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Reduction in horizontal transfer of conjugative plasmid by UV irradiation and low-level chlorination

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ABSTRACT

The widespread presence of antibiotic resistance genes (ARGs) and antibiotic resistant bacteria (ARB) in the drinking water system facilitates their horizontal gene transfer among microbiota. In this study, the conjugative gene transfer of RP4 plasmid after disinfection including ultraviolet (UV) irradiation and low-level chlorine treatment was investigated. It was found that both UV irradiation and low-level chlorine treatment reduced the conjugative gene transfer frequency. The transfer frequency gradually decreased from 2.75×10^{-3} to 2.44×10^{-5} after exposure to UV doses ranging from 5 to 20 mJ/cm^2. With higher UV dose of 50 and 100 mJ/cm^2, the transfer frequency was reduced to 1.77×10^{-6} and 2.44×10^{-8}. The RP4 plasmid transfer frequency was not significantly affected by chlorine treatment at dosages ranging from 0.05 to 0.2 mg/l, but treatment with 0.3–0.5 mg/l chlorine induced a decrease in conjugative transfer to 4.40×10^{-5} or below the detection limit. The mechanisms underlying these phenomena were also explored, and the results demonstrated that UV irradiation and chlorine treatment (0.3 and 0.5 mg/l) significantly reduced the viability of bacteria, thereby lowering the conjugative transfer frequency. Although the lower chlorine concentrations tested (0.05–0.2 mg/l) were not sufficient to damage the cells, exposure to these concentrations may still depress the expression of a flagellar gene (*FlgC*), an outer membrane porin gene (*ompF*), and a DNA transport-related gene (*TraG*). Additionally, fewer pili were scattered on the bacteria after chlorine treatment. These findings are important in assessing and controlling the risk of ARG transfer and dissemination in the drinking water system.

1. Introduction

Antibiotic resistant bacteria (ARB) and antibiotic resistant genes (ARGs) have been extensively detected in drinking water systems (Zhang et al., 2009; Bouki et al., 2013; Bergeron et al., 2015), and their widespread presence in drinking water constitutes a major public health issue. Flemming and Ridgway (2009) suggested that most microorganisms (95%) can aggregate as biofilms on the surface of pipelines despite the presence of chlorine. The ubiquitous presence of bacteria in a planktonic state or in biofilms found in both water treatment processes and in pipelines easily transmit resistance through horizontal gene transfer (HGT) (Madsen et al., 2012). Additionally, some of the microorganisms can be pathogens, which further increases the risks and dangers posed to human beings.

Bacterial antibiotic resistance can be conferred either by spontaneous mutation or by horizontal transfer. Mutagenic factors, such as disinfection by-products, are believed to induce antibiotic resistance in water treatment processes (Lv et al., 2014). However, in many cases, bacteria cannot adapt to such harsh conditions, and instead, they receive mobile genetic elements (MGEs) that carry the resistance genes from other bacteria, which is known as HGT (Ojala et al., 2014). Three genetic mechanisms are responsible for HGT of ARGs: 1) conjugative transfer by MGEs such as plasmids, transposons, and integrons; 2) transformation of naked DNA in either naturally competent bacteria or bacteria with competency induced by environmental factors; and 3) transduction by bacteriophages (Zhang et al., 2009; Dodd, 2012). Bacterial conjugation promotes the horizontal transfer of genetic materials when donor and recipient cells are in physical contact (Ghigo, 2001). Plasmid-based conjugative transfer has been widely recognized to occur in environmental microbial communities and in laboratory conditions (Anjum et al., 2011; Yang et al., 2013; Bellanger et al., 2014). Therefore, conjugative transfer may be particularly important in

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disseminating ARGs in drinking water system.

Disinfection technologies (such as UV irradiation and chlorination disinfection), are widely applied to ensure microbial safety in water systems. Over the past few decades, UV treatment has been an increasingly popular technology for water disinfection because microorganism inactivation is achieved without the production of genotoxic byproducts (Guo et al., 2013; Lee et al., 2015). In addition, chlorine, as a common disinfectant, was introduced into urban water supplies in the early 19th century to eliminate waterborne pathogens based on its effectiveness, easy of use, and low cost (Ferey et al., 2000; Simoes et al., 2010). However, despite the application of these different disinfection processes, bacterial regrowth is a common problem during water distribution. As a result, maintaining residual chlorine (>0.05 mg/l) is a common practice for controlling microbial regrowth in pipelines.

Previous studies have typically focused on the direct influence of disinfection on ARB and ARGs, and have revealed that disinfection processes (e.g., chlorination and UV disinfection) can substantially remove ARB and ARGs during water treatment (Huang et al., 2011; Guo et al., 2013). However, it has been shown that some of the ARB or ARGs perform resistance against disinfectants (Xi et al., 2009; Shi et al., 2013). Consequently, these persistent bacteria can survive and spread resistance to downstream communities, especially in water supply pipelines. Moreover, intact remnants of DNA may be transferred to surrounding populations via horizontal transfer. Thus, transfer of ARGs among bacteria after disinfection in drinking water terminal systems is a major public concern with direct consequences for consumers. To date, there have been no reports regarding the effects of UV irradiation and low-level chlorine treatment (0.05–0.5 mg/l, simulating residual chlorine in pipelines) on the risk of HGT of ARGs. In this study, we established a conjugative model system under laboratory conditions and investigated changes in the conjugative transfer frequency after UV irradiation and low-level chlorination as well as the underlying mechanisms.

2. Materials and methods

2.1. Bacteria strains and plasmid

The donor strain used in this study was *Escherichia. coli* HB101, which harbored the RP4 plasmid that confers resistance to kanamycin, tetracycline, and ampicillin. These resistances were encoded by the *aphA*, *tetA* and *tetR*, and *bla* genes, respectively. This broad-host-range plasmid RP4 was classified as medium-copy plasmid, and could have a copy number of about 4–7 in each cell (Priefer et al., 1985). Another *E. coli* K12 strain resistant to rifampicin was used as the recipient bacteria. Their resistance was conferred by mutations in *rpoB*. Both the donor and recipient strains were gifted by Professor Junwen Li and Dr. Zhigang Qiu of the Institute of Health and Environmental Medicine. The donor and recipient strains were pre-cultured separately in Luria–Bertani (LB) broth supplemented with appropriate antibiotics (80 mg/l kanamycin, 50 mg/l tetracycline, and 60 mg/l ampicillin for donor strains, and 100 mg/l rifampicin for recipient strains) on a 180 rpm shaking incubator at 37 °C. After overnight incubation, the cell density reached approximately 10^9 CFU/ml, and the cells were washed twice with 0.9% sterile saline. Cultures were centrifuged at 4500 g for 10 min. Samples of donor and recipient strains were re-suspended and then diluted to approximately 10^8 CFU/ml with pure or 1/500 LB broth.

2.2. Conjugation and disinfection experiment

For UV treatment, donor cells resuspended in 0.9% sterile saline were exposed to the UV irradiation at different fluences (0, 5, 10, 15, 20, 50, or 100 mJ/cm^2) according to the standard collimated beam test protocol described by Guo et al. (2013) and Zhang et al. (2015). After UV irradiation, donor cells were mixed with recipient cells (resuspended by pure LB broth) at a 1:1 ratio (10 ml each). All steps were carried out in the dark to prevent photoreactivation. The mixtures were grown at 37 °C with shaking at 80 rpm for 24 h.

During chlorination, LB broth was diluted 500-fold (total organic carbon (TOC) = 10 mg/l) to avoid chlorine consumption by a high concentration of organic compounds. Solutions of resuspended donor and recipient cells (10 ml each) were mixed together at a 1:1 ratio and then cultured under the same conditions applied for UV treatment. Sodium hypochlorite was introduced to establish different doses of free chlorine (0, 0.05, 0.1, 0.2, 0.3, and 0.5 mg/l Cl_2), and cells were cultured for 0–48 h (Huang et al., 2011, 2013). The medium was replaced by fresh medium (1/500 LB) supplemented with the appropriate chlorine concentration every 12 h. Finally, chlorination was terminated with the addition of 1.5% sodium thiosulfate solution before plating.

2.3. Transconjugant identification and mechanism analysis

After mating periods of 6, 12, 24, or 48 h, 1 ml of the donor and recipient mixture was collected from each disinfection system. The cultures were serially diluted and spread on LB medium plates containing different antibiotics. The numbers of donors and recipients were determined by counting colonies on LB agar supplemented with 80 mg/l kanamycin, 50 mg/l tetracycline, and 60 mg/l ampicillin or 100 mg/l rifampicin. The recipients possessing the RP4 plasmid were recognized as transconjugants. The transconjugants were selected and counted on LB agar plates containing 80 mg/l kanamycin, 50 mg/l tetracycline, 60 mg/l ampicillin, and 100 mg/l rifampicin. To confirm that the donor and recipient strains could be distinguished, they were cross plated. Transconjugant colonies were randomly selected, and then confirmed using colony polymerase chain reaction (PCR)-based detection technology. The PCR reactions and amplification conditions recommended by Yang et al. (2013) were used. PCR products were detected by electrophoresis in 0.8% agarose gel. Electrophoresis was carried out at 25 °C at 180 V for 20 min in Tris-acetate-EDTA (TAE) buffer. Finally, the PCR products (140 bp) were sent to Sangon Biotech (Shanghai, China) for sequencing. The retrieved sequences were aligned with those found in the NCBI database using the Blast tool (http://www.ncbi.nlm.nih.gov/blast).

To analyze the mechanisms responsible for the effects of UV irradiation and chlorine treatment on conjugative gene transfer, different donor/recipient rates (e.g., 10^3, 10^4, 10^5, 10^6, 10^7, and 10^8 CFU/ml donor cells mixed with 10^8 CFU/ml recipient cells) were inoculated, and the corresponding transfer frequencies were calculated. Additionally, the expression of three genes, i.e., a flagllum gene (*FlgC*), an outer membrane protein gene (*ompF*), and a transfer regulated gene (*TraG*) were assessed. *FlgC* encodes a flagellar protein. For conjugation to occur, the donor and recipient cells must be in physical contact (Dodd, 2012). Bacterial mobility is regulated by polar flagella, which affects bacterial collision and attachment. Thus, the transfer frequency is influenced by the expression of *FlgC*. *OmpF* is a common porin protein, and occlusion of *ompF* may interrupt substance import, including nucleic acid intake during conjugative transfer (Spector et al., 2010). *TraG* is essential for the conjugative process, because it directly determines the mating between the donors and recipients (Hamilton et al., 2000). RNA extraction was carried out using the EasyPure™ RNA kit (TransGen Biotech, China) according to the manufacturer's instructions. Because RNAs with higher turnover rates would disappear more rapidly, reverse transcription was performed immediately using a reverse transcription (RT)-PCR kit (Promega,