Gradually Evolving Escherichia coli to Grow in 10% NaCl in 6 Months

JZR Lim, JA How, DJW Goh, WC Ng, MHT Ling
School of Chemical & Life Sciences, Singapore Polytechnic,Singapore

Introduction

❑To observe the evolution of *Escherichia coli*

❑Adaptation and evolution of *E. coli* to antibiotics and drugs widely studied

❑Non-antibiotic agents, such as salt, are less understood

❑Salt, common food additive, inhibits growth of microorganisms

❑*E. coli* is a textbook example of non-halophillic bacteria

❑Used as an indicator for faecal contamination in water

❑Preservatives are used to inhibit *E. coli* growth
Significance of *E. coli* adapting to salt

- Will it be resistant to other preservatives?
- Is the ability of *E. coli* to grow in saline underestimated?

Objectives

☐Adapt *E. coli* up to 10% NaCl

☐Observe growth kinetics of *E. coli*

☐Observe the genetic changes of the *E. coli* as it adapts to stress caused by NaCl

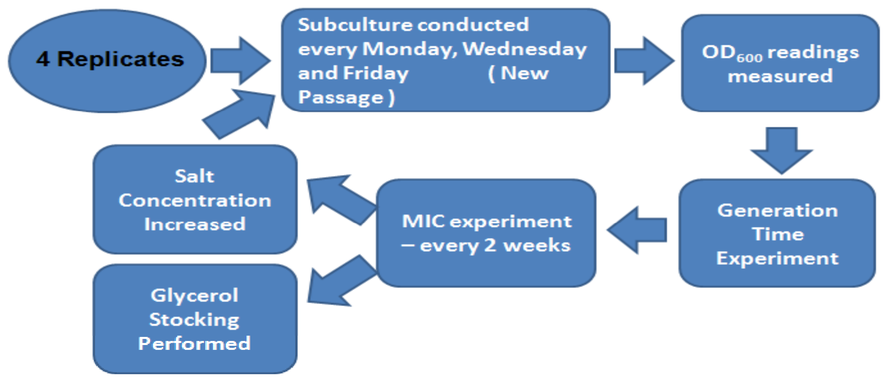
Hypothesis

1. *E. coli* can be adapted to grow at 10% NaCl

2. *E. coli* should be able to adapt to 5-10% NaCl but speed of adaptation is expected to decrease as concentration of salt increases

3. DNA of progeny *E. coli* is likely to differ from ancestor strains as it adapts to the increase in NaCl concentration

Procedures



Hypothesis 1: VALID

Culturing at 11% NaCl

- 1% inoculation of culture into 11% NaCl and incubated for 21 to 23 hours at 37°C. OD600 readings were taken after incubation
- Done as part of minimum inhibitory concentration from 0% NaCl to 11% NaCl
- OD600 readings suggests that *E. coli* can grow in 11% NaCl after 54th passage
- Implies that the cells are able to grow at 10% NaCl

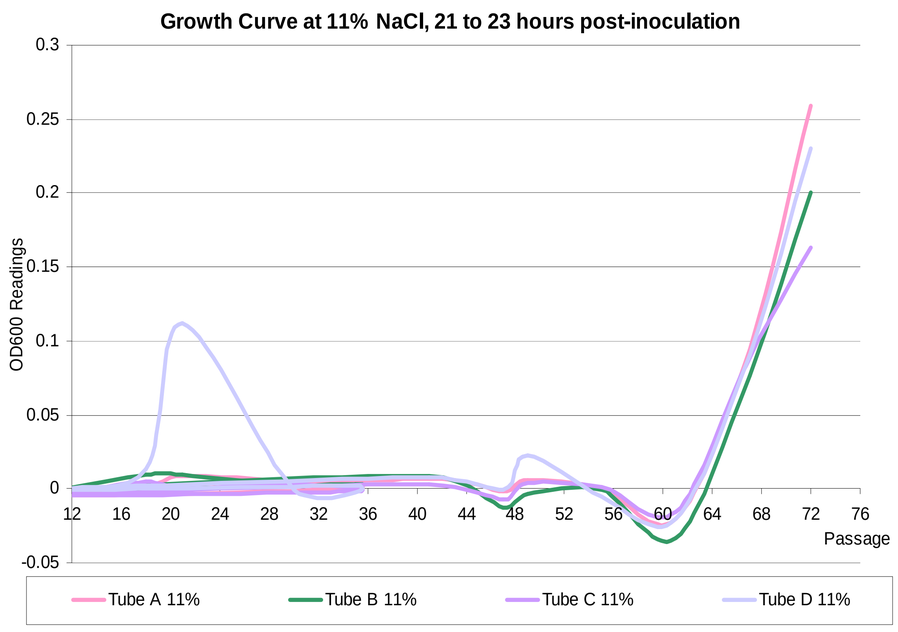
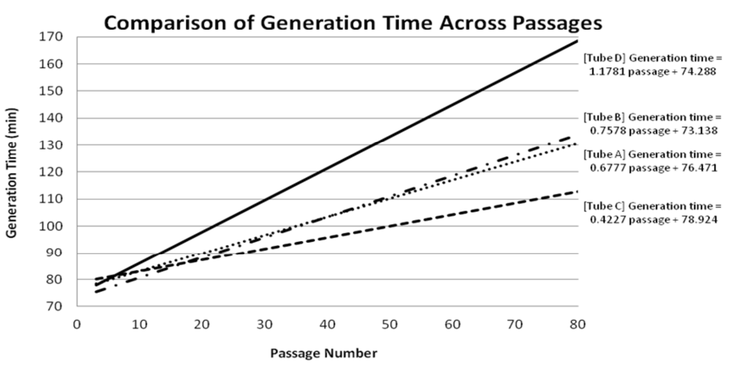
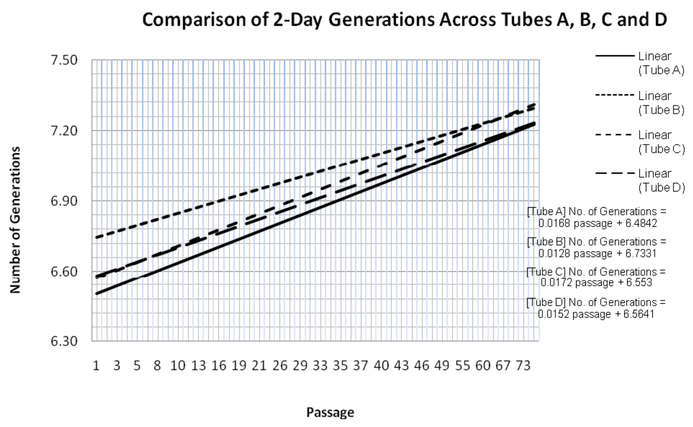


Figure 1: Growth Curve at 11% NaCl, 21 to 23 hours post-inoculation.

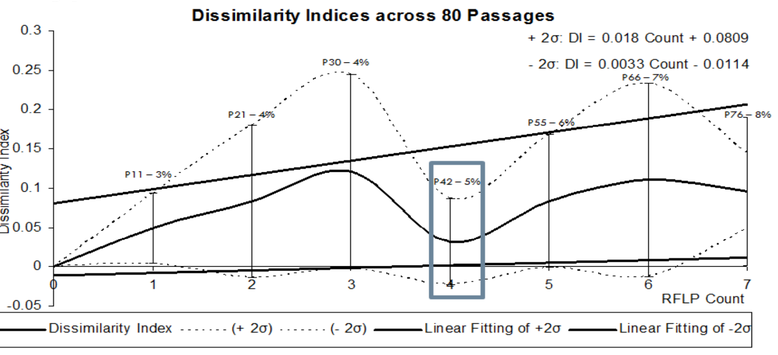
Hypothesis 2: VALID





- Increase in generation time is linked to increase in generations
- Judging from number of generation increases suggests that *E. coli* are able to adapt

Hypothesis 3: VALID



As NaCl increases, dissimilarity index increases

Critical weeding point at 5% NaCl (P42)

ANOVA Test

Source	df	Significance
Treatment	6	0.010
(Tube) Replicates	3	0.559
Treatment x Tube	18	0.539

Tests between – Subjects effects in relation to generation time

- Based on 2 way ANOVA test, only treatment is significant

Conclusion

- *E. coli* is able to adapt to10 % NaCl concentration over 80 passages
- Generation time increases when NaCl increases
- Genome of progeny *E. coli* is different from the ancestor *E. coli*
- Decrease in genetic dissimilarity correlated with increase in COV

Past Research

- Hrenovic and Ivankovic (2009)
- Survival of *E. coli* at various concentrations of sodium chloride
- *E. coli* in nutrient broth able to reproduce actively up to 5% NaCl
- No significant growth at 7% NaCl
- Total die off at 20% NaCl after 72 hrs
- Total die off at 30% NaCl after 48 hrs

References

HRENOVIC, J. & IVANKOVIC, T. (2009) Survival of *Escherichia coli* and *Acinetobacter junii* at various concentrations of sodium chloride. *EurAsian Journal of Biosciences*, 3**,** 144–51.

DOUDOROFF, M. (1940) Experiments on the adaptation of Escherichia coli to sodium chloride. *The Journal of General Physiology*, 23**,** 585.

DOUDOROFF, M. (1940) Experiments on the adaptation of Escherichia coli to sodium chloride. *The Journal of General Physiology*, 23**,** 585.