Ecological Specialisation of *Escherichia coli* within 1000 Generations

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INTRODUCTION

➢Adaptive mechanisms of *E. coli* in food preservatives/ additives not widely studied

➢Food additives commonly used

➢Investigate relationship between such chemicals (Monosodium glutamate, Benzoic Acid, Common Salt) and *E. coli*

➢Continuation of previous Final Year Project (Passage 1-70)

OBJECTIVES

➢Further studies on changes in growth kinetics/ genetic changes of *E. coli* ATCC 8739
- •Further 83 passages – 71st to 153rd passage

➢To find out possible trends:
- •Changes in Adaptation rates in various media
- •Genetic similarities/ differences
- •Contrast adaptation rates between earlier and later passages

MATERIALS AND METHOD

Experimental procedures

Inoculating into 8 different treatment supplementation

Subculturing (100x dilution) of cells from previous culture performed 3 times/week

OD_{600} readings taken before every subculture (including 5 & 7 Days culture)

Generation Time Readings taken every 3rd passage (Friday), Swap experiment fortnightly

PCR-RFLP Analysis done on every 12th passage

Composition of 8 media

[High] Media	[Low] Media
H MSG [0.025% (w/v)]	L MSG [0.0025% (w/v)]
H BA [0.025% (w/v)]	L BA [0.0025% (w/v)]
H Salt [1% (w/v)]	L Salt [Nutrient Broth]
H COMB	L COMB

Swap Experiments
➢ Swap A: [High] ↔[Low] treatments swaps
➢ Swap B: [Low] treatments → [Low] Combination swaps
➢ Swap C: [High] treatments → [High] Combination swaps
➢ Swap D: [High] ↔ [Low] treatment swaps

HYPOTHESIS 1

➢Decreased rate of adaptation in later passages (Passages 71-153)

Comparison of gradient of generation time between earlier and later passages
Generation time of treatments are decreasing

Gradient Of	H MSG	L MSG	H BA	L BA	H SALT	L SALT	H COMB	L COMB
Passage 0 - 70	-0.91	-1.87	-1.15	-1.39	-1.12	-1.24	-2.02	-1.22
Passage 71 - 153	0.03	-0.058	0.042	0.109	-0.149	0.175	-0.564	0.223

Gradient of Passages 71-153 is significant (p-value <0.05) as compared to Passages 1-70 → Significant decrease in rate of adaptation

Results indicate
- Slower decline of generation time in later passages
- Decreasing rate of adaptation present
- Most adaptive mutation occurred in the first 500-700 generations
- **Hypothesis 1 is ACCEPTED**

ECOLOGICAL SPECIALISATION

Suggested from hypothesis 2 and 3:

➢ Cells adapt specifically to their own growth environments
➢ Specific adaptation → Adaptive mutation → Genetic differences between cells of different treatments → **Diverged DI**
➢ Pair-wise comparison chart
- Compare DI of each treatment set
- To deduce if genomic differences in each pair are due to a consequent effect from the resulting effects

➢Example of pair-wise comparison

❑ **L MSG/ L SALT**
- **L MSG = NB + MSG**
- **L SALT = NB**
- **L MSG/L SALT = MSG (Resulting effect in comparison)**

❑ **L BA/ L COMB**
- **L BA = NB + BA**
- **L COMB = NB + BA + MSG**
- **L BA/ L COMB = MSG (Resulting effect in comparison)**

➢ Since MSG is similar in both sets, it can be used as e resulting effect in comparison

➢ Comparison of significance of p-value for Passage 1-70 and Passage 71-153
- DI value of two treatment sets plotted for correlation coefficient (CC) value
- p-value was calculated from the CC value

		Passage 1-70		Passage 71-153	
PCR/RFLP Comparison	Resulting Effect	p-value	Significant	p-value	Significant
LMSG/LS, LBA/LC	MSG	0.173	No	$2.26x10^{-04}$	Yes
LMSG/LC, LBA/LS	BA	0.431	No	$1.31x10^{-04}$	Yes
LMSG/LBA, LS/LC	BA + MSG	0.156	No	$1.48x10^{-04}$	Yes
HMSG/HS, HBA/HC	10MSG + S	0.091	No	$3.71x10^{-04}$	Yes
HMSG/HC, HBA/HS	10BA + S	0.019	Yes	$1.57x10^{-03}$	Yes
HMSG/H BA, HS/HC	10MSG + 10BA	0.434	No	$2.08x10^{-04}$	Yes

➢ p-value significant = cells are **NOT** correlated and adapt to individual treatment
➢ p-value not significant = cells are correlated and adapting based on the similar resulting factor

Since all results show significance in p-value, ecological specialisation may be present

HYPOTHESIS 2

➢Different chemical concentration poses different type of stress

Hypothetical Scenario: Different chemical concentrations should induce different level of stress
- High treatment media = Higher stress
- Low treatment media = Lower stress
- [High] (1%) ↔ [Low] (0%) swap treatment done

Based on previous FYP results, different chemical concentration shown to induce different **type** of stress instead → New swap experiment to confirm hypothesis
- [High] salt 1%)→ [Higher] salt(2%)
- [Low] salt(0%)→ [Higher] salt(2%)

➢**Rate of decrease of generation time in L salt cells is faster than H salt cells → Experimental results similar to previous FYP findings**

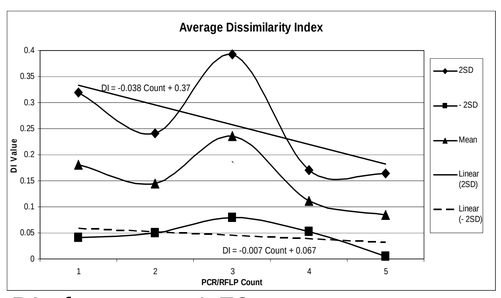
➢**Hypothesis 2 is ACCEPTED**

HYPOTHESIS 3

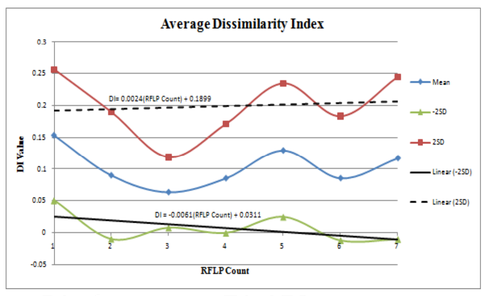
➢ PCR-RFLP profiles within treatments of a passage are likely to show genetic similarity

Detect genetic changes across time
- RFLP done using MspI, TaqI and HinfI after PCR
- Differences between the two samples
  - o Estimated by Nei-Li's Dissimilarity Index

Comparison with previous data shows genetic divergence as compared to genetic convergence shown previously



DI of passage 1-70



DI of passage 71-153

Divergence of upper and low class limits
- Suggests increase in genetic distances

➢ **Hypothesis 3 is REJECTED**

CONCLUSION

➢ Slower rate of adaptation

➢ Different chemical concentration causes different type of stress

➢ Presence of ecological specialisation

FUTURE WORKS

➢ Conduct more swap experiments of other chemicals into a higher concentration

➢ More enzymes and primers to increase coverage of the genome
- •Adaptive mutations may not occur on amplified fragments

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