High Throughput Phenotyping of *E. coli* Growth and Extracellular pH

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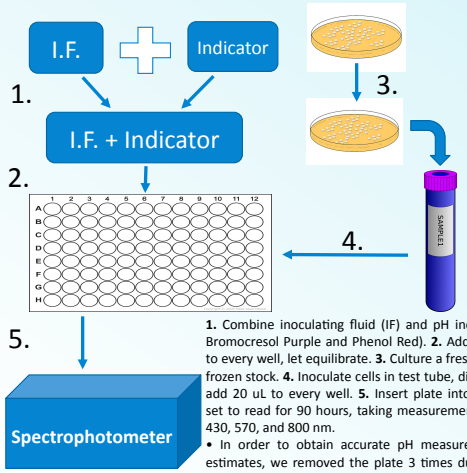
Abstract

Intracellular metabolism has a direct influence on extracellular pH. Examining this relationship more closely may yield important insight into metabolism and improve metabolic modeling. These insights may improve our understanding of human associated microbes that affect health via manipulating extracellular pH such as those in the oral and skin microbiomes[1,2]. Despite the importance of this relationship, it has yet to be fully characterized in the well studied model organism *E. coli* K12. We hypothesized that high throughput phenotyping of *E. coli* growth and extracellular pH will reveal more about this relationship. Using the Biolog PM1 96-well plate, we characterized the growth and extracellular pH when *E. coli* was cultured aerobically on 96 different carbon sources. Notably we were able to group the results from media with similar chemical compositions and thus identify trends across and within media. We noticed that an inverse relationship exists between growth rate and pH among various sugars we tested such as glucose, whereas growth on organic acids such as acetic acid yielded a direct relationship. Previously studied metabolic models yield similar results[3], however the lack of high-throughput data leaves much left to be characterized and analyzed. Future efforts will utilize high throughput phenotyping to study a larger number of organisms under a more diverse set of conditions such as anaerobic cultivation, and growth under different limiting resources. In conjunction with existing metabolic models, this data will improve our understanding of the important relationship between intracellular metabolism and extracellular pH.

Introduction

Currently, there is not enough information regarding the relationship between cellular metabolism and extracellular pH. New information can be used to better understand the effect of microbes on the human body by updating current metabolic models. High throughput phenotyping (HTP) is one of the most powerful tools currently aiding in microbe characterization and therefore metabolic model construction[4]. In order to obtain HTP, the Biolog Phenotypic Microarray plate with 96 different carbon sources was used. As one of the most well characterized bacterial models, and known for its relatively simple lab reproducibility, *Escherichia coli K12* was chosen as a suitable microbe. Furthermore, E. coli has the ability to respire with or without oxygen and has a wide pH range for survival, making the characterization of E. coli a very versatile tool for comparisons to many other microbes. Studying the hydrogen ion flux as E. coli metabolizes has painted a clearer picture of how an intracellular function affects the extracellular environment of a microbe.

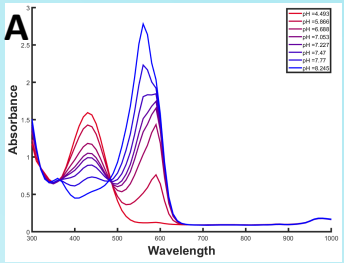
Methods



1. Combine inoculating fluid (IF) and pH indicator (a mixture of Bromocresol Purple and Phenol Red). **2.** Add 100uL indicator + IF to every well, let equilibrate. **3.** Culture a fresh plate of *E. coli* from frozen stock. **4.** Inoculate cells in test tube, dilute to OD600 of 0.3, add 20 uL to every well. **5.** Insert plate into spectrophotometer, set to read for 90 hours, taking measurements every 0.5 hour at 430, 570, and 800 nm.

- In order to obtain accurate pH measurements and not just estimates, we removed the plate 3 times during the experiment (and once at the beginning and end) to measure pH with a pH meter.

Results



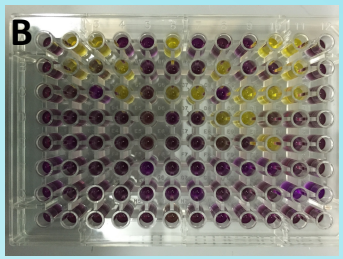


Fig 1:
A) Graph of pH indicator absorption spectrum.
- The 430 and 570 nm readings were used to measure pH using the pH indicator (dye). This is based on the pH indicator dye: the media transitions from yellow to purple as the pH increases. Therefore as the pH increases, the purple absorbance is decreasing and the yellow absorbance is increasing. So the ratio of yellow to purple absorbance will increase as the pH increases.
- 800 nm reading was used to measure bacterial growth as the absorbance at this wavelength was not affected by pH changes, and thus is directly proportional to biomass (growth)

B) Photograph of plate following experiment
- Yellow wells resulted from carbon sources that drove *E. coli* to lower the pH while purple wells resulted from carbon sources that did the inverse.

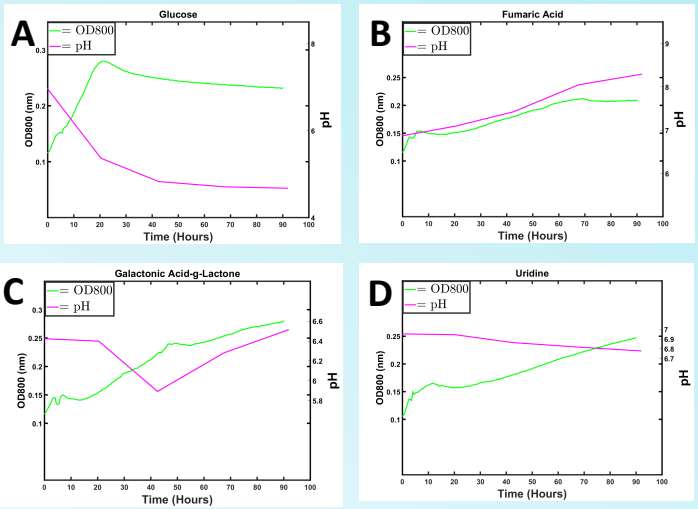


Fig 2: E.coli pH and absorbance at OD800 nm over 90 hours.
A) Glucose growth corresponded with a drop in extracellular pH
B) Fumaric acid growth corresponded with an increase in extracellular pH
C) Galactonic Acid-g-Lactone exhibited interesting pH dynamics with an initial decrease followed by an increase
D) Uridine growth corresponded with minimal pH change

Conclusions

- We observed that, experimentally and on a model basis, intracellular metabolism and extracellular pH have an inverse relationship when *E. coli* is introduced to sugars such as glucose (Fig. 2a). We observed the opposite effect when *E. coli* was grown on acidic media such as fumaric acid (Fig 2b). As growth rate increased, so too did the pH, revealing a direct relationship. Some media displayed both direct and inverse relationships, such as Galactonic acid-g-Lactone (Fig. 2c). The exponential growth phase for Galactonic acid-g-Lactone was characterized by a drop in pH (inverse) as it fed on Lactone, and then a rise in pH as E. coli switched metabolites to Galactonic acid, resulting in an increasing pH (direct). Still, other media such as Uridine grew for all 90 hours and changed pH by a measure of only ~ 0.2 (Fig. 2d).
- Interestingly enough *E. coli* prefers an optimal pH of ~ 7 to proliferate[5], yet when grown on a variety of carbon sources, *E. coli* alters its pH in a myriad of ways as opposed to remaining at a stable, uniform pH. Going forward, we plan to further investigate the metabolic constraints that underlie this phenomenon. This data will be used to continue to improve metabolic models such that they will more accurately predict intracellular metabolism in relation to extracellular pH. Future HTP will include the characterization of other microbes that have an effect on human health.

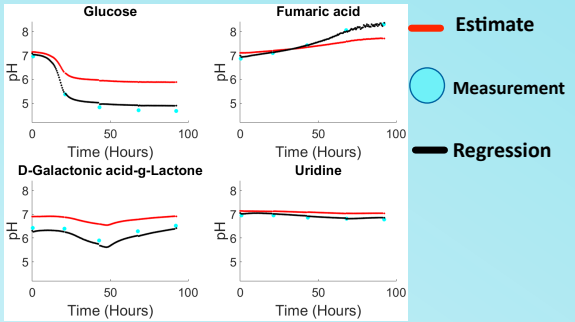


Fig 3: The pH estimate based on the pH indicator dye (red) is plotted against actual pH measurements (light blue). The pH indicator dye was a relatively accurate tool for predicting pH. A regression line (black) shows the predicted pH response based on estimate data.

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Acknowledgements

This work was funded by the Boston University Bioinformatics BRITE REU program. Specifically, I'm very gracious for Gary Benson's instruction over the BRITE 2016 summer program. A very special thank you is extended to David Bernstein, Alan Pacheco, and Meghan Thommes for their invaluable guidance throughout this process.