Direct dilution of cell aliquot for high temporal resolution bacterial surface charge measurement

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Conflict of Interest

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Abstract

Bacterial surface charge (SC) mediates important cell-environment and microbe-host interactions, and its accurate and precise measurement by microelectrophoresis requires removing metabolites adhered to the cell surface - where repeated centrifugation and washing by buffers is the gold standard approach. Unfortunately, the need for time-consuming centrifugation limits the temporal resolution of sampling and interrogation of experimental dynamics; especially for samples requiring immediate treatment post sampling. Herein, the feasibility of diluting cell aliquots with buffer as a one-step sample preparation technique for SC measurement was investigated by characterising the effects of dilution factor, type of cation, and buffer conductivity on measuring SC of *Escherichia coli* DH5α grown in LB medium. Results indicated that dilution factor was critical to accurate SC measurement since low signal-to-noise ratios in high or low cell concentration samples generated substantial error. Type of buffer cation was also important since putative binding of high affinity cations to the cell surface underestimated SC of negatively-charged bacteria. Finally, although high conductivity buffers enabled greater removal of adsorbed metabolites through increased charge screening, a broader statistical distribution of measured SC was also observed – which, at extreme conductivity values, led to inaccurate data, probably due to removal of both intrinsic cell surface ions and exogenous adsorbed metabolites. Altogether, one-step dilution of cell aliquot with deionized water reliably reproduced *E. coli* SC values obtained via the gold standard approach; however, since the ensemble of secreted metabolites is bacteria/medium specific, distinct diluent and optimal parameters exist for each system. The described methodology may find use in preparing samples for cell surface characterisation studies, where it would help reduce sample preparation time – and thus, improve temporal resolution at which scientific questions can be probed and answered.

Keywords: adsorption; desorption; zeta potential; microelectrophoresis; ionic strength; non-specific adsorption

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