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Differences in photoelectrocatalytic inactivation processes between *E. coli* and its isogenic single gene knockoff mutants: Destruction of membrane framework or associated proteins?

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ABSTRACT

Fatty acids (FAs) are the main components of bacterial cell membranes (phospholipid bilayer). FA profiles and responses to photoelectrocatalytic (PEC) treatment were comparatively investigated using an *Escherichia coli* parental strain BW25113 and two isogenic FA synthesis deficient mutants. Both mutants, which have higher ratios of unsaturated FAs (UFAs) to saturated FAs (SFAs), were more susceptible to PEC inactivation than the parental strain. PEC treatment can elevate the proportion of bacterial SFA, especially for the mutants, indicating that UFAs are more sensitive to PEC treatment. Collective data from the cytoplasmic K^+ leakage, bacterial fluorescent, and scanning electron microscopic analyses showed that the cytoplasmic membrane framework was damaged by PEC treatment. Interestingly, compared with the membrane framework damage, the functional disruption of membrane proteins was observed much earlier. For example, significant decreases in bacterial respiration rates and adenosine triphosphate (ATP) generation potential were seen in the initial stage of PEC treatment. As such, the disruption of the bacterial energy metabolism system caused by membrane protein damage was more likely the initial lethal step during PEC bacterial inactivation. The clear understanding of PEC inactivation mechanisms can help its practical applications.

1. Introduction

Photocatalytic (PC) and photoelectrocatalytic (PEC) technologies have been studied extensively, and their applications to water disinfection are promising due to the solar energy-driven potential and self-cleaning capacity [1–5]. PC and PEC bacterial inactivation processes do not produce carcinogenic byproducts during disinfection, like conventional processes like chlorination do [6]. However, PC and PEC treatment inactivation mechanisms are still not well established, which hinders the development of mechanism-based kinetic models, as well as practical applications. The bactericidal agents produced in PC and PEC systems include different reactive species (RSs) such as H_2O_2, $O_2^{\bullet -}$, h^+, and $^{\bullet}OH$ [7,8], however, the targets of the RS attacks and the specific lethal steps of PC and PEC treatments are not well understood. For Gram negative bacteria, the outer membrane, cell wall, and cytoplasmic membrane are located at the outside of the cell, and thus are the most probable targets of attack. The damaged bacterial envelopes seen in SEM (scanning electron microscopic) and TEM (transmission electron microscopic) images of PC and PEC treated samples support this hypothesis [9–12].

Some reports have argued that the cell wall is rather porous and might allow RSs to pass through, making this layer not the actual initial attack target [13]. On the other hand, the bacterial cytoplasmic membrane, consisting mainly of the phospholipid bilayer, has been shown to be susceptible to RSs attack, and the oxidative

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damage to the cytoplasmic membrane may alter its permeability. This leads to the entry of RSs into intracellular compartments as well as cytoplasmic component leakage [14–16]. However, whether the cytoplasmic membrane framework (phospholipid bilayer) is the initial lethal target of RSs attack during PC and PEC inactivation is still debated.

Additionally, different proteins associated with the cytoplasmic membrane (phospholipid bilayer) play essential roles in various cellular functions, such as substrate transportation and energy metabolism. For example, the electron transport chain for bacterial respiration, including dehydrogenase and a series of coenzymes, is located at the bacterial cytoplasmic membrane [17]. Similar to the cytoplasmic membrane framework, these membrane proteins are also exposed to RSs attack during PC and PEC treatments [1]. Some research suggests that the oxidative damage of these proteins may be essential steps of bacterial inactivation [18]. Nevertheless, the impact of cytoplasmic membrane destruction and membrane protein disruption on bacterial inactivation has not been widely studied during PC or PEC treatment. Thus, it is not clear whether the two types of damage mechanisms mentioned above are lethal steps during PC and PEC inactivation.

This work explored the progression of bacterial membrane changes, to better understand the lethal steps of bacterial inactivation during PEC treatment. The study used an *Escherichia coli* parental strain and its two isogenic mutants; these were effective in studying disinfection mechanisms [19]. Therefore, in this work, the *E. coli* isogenic mutants deficient in fatty acid (most important content of membrane framework) synthesis, together with their parental strain *E. coli* BW25113, were used to investigate the cytoplasmic membrane lipid oxidation mechanism. Does the cytoplasmic membrane oxidative damage occur? Which is the major target of RSs attack, the cytoplasmic membrane framework (phospholipid bilayer) or the membrane associated proteins? Is the decomposition of the cytoplasmic membrane framework or the disruption of membrane associated proteins directly responsible for the bacterial inactivation? Answering these questions would deepen our understanding of PC and PEC disinfection mechanism and further help to develop new high efficiency bactericidal techniques.

2. Experimental section

2.1. Photoelectrocatalytic inactivation apparatus setup

A PEC reactor (50 mL volume) was used [20] to perform inactivation experiments, with a TiO_2 nanotube array photoanode [21]. Detailed experimental conditions are described below; materials included a counter electrode, platinum foil; reference electrode, saturated Ag/AgCl; bias potential of the anode, 1 V *versus* Ag/AgCl; light source, LED lamp with maximum emission at 365 nm and the light intensity adjusted to 27 mW cm^{-2}.

2.2. E. coli *strains and bacterial suspension preparation for inactivation*

Table S1 lists the three *E. coli* strains used for PEC inactivation. All three strains were purchased from Coli Genetic Stock Center (CGSC, Yale University, New Haven, CT, USA). *E. coli* JW3935-4 (Δ*fabR*) and *E. coli* JW1077-1 (Δ*fabH*) were the isogenic mutants of parental strain *E. coli* BW25113 with single gene knocked out. The *fabR* encodes the transcription inhibitor of *fabA* and *fabB*, both of which are essential for unsaturated fatty acid (UFA) synthesis, and the *fabR* deficient strain has elevated UFA fraction compared with wild type strain [22]. The *fabH* encodes β-ketoacyl-acyl carrier protein

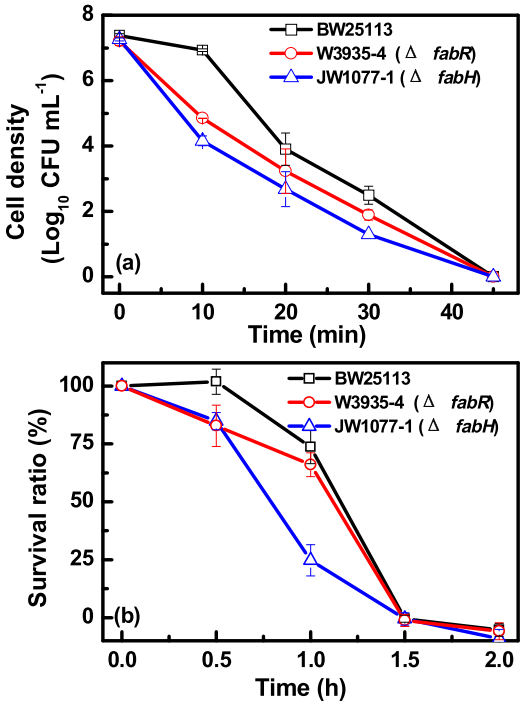

Fig 1. The PEC inactivation performances of parental strain *E. coli* BW25113 and isogenic mutants deficient in fatty acid biosynthesis (*E. coli* JW3935-4 and *E. coli* JW1077-1). (a) The initial bacterial density of the suspension is ~2×10^7 CFU mL^{-1}, and the survived bacteria determined by the colony counting method; (b) the initial bacterial density of the suspension is ~2×10^8 CFU mL^{-1}, and the cell viability determined by Live/Dead Fluorescent Kit.

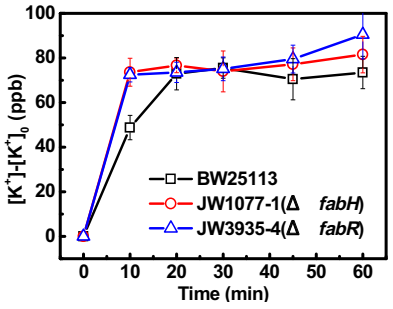

Fig. 2. K^+ leakage from bacterial cells during PEC inactivation (~2×10^7 CFU mL^{-1}) of parental strain *E. coli* BW25113 and two isogenic mutants. The $[K^+]$ represented the K^+ concentration at sampling time point, and $[K^+]_0$ represented the K^+ concentration at time of 0 min for each bacterial strain.

synthase III (KAS III), which catalyzes the condensation reaction during the initial step of fatty acid biosynthesis [23].

Previous studies found that the KAS III deficient *E. coli* strain had a higher UFA proportion compared with the wild type strain, especially the monounsaturated fatty acid with 18 carbons (18:1) [24]. The bacterial strains were stored in 25% sterilized glycerin at −80 °C. To prepare the bacterial suspension for PEC inactivation, the individual bacterial strain was streaked on a nutrient agar plate and incubated to acquire isolated colonies. The colony was inoculated into Nutrient Broth (NB) and incubated overnight. The bacterial suspension was diluted with fresh NB by 1:100, and then incubation continued for 6 h at 37 °C and 200 rpm to log phase. The bacterial cells were harvested by centrifugation at 5000 rpm for 5 min, washed twice with sterilized water, and re-suspended in 0.2 M $NaNO_3$. Unless otherwise noted, the bacterial density of the suspension used for PC and PEC inactivation in this study was ~2×10^7 CFU mL^{-1} (colony forming unit per mL).