Zeta potential of bacterial cells: Effect of wash buffers

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Submitted to *PeerJ* Preprint Server

Conflict of Interest

The authors declare no conflict of interest.

Author's contributions

Wenfa Ng conceived the idea, designed and performed the experiments, analyzed the data, and wrote the abstract. Yen-Peng Ting mentored Wenfa Ng, analysed the data and wrote the abstract.

Funding

The authors would like to thank the National University of Singapore for financial support.

Comment

A poster describing this work was presented at the 17th Regional Symposium on Chemical Engineering, 22-23 November 2010, Bangkok, Thailand. The abstract reported in this preprint is an improved version of the one published in the hardcopy conference proceedings.

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Abstract

Zeta potential - defined as the electrical charge at the shear plane - is widely used as a proxy for cell surface charge. Consequent of its definition, nonspecific adsorption of ions on the cell surface may alter the value - and polarity - of the measured zeta potential, thereby, leading to erroneous results. Multiple wash and centrifugation steps are commonly used in preparing cells for zeta potential analysis – where various wash buffers (such as 9 g/L sodium chloride and 0.1M sodium nitrate) help remove ions and charged molecules nonspecifically bound to the cell surface. Nevertheless, little information on the wash buffers' relative efficacies in removing nonspecifically bound ions hamper the comparison of zeta potential results across laboratories even for the same bacterial strain cultured under identical conditions. Thus, the present study sought to evaluate the effect of various wash buffers on zeta potential of bacterial cells grown in two culture media differing in salt content – thereby, allowing potential differential efficacy of buffers in removing nonspecifically adsorbed ions and metabolites to be discerned. Preliminary data revealed that for *Escherichia coli* DH5α (ATCC 53868) grown in LB Lennox (supplemented with 2 g/L glucose), the zeta potential-pH profile was not significantly different over the pH range from 2 to 12 for deionized water, 9 g/L sodium chloride, and phosphate buffer saline (PBS) wash buffers. As the glucose supplemented LB medium was a low salt medium without a phosphate buffer, it was unlikely that nonspecific adsorption of ions on the cell surface was extensive – thus, supporting the observation that the various wash buffers used did not have differential effect on zeta potential measurement. On the other hand, the zeta potential-pH profile of *E. coli* grown in a semi-defined medium with a high capacity phosphate buffer system, was significantly different over the pH range from 1 to 12 for deionized water, 9 g/L sodium chloride, 0.1M sodium nitrate, 0.1M sodium acetate, and 0.1M sodium citrate with the extent of difference positively correlated with wash buffers' ionic strength. A similar relationship was also observed between the measured point of zero charge (pH_{zpc}) and ionic strength of wash buffer, which, taken together, suggested that charge screening might be an important mechanism for removing the adsorbed ions. Collectively, although the experimental data suggests possible use of high ionic strength wash buffer in removing nonspecifically adsorbed ions from bacterial cell surface prior to zeta potential analysis, possible structural damage to the surface from removing intrinsic ions - necessary for stabilizing the bacterial cell wall - could not be discounted.

Keywords: zeta potential, wash buffer, surface charge, nonspecific adsorption, *Escherichia coli*

Subject areas: Microbiology; Environmental Sciences; Biotechnology