Polar Aging of *Escherichia coli* under Neutral and Acidic Conditions

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Abstract

Escherichia coli cells divide by binary fission into two morphologically identical daughter cells. The daughter cells then continue to grow and divide over time, forming a colony of *E. coli*. As cells divide, each pole can be designated an “old” pole or a “new” pole depending on whether it was inherited from the parent cell or newly formed at the septum during division. The old pole and new pole are biologically different in their composition, since the old pole retains the membrane and intracellular components of the parent cell, whereas the new pole is formed of newly generated membrane and cell components. Environmental factors like pH stress can influence how cells age and divide their cellular components. External pH stress is considered to be around pH 6.0 and lower for *E. coli*, while neutral pH levels promote healthy cell growth. Previously, we have observed *E. coli* aging in pH 6.0 and pH 7.5 media and tracked cell poles over six generations. Additional lineages were constructed from experimental replicates of cells aging under both the acidic and neutral pH conditions. Brightfield and fluorescence images were taken periodically to track individual cells and observe colony growth over time. Intracellular pH levels are visualized using ratiometic pHluorin. Cell lineages were compiled and existence time measurements were consolidated for upcoming analysis.

Introduction

- *Escherichia coli* divide into morphologically identical cells via binary fission, generating two symmetrical daughter cells (1)
- There are no visually apparent aging effects due to symmetrical division and lack of distinct maturity phases in *E. coli* (1,2)
- Cell parts that are inherited from the parent cell at the old pole include the old cell wall, proteins, and other cellular house-keeping components (2)
- Over time, as multiple cell divisions result in colony formation, each cell contains a different amount of old and new cell parts, and different degrees of aging parts (1)
- Cytoplasmic pH of individual cells can be visualized though expression of ratiometric GFP pHluorin, pH-sensitive fluorescent protein induced by L-arabinose in *E. coli* strain JLS1105 (4)
- We investigate if bacterial aging in a stressful condition such as acid stress of pH 6.0 can lead to a fitness advantage exhibited through reproductive ability
- We predict that cells that contain newer components versus older parts may divide at different rates, or exhibit differing abilities to regulate intracellular pH

Methods

Cell preparation: *E. coli* strain JLS1105 (W3110 pGFPR01), which expresses ratiometric pHluorin, was used to observe cell growth and division. Cells were cultured in 2 mL LBK pH 7.5 100 mM MOPS with 40 µL of 20% L-Arabinose and 2 µL of 100 mg/mL ampicillin for a period of 12-14 hours at 37°C. Perfusion media 500 mL LBK pH 7.5 100 mM MOPS was warmed in 37°C incubator and 250 µL of 100 mg/mL ampicillin and 10 mL of 20% L-Arabinose were added to media for concentrations of 50 µg/mL ampicillin and 0.4% L-Arabinose.

Fluorescence microscopy: 0.35% agarose was heated and 1 mL aliquots were cooled at 43°C. An approximately 1:36 dilution of the culture (28 µL) was added to the 1 mL of agarose and spread into three 5 µL strips on a glass coverslip. FCS3 chamber was assembled with coverslip and cells were viewed using an Olympus BX61WIF-5 microscope and MetaMorph’s Metafluor program. The chamber was perfused with aforementioned perfusion media throughout the experiment. (4)

Pole tracking and lineage construction: Brightfield images were viewed in chronological order. A color-coded dotting system was used to track each old pole and label new poles as cells divided. Each color corresponds to a specific generation and each cell has two dots, one for each pole. Dotting analysis of brightfield images was converted into a graphic representation of a lineage using rectangles to represent cells, with time intervals labeled.

Results

Cell Tracking at pH 7.5

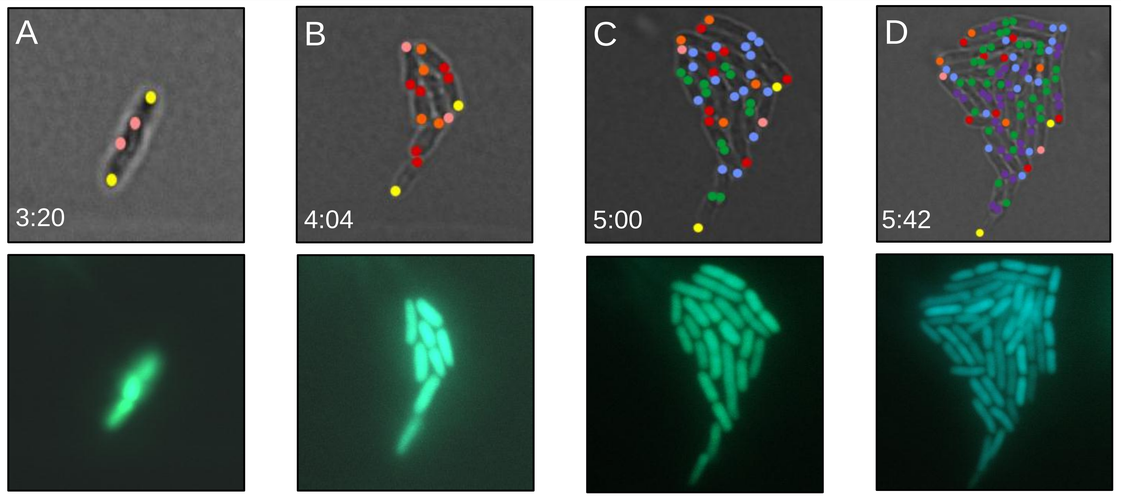

Figure 1. Brightfield and fluorescent images in pH 7.5 from July 17th, 2014. Images A-D show successive divisions of an *E. coli* colony, beginning with two sister cells in image A and reaching six generations of growth by image D. Coloration of dotting corresponds to the relative age of each pole, whether inherited from the parent cell or newly formed at the septum. The sixth generation (image D) resulted in a total of 48 cells after 2.37 hours. Fluorescence images are directly below the corresponding brightfield image. Fluorescence remains consistently green, indicating neutral pH, for all cells except the final image at 5:42 p.m., which ends with slight intracellular acidity.

Cell Lineage

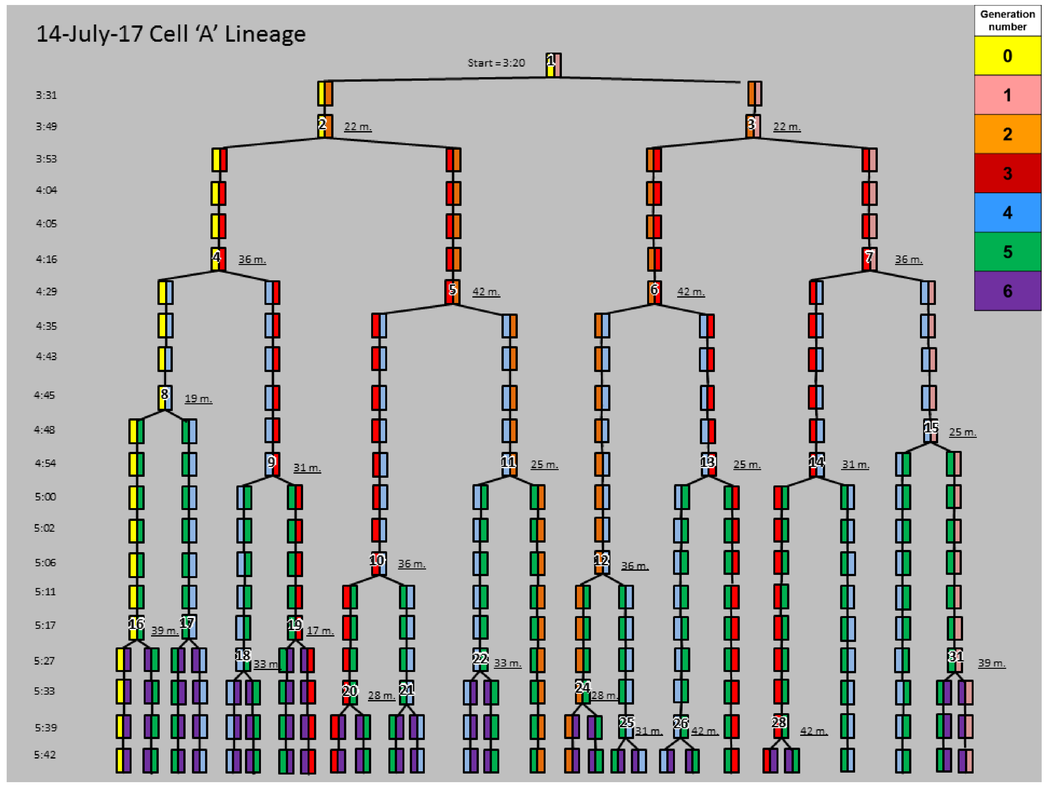

Figure 2. Cell Lineage in pH 7.5 from July 17th, 2014 Cell ‘A’ spanning six generations. Over the course of 2.37 hours, the initial sister ‘cell A’ divided multiple times, and individual cell divisions were tracked and illustrated using rectangles to represent individual cells. Colors correspond to the dotting analysis images, and existence times are labeled to the immediate right of each cell.

Growth Curve of JLS1105 in Potassium Benzoate

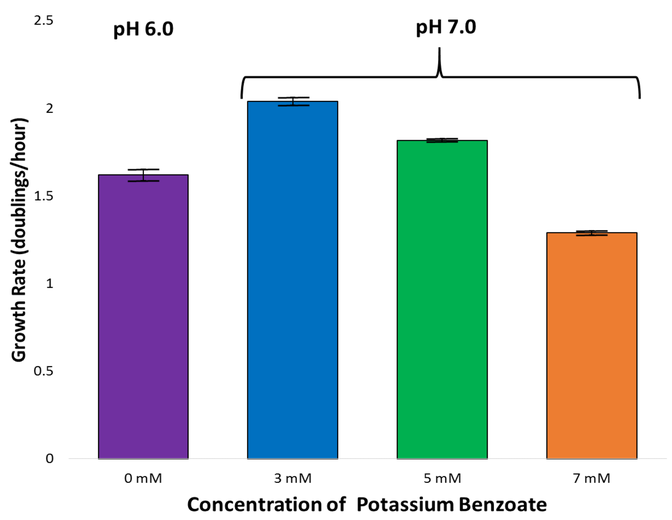

Figure 3. Growth curve of JLS1105A w/GFP pHluorin in varying concentrations of potassium benzoate media. Cell cultures were tested in 3 mM, 5 mM, and 7 mM potassium benzoate at pH 7.0 and 0 mM potassium benzoate at pH 6.5. Growth rate (doublings/hour) decreased as potassium benzoate concentration increased for pH 7.0, whereas 0 mM potassium benzoate pH 6.0 produced growth rates between those of 5 mM and 7 mM concentrations. This indicates that the amount of potassium benzoate stress that would allow for adequate growth for microscopy is approximately 6 mM.
Error bars=SEM, n=3.

Discussion

- Ten lineages are complete for both the acidic (pH 6.5) and neutral (pH 7.5) conditions, extending to the sixth generation and beyond
- Before statistical analysis can be completed, additional replicates are needed for each pH condition, resulting in a minimum of 12 total lineages for each condition
- Analysis ideas include tracking the extreme old pole versus the extreme new pole, and comparing division rate (number of divisions/total time) and average generation times (time/generation)
- Variation in existence times across experiments make comparison of divisions per experiment difficult, so alternative analysis could compare sister cells or cousin cells for division rate, which increases the sample size to be analyzed but may not result in a significant difference between the sister pairs
- Additional areas of focus include improving cell viewing techniques to optimize the number of successful experiments, specifically in the preparation of the agarose strips, as cell overlap often limits tracking abilities

Current and Future Directions

- As the process is optimized and more experiments conducted, the brightfield images will continue to be dotted, transferred to lineage format, and integrated with the current compilation of lineages
- There may or may not be a significant difference between inheriting the old versus new pole in neutral condition compared to acidic condition
- Future research may include observation of *E. coli* aging in a permeant acid (potassium benzoate) to investigate effects of intercellular pH stress on cell division and aging

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