Escherichia coli Adapts to Food Additives within 180 Generations

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INTRODUCTION

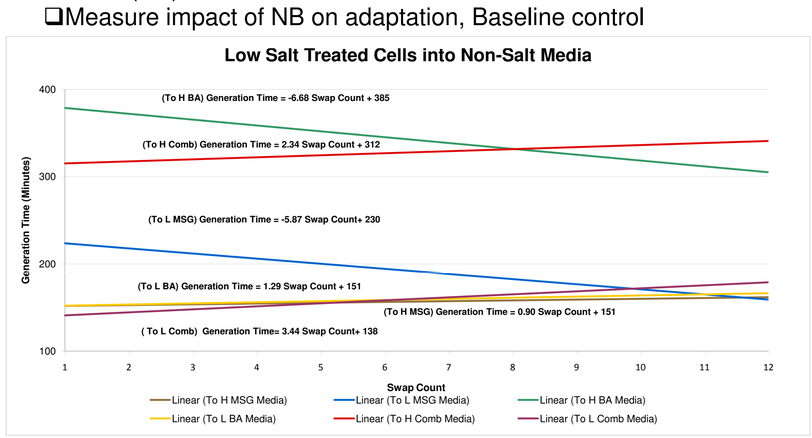
- *Escherichia coli, a* normal gut flora in humans
  - Produce Vitamin K
  - Prevent pathogenic bacterial growth
- Any changes to *E. coli* will affect human body
- Constantly exposed to food additives
  - Less studied compared to medicine, eg. antibiotics
- Thus, important to study relationship between *E. coli* and additives

OBJECTIVES

- Observe evolution of *E. coli* in different additives
  - Monosodium Glutamate (MSG)
  - Sodium Chloride (SALT)
  - Benzoic Acid (BA)
- Characterize adaptation of *E. coli* at genomic level
  - Polymerase Chain Reaction (PCR)
  - Restriction Fragment Length Polymorphism (RFLP)

WILL NUTRIENT BROTH IMPACT ADAPTATION?

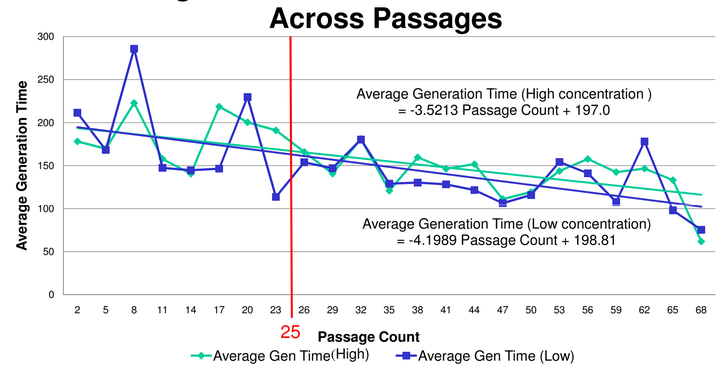
- Swap A
  - SALT (NB) cells → 6 other non-salt media
  - Measure impact of NB on adaptation, Baseline control



- Nutrient broth (Low SALT) unlikely to impact cell adaptation
  - p-value > 0.25, not significant
    - Statistically parallel to x-axis
    - Regression gradients = 0
  - **NB does not prime cells for other treatments**

HYPOTHESIS 1

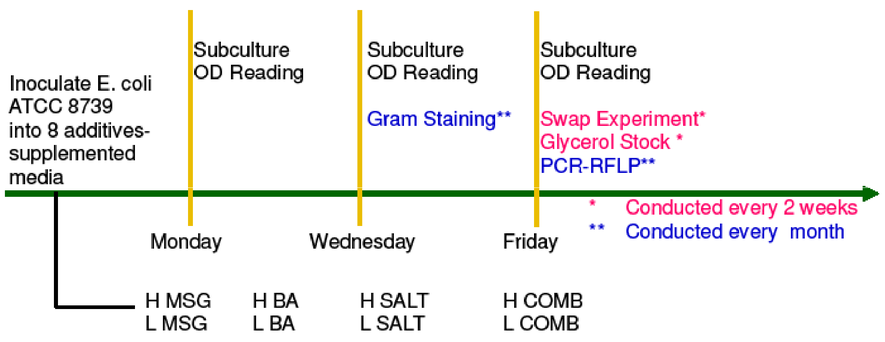
Extended exposure of *E. coli* to additives → Increase in fitness → Grow faster in their treatment → Decrease in generation time



- All treatments show decreasing generation time trend
  - Indicate shorter generation time
  - **Result agree with Hypothesis 1**

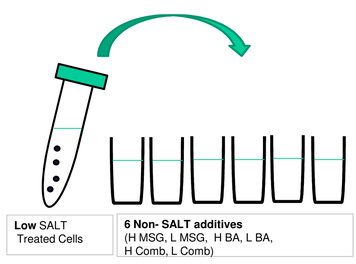
MATERIALS AND METHODS

Timeline of Experiments

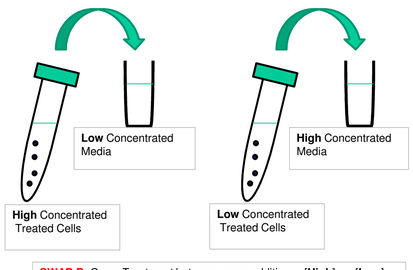


- 8 additives-supplemented media
  - High Concentration of MSG (H MSG, 0.025%)
  - Low Concentration of MSG (L MSG, 0.0025%)
  - High Concentration of BA (H BA, 0.025%)
  - Low Concentration of BA (L BA, 0.0025%)
  - High Concentration of SALT (H SALT, 1%)
  - Low Concentration of SALT (L SALT, 0.8775%)
  - High Concentration of Combination (H MSG + H BA + H SALT= H COMB)
  - Low Concentration of Combination (L MSG + L BA + L SALT = L COMB)

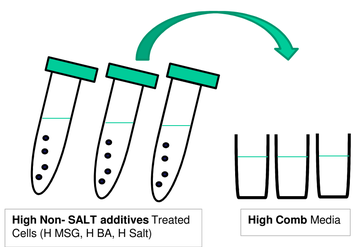
SWAP EXPERIMENT



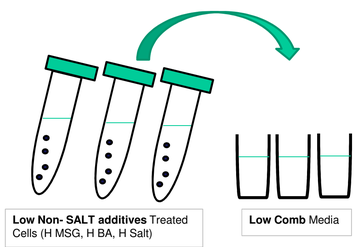
SWAP A: Low SALT into 6 non-salt media



SWAP B: Cross Treatment between same additives, [**High**] ↔ [**Low**]



SWAP C: High Non-Salt additives into High Comb Media

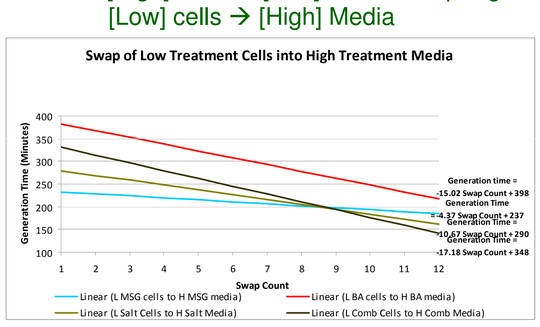


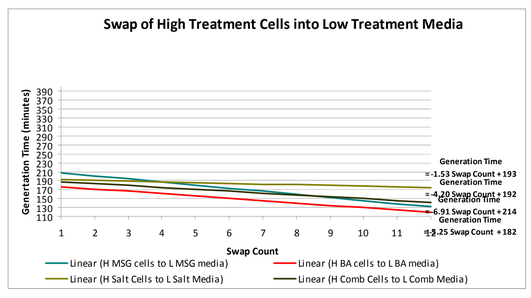
SWAP D: Low Non-Salt additives into Low Comb Media

HYPOTHESIS 2

- Types and level of stress induced by additives
  - High concentration and low concentration causes same type of stress
  - High concentration induce more stress than low concentration
  - Expected Results: [High] cells → [Low] Media steeper gradient than [Low] cells → [High] Media

→ Hypothesis 2 : INVALID



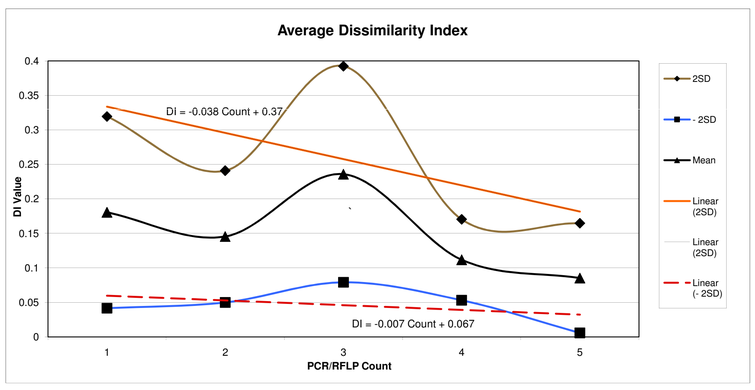


- Gradient of [Low] cells → [High] Media **steeper** than [High] cells → [Low] Media
- Experimental results show **OPPOSITE**.
- [High] cells → [Low] Media:-1.53 to -6.91
- [Low] cells → [High] Media: -4.37 to -17.18

PCR-RFLP

Detect genetic changes across time

- RFLP done using MspI, TaqI and HinfI after PCR
- Differences between genome of two samples
  - Estimated and Examined by Nei-Li's Dissimilarity Index (DI)

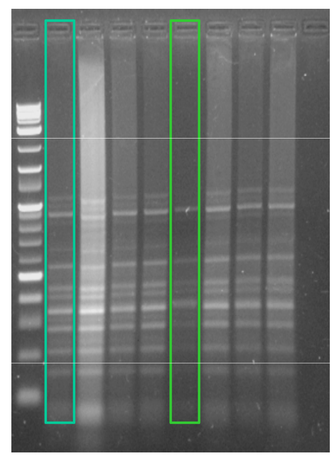


- Convergence of upper and lower class limit
  - Original cells are identical (from same ancestral cells)
  - Genetic distance increases then decreases

→ Suggests genetic involvement in adaptation

NEI-LI DISSIMILARITY INDEX

$$DI = 1 - \frac{2 \times \text{Number of regions where both species are present}}{\left[2 \times (\text{Number of regions where both species are present}) + \text{Number of regions where only one species is present}\right]}$$



H MSG	H SALT
3.8	--
4.0	--
4.1	--
4.2	4.2
4.4	--
5.0	--
5.4	5.4
5.9	5.9
6.0	6.0
6.4	6.4
6.5	--
6.8	6.8
7.2	--
7.3	7.3
7.7	7.7

2 Species present: 8
1 Species present: 7

$$DI = 1 - \frac{2 \times 8}{(2 \times 8) + 7} = 0.304$$

CONCLUSION

1. Nutrient Broth (Low SALT) does not prime cells for other treatments
2. *E. coli* cells grow better over time with less fluctuations after 180 generations
3. Adaptation may involved genetic changes

FUTURE WORK

- Detect adaptations to cells by measuring the maximum inhibitory concentration for each additives
- Investigate the kinetics of *E. coli* growth in different media
- Increase salt tolerance of *E. coli* to 10%.

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- SEZONOV, G., JOSELEAU-PETIT, D. & D'ARI, R. (2007) Escherichia coli physiology in Luria-Bertani broth. *J Bacteriol,* 189, 8746-9.