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Bacteria surface charge in "layers": revealed by wash buffers of different ionic strength

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Abstract

Bacteria surface charge derives its meaning from the cell's environment; thus, there is no specific surface charge. Determining the surface charge of bacteria in its native environment requires measuring the proxy variable, zeta potential, using cells obtained from field studies. However, lack of adequate cell mass and concerns over measurement of a mixed species consortia rather than a specific species meant that bacteria surface charge measurement require biomass obtained from pure culture. Often grown in rich medium where myriad proteins and ions nonspecifically adsorbed onto the cell envelope or peptidoglycan layer, standard procedures for preparing the cell mass incorporate repeated steps of washing and centrifugation with various wash buffers, the efficacies of which are poorly understood. This report describes a systematic study on how wash buffers of different composition and salinity affect the efficiency of removing nonspecifically adsorbed biomolecules and ions from *Escherichia coli* DH5α (ATCC 53868) cultured aerobically (shake flask, 37 ºC and 230 rpm) in LB Lennox medium. Using zeta potential-pH profiles over pH 1 to 12 as readout, proxy measurement of wash buffers' efficacies showed that efficiency of removing nonspecifically adsorbed ions and metabolites positively correlates with wash buffer ionic strength. More importantly, 0.15M ionic strength (i.e., 9 g/L NaCl and phosphate buffer saline) seems to be the minimum below which there appeared to be little removal of nonspecifically adsorbed biomolecules. On the other hand, high ionic strength of 0.6M (e.g., 0.1M sodium citrate) significantly changed the point of zero charge (pH_{zpc}), a reference marker for removing ions intrinsic to the cell envelope; thus, indicating significant cell surface damage. Collectively, results obtained inform wash buffer choice with regards to preserving cell envelope integrity. But, is there a true cell surface charge? Yes, but how do we define it in number of "layers" of adsorbed biomolecules? Philosophically, cells in culture broth are coated with layers of metabolites, proteins and ions; hence, desire to reveal the true surface charge is essentially a decoating process, where wash buffers of increasing ionic strength remove each layer via charge screening. However, where is the endpoint? This research offers a different perspective and answer: i.e., ionic strength of wash buffers chosen should be similar to that of the environment the research is seeking to address. Imagine a single bacterium suspended in LB medium, where there is constant adsorption and desorption of biomolecules as the cell grows: what is its relevant surface charge? It is the one where the loosely associated ions and metabolites is removed. Thus, deionized water wash provides a good estimate of the bacteria surface charge as grown in specific medium.

Keywords: zeta potential, shear layer; cell surface; bacteria; wash buffer; adsorption; biosorption;

Subject areas: microbiology; bioengineering; environmental sciences; biophysics; biotechnology;

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New in this version

Language and sentence structure was improved in this version.

Conflicts of interest

The author declares no conflicts of interest.

Author's contributions

Wenfa Ng developed the idea, designed and performed the experiments, analyzed the data, and wrote the abstract. Prof. Yen-Peng Ting of the Department of Chemical and Biomolecular Engineering at the National University of Singapore, mentored Wenfa Ng and discussed the data.

Author's comment

This is a fresh look at existing data and formulation of new ideas concerning how wash buffers of increasing ionic strength would be able to remove successive layers of nonspecifically adsorbed ions and metabolites on the bacteria cell surface. An earlier preprint describes some preliminary data of the systematic study on efficacy of wash buffers in removing loosely associated metabolites bound on the cell envelope of *Escherichia coli* DH5α (ATCC 53868). The preprint can be found at *PeerJ Preprints*: https://peerj.com/preprints/110v4/

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