P17-13 : Optimized Flow Cytometry Protocol to Quantify Green Fluorescent Protein-expressing Escherichia coli Cells Inoculated into Sewage Sludge

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Exogenous bacteria have been widely used as microbial inoculant in many fields for different purposes such as biodegradation, agriculture, or enhancement of energy production. Some application of inoculants into certain environments has resulted in successful outcomes and some may not. Therefore, accurate quantification of inoculant and indigenous bacteria in a sample must be required by using precise methods. In this study, *Escherichia coli* BW25113 expressing enhanced green fluorescence protein (EGFP) was used as an indicator to evaluate the survival of the cells inoculated into sewage sludge. Flow cytometry (FCM) was used to trace the persistence of EGFP-expressing *E. coli* and then the live or dead cells in sewage sludge were also analyzed using syto9 and propidium iodide dyes. The EGFP has similar excitation wavelength (488nm) to syto9 dye used in this study. Thus, the analysis of *E. coli* EGFP cells in sewage sludge has its own optimum gating protocol to accurately quantify the number of EGFP-expressing cells by its fluorescence emission, and also quantify the live and dead cells of indigenous bacteria which was comparable to the conventional methods, plating count and fluorescence microscope. In conclusion, FCM provides potential approach for rapid, high sensitivity, and reproducible assessment of exogenously-added *E. coli* cells and indigenous bacteria in sewage sludge.

keywords:inoculant,flow cytometry,enhanced green fluorescence protein,fluorescence microscope,plating count