Investigation of Single-Cell pH Homeostasis Using Fluorescence Ratio Imaging Microscopy

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Abstract

Escherichia coli cells experience rapid fluctuations in pH during intestinal colonization, and mapping their response to these changing environments is vital to understanding their survival in unfavorable environments. Cytoplasmic pH of individual adherent cells can be measured under fluorescence microscopy using a pH-sensitive variant of green fluorescent protein (GFP). Tracking the internal pH of cells exposed to rapid acid shock allowed characterization of responses. A great amount of heterogeneity existed within single cultures, with adjacent cells experiencing very different responses to external acidification. Some cells quickly recovered pH homeostasis, while others seemed unable to recover from rapid acid shock. This technique was also used in order to visualize the distribution of pH within an adherent biofilm.

Introduction

The gram-negative bacterium *Escherichia coli* experiences rapid and drastic shifts in pH during its passage through a host's digestive system, and must be able to grow in ranges from pH 4.5 to pH 9 (Wilks and Slonczewski, 2007). How *E. coli* responds to rapid pH shifts is of interest in understanding intestinal colonization by pathogenic bacteria.

A new way to measure cell pH allows single-cell measurements by using a GFP variant (ratiometric GFP) with an excitation spectrum dependent on pH (Olsen et al., 2002). The ratio of fluorescence intensities of two major peaks in the spectrum can be used as a reliable measure of intracellular pH (Miesenböck et al., 1998).

E. coli is extremely acid resistant and can survive at pH values as low as 2 for hours. Several different mechanisms protect *E. coli* from acid stress, one involving an ATPase and the others relying on decarboxylation and antiporter activity (Foster, 2004). These mechanisms, in combination, allow for the acid resistance and rapid pH recovery shown by *E. coli* cells.

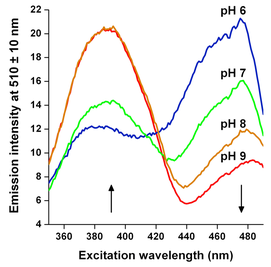


Figure 1. Ratiometric GFP fluorimetry. *B. subtilis* MMB1437 (AG174 pMMB1437) grown to OD_{600} = 0.4 in LBK was resuspended in buffered minimal media with 40 mM benzoate and methylamine to collapse ΔpH from pH 6 to pH 9 and assayed in a spectrofluorimeter.

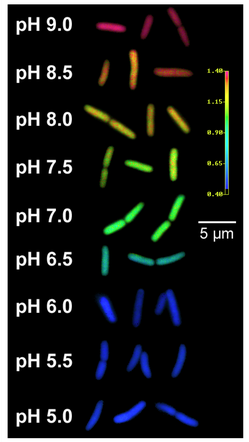
Materials and Methods

Cultures: Cells were grown overnight in unbuffered Luria broth with KCl (LBK) with 0.2% L-arabinose and 50 µg/mL ampicillin at 37°C, and rotating for 16-20 hrs. Cultures were then diluted 1:50 into baffled flasks with LBK at pH 7.5 with 0.2% L-arabinose and 20 µg/mL ampicillin. Log phase cultures were grown to an OD_{600} of 0.7. Stationary phase cultures and biofilms were allowed to grow overnight, in solution and in the FCS3 Stage Adaptor Flow Cell chamber (Bioptechs),respectively.

Standard Curve: Cultures were resuspended at an OD_{600} of 2 in M63A minimal media (7.45 g/liter KCl, 2 g/liter casein hydrolysate, 2 g/liter $(NH_4)_2SO_4$, 0.4 g/liter KH_2PO_4, and 0.4 g/liter K_2HPO_4) (Miller, 1972) buffered at pH 5.0 (50 mM HOMOPIPES), 5.5 (50mM MES), 6.0 (50mM MES), 6.5 (50mM PIPES), 7.0 (50mM MOPS), 7.5 (50mM MOPS), 8.0 (50mM TAPS), 8.5 (50mM TAPS) and 9.0 (50 mM AMPSO). An aliquot of cell culture was placed on a 40 mm round coverslip coated in 0.01% alpha poly-L-lysine. Coverslips were then placed in a flow cell chamber. Fresh M63A medium buffered at the same pH was perfused through the chamber via gravity feed. Cells were observed using an Olympus BX61WIF-5 microscope (1000x oil). Excitation values of 410 and 470 nm were defined using filters D410 and D470 (Chroma Technology Corp), contained within a Lambda 10-3 filter wheel upon a xenon arc lamp (Sutter Instrument LB-LS/OF17). Excitation intensity ratios were calculated using the software MetaFluor 7.6.5.0. Multiple images were taken before the perfusing media was switched to M63 buffered at the same pH containing 40 mM Potassium Benzoate and 40 mM Methylamine to collapse ΔpH. Several images were taken of cells without a ΔpH in order to establish ratio intensity values corresponding to definite pH values.

pH Shifts: Cultures were prepared and observed in the same manner as for the standard curve. Multiple images of a single field of view were taken while perfusing with pH 7.5 media before a switch was made to media buffered at pH 5.5. Images were taken every 2-5 seconds, refocusing as necessary.

Results



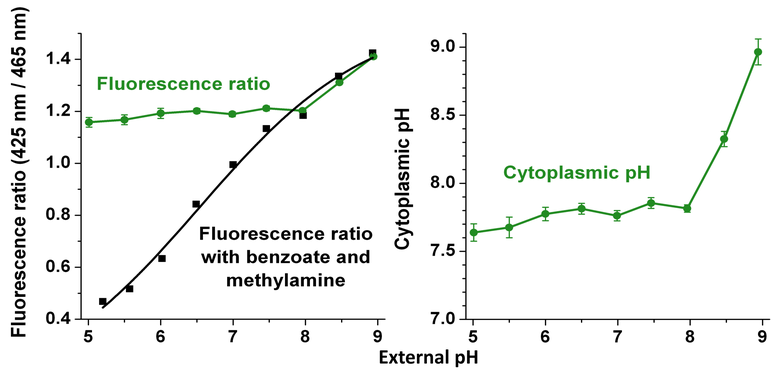


Figure 1: Left: False-color images of cells with cytoplasmic pH values shown. Middle: Standard Curve of JLS 1105. Right: Cytoplasmic pH of cells maintaining ΔpH.

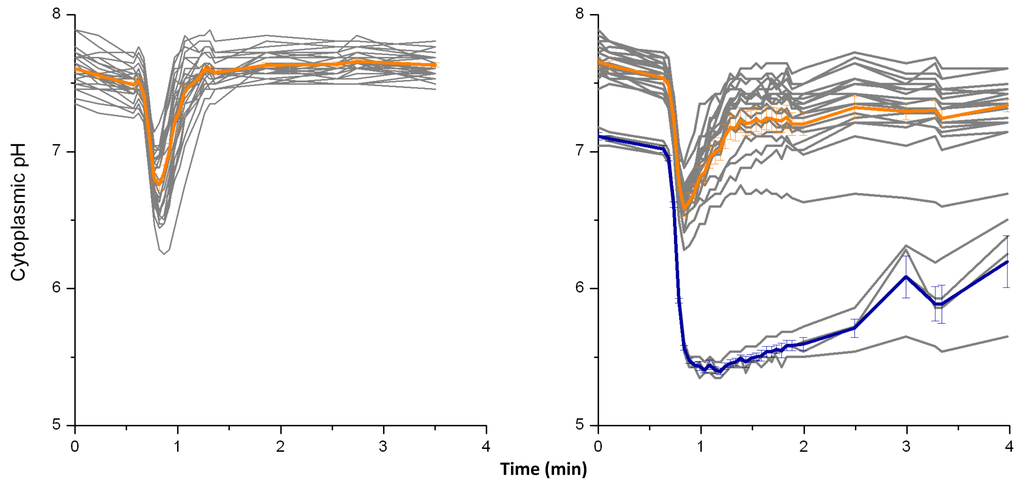


Figure 2: Cytoplasmic pH of log phase *E. coli* cells shows variable responses to external acid shift. Error bars represent ± SEM of averages. Left graph shows average (orange) of all cells. Right graph shows average of quickly recovering cells (orange) and of slow responders (blue).

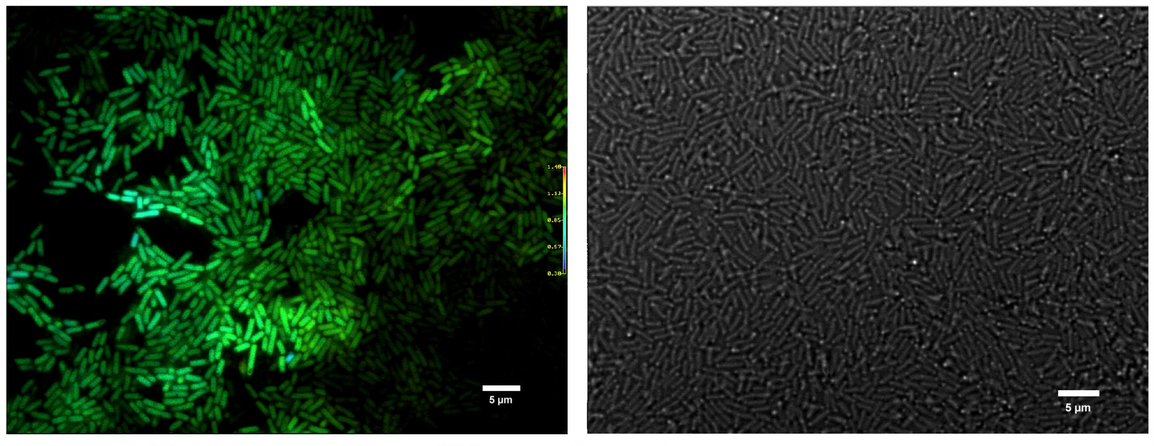
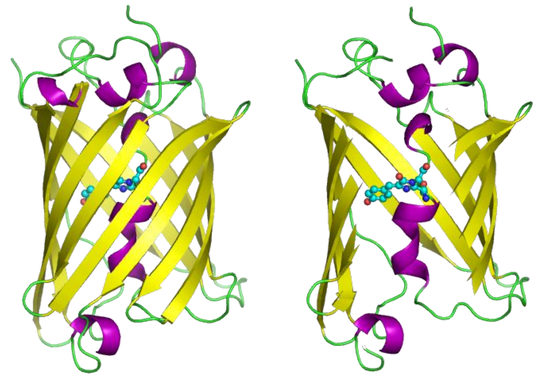


Figure 3: Biofilm visualized using ratiometric GFP Fluorescence Microscopy: Image on the left uses false-color display to show ratio intensity values (425/465). Image on the right shows the same field of view under brightfield illumination.



Left: Green Fluorescent Protein (GFP). Right: GFP with section removed to show fluorophore.

Conclusions

•A standard curve was generated for ratiometric GFP with an effective range from pH 5.5 to 9.0 (Fig. 1). The typical cytoplasmic pH of cells maintaining a pH gradient was also established for this range (Fig. 1).

•Cytoplasmic pH was measured over time in single adherent cells using ratiometric GFP and fluorescence microscopy. The responses of multiple cells to rapid environmental acidification were tracked over the course of several minutes (Fig. 2).

•Logarithmic and stationary phase cells showed similar responses to rapid acid shift. Both types of cultures showed different responses, but there was no response limited to just one culture type.

•A great deal of heterogeneity existed between cultures and even within cultures, both in log phase and stationary phase. Cells showed variable levels of resistance and recovery to rapid acidification of their environment (Fig. 2).

•Within a biofilm, cells showed diverse pH values. Most cells maintained homeostasis normally, but areas of both higher and lower pH were observed (Fig. 3). This could be due to varying degrees of access to fresh media, dependent on the biofilm architecture.

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References

1. Miller, JH. 1972. Experiments in molecular genetics. Cold Spring Harbor Laboratory, Cold Spring Harbor, New York
2. Miesenböck, G., D.A. De Angelis, and J.E. Rothman. 1998. ***Nature* 394:**192-195.
3. Olsen, K.N., B.B. Budde, H. Siegumfeldt, K.B. Rechinger, M. Jakobsen, and H. Ingmer. 2002. ***Appl. Environ. Microbiol.* 68:** 4145-4147.
4. Wilks, J.C., and J.L. Slonczewski. 2007. ***J. Bacteriol* 189:** 5601-5607.
5. Foster, JW. 2004. **Nature Reviews Microbiology 2:** 898-907.