ARB was longer than that of ASB, and the ARB survival times were obviously different from each other under photocatalysis. The *E. coli* DH5α (TET) and *E. coli* DH5α (SMZ) cells could survive up to 50 min during the photocatalytic inactivation process. Although the other ARB could survive up to about 40 min of photocatalytic oxidation, *E. coli* DH5α (CTX) and *E. coli* DH5α (PB) could survive longer time than *E. coli* DH5α (NAL) and *E. coli* DH5α (RA) (Fig. 1a and 1c). At the same time, the survival times of *E. coli* DH5α (CTX) and *E. coli* DH5α (PB) was found to be similar, as were the survival times of *E. coli* DH5α (NAL) and *E. coli* DH5α (RA) (Fig. 1c). In addition, the bacterial inactivation efficiency of UVA irradiation and the presence of TiO_2 alone in the system did not obviously affect the survival of the selected ARB and ASB (Fig. S1). The antibiotic resistance level of the ARB cells increased greatly under photocatalytic oxidation (Fig. 1b). Moreover, the antibiotic-resistance level of different ARB increased up to 16-fold for CTX, 64-fold for PB, 32-fold for TET, 16-fold for SMZ, 32-fold for NAL and 16-fold for RD within 40 min of photocatalytic oxidation (Fig. 1d).

Moreover, six selected ARGs were obviously reduced through the process of heterogeneous photocatalytic oxidation and some ARGs, *mcr-1* and *sul3*, could be eliminated within 24 h (Fig. 1e). However, no significant change was observed in the concentrations of the tested ARGs during the ARB inactivation process. Among the three bacterial HGT pathways, conjugation of ARGs is the most frequent and efficient in the environment (Cai et al., 2021; Massoudieh et al., 2007). In this study, the number of transconjugants notably increased for all ARB, except *E. coli* DH5α (NAL) and *E. coli* DH5α (RA), during the initial stage of exposure (i.e., 10 min). The frequency of conjugative transfer from *E. coli* DH5α (TET) to *E. coli* C600 (SM) continuously increased and peaked at 20 min. However, the number of transconjugants for the other ARB dramatically decreased and was below the initial level at 20 min. Conjugational transfer after 30 min of photocatalytic oxidation could be detected for *E. coli* DH5α (TET), *E. coli* DH5α (PB) and *E. coli* DH5α (SMZ). Only transconjugants of *E. coli* DH5α (TET) and *E. coli* DH5α (SMZ) were observed at 40 min (Fig. 1f).

The above results indicate that all six ARB had stronger tolerance than ASB to the heterogeneous photocatalytic oxidation process (Fig. 1). Oxidation of ARB through photocatalysis may induce bacterial stress responses, which can confer more advantages for bacterial cell survival and enhance the conjugative transfer (Beaber et al., 2004; Makwana et al., 2015; Yin et al., 2020). It is worth nothing that the bacterial characteristics of photocatalytic oxidation for the six ARB were obviously different. Antibiotics targeting synthesis of the cell wall and cell membrane could confer more advantages to bacterial cell survival (Fig. 1a and 1c) and higher conjugative transfer frequency (Fig. 1f) than those targeting synthesis of DNA and RNA. Giannakis *et al.* and Kubacka *et al.* also reported similar results (Giannakis et al., 2018; Kubacka et al., 2014). This may be relative to the mechanism involving inactivation of bacterial cells using photocatalytic oxidation. The cell wall and cell membrane were first attacked by ROS generated during photocatalytic oxidation, while the released proteins were also decomposed at the same time (Sun et al., 2014). We thus hypothesized the reason for these differences may be related to the antibiotic resistance action targets and the sequence of photocatalytic oxidation. The antibiotics with similar targets, such as the cell wall and cell membrane, almost simultaneously functioned when the ARB were exposed to photocatalytic oxidation, while the differences between *E. coli* DH5α (PB) and *E. coli* DH5α (CTX) might be related to the antibiotic resistance action target peptidoglycan (Saikachi et al., 2021). Response protection of relevant antibiotic resistance action targets triggered by the process of photocatalytic oxidation might exist, leading to differences in the survival of ARB and the frequency of HGT.

3.2. Response of bacterial antibiotic resistance action targets

To explore the response of bacterial antibiotic resistance action targets under photocatalytic oxidation, SEM (Fig. 2), live/dead bacteria staining (Fig. 3e), lactic dehydrogenase (LDH) and cell membrane potential (Fig. 3c and 3d) experiments were carried out. Results of SEM analysis indicate that *E. coli* DH5α (NAL) and *E. coli* DH5α (CTX) maintained normal cell morphology better than ASB during photocatalytic oxidation. Most of the *E. coli* DH5α (NAL) showed shrinkage and lysed after 25 min of photocatalytic oxidation. Although *E. coli* DH5α (CTX) cells also showed local shrinkage, the cell morphology remained basically unchanged. However, differences in the response of bacterial antibiotic resistance action targets from ARB could be found during photocatalytic oxidation. Similar results for the cell membrane were also detected through LDH, live/dead bacteria staining and cell membrane potential experiments. During photocatalytic oxidation, the fluorescence intensity of *E. coli* DH5α decreased, indicating that cell membrane permeability decreased. Then, with the death of a large number of bacterial cells, cell membrane rupture and protein extravasation caused the release of proteins, gradually increasing their concentration in the cell suspension. The proteins partially combined with the dye to reduce the concentration, resulting in an increase in the fluorescence concentration until the fluorescence stabilized within 40 min of photocatalytic oxidation, indicating that *E. coli* DH5α cells were destroyed. Under photocatalytic oxidation, the bacterial cell membrane potential of *E. coli* DH5α (NAL) and *E. coli* DH5α (PB) tended to be more stable than that of *E. coli* DH5α. The reason that the fluorescence intensity of *E. coli* DH5α (NAL) increased faster than that of *E. coli* DH5α (PB) after 30 min of photocatalytic oxidation was mainly due to the decomposition of bacterial cells, causing the dye to combine with a large number of exosmotic proteins, such as LDH.

These data could indicate that the stress response provides a survival advantage for ARB. Photocatalytic oxidation led to dramatic destruction of the ASB, as demonstrated by deeper sinking and shriveled cell walls observed. In contrast, the damage to *E. coli* DH5α (CTX) was not so obvious (Figs. 2 and 3e). As reported, ARB induce stress responses when encountering oxidation pressure mediated by ROS (An et al., 2016; Sun et al., 2017) and most ARB cells can temporarily maintain viability and avoid destruction by a limited amount of ROS generated in the photocatalysis system (Geeraerd et al., 2000; Geeraerd et al., 2005). Although all the ARB induced the stress response, differences in the degree of response were observed. The reason for the differences in response is related to the inactivation sequence of photocatalytic oxidation on the bacterial cells (Qiu et al., 2020). The response of *E. coli* DH5α (CTX) was first activated to maintain cell morphology when the cell wall was attacked by ROS generated due to photocatalytic oxidation, which was different than the response of *E. coli* DH5α (NAL) (Fig. 2).

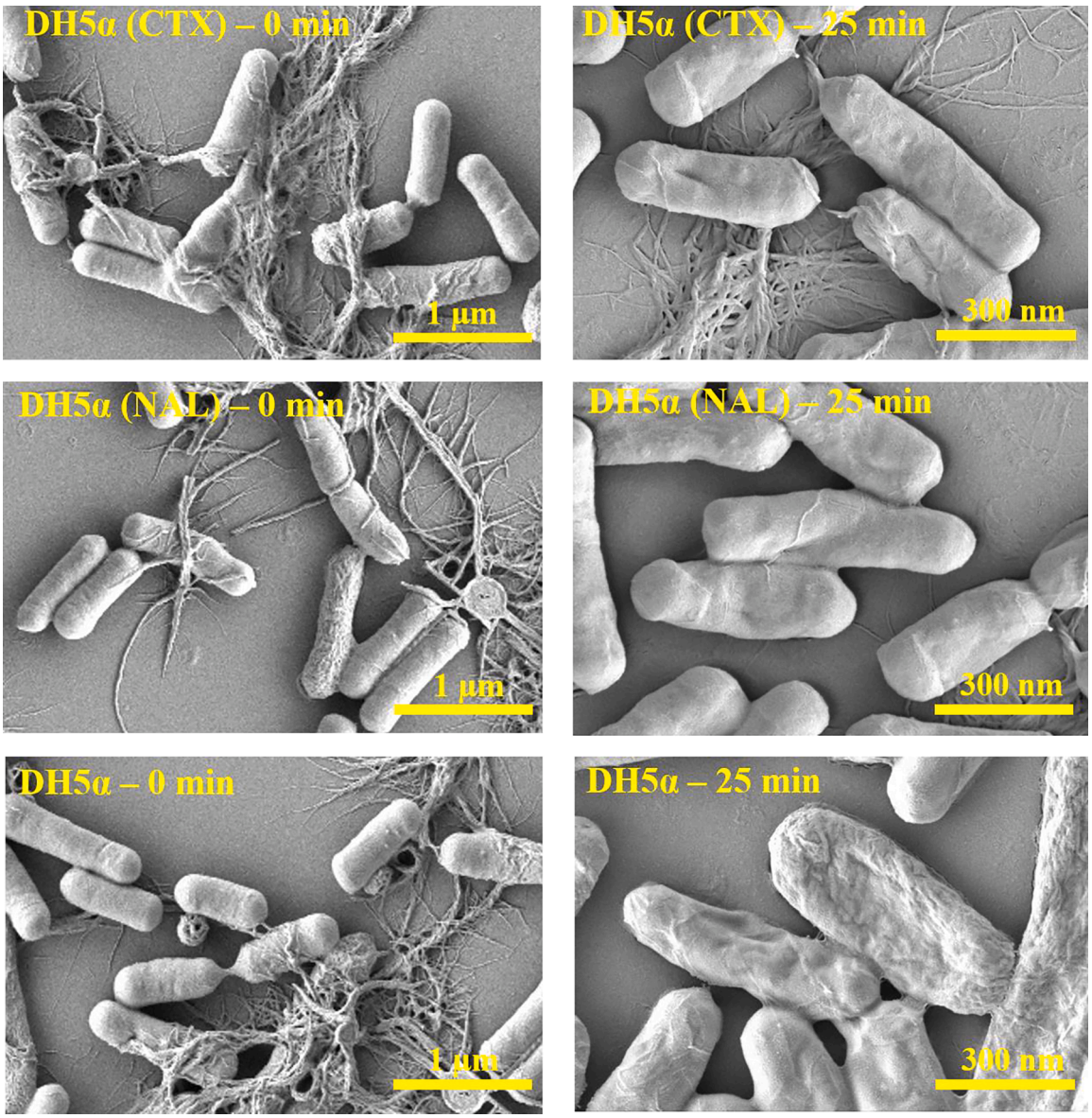

Fig. 2. The scanning electron microscopic of *E. coli* DH5α (CTX), *E. coli* DH5α (NAL) and *E. coli* DH5α under the exposure to photocatalytic oxidation.

Comparatively, damage related to cell membrane integrity loss and increased permeability caused by photocatalytic oxidation was more obvious in *E. coli* DH5α (NAL) than *E. coli* DH5α (PB) (Fig. 3c and 3d). *E. coli* DH5α (PB) might initiate a strong response of antibiotic resistance action targets, resulting in a stronger affinity to the MCR-1 protein and lipid A during photocatalysis, with the lipopolysaccharides and proteins contributing to the stiffness of the outer membrane (Li et al., 2017), which provides an advantage for survival under adverse environmental conditions (Rojas et al., 2018).

3.3. Stress response of arb

Stress response plays a crucial role in the antimicrobial resistance of Gram-negative bacteria (Poole, 2012). Exposure to photo-generated ROS causes bacteria to respond to stress by producing CAT and SOD antioxidant enzymes that detoxify superoxide ($O_2^{\cdot-}$) and H_2O_2 (Cabiscol et al., 2000; Fraud and Poole, 2011; Vega et al., 2013).

Exposure to photocatalytic oxidation first caused an increase followed by a decrease in the relative levels of ROS in the bacterial cultures of the six investigated ARB strains (Fig. 4a). Compared to the ASB, a higher level of intracellular ROS could be detected in all the selected ARB cells, which remained high during photocatalytic oxidation. At the same time, both the SOD and CAT activities first increased and then decreased during photocatalytic oxidation (Fig. 4b and 4c). Similar results were found in earlier work (Chen et al., 2019; Sun et al., 2017). However, the levels of CAT and SOD, as well as the level of the intracellular ROS, were lower in *E. coli* DH5α (PB) and *E. coli* DH5α (CTX) than those of other the ARB. It is worth noting that the resistance action targets of *E. coli* DH5α (PB) and *E. coli* DH5α (CTX) are the cell envelope and cell wall, respectively. When ROS cause a stress response in the bacterial cells, due to antibiotics targeting the cell wall and cell membrane, the ARB initiate a protective response. This protective response accelerates cell repair and maintains the fitness of the membrane (Jiang et al., 2011). Compared the other ARB, this resulted in a low level of intracellular ROS being detected in *E. coli* DH5α (PB) and *E. coli* DH5α (CTX) cells and a further reduction in CAT and SOD used to resist intracellular ROS. β-Lactamases resist the combination between β-lactam and penicillin-binding proteins, preventing damage to the cell wall and maintaining cell morphology (Kim et al., 2014). Their production was also found, indicating that *E. coli* DH5α (CTX) had a strong protective response of the antibiotic resistance action target under photocatalytic oxidation (Fig. 3a).

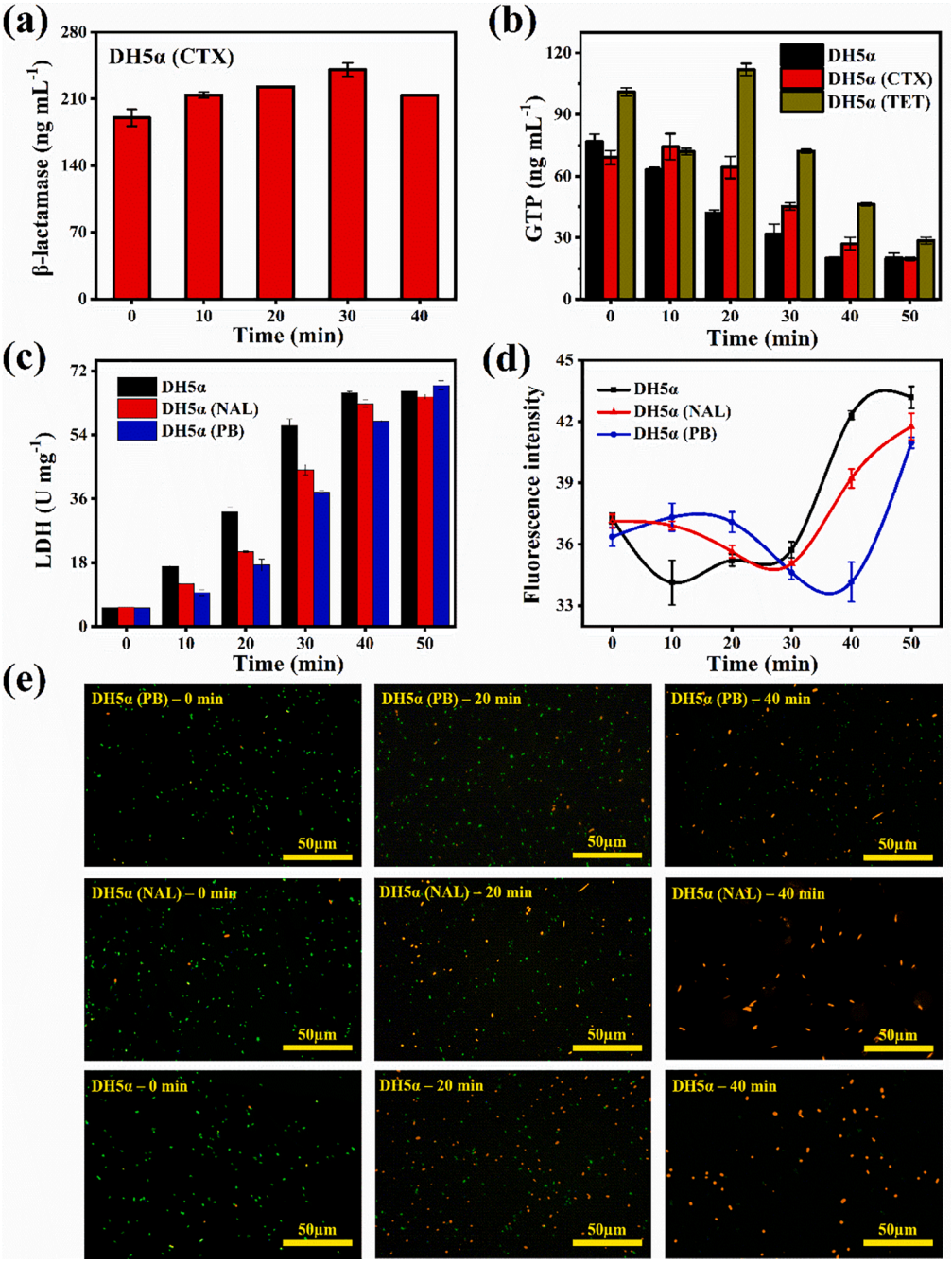

Fig. 3. (a) The β-lactamase level of *E. coli* DH5α (CTX); (b) The guanosine triphosphate (GTP) of *E. coli* DH5α (CTX), *E. coli* DH5α (TET) and *E. coli* DH5α; (c) Lactic dehydrogenase (LDH) level of *E. coli* DH5α (PB), *E. coli* DH5α (NAL) and *E. coli* DH5α; (d) Cell membrane potential experiments of *E. coli* DH5α (PB), *E. coli* DH5α (NAL) and *E. coli* DH5α; (e) The live/dead bacteria staining of *E. coli* DH5α (PB), *E. coli* DH5α (NAL) and *E. coli* DH5α, under the exposure to photocatalytic oxidation.

Compared with *E. coli* DH5α (PB) and *E. coli* DH5α (CTX), the stress response degrees of *E. coli* DH5α (NAL) and *E. coli* DH5α (RA) were limited under photocatalytic oxidation. The cellular stress response is graded and the properties vary with the extent of cell damage (Somero, 2020). Somero found that the response targeting DNA and RNA might happen at a later stage of the stress. In addition, time is required for cell repair when DNA is subjected to a high level of endogenous damage caused by a lack of DNA damage response in ARB (Ciccia and Elledge, 2010; Curtin, 2012). Thus, the degree of photocatalytic oxidation on the antibiotic resistance action targets of *E. coli* DH5α (NAL) and *E. coli* DH5α (RA) was limited and the protective response was not obvious.

For *E. coli* DH5α (TET), the intracellular ROS was maintained at a high level during the reaction process, resulting in a strong stress response and, consequently, production of a large amount of SOD and CAT (Fig. 4). These results were similar to the work by Lushchak, who found that the expression products of certain genes were helpful under continuous exposure to enhanced ROS levels (Lushchak, 2011). The GTP results also indicate that photocatalytic oxidation caused a strong stress response in *E. coli* DH5α (TET), which required more energy to synthesize relative antioxidant enzymes to combat the adverse environment (Fig. 3b). At the same time, the presence of GTP also had a synergistic effect with TetM protein in the cells, which improves the antibiotic tolerance of *E. coli* DH5α (TET) (Arenz et al., 2015; Burdett, 1996).

Changes in the stress response of *E. coli* DH5α (SMZ) during photocatalysis also showed the same trend as that of *E. coli* DH5α (TET) (Fig. 4). This indicates that the protective response of antibiotic

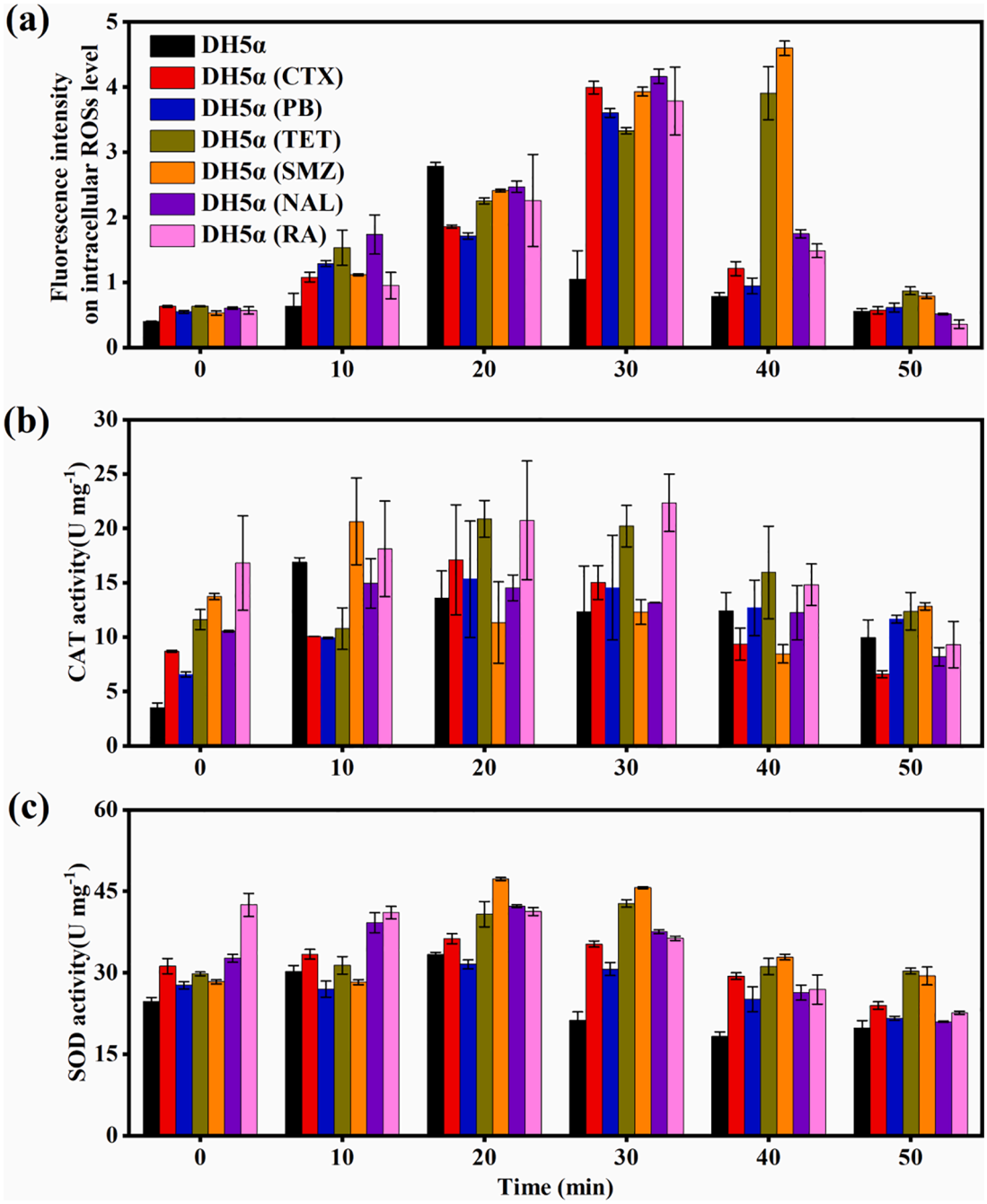

Fig. 4. (a)The intracellular reactive oxygen species (ROSs) by fluorescent intensity of probe 2,7-dichlorodihydrofluorescein diacetate (DCFH-DA); (b) The antioxidant enzymatic activities of catalase (CAT); (c) The antioxidant enzymatic activities of superoxide dismutase (SOD).

targeting folate synthesis could confer more advantages by modulating energy metabolism to provide ATP and restoring the integrity of macromolecular systems (Kok et al., 2020). This is consistent with the bacterial inactivation results, where the bacteria cells showed less lethal injuries and kept their activity for a long time. As reported, *E. coli* DH5α (SMZ) was in the stress response state during most of the treatment, reducing the damage caused by the generated ROS (Sun et al., 2017).

Based on the above results, the stress response of antibiotic resistance action targets on *E. coli* DH5α (CTX) and *E. coli* DH5α (PB) cells was first triggered by extracellular ROS to combat oxidative damage. Subsequently, the cellular ROS increased dramatically causing a strong stress response of *E. coli* DH5α (TET) and *E. coli* DH5α (SMZ) as well as inducing a global response to combat the damage. However, the low degree response of photocatalytic oxidation on the antibiotic resistance action targets of *E. coli* DH5α (NAL) and *E. coli* DH5α (RA) was relative to its hysteretic graded stress response and the unobvious protective response of antibiotic resistance action targets.

3.4. Gene regulation mechanism of ARB

To clarify the underlying mechanisms of the bacterial cells with different antibiotic resistance action targets to stress from photocatalytic oxidation, the expression of genes associated with antibiotic resistance action targets were compared. The expression of all the studied genes is illustrated in a heat map (Fig. 5). For the ASB, the level of gene expression associated with antibiotics targeting the cell wall, cell membrane, protein synthesis, folate synthesis, DNA synthesis and RNA synthesis was low under exposure to photocatalytic oxidation. In

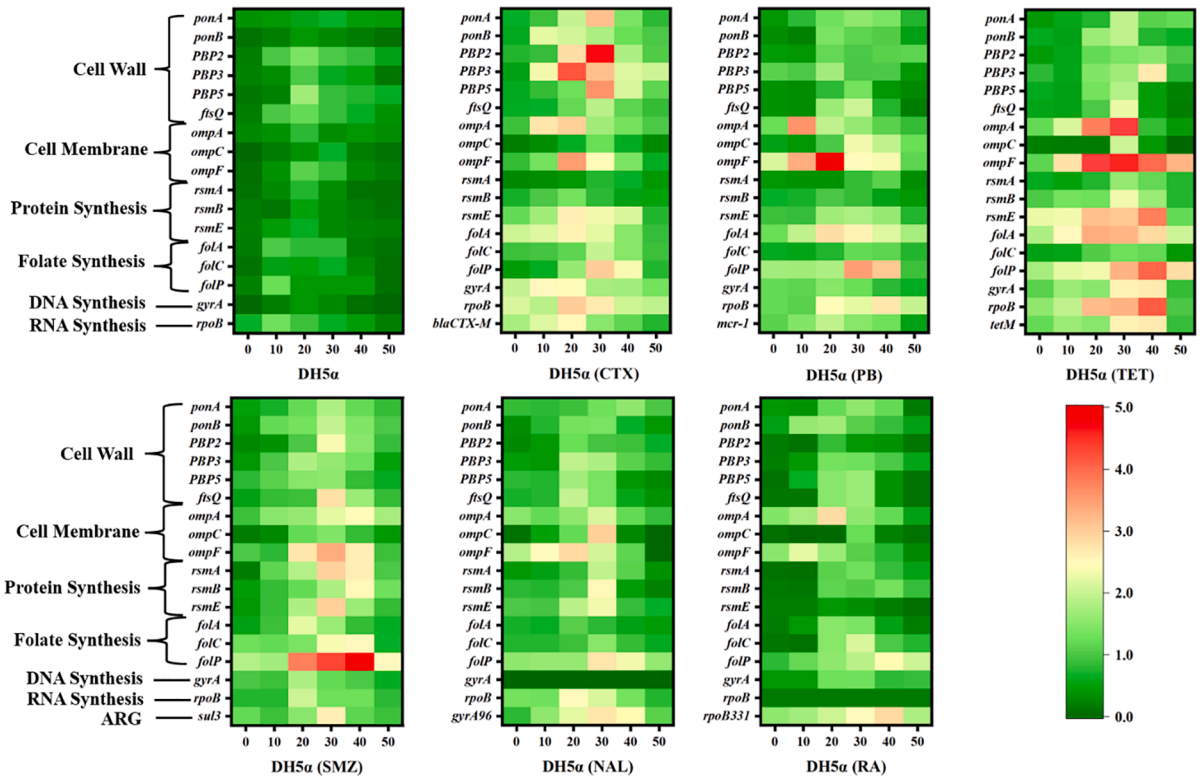

Fig. 5. Gene expression profiles of both ARGs and antibiotic resistance action target genes involved in the synthesis of cell wall, protein, DNA, RNA, folate and cell membrane regulation upon exposure to photocatalytic oxidation. X-axis: the monitoring time in minutes; Y-axis: clusters of target genes and list of genes tested, Y-axis right: the figure legend bar (depicted as a green-red color scale. Red spectrum color indicates strong up-regulated expression; green spectrum color indicated weak up-regulated expression).

contrast, when the ARB were subjected to photocatalytic oxidation, the expression of genes related to antibiotic resistance action targets increased about 2 − 8-fold, which was notably higher than that of ASB. Furthermore, the expression of genes for antibiotic resistance action targets varied among individual ARB and the relative target gene expression in the specific ARB was obvious, which could be attributed to the different antibiotic resistance action target mechanisms of the stress response.

Compared with *E. coli* DH5α (NAL) and *E. coli* DH5α (RA), the gene expression of *E. coli* DH5α (CTX) and *E. coli* DH5α (PB), in which the cell wall and cell membrane were respectively targeted, were obviously up-regulated with an initial 30 min of photocatalytic oxidation, especially their respective relative target genes. These results indicate that the stress response of antibiotic resistance action targets depends on the bacterial inactivation mechanism and the graded bacterial stress response during photocatalytic oxidation, causing the stress response mechanism of bacterial cells with targeted DNA and RNA to be unobvious and mainly occurring later in the stress period (Kültz, 2020; Sun et al., 2017).

Most of the tested genes were up-regulated in *E. coli* DH5α (TET) cells under photocatalytic oxidation. This indicates that the damage to *E. coli* DH5α (TET) can quickly induce global regulators, mediating different stress response systems via the cross-protection mechanism (Mitchell et al., 2009; Young et al., 2013). As for *E. coli* DH5α (SMZ), enhanced tolerance was related to the overexpression of *sul3* and related genes targeting folate synthesis. Folate is necessary for carbon transfer reactions during the biosynthesis of a spectrum of biomolecules, including amino acids and nucleotides (Capasso and Supuran, 2014). The enhanced expression of folate synthesis-related genes indicates that *E. coli* DH5α (SMZ) triggered a strong stress response mechanism for antibiotic resistance action targets under photocatalytic oxidation.

Based on the above results, the extracellular ROS generated by photocatalytic oxidation first caused the stress response mechanism of *E. coli* DH5α (CTX) and *E. coli* DH5α (PB), in which the cell wall and cell membrane were targeted, to repair the damaged cell wall and maintain membrane permeability. Correspondingly, the stress mechanism of *E. coli* DH5α (TET), in which protein synthesis was targeted, was also triggered and enhanced by the increased cellular ROS level, especially after the activated SOS response mechanism, which produced a large number of antioxidant enzymes to ensure survival of the bacterial cells. In addition, the overexpression of folate caused by the stress response mechanism of *E. coli* DH5α (SMZ) played a key role in maintaining normal metabolism of the bacterial cells and ensuring stability of hereditary substances. As for *E. coli* DH5α (NAL) and *E. coli* DH5α (RA), due to the graded cellular response of ARB, the stress response mechanism of bacterial cells with targeted DNA and RNA was unobvious and caused weak tolerance under photocatalytic oxidation. Overall, these results show in detail the effects of photocatalytic oxidation on the stress response mechanism of bacterial cells with different antibiotic resistance action targets (Fig. 6). Moreover, strong evidence was provided for a response mechanism that can enhance antibiotic tolerance and accelerate the repair of bacterial cells under photocatalytic oxidation, conferring more advantages to combat adverse environments.

4. Conclusions

This study mainly demonstrates that ARB with different antibiotic resistance action targets will respond differently to stress caused by photocatalytic oxidation, resulting in varying bacterial inactivation efficiencies, antibiotic resistance, cell morphology and conjugational transfer characteristics, antioxidant enzyme activities and cellular protein contents. The stress response mechanism of *E. coli* DH5α (TET) and *E. coli* DH5α (SMZ), in which synthesis of protein and folate is targeted, could rapidly induce global regulators through the overexpression of the

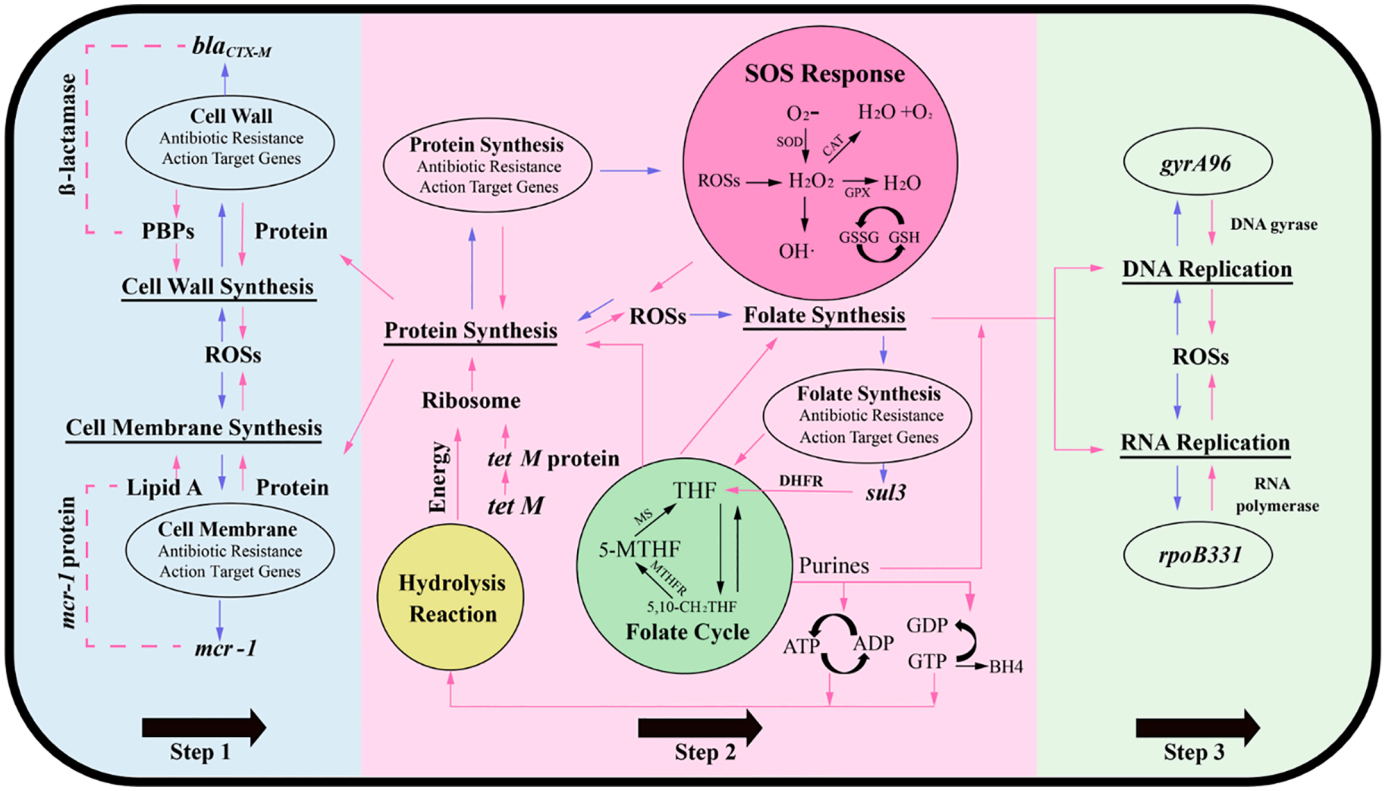

Fig. 6. The stress response mechanism of six ARB with different antibiotic resistance action targets.

respective antibiotic resistance action target genes. This mediates different stress response systems via the cross-protection mechanism, which enhances the antibiotic tolerance of bacteria in adverse environments. Moreover, the photocatalytic bacterial inactivation mechanism of different bacterial cells and the graded response of the cellular stress mechanism related to antibiotic resistance action targets, resulting in a stronger tolerance of *E. coli* DH5α (PB) and *E. coli* DH5α (CTX) than that of *E. coli* DH5α (NAL) and *E. coli* DH5α (RA). This new perspective reveals more detailed information on the practical application of photocatalytic oxidation for inactivating ARB.

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version.

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.

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Supplementary materials

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