Monitoring individual cell death using time-lapse microscopy: Application to stochastic modeling of microbial inactivation

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Traditionally, microbial inactivation studies are conducted with large microbial populations, without considering the individual cells. Although recent studies have reported the importance of individual cell variability in microbial growth, very limited information is available regarding individual cell heterogeneity in microbial inactivation. In the present work, single cell inactivation of *Salmonella enterica* ser. Agona was studied using an inverted confocal laser scanning microscope. Direct monitoring of cell death was achieved with the time-lapse method and the use of Propidium Iodide (PI), a fluorescent nucleic acid dye that is permeant only in cells with damaged membrane thus, enabling the discrimination between dead and alive cells. The cells of the pathogen were surface plated on solid laboratory medium, where micro-colonies were formed, and then covered with the inactivation solution containing PI. A sequence of frames for the selected micro-colony with time was obtained, allowing the monitoring of each cell inactivation, through time-lapse videos. With the aid of an in-house program, the coverage of the cell surface with PI was estimated and, after defining the threshold value for cell coverage corresponding to cellular death, the individual cell time of inactivation was calculated. The results showed that individual cell time of death in a micro-colony is characterized by high heterogeneity, indicating single cell inactivation behavior as a source of biological variability in microbial inactivation. The estimated probability distribution of inactivation times was used in a stochastic modeling approach for evaluating and describing the individual cell heterogeneity as source of variability in microbial inactivation. For small populations, the D-value used in deterministic inactivation models was found to be better characterized by a probability distribution rather than a uniform value.

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