学 位 論 文 内 容 の 要 旨

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学 位 論 文 題 名

The effect of water disinfection on the adaptive evolution of waterborne viruses
(水の消毒が水系感染症ウイルスの適応進化に与える影響に関する研究)

The use of reclaimed wastewater for non-potable reuse such as irrigation and recreational purpose is becoming a necessity due to freshwater scarcity for drinking water resources. An important point that the reuse of reclaimed wastewater needs more cautions due to a higher concentration of enteric viruses which excreted from human feces and goes to the influent of wastewater treatment. Although guidelines are available for necessary log_{10} reduction removal to achieve a safe level based on 10^{-6} DALY lost per person per year as a tolerable additional disease burden for the safe use of wastewater for enteric virus; a detail of CT value needed to obtain certain log_{10} inactivation for the enteric viruses listed as water contaminant using a disinfectant is not clearly described. A CT value described the disinfection strength calculated by concentration times time needed for specific log_{10} reduction. Using meta-analysis review concept and applying a regression model for the selected data set, we proposed credit value of virus inactivation to the disinfection process in the international and domestic guidelines for wastewater reclamation and reuse.

In our study, we extracted published experimental data on enteric virus inactivation using free chlorine and monochloramine and applied the Tobit analysis and simple linear regression analysis to calculate the range of CT value needed for 2 log_{10} inactivations. Data were selected from peer-reviewed papers containing kinetics data of virus infectivity and chlorine residual in water. Coxsackievirus and echovirus require higher CT values (lower susceptibility) for 2 log_{10} inactivations than adenovirus and human norovirus surrogates (murine norovirus). The influential factors for the required CT value are virus type, pH, water temperature and water matrix. This systematic review reveals that enteroviruses are appropriate as representative among enteric viruses in water in terms of the susceptibility to free chlorine and monochloramine because of the relatively lower susceptibility than calicivirus and adenovirus.

Enteric viruses included human norovirus has a high genomic diversity of norovirus owing to the high mutation rate raises a concern of the emergence of higher resistance population to water disinfection. Free chlorine, one of the major water disinfectants, acts as a selective pressure on norovirus evolution. Using the murine norovirus S7-PP3 as human norovirus surrogate, two independent cycle experiments with ten-time repetition of free chlorine exposure and propagation in host cells (RAW 264.7), followed by a capsid gene analysis using next-generation sequencing. As a control, a cycle experiment with 10,000-fold dilution instead of free chlorine exposure was conducted. The chlorine-treated populations showed lower susceptibility to free chlorine and a higher number of synonymous and nonsynonymous mutation compared to the control population. A nonsynonymous mutation at nucleotide (nt) position of 7280 was observed only in the chlorine-treated population for both trials,

which indicated that it may relate to better survival during the free chlorine exposure. The principal coordinate analysis showed that the chlorine-treated populations at the 5th and 10th cycles were clustered separately from the control populations at the identical cycle numbers, although the original population and all the populations at the 1st cycle were clustered together.

The difference of evolutionary direction of the chlorine-treated population suggested that the free chlorine treatment has the effect of selection pressure on murine norovirus evolution. In addition, all clones isolated from the chlorine-treated population with a mutation at nt T7280C[VP2:F200S] had lower susceptibility to free chlorine than clones from the control population, which suggested that this mutation increases the stability of the viral capsid from disassembly due to chlorine exposure. In term of genetic diversity at chlorine treated population, there are increasing from cycle one to five before decreasing in cycle 10 which suggested that free chlorine selective pressure exert genetic diversity changes in MNV population. Furthermore, lower number of evolutionary rates of chlorine treated population compared to control population suggested that selective pressure by chlorine may delay the evolution process of murine norovirus.