Elimination of transforming activity and gene degradation during UV and UV/H2O2 treatment of plasmid-encoded antibiotic resistance genes

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Abstract
To better understand the elimination of transforming activity of antibiotic resistance genes (ARGs), this study determined deactivation of transforming activity of an ARG and the ARG degradation during UV and UV/H2O2 treatment of plasmid pUC19 containing a ampicillin resistance gene (ampR). The fluence-based rate constant (k) of ~6.2x10^-2 cm^2/mJ was determined for the decrease of transforming activity during UV treatment. This k value for pUC19 was much lower than the k calculated for cyclobutane-pyrimidine dimer (CPD) formation in the entire plasmid, indicating a significant role of CPD repair in the host cells. The degradation rate constants (k) of ampR measured by qPCR increased with the increasing target amplicon size and were close to the k calculated for the CPD formation in the given amplicons. Further analysis of the degradation kinetics of plasmid-encoded genes revealed that qPCR detected most UV-induced DNA damage. Our results indicate that calculated CPD formation rates and qPCR analyses are useful for predicting and/or measuring the rate of DNA damage and predicting the efficiency of transforming activity elimination for plasmid-encoded ARGs during UV-based water disinfection and oxidation processes.

Theoretical background - Reasons for performing this work and progress beyond the state of the art
There has been growing interest in the efficiency of wastewater disinfection and oxidation processes to lower the levels of ARB and ARGs, in addition to micropollutant elimination. ARGs in wastewaters exist in different forms such as intracellular (within bacteria) and extracellular, as free DNA and viruses. ARGs can transfer resistance by HGT mechanisms such as conjugation, transduction, and transformation. Considering the potential for ARG transfer via transformation, it is necessary to assess the efficiency of disinfectants at destroying ARGs and eliminating their associated transforming activities.

The qPCR method has been employed to assess the efficacy of disinfection processes for ARG elimination by quantifying target qPCR amplicons. As an alternative approach, the transforming activity of ARGs can be directly measured by transformation assays. Nevertheless, few studies have applied such an ARG transformation assay to assess the efficacy of disinfection processes for the elimination of antibiotic resistance. Furthermore, the relationship between the qPCR method and the transformation assay for determining a biologically active ARG concentration is still poorly understood.
To elucidate the efficiency of deactivation and degradation of ARGs during water disinfection and oxidation processes, in this study, we determined and compared the changes in transforming activity and ARG concentrations during UV\textsubscript{254nm} (hereafter UV) or UV/H\textsubscript{2}O\textsubscript{2} treatment of plasmid-encoded ARGs in bench scale disinfection experiments.

**Experimental setup and main methods used in this work**

A quantitative transformation assay employing *Escherichia coli* as the recipient was conducted to determine the transforming activity of the target plasmid. ARG concentrations were determined by qPCR with different amplicon sizes to determine the DNA damage in different parts of the target plasmid. In addition, agarose gel electrophoresis was carried out to determine structural changes of the plasmid. Both extracellular and intracellular forms of plasmids were treated to test the effects of cellular components on the efficiency of ARG elimination. The results were evaluated with respect to factors affecting the efficiency of elimination of ARGs’ transforming activities; for example, DNA repair, plasmid characteristics, the type of DNA damage, and sensitivity of DNA polymerase.

**Important findings**

Under typical UV fluences for disinfection purposes (e.g., 40 mJ/cm\textsuperscript{2}), a ~1 log reduction in the transforming activity of a plasmid-encoded ARG is expected. To achieve more extensive elimination of the transforming activity (e.g., >4 log reduction), UV fluence of more than 150 mJ/cm\textsuperscript{2} is required. Addition of H\textsubscript{2}O\textsubscript{2} (i.e., the UV/H\textsubscript{2}O\textsubscript{2} advanced oxidation process) does not significantly enhance the efficiency of elimination of the transforming activity.

Efficiency of elimination of the transforming activity for a plasmid-encoded ARG during UV treatment depends on the rate of formation of CPDs in the plasmid and the repair of such DNA damage during the transformation process in host cells. Significant capacity for CPD repair is present in the *E. coli* recipient strain (DH5a) used in this study and is also expected in many wild-type bacterial cells. CPD formation is the major DNA damage mechanism and responsible for the elimination of transforming activity of extra- and intracellular plasmids during UV and UV/H\textsubscript{2}O\textsubscript{2} treatment.

**Biography - Yunho Lee Lee**

Yunho Lee is an associate professor in the School of Environmental Science & Engineering at Gwangju Institute of Science and Technology (GIST), Korea. He received a Ph.D. in the Chemical Engineering Department at Seoul National University, Korea in 2005 and did postdoctoral research at the Swiss Federal Institute of Aquatic Science and Technology (Eawag) before joining GIST in 2011. His research focuses on characterizing and optimizing oxidative water treatment processes for drinking water and municipal and aquaculture wastewater for abating organic and biological contaminants of concern with minimized toxic by-product formation.