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Photocatalytic inactivation of *Escherichia coli*—The roles of genes in β-oxidation of fatty acid degradation

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ABSTRACT

A genetic study on the relationship between cell membrane fatty acid profile and photocatalytic inactivation efficiency of *Escherichia coli* was conducted. Photocatalytic inactivation of a parental strain (*E. coli* BW25113) and its seven isogenic mutants with deletion of single gene involving in β-oxidation of fatty acid degradation (fad) were compared. Most of the mutants involved in fatty acid degradation did not show significant difference in susceptibility towards photocatalytic inactivation compared with the parental strain, except that *E. coli* JW1176 (*fad*R mutant) and *E. coli* JW3935 (*fab*R mutant) showed a lower and higher sensitivity than the parental strain, respectively. Fluorescence microscopic analysis showed that the loss of cell permeability preceded the inactivation of bacterial cells. The results of temperature pre-treatment and fatty acid profiles of the parental strain and *fad*R mutant indicated that the alteration in cell membrane fatty acid composition played an important role, but not the most crucial one to affect the susceptibility of bacterial cell towards photocatalytic inactivation. The results in this study demonstrated the importance of cell membrane in the bacterial defense system against photocatalytic inactivation.

1. Introduction

Photocatalytic inactivation of biohazards has been considered as a promising technology for water disinfection since the first application of semiconductor on sterilization of microbial cells [1]. Afterward, photocatalytic disinfection is extensively investigated and receives an increasing research interest. Recently, the bacterial cell membrane was considered to be the primary target attacked by reactive oxygen species (ROSs), including superoxide ($\bullet O_2^-$), hydrogen peroxide (H_2O_2) and hydroxyl radical (•OH), generated by photocatalysis [2–5]. These ROSs causes oxidative damages to the bacterial cell envelope (including outer membrane, cell wall, cell [or inner] membrane) and nuclei acids [6,7].

Most of the studies supported that •OH was the major ROS for photocatalytic bacterial inactivation [4,8–10]. The intact bacterial cell envelope initially blocked the attack of ROSs but eventually the outer membrane was decomposed by the ROSs, followed by the lipid peroxidation of the polyunsaturated phospholipids of the cell membrane, which was suggested to be the underlying mechanism [2,3,5,11–13]. Subsequent loss of normal functions of the bacterial cell such as respiratory activity and loss of fluidity, and oxidative attack of the intracellular components results in the cell death [2,5,11,12]. The •OH can attack the unsaturated lipids and form lipid radicals. In the presence of O_2, the lipid radicals react with O_2 to generate lipid peroxyl radicals, which are readily to further react with nearby unsaturated lipid, producing a new lipid radical and lipid hydroperoxide. These reactions initiate a reaction cycle which terminates as two radicals bind into non-radical species [3]. During this process, the formed lipid peroxides will be oxidized by lipid radicals and other by-products such as malondialdehyde [2,3,14,15]. Consequently, it causes the damage of bacterial cell membrane and change in the membrane permeability. With the loss of cell membrane selective permeability, there could be either influx of photocatalyst or efflux of cellular contents [5], hence allowing the attack of ROSs towards the intracellular components [2,3,12]. Therefore, the fatty acid composition of the cell membrane, especially the relative amounts of unsaturated and saturated fatty acids, would be a potential factor for determination of the cell susceptibility towards the attacks of ROSs during photocatalytic inactivation [4,6,16]. However, Dalrymple et al. [3] suggested that

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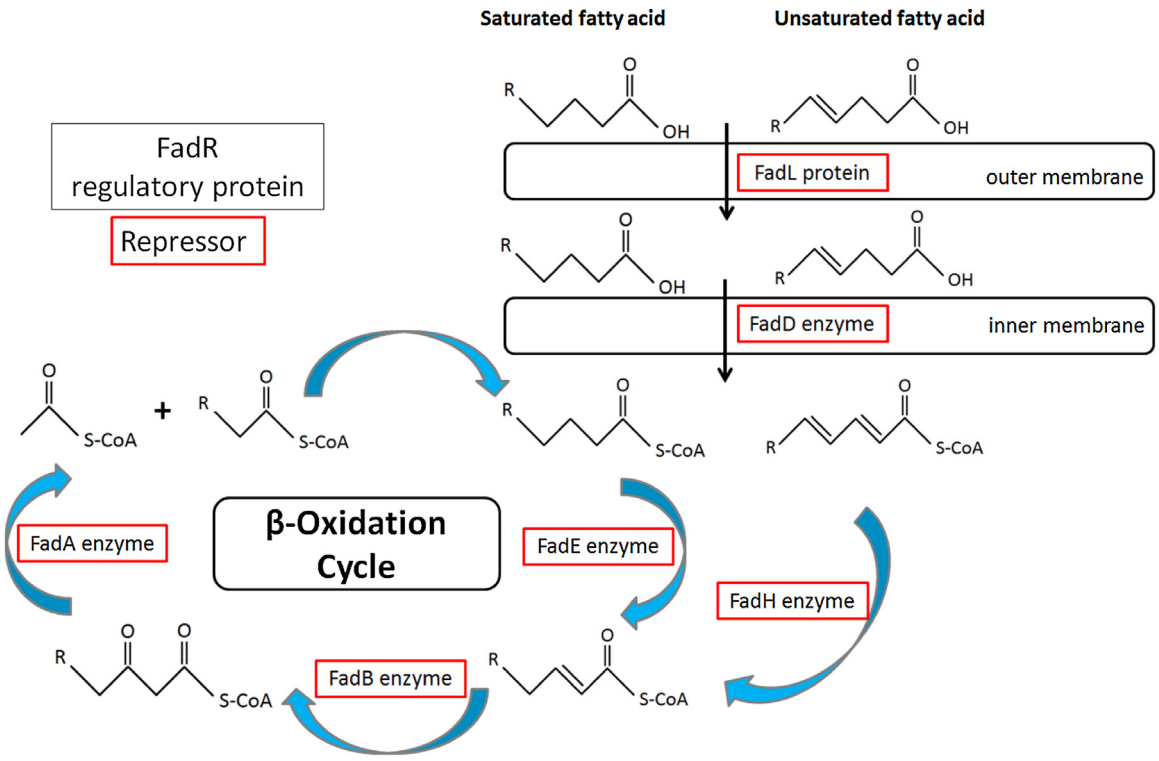

Fig. 1. Simplified schematic diagram of fatty acid degradation of *E. coli* (modified from Fujita et al. [19]).

the amount of monounsaturated fatty acids of cell membrane was not critical in photocatalytic disinfection. This leaves an unsolved issue on whether the cell membrane fatty acid profile has significant effect on photocatalytic bacterial inactivation.

Referring to Fujita et al. [19], a modified schematic diagram of the fatty acid degradation of *Escherichia coli* is illustrated in Fig. 1. The degradation process is regulated by the FadR regulatory protein. Long-chain fatty acids are first transported through the outer membrane under the control of transport protein FadL, and the enzyme FadD converts the fatty acid into acyl-CoA at the inner membrane. The β-oxidation pathway then starts under catalysis of FadE (for saturated fatty acid) and FadH (for unsaturated fatty acid) enzymes, followed by FadB and FadA enzymes. While coenzyme A (CoA) level was reported to affect the resistance of *E. coli* towards photocatalytic inactivation [4], the fatty acid degradation, which involves the production of CoA-containing intermediates and products (Fig. 1), is probably influential to the photocatalytic inactivation of *E. coli*.

The present study aims to investigate whether and how the genes responsible in β-oxidation of fatty acid degradation of *E. coli* play a role in affecting the photocatalytic inactivation. A previous study on selected *E. coli* mutants reported that the genes responsible for fatty acid biosynthesis and CoA production in *E. coli* were important in affecting the photocatalytic inactivation efficiency [4]. In addition to easy comparison of various gene products taking part in fatty acid degradation during bacterial inactivation, this genetic approach can avoid multiple physiological differences between bacterial strains because the mutants are isogenic (i.e. only a single gene different). As a result, to verify and study the role of fatty acid profile of cell membrane in bacterial defence system, single-gene deleted *E. coli* mutants with the mutations responsible for β-oxidation in fatty acid degradation [19] were used in the present study to compare their photocatalytic inactivation efficiencies with their parental strain, *E. coli* BW25113. *E. coli*, a common waterborne bacterium, was selected due to its best-studied genetics [4,15,19–21]. The cell permeability of the parental and mutant strains during the photocatalytic inactivation process was revealed. Since previous studies reported that temperature could alter the fatty acid composition of bacterial cells and significantly influence the photocatalytic inactivation [4,22,23], the present study also investigates the inactivation efficiency and fatty acid profiles after temperature pre-treatment to study the influence of the genes towards photocatalytic inactivation.

2. Materials and methods

2.1. Bacterial strains

Parental strain, *E. coli* BW25113, and its seven single-gene knockout mutants (*fad*A, *fad*D, *fad*E, *fad*H, *fad*L, *fad*R and *fab*R, Table 1), belonging to the Keio Collection [20], were purchased from the Coli Genetics Stock Center (CGSC), Yale University. For clearer and easier understanding, all the mutant strains will be mentioned as the abbreviated name as shown in Table 1 in this study. The Keio Collection was originated from the ancestral strain *E. coli* K-12 [20]. After a series of generalized transductions and allele replacements, *E. coli* BW25113 was derived, followed by the derivation of a set of mutants with precisely defined single-gene deletions [20]. The selected mutants differ from the parental strain by only one single gene (Table 1) and each individual deleted gene plays important role in β-oxidation of fatty acid degradation pathway [19]. Briefly, the *fad*L mutant lacks the fatty acid transport protein across the outer membrane of the bacteria. For the *fad*D mutant, there is absence of FadD protein (acyl-CoA synthetase), which is responsible for converting fatty acids into long-chain acyl-CoAs, the initial reactant in β-oxidation cycle. For the *fad*E, *fad*H and *fad*A mutants, there are absences of the acyl-CoA dehydrogenase, 2,4-dienoyl-CoA reductase and 3-ketoacyl-CoA thiolase, respectively. These enzymes are responsible for the conversion of long-chain acyl-CoAs into shorter ones during β-oxidation. In the *fad*R and *fab*R mutants, the regulatory FadR and FabR proteins responsible

Table 1
The genetic information of *Escherichia coli* parental strain (*E. coli* BW25113) and its single-gene deleted mutants (purchased from Coli Genetics Stock Center (CGSC), Yale University).

CGSC no.	*E. coli* strain no.	Abbreviated name	Deleted gene	Function of deleted gene	Impact of deleted gene on photocatalytic inactivation
NA	BW25113	NA	NA	NA	NA
11644	JW 5578	*fad*A mutant	*fad*A	3-Ketoacyl-CoA thiolase	None
9503	JW 1794	*fad*D mutant	*fad*D	Acyl-CoA synthetase	None
11134	JW 5020	*fad*E mutant	*fad*E	Acyl-CoA dehydrogenase	None
10330	JW 3052	*fad*H mutant	*fad*H	2,4-Dienoyl-CoA reductase	None
9875	JW 2341	*fad*L mutant	*fad*L	Fatty acid transport protein	None
9082	JW 1176	*fad*R mutant	*fad*R	fad repressor; fabAB activator	Higher resistivity
10840	JW 3935	*fab*R mutant	*fab*R	fabAB repressor	Higher sensitivity

NA = Not applicable.

for β-oxidation cycle and fatty acid biosynthesis cycle are absent, respectively [19].

The bacterial strains were cultured and harvested by the methods modified from that reported by Leung et al. [16]. Briefly, the bacterial cells were cultured in Nutrient Broth (NB, Lab M Ltd., Lancashire, UK) and agitated at 180 rpm for 18 h at 37 °C. Then, bacterial culture was washed for 3 times, each time with sterilized saline solution (0.9% NaCl) and by centrifugation at 13,000 rpm for 1 min at 22 °C (room temperature) by a microfuge. The cell pellet was re-suspended in saline solution with concentration of about 2×10^9 colony forming unit (cfu)/mL. The final cell density in the reaction mixture during experiment was adjusted to 2×10^7 cfu/mL.

2.2. Photocatalytic inactivation

The photocatalytic inactivation of the bacterial cells was carried out in a reactor holding 2 UVA lamps [16]. The UVA (λ = 365 nm, 15 W, 60 Hz, Cole-Parmer, USA) intensity was fixed at 0.274 mW/cm^2, measured by an UVX digital radiometer (UVP, Inc. Upland, CA, USA). Saline solution was used to make up the 50 mL-reaction mixture containing 100 mg/L TiO_2 (P25, Degussa Corporation, Germany) and 2×10^7 cfu/mL bacterial cells. Magnetic stirring of the reaction mixture was applied throughout the experiment. In the temperature pre-treatment experiment, the harvested cells (2×10^9 cfu/mL) in saline solution were pre-incubated at different temperatures (22, 30 and 37 °C) for 2 h before photocatalytic inactivation with reference to our previous study [4]. Dark control (TiO_2 added without UVA irradiation) and light control (UVA irradiation without addition of TiO_2) were also conducted. The cell inactivation abilities of various treatments were estimated by means of colony-counting on NB plate. For each sampling, 1 mL of sample was collected from the reaction mixture and immediately diluted with serial dilutions. 0.1 mL of sample from each single dilution was spread on a NB agar plate. All the spread plates were then incubated at 37 °C for colony-counting after 24 h.

2.3. Cell permeability

Cell permeability of the bacterial cells was determined by fluorescence spectroscopy [10]. Samples of bacterial cells during photocatalytic inactivation were collected and fluorescently stained with dyes of LIVE/DEAD® BacLight™ Bacterial Viability Kit (L7012, Molecular Probes, Inc., Eugene, OR, USA) according to procedures recommended by the manufacturer. The experiment was carried out using the staining reagent containing mixture of SYTO 9 dye and propidium iodide (PI). SYTO 9 labels bacterial cells with intact cell membrane (live) fluorescent green (Excitation/emission: 485 nm/530 nm) whereas PI penetrates only damaged membranes and stains the bacterial cells (dead) fluorescent red (Excitation/emission: 485 nm/630 nm). After incubation in dark for 15 min, the stained samples were examined by a fluorescence spectroscope (Nikon ECLIPSE 80i, Japan) equipped with a filter block N UV-2A consisting of excitation filter Ex 330-380 (Nikon, Japan) and Spot-K slider CCD camera (Diagnostic instruments. Inc., USA).

2.4. Fatty acid profile

Fatty acid profiles of the bacterial cells after temperature pre-treatment were analysed by an Agilent HP 6890 Series II gas chromatograph (Hewlett Packard, Palo Alto, USA) coupling with a HP 7863 autosampler and a HP flame ionization detector (FID) [16]. With the Sherlock Microbial Identification System Version 4.5 software, the fatty acids were identified by comparison of the retention times with Calibration Standard 1 No. 1200-A (Microbial ID, Newark, USA) and from a database in the "TSBA50 5.00 Library", which was provided by Microbial ID, Inc. The relative amount of different fatty acids in bacterial cells was determined.

2.5. Inactivation kinetics

The rates of inactivation of *E. coli after* temperature pre-treatment were compared by calculating the inactivation kinetics according to the model proposed by Geeraerd et al. [17]. The inactivation kinetics of the parental strain and mutants were fitted into the linear-tail model (Eq. (1)) with the software GInaFit [18].

$$\log(N) = \log[(10^{\log N_0} - 10^{\log N_{res}}) \times e^{-K_{max}t} + 10^{\log N_{res}}] \quad (1)$$

where K_{max} = specific inactivation rate (min^{-1}). N_{res} = residue population density (cfu/mL).

3. Results and discussion

3.1. Photocatalytic inactivation

The photocatalytic inactivation of each single-gene knockout mutant strain was compared with that of the parental strain (*E. coli* BW25113). It was found that the photocatalytic inactivation of most of the mutants (*fad*A, *fad*D, *fad*E, *fad*H and *fad*L mutants), except *fad*R and *fab*R mutants, was comparable to that of the parental strain (data not shown). Fig. 2 shows the photocatalytic inactivation of *fad*R and *fab*R mutant strains and the parental strain (*E. coli* BW25113) by TiO_2 suspension under irradiation of UVA. There was no significant reduction of viable colony counts in the dark and light controls for both mutants within 80 min (data not shown). Without significantly affecting the viability of the bacterial cells by UVA radiation reflects that the bacterial cells could adapt to UVA radiation, as indicated by Berney et al. [24]. Besides, the TiO_2 alone also did not cause toxic effect to both bacterial strains. In the presence of both UVA and TiO_2, *fab*R mutant and parental strain population started to decrease while *fad*R mutant did not show significant change in the first 20 min (Fig. 2). After that, the inactivation of *fad*R mutant was

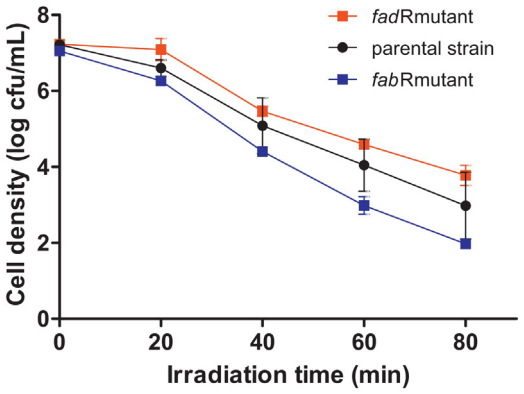

Fig. 2. Comparison of photocatalytic inactivation efficiencies of parental strain, *fad*R mutant and *fab*R mutant. TiO_2 concentration = 100 mg/L, reaction volume = 50 mL, agitation = 300 rpm, temperature = 22 °C, initial cell density = 2×10^7 cfu/mL, bacterial detection limit = 1 cfu/mL, error bar = standard deviation of 3 replicates.

observed and the photocatalytic inactivation of the three strains continued. At 80 min, the *fab*R mutant had the greatest inactivation efficiency with a 5 log cfu/mL reduction in cell density while the *fad*R mutant only had a decrease of 3 log cfu/mL. When compared to the parental strain which had 4 log cfu/mL of reduction, the *fab*R mutant and *fad*R mutant showed higher and lower photocatalytic inactivation efficiencies than the parental strain, respectively.

Since the individual mutants in β-oxidation cycle did not show great difference in inactivation efficiency, it indicates that most of the genes in fatty acid degradation do not have great influence on the bacterial defense system against photocatalytic inactivation. Indeed, the regulatory role of *fadR* gene product (FadR protein) is more crucial in affecting the photocatalytic inactivation efficiency.

The *fad*R gene encodes the regulatory protein for fatty acid metabolism and this protein product (FadR protein) is the repressor of the expression of the genes involved in β-oxidation cycle of the fatty acid degradation [19]. In the absence of the FadR protein, its repressive function is lost and there are more expressions of enzymes, which are encoded by *fad*A, *fad*D, *fad*E, *fad*H and *fad*L genes, involved in the fatty acid degradation. More active intermediates (such as acyl-CoAs) could be formed in the presence of the enzymes such as acyl-CoA synthetase (encoded by *fad*D), so it was expected the ROSs could attack the cell of *fad*R mutant more easily. However, the result (Fig. 2) was the opposite case that the *fad*R mutant was more resistant than the parental strain towards photocatalytic inactivation.

To account for this, it should be noted that apart from the role of repressor in fatty acid degradation, FadR protein also is an activator for genes expression in the unsaturated fatty acid biosynthesis process (*fab*A and *fab*B) [19,25,26]. In the absence of this activator, gene products involved in fatty acid biosynthesis are reduced, causing a reduction in the amount of unsaturated fatty acids [27]. Since the unsaturated fatty acids are the key targets of ROSs [2,4,5,28,29], lower proportion of unsaturated fatty acids should reduce the lipid peroxidation by ROSs, causing a higher resistance of the cells of *fad*R mutant towards photocatalytic inactivation. It was consistent with the lower susceptibility of *fad*R mutant shown in Fig. 2.

To further investigate how the role of FadR protein in fatty acid biosynthesis affects the photocatalytic inactivation of *E. coli*, the *fab*R mutant, in which the single knocked-out gene *fab*R is responsible for encoding the repressor regulatory protein (FabR) involved in unsaturated fatty acid biosynthesis of *E. coli* [19], was examined under photocatalytic inactivation (Fig. 2). FabR was believed to play a role in modulation of physical properties of the bacterial cell membrane by alteration of unsaturated fatty acid production [30]. The result showed that the *fab*R mutant was more sensitive than the parental strain towards photocatalytic inactivation. Since the presence of FadR protein is required for the negative regulation of FabR protein [19], FabR protein in the *fad*R mutant could not exert any effect in the unsaturated fatty acid biosynthesis, hence the photocatalytic inactivation. While in the *fab*R mutant, the role of FadR protein could be reflected by the FabR protein regulation during photocatalytic inactivation. Without the repression from the FabR protein, more unsaturated fatty acids are produced in the *fab*R mutant under the activation of biosynthesis pathway by FadR protein [19]. As a result, more unsaturated fatty acid were available in bacterial cell membrane, causing the *fab*R mutant to be more susceptible than the parental strain towards the ROSs attacks during photocatalytic inactivation (Fig. 2).

From the results in Fig. 2, it implies that in the β-oxidation cycle of fatty acid degradation, the regulatory *fad*R gene plays the most important role in affecting the photocatalytic inactivation efficiency, but single gene which encodes single enzyme within the pathway is not significantly influential to photocatalytic inactivation by the TiO_2–UVA system. Meanwhile, the role of *fad*R gene as an activator in the fatty acid biosynthesis pathway overwhelmed the role as a repressor in fatty acid degradation pathway in determining the bacterial susceptibility towards photocatalytic inactivation.

3.2. Cell permeability

To investigate the change of cell membrane permeability throughout the photocatalytic inactivation process, fluorescent assay was conducted. Fig. 3 shows the fluorescent images of the stained bacterial cells of parental strain (Fig. 3a) and *fad*R mutant (Fig. 3b) during the photocatalytic inactivation at 22 °C.

It obviously shows that at the beginning of the photocatalytic inactivation process the cells were intact (all cell stained green) and the cells of both bacterial strains turned from green to red within first 20 min. With the increase of the reaction time up to 20 min, the cell membrane was damaged and the bacterial cell lost its cell membrane permeability. It shows that prior to any inactivation, there was a loss of cell membrane permeability of the bacterial strains as early as 20 min (Fig. 3). This result indicates that the cell membrane is likely to be the primary target of the attack by ROSs in previous studies [4,15,31,32].

3.3. Effect of temperature pre-treatment

Fig. 4 shows the photocatalytic inactivation of *fad*R mutant and parental strain by TiO_2 suspension under irradiation of UVA after pre-incubating the harvested cells at different temperatures for 2 h. Dark and light control experiments in sterile saline solution showed that cells of both strains were not inactivated after pre-incubation at different temperatures (data not shown).

It was observed that the photocatalytic inactivation efficiencies of both the parental strain and *fad*R mutant were higher when the bacterial cells were pre-incubated at 22 °C than those at higher temperatures (Fig. 4). The kinetics of inactivation of the bacterial strains were well-fitted into the "linear-tail" model proposed by Geeraerd et al. [17,18], with adjusted $R^2 > 0.9$ for most of the cases (Table 2). The rate of inactivation (K_{max}) decreases when the temperature of pre-incubation increases (Table 2). This result is consistent with the findings of Gao et al. [4]. After pre-incubation at 22 °C, the *fad*R mutant was completely inactivated (K_{max}: 0.56/min) within 40 min, whereas the bacterial cells of parental strain only had a 5 log decrease (K_{max}: 0.45/min) in population (Fig. 4). The photocatalytic inactivation of the parental strain after incubation at 30 and 37 °C were similar (K_{max}: 0.39/min and 0.36/min, respectively), with about 4 log decreases in cell population after 80 min. Similar trends were also found in photocatalytic inactivation of the *fad*R mutant after pre-incubation at 30 °C (completely inactivated in 60 min,

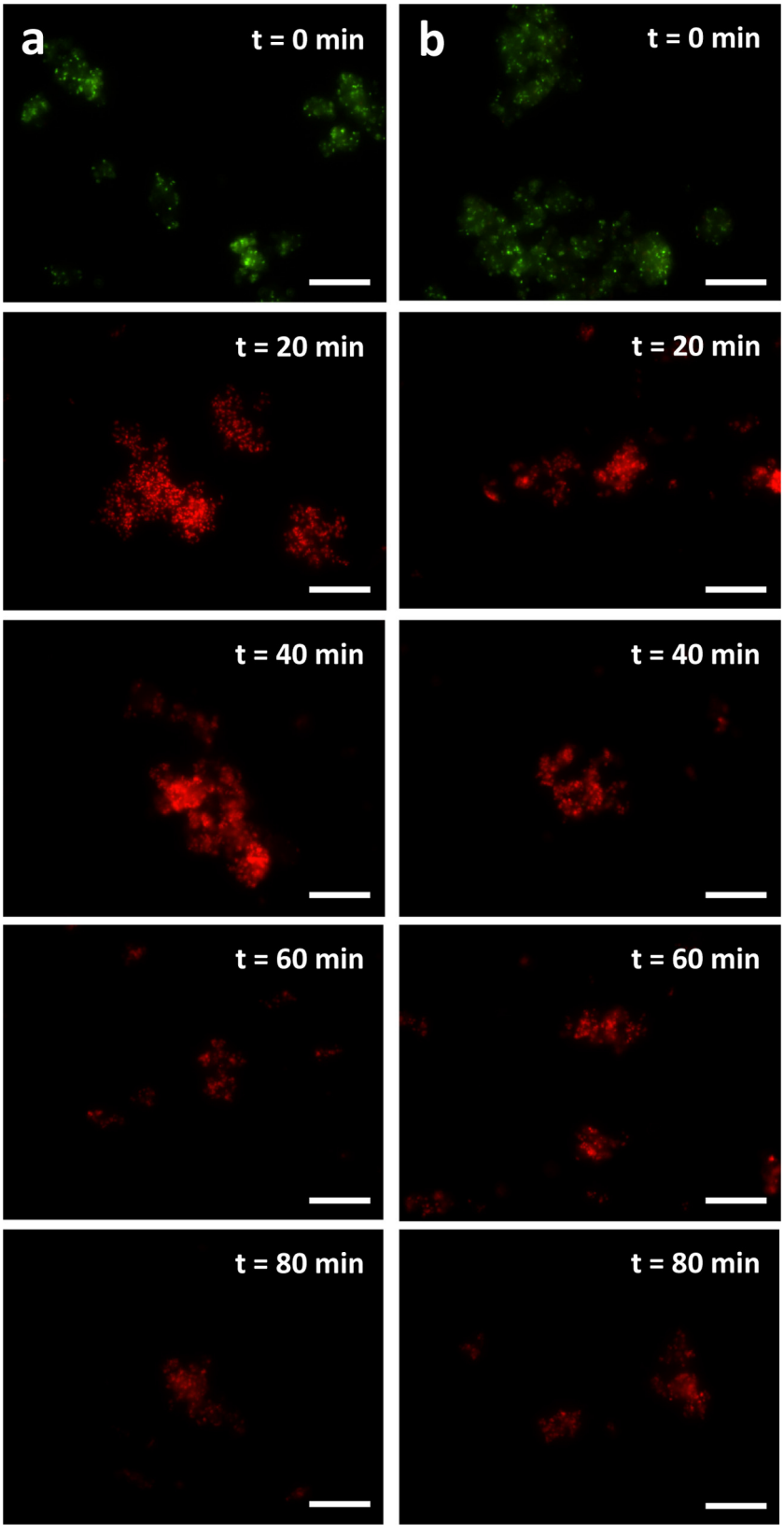

Fig. 3. Fluorescent microscopic images of (a) parental strain (2×10^7 cfu/mL, 50 mL) and (b) *fad*R mutant (2×10^7 cfu/mL, 50 mL) photocatalytically inactivated with 100 mg/L of TiO_2 suspension under UVA (λ = 365 nm) irradiation during reaction time (t) = 0, 20, 40, 60 and 80 min. (Scale bars: 20 μm).

K_{max}: 0.27/min) and 37 °C (cell population reduced to 1 log cfu/mL, K_{max}: 0.25/min). Generally, at each pre-incubation temperature, the photocatalytic inactivation efficiency of *fad*R mutant was higher than that of parental strain. However, this difference in photocatalytic inactivation efficiency was contrary to previous experiment (Fig. 2). The observed temperature effect could be due to the change in cell membrane fatty acid composition after the pre-incubation at different temperatures. Previous studies showed

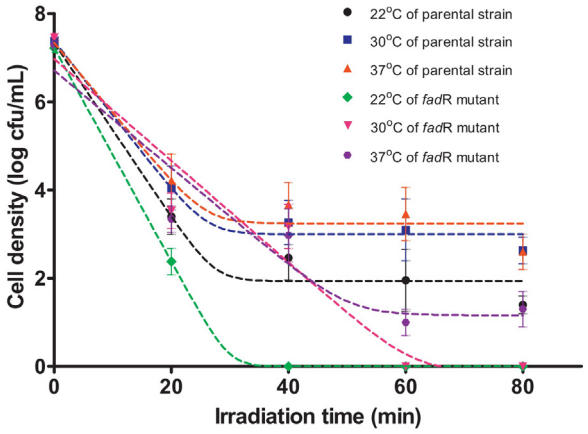

Fig. 4. Photocatalytic inactivation of parental strain and *fad*R mutant after pre-incubation at different temperatures. TiO_2 concentration = 100 mg/L, reaction volume = 50 mL, agitation = 300 rpm, temperature = 22 °C, initial cell density = 2×10^7 cfu/mL, bacterial detection limit = 1 cfu/mL, error bar = standard deviation of 3 replicates. The points are fitted into the "linear-tail" model proposed by Geeraerd et al. [17,18].

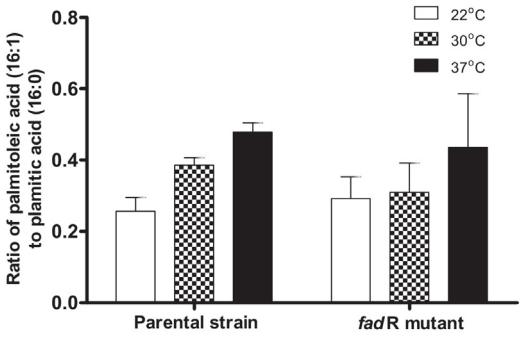

Fig. 5. Ratio of palmitoleic acid (16:1) to palmitic acid (16:0) after pre-incubation of parental strain and *fad*R mutant under different temperatures.

that temperature could lead to the change in the relative amount of unsaturated and saturated fatty acids of bacterial cell membrane [23] and the ratio of unsaturated to saturated fatty acid in cell membrane could influence the susceptibility of the bacterial cells towards photocatalytic inactivation [4,21]. When there was an increase in unsaturated fatty acid, the cell membrane became less rigid and viscous [33]. The bacterial cells then become easier to be attacked by the ROSs, leading to a higher sensitivity.

3.4. Fatty acid profile

To investigate the effect of the pre-incubation temperature and the fatty acid composition of the bacterial cells, the fatty acid profiles of the parental strain and *fad*R mutant were examined. Since palmitic acid (16:0) is a representative fatty acid commonly found in high amount in *E. coli* [4,34], and the palmitoleic acid (16:1) is the only unsaturated fatty acid found in the samples, the relative amount of unsaturated to saturated fatty acids in each sample therefore was represented by the ratio of palmitoleic acid (16:1) to palmitic acid (16:0). The ratios of the parental strain and *fad*R mutant after pre-incubation at different temperatures were illustrated in Fig. 5. The results show that for both strains, the ratio of palmitoleic acid (16:1) to palmitic acid (16:0) increased with the increase of pre-incubation temperature (Fig. 5). These also indicate that the temperature pre-treatment altered the fatty acid composition of the bacterial cells.

In general, at lower temperature, there would be an increase in relative amount of unsaturated fatty acid compared to saturated fatty acid in cell membrane due to homeoviscous adaptation [4,23]. However, the trend for the increasing ratios of unsaturated to saturated fatty acids at higher pre-treatment temperature is not consistent with this general trend. One of the reasons could be that homeoviscous adaptation occurs during the growth of bacterial cells in nutrient broth (NB) at different temperatures [23]. In the present study, the bacterial cells were pre-incubated in sterile saline solution, so the change of fatty acid composition of the cell membrane could be different from that during bacterial growth in nutrient broths. In addition, the palmitoleic acid (16:1) used for determining the ratio in the present study was different from the oleic acid (18:1) in the study of Gao et al. [4]. Since the only unsaturated fatty acid detected in recent study was palmitoleic acid (16:1), the ratios determined was not used for a good comparison with the ratios calculated from oleic acid (18:1).

Previously, it was expected that the temperature pre-treatment contributed to the higher sensitivity of the *fad*R mutant than the parental strain towards photocatalytic inactivation (Fig. 4). More unsaturated fatty acids in the cell membrane increase vulnerability of the bacterial cells towards the ROSs attack [2,3,34]. However, after 2 h pre-incubation, the ratios of unsaturated fatty acid to saturated fatty acid between the two bacterial strains were not significantly different at each pre-incubation temperature (Fig. 5). The results suggest that even it was found that the fatty acid composition of the cell membrane could be influenced by the pre-incubation temperature, the relative amount of unsaturated and saturated fatty acids of the cell membrane is not the only critical factor in determining the photocatalytic inactivation efficiency of bacterial cells, as previously suggested by Dalrymple et al. [3]. Some other hidden factors, which may need investigation in further studies, could possibly contribute to the inactivation efficiency difference between the strains.

Indeed, the present findings revealed the importance of the physiological conditions of bacterial cell membrane in photocatalytic inactivation. With the Gram-negative *E. coli* as a model for the study of photocatalytic inactivation on other pathogenic Gram-negative bacteria in water systems, such as *Legionella* sp. which causes Legionnaires' diseases and Pontiac fever [35], the results in the present study can provide some implications on the application of photocatalytic disinfection and clean-up processes.

Table 2
Inactivation rates and adjusted R^2 of the kinetic model of photocatalytic bacterial inactivation by TiO_2/UVA system after pre-treatment at different temperatures[a].

	Pre-treatment temperature (°C)	K_{max} (min^{-1})	Adjusted R^2
Parental strain	22	0.45 ± 0.09	0.9496
	30	0.39 ± 0.06	0.9702
	37	0.36 ± 0.10	0.9035
*fad*R mutant	22	0.56 ± 0.00	1.0000
	30	0.27 ± 0.07	0.8804
	37	0.25 ± 0.09	0.8292

±Denote the standard error.

[a] The kinetics of bacterial inactivation are fitted into a "linear-tail" model proposed by Geeraerd et al. [17,18].

4. Conclusions

This study was a systematic genetic study on the importance of fatty acid composition regulation of bacterial cell membrane towards photocatalytic inactivation by TiO_2–UVA system. All examined mutants showed no significant effect in photocatalytic inactivation except that the *fad*R mutant was found to be more resistant and the *fab*R mutant was more sensitive towards photocatalytic inactivation than the parental strain. With the dual

regulatory roles of *fad*R gene, its role as a repressor in β-oxidation of fatty acid degradation pathway was not crucial, while its role as an activator in fatty acid biosynthesis pathway was more important in affecting the photocatalytic inactivation efficiency. The cell membrane permeability experiment confirms the role of cell membrane as the primary target of ROSs during photocatalytic inactivation. With the alterations of fatty acid composition of the bacterial cell membrane after temperature pre-treatment experiment, it was shown that the relative amount of unsaturated and saturated fatty acids in bacterial cell membrane play an important, but not the most crucial role in determining the photocatalytic inactivation efficiency of the *E. coil* strains. Further studies are necessary to investigate the other critical factors affecting the photocatalytic inactivation efficiency of *E. coli.*

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References

[1] T. Matsunaga, R. Tomoda, T. Nakajima, H. Wake, FEMS Microbiol. Lett. 29 (1985) 211–214.
[2] P.C. Maness, S. Smolinski, D.M. Blake, Z. Huang, E.J. Wolfrum, W.A. Jacoby, Appl. Environ. Microbiol. 65 (1999) 4094–4098.
[3] O.K. Dalrymple, W. Isaacs, E. Stefanakos, M.A. Trotz, D.Y. Goswami, J. Photochem. Photobiol., A: Chem. 221 (2011) 64–70.
[4] M. Gao, T. An, G. Li, X. Nie, H.Y. Yip, H. Zhao, P.K. Wong, Water Res. 46 (2012) 3951–3957.
[5] Z. Huang, P.C. Maness, D.M. Blake, E.J. Wolfrum, S.L. Smolinski, W.A. Jacoby, J. Photochem. Photobiol., A: Chem. 130 (2000) 163–170.
[6] O.K. Dalrymple, E. Stefanakos, M.A. Trotz, D.Y. Goswami, Appl. Catal., B: Environ. 98 (2010) 27–38.
[7] W.G. Wamer, J.J. Yin, R.R. Wei, Free Radical Biol. Med. 23 (1997) 851–858.
[8] M. Cho, H. Chung, W. Choi, J. Yoon, Water Res. 38 (2004) 1069–1077.
[9] A.A. Khodja, A. Boulkamh, C. Richard, Appl. Catal., B: Environ. 59 (2005) 147–154.
[10] L.S. Zhang, K.H. Wong, H.Y. Yip, C. Hu, J.C. Yu, C.Y. Chan, P.K. Wong, Environ. Sci. Technol. 44 (2010) 1392–1398.
[11] K. Sunada, T. Watanabe, K. Hashimoto, J. Photochem. Photobiol., A: Chem. 156 (2003) 227–233.
[12] D.M.A. Alrousan, P.S.M. Dunlop, T.A. McMurray, J.A. Byrne, Water Res. 43 (2009) 47–54.
[13] A.G. Rincón, C. Pulgarin, Sol. Energy 77 (2004) 635–648.
[14] D. Wu, Hong Yu, D. Jin, X. Li, J. Photochem. Photobiol., A: Chem. 217 (2011) 177–183.
[15] S. Pigeot-Rémy, F. Simonet, D. Atlan, J.C. Lazzaroni, C. Guillard, Water Res. 46 (2012) 3208–3218.
[16] T.Y. Leung, C.Y. Chan, C. Hu, J.C. Yu, P.K. Wong, Water Res. 42 (2008) 4827–4837.
[17] A.H. Geeraerd, V.P. Valdramidis, J.F. van Impe, Int. J. Food Microbiol. 59 (2000) 185–209.
[18] A.H. Geeraerd, V.P. Valdramidis, J.F. van Impe, Int. J. Food Microbiol. 102 (2005) 95–105.
[19] Y. Fujita, H. Matsuoka, K. Hirooka, Mol. Microbiol. 66 (2007) 829–839.
[20] T. Baba, T. Ara, M. Hasegawa, Y. Takai, Y. Okumura, M. Baba, K.A. Datsenko, M. Tomita, B.L. Wanner, H. Mori, Mol. Syst. Biol. 8 (2006) 1–11.
[21] Y.W. Cheng, R.C.Y. Chan, P.K. Wong, Water Res. 41 (2007) 842–852.
[22] B. Gügi, N. Orange, F. Hellio, J.F. Burini, C. Guillou, F. Leriche, J.F. Guespin-Michel, J. Bacteriol. 173 (1991) 3814–3820.
[23] M. Sinensky, Proc. Nat. Acad. Sci. U.S.A. 72 (1974) 522–525.
[24] M. Berney, H. Weilenmann, T. Egli, J. Photochem. Photobiol., B: Biol. 86 (2007) 149–159.
[25] M.F. Henry, J.E. Cronan, J. Mol. Biol. 222 (1991) 843–849.
[26] J.W. Campbell, J.E. Cronan, J. Bacteriol. 183 (2001) 5982–5990.
[27] K. Magnuson, S. Jackowski, C.O. Rock Jr., J.E. Cronan, Microbiol. Rev. 57 (1993) 522–542.
[28] J. Kiwi, V. Nadtochenko, J. Phys. Chem. B 108 (2004) 17675–17684.
[29] V.A. Nadtochenkoa, A.G. Rincon, S.E. Stanca, J. Kiwi, J. Photochem. Photobiol., A: Chem. 169 (2005) 131–137.
[30] Y.M. Zhang, H. Marrakehi, C.O. Rock, J. Biol. Chem. 277 (2002) 15558–15565.
[31] T. Saito, T. Iwase, J. Horie, T. Morioka, J. Photochem. Photobiol., B: Biol. 14 (1992) 369–379.
[32] C. Pablos, R. van Grieken, J. Marugán, B. Moreno, Catal. Today 161 (2011) 133–139.
[33] H. Träuble, P. Overath, Biochim. Biophys. Acta 307 (1973) 491–512.
[34] G.A. Pradenas, B.A. Paillavil, S. Reyes-Cerpa, J.M. Pérez-Donoso, C.C. Vásquez, Microbiology 158 (2012) 1279–1283.
[35] B.R. Kim, J.E. Anderson, S.A. Mueller, W.A. Gaines, A.M. Kendall, Water Res. 36 (2002) 4433–4444.