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One-step cell lysis suitable for quantitative bacteria detection in inhibitor-laden sands

Hyun Jeong Lim, Jung-Hyun Choi, and Ahjeong Son
Department of Environmental Sicence and Engineering, Ewha Womans University, Seoul, Korea, Republic Of

Complexity and heterogeneity of soils often hinder effective DNA extraction from the soil matrix. In particular, conventional DNA extraction techniques require extensive purification which makes DNA extraction time-consuming and labor-intensive. Other drawbacks include lower recovery yield, degradation, and damage of DNA, which are also caused by intensive purifications during DNA extraction. Therefore a rapid and simple and yet effective DNA pretreatment method is preferred for environmental monitoring and screening. This study has evaluated the feasibility of simple physical pretreatment for effective cell lysis of bacteria in sands. Bead beating method was selected as an effective physical cell lysis method in this study. We examined the capability of this physical lysis for *Pseudomonas putida* seeded sands without additional chemical purification steps. The lysate from the method was analysed by the quantitative polymerase chain reaction (qPCR) assay and subsequently compared to that by commercial DNA extraction kit. The best lysis condition (treatment with 0.1 mm glass beads at 3000 rpm for 3 minutes) was selected. The qPCR results of bead beating treated samples showed the better performance than that of conventional DNA extraction kit. Moreover, the qPCR assay was performed to the sands laden with qPCR inhibitors (humic acids, clay, and magnesium), which generally present in environmental samples. Further experiments with the sands containing less than 10 μg/g of humic acids and 70% of clay showed successful quantification results of qPCR assay. In conclusion, the bead beating method is useful for simplified DNA extraction prior to qPCR analysis for sand samples of particular composition. It is expected that this approach will be beneficial for environmental in-situ analysis or immediate pre-screening. It also provides the groundwork for future studies with real soil samples that have various physico-chemical properties.