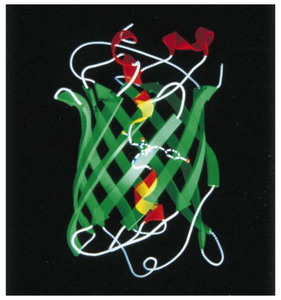

Measurement of cytoplasmic pH of *Escherichia coli* with fluorescence ratio imaging microscopy

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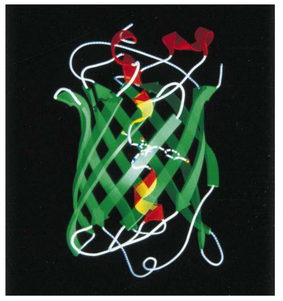

Abstract

Escherichia coli, gram-negative bacteria, are of significant interest for their ability to protect themselves from pH stress over a range of pH 5 to pH 9, by maintaining a pH homeostasis. Ratiometric GFP, when expressed in the bacteria, permits us to perform fluorescence ratio imaging that allows for direct measurement of cytoplasmic pH response to different pH stresses in a single cell. Cytoplasmic pH of individual *Escherichia coli* was measured under the LED source fluorescence microscopy. Adherent cells of *E. coli* maintained exceptionally high pH homeostasis (pH 7.8-8.0) in media when exposed to external pH 6-8, which is consistent with previous research. Individual cells observed in media with benzoate/methylamine, which allowed the external and internal pH to equilibrate, showed an initial drop followed by a recovery in pH within 5 min when exposed to a shift from external pH 7.5 to pH 5.5 using the perfusion apparatus. These results have provided us with accurate representation of cell's response to pH, providing a better understanding on the bacteria's regulatory mechanism. Further research could include sporulation in *Bacillus subtilis,* comparing pH homeostasis between the large mother cell and the smaller forespore compartment within the same cell.

Introduction

• *Escherichia coli* is a model system for gram-negative bacteria that are able to protect themselves from pH stress over a range of pH 5 to pH 9, by maintaining a pH homeostasis. *E. coli* cells are able to restore their internal pH due to a combination of constitutive and regulated mechanism which they employ to protect themselves against these environmental changes. (Slonczewski et al. 2009).

• Ratiometric GFP, when expressed in the bacteria, will permit us to perform fluorescence ratio imaging that will allow for direct measurement of cytoplasmic pH response to different pH stresses in a single cell.

• Previous experiments for bacterial pH-dependent have been observed using GFP fluorimetry of suspended cells which has its limitation in that it does not measure cytoplasmic pH independent of external pH (Wilks & Slonczewski. 2007). However, the microscopy will enable us to address the physiology and diversity of single cells on a substrate (Brehm-Stecher and Johnson. 2004).

Materials and Methods

Fluorescence: *Escherichia coli* strain W3110 was transformed with a reporter plasmid (*bla* GFP-pHluorin). Cells were grown overnight in Luria broth with KCl (LBK) unbuffered, and rotating for 16-20 hrs. Cultures were then diluted 1:100 into baffled flasks with fresh unbuffered LBK and grown up to OD_{600} of 0.4 at 37°C

Fluorescence microscopy: *E. coli* cells were spotted on cover slips coated with alpha-poly (L)-lysine to adhere cells (Colville et al., 2010). The cover slips were placed on a FCS3 Stage Adaptor flow cell chamber (Bioptechs); M63 fresh medium was perfused with a peristaltic pump. Cultures of *E. coli* were assayed microscopically (1000 X oil) using an Olympus BX61WIF-5 with CoolLED light sources for excitation at 425 nm and 465 nm. Excitation ratios were computed using Metafluor.

Results

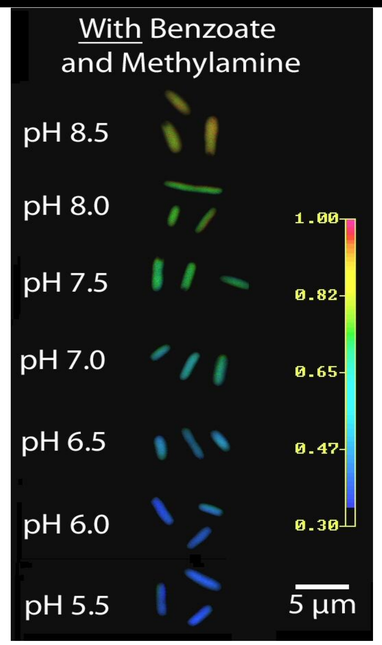

Figure 1 Effect of benzoate and methylamine on cytoplasmic pH of *E. coli*. The cytoplasmic pH of *E. coli* suspended in M63 minimal media containing 50mM methylamine and 50mM benzoate at pH 5.5 (50mM MES), 6.0 (50mM MES), 6.5 (50mM PIPES), 7.0 (50mM MOPS), 7.5 (50mM MOPS), 8.0 (50mM TAPS) and 8.5 (50mM TAPS) was observed. Cells were grown up in unbuffered LBK media to OD_{600}=0.4 and were loaded on to an alpha poly-(L) - lysine coverslip. After the minimal media perfused on the entire coverslip, images of 22 individual cells were taken at random using the microscopy.

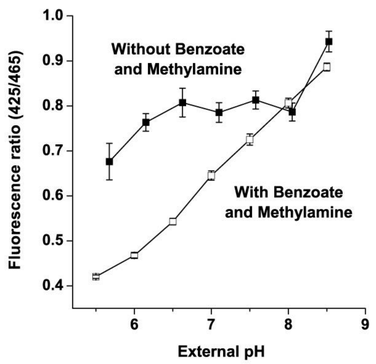

Figure 2
***E. coli* are able to maintain pH homeostasis in an external pH ranging from 5.5-8.0.** The cytoplasmic pH of *E. coli* suspended in M63 minimal media with 50mM methylamine and 50mM benzoate or without 50mM methylamine and 50mM benzoate at pH 5.5 (50mM MES), 6.0 (50mM MES), 6.5 (50mM PIPES), 7.0 (50mM MOPS), 7.5 (50mM MOPS), 8.0 (50mM TAPS) and 8.5 (50mM TAPS) was observed. Cells were grown in LBK to OD_{600}=0.4 and loaded onto an alpha poly-(L) - lysine coverslip, then perfused with M63 medium. Images of 22 individual cells were compiled.

Results cont.

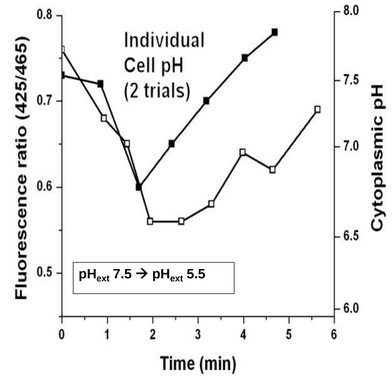

Figure 3 The effect of an acid stress on cytoplasmic pH of *E. coli*. The cytoplasmic pH of *E. coli* suspended in M63 minimal media without methylamine/benzoate at pH 7.7 (50mM MOPS). Cells were grown up in unbuffered LBK media to OD_{600}=0.4 and were loaded on to an alpha poly-(L) - lysine coverslip. After the pH 7.5 minimal media perfused on the entire coverslip, an image of a single cell was taken and then the coverslip was suspended with M63 pH5.5 (50mM MES). Images were then taken at regular intervals.

Discussion

•Cytoplasmic pH of individual *E. coli* cells under different pH is seen under the fluorescence microscopy with ratiometric GFP. (Figure 1)

• Cytoplasmic pH was observed in M63 media with benzoate/ methylamine suspension respond consistently according to the external pH. (Figure 2)

• pH homeostasis was maintained in E. coli cells suspended in media without benzoate/methylamine when exposed to external pH ranging from pH 5.5-8.0. This is compatible to the results obtained from suspended cells using fluorimetry (Wilks & Slonczewski. 2007). (Figure 2)

• Individual cells exposed to a shift from external pH 7.5 to pH5.5 using the perfusion apparatus showed an initial drop followed by a recovery in pH.

Future experiments: One future investigation could involve sporulation in *Bacillus subtilis.* Sporulation involves the cell differentiating into a large mother cell and a smaller forespore compartment each producing their own rationmetric GFP. This allows us to not only compare different cells but also to compare within the cell itself. (Sonenshein et al.2002). Another aspect that could be tested is the ability of cells of different sizes to maintain homeostasis. As pH homeostasis depend greatly on ion pumps thus larger cells with high surface area to volume ratio should maintain pH regulation better than smaller cells.

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Acknowledgements

This research could not have been carried out without the help and guidance of many people. I would like to thank Dr. Slonczewski for her mentorship and patience on this project and to Ryan Kitko for his excellent assistance in lab. This project was funded through the MCB-0644167 from the National Science Foundation and the Summer Science Scholar Program at Kenyon College.