外国語要約

Analysis of chlorine damage mechanism using fluorescent protein-expressing *Escherichia coli*

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「Water disinfection is important to suppress infection pathogenic microorganisms. In Japan, chlorine disinfection method has been mainly used. However, the chlorine resistance pathogenic microorganism *Cryptosporidium* caused serious outbreak in waterworks in 1996. It was suggested that rapid suppress of pathogenic microorganism was always necessary. In addition, evaluation methods for disinfection effect still have many problems. Indicator microorganisms is commonly used to evaluate disinfection effects. It is necessary to fully verify whether the effects on the indicator microorganisms are the same for the pathogenic microorganisms. Furthermore, viable but non-culturable (VBNC) microorganisms or microorganisms that are damaged a part of the cell can misjudge the cell dead. Therefore, it is possible to overestimate disinfection effect depending on conventional way. The better disinfection treatment condition should be proposed based on the knowledge about damage mechanisms. Similarly, this concept will be applied to determine the most appropriate evaluation method. This study focused on bacteria in microorganisms, and attempted to establish the more specific analytical method to evaluate where and how much cell are damaged by disinfection using fluorescent protein. Fluorescent protein is a good indicator due to its fluorescence emission. In addition, it has a benefit of localization because it can be expressed in specific sites of cells. In this study, the fluorescent protein was expressed at the cytoplasm of *Escherichia coli*. After chlorination, we investigated whether the degree of cell wall and cell membrane damages were able to evaluate by measuring the fluorescence intensity of leaked fluorescent protein. The decrease after leaked was also considered. And, the variation in the fluorescence intensity inside the cells were also measured. Firstly, fluorescent protein was expressed at the cytoplasm of *E. coli* by transformation. It was possible to express the fluorescent protein to two strains other than the strain with high transformation efficiency. These strains were proposed as one of the strains in various transformation studies. Using improved plasmid DNA, *E. coli* strain with high transformation efficiency expressed the fluorescent protein at the periplasm. Since the same fluorescent protein could be expressed in each site, this strain was also used for detailed site-specific damage assessment. By varying sample pH, the damage mechanisms for each form of free chlorine were investigated. At pH 9.0, hypochlorite ion was the main form, leakage due to inactivation was remarkably confirmed. Cell membrane damage due to the sample pH was also partially confirmed. The results suggested that in such condition, cell wall and cell membrane of *E. coli* was damaged by hypochlorite ions and inactivated. In addition, the relationship between inactivation and cell

damage could be quantified by considering the residual ratio of fluorescent protein as the cell damage rate, indicated that cell damage occurred more slowly than inactivation. On the other hand, at pH 7.4, small leakage due to inactivation was observed in all strains as the proportion of hypochlorous acid increased. The results suggested that hypochlorous acid inactivated *E. coli* by penetrating inside before hypochlorite ion had any effect. In both cases, the changes in the fluorescence intensity inside the cells were consistent with the leakage results. The leakage rate of fluorescent protein expressed in the cytoplasm and the periplasm was different to be specific, the rate was higher in periplasm. This result indicated that only the outer membrane of *E. coli* was damaged. Hence, detailed analysis of the site damage was possible using this method. Compared with each *E. coli* strain, leakage rates were different due to strains. It was found that the degree of damage to the cell wall and cell membrane of *E. coli* differed depending on the strain. In conclusion, the research has established a method to analyze damage mechanism by chlorination. It is expected that the damage mechanism by disinfection treatment can be analyzed further by combining with other methods, and it is able to apply to other disinfection treatments.」