

# Evolving in an Unpredictable World: The *E. coli* Story

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## Summary

### Background

Temporally heterogenous environments are likely to select for Generalist populations(1,2,4). However, the fitness advantage of Generalists in uncorrelated, abiotic, novel environments is not well characterized(6).

### Results

- Randomly fluctuating, complex, stressful environments select for populations which, compared to the control populations, grow better in some of the novel environments, without any trade off in others. Such populations are called Generalists.
- The fitness advantage of the Generalists is retained even after acclimation.
- Evolution of Generality does not lead to a cost ( growth disadvantage) in face of long term directional selection in the novel environments.
- Active efflux by RND multi-drug pump is a possible mechanism for Generality.

## Experiment

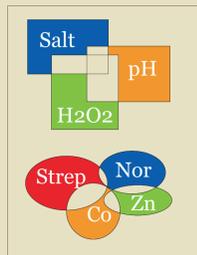
### Selection Environment

- Model Organism - *E. coli*
- Culture Conditions - Nutrient broth, 37°C, 150 rpm, passage every 24 hours
- Different combinations of sub-lethal concentrations of NaCl, pH and Hydrogen peroxide were used to create 72 different environments. These were arranged in a random sequence of 30, resulting in 'Randomly fluctuating, Complex, Stressful Selection Regime'.

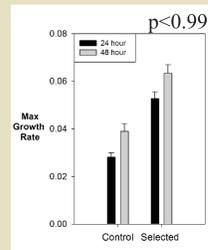
- Control populations were passaged in nutrient broth for same duration.

### Fitness Assessment

- Five novel stresses used for fitness assessment of Selected populations - Cobalt, Zinc, Streptomycin, Norfloxacin, Ethidium bromide.
- These stresses were neither part of the selection regime, nor are they correlated to the stresses which were part of the selection regime.

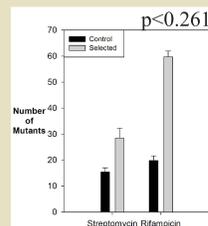


## Routes to Generality



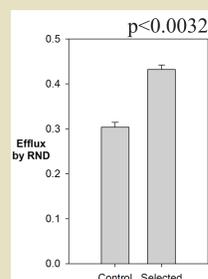
Maximum growth rate of Control (Black bars) and Selected populations (Grey bars) was estimated in the five novel environments after two different durations of relaxation in nutrient broth. We expect the growth rate to decline from 24 hours of relaxation to 48 hours of relaxation, if the Generalist property is not heritable and just a physiological carry over from the selection environment. But maximum growth rates are comparable for the two relaxation times.

**Generality is stably heritable.**



To check whether Generalists are hypermutators, number of mutant colonies were counted in two antibiotic backgrounds, Streptomycin and Rifampicin. In both the cases Selected populations (Grey bars) show greater number of mutant colonies compared to the Controls (Black bars). But the increase is two to three fold which is not a characteristic of hypermutators (6).

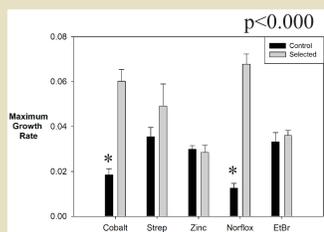
**Observed Generality can not be attributed to hypermutation.**



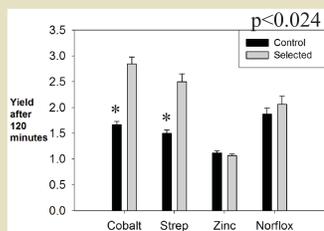
Active efflux by RND (resistance nodulation division) multidrug pump was quantified in fluorescence based assay. Measure of efflux, represented after normalization for cell numbers and arcsine square root transform shows that Selected populations have significantly greater efflux activity than the Controls.

**Efficient efflux could be responsible for the observed Generality.**

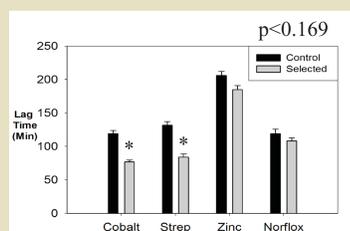
## Performance of the Selected lines in the novel environments after 30 days (~500 generations) of selection



Maximum growth rate of the Selected and Control populations was measured in five novel environments. Selected populations (Grey bars) showed significantly higher growth rate than Controls (Black bars) in Cobalt and Norfloxacin, without any growth disadvantage in the other three novel environments.



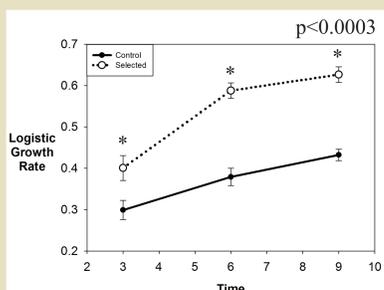
Growth of individual cells was tracked and number of progeny cells produced by each individual after 120 minutes was scored as yield. Selected individuals (Grey bars) showed significantly higher yield than Controls (Black bars) in Cobalt and streptomycin and similar yields in Zinc and Norfloxacin.



Growth of individual cells was tracked and time taken till first division was scored as lag time. Selected individuals (Grey bars) showed significantly lower lag than Controls (Black bars) in Cobalt and streptomycin and similar lags in Zinc and Norfloxacin.

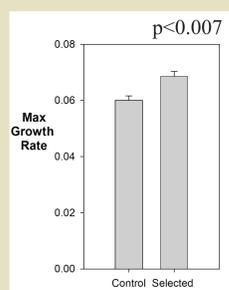
**Selected Populations and Individuals show fitness advantage in some novel environments without any trade-off in other environments(3). Generalists have evolved!**

## Generalists in the face of Acclimation and Directional Selection



Control and Selected populations were acclimated to five different novel environments, separately; for durations of 3, 6 and 9 hours. Estimated logistic growth rate of Selected populations (Dotted line) is significantly higher than Controls (Solid line) at each of the acclimation point when measured in the environment same as that of acclimation.

**Generalist's advantage is retained after acclimation.**



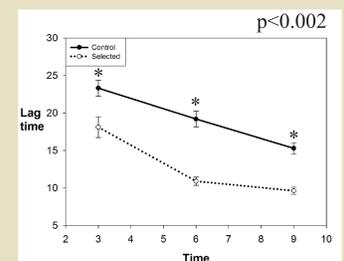
Control and Selected populations were directionally selected in four different novel environments separately, for the duration of 10 days. Selected populations show significantly higher maximum growth rate than control populations, when assayed in the novel environmental background.

**No cost for Generality in long term selection.**

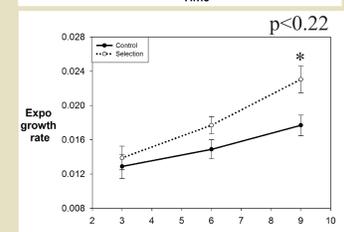
## Components of Logistic growth rate

For determining the relative contribution of different phases in the bacterial growth trajectory after acclimation, we dissected the growth data in three components -

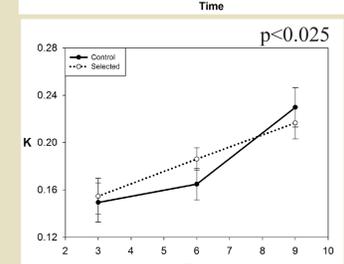
(A) **Lag time** - Selected populations (Dotted line) showed significantly lower lag than Controls (Solid line) at all the three acclimation durations.



(B) **Exponential Growth rate** - Selected populations showed significantly higher growth rate than controls after 9 hours of acclimation but not at the shorter acclimation duration.



(C) **Carrying Capacity** - The final density reached by the Selected and Control populations does not differ for any of the acclimation periods.



**The growth advantage of Generalists during acclimation is mainly in the early growth phase.**

**Fitness advantages can differ across the phases of growth trajectory and analyzing them separately can provide better insight!**

## Implications

Multi-drug resistance in bacteria is supposed to evolve due to use/abuse of antibiotics. Our results suggest that there are alternate ways of evolution of multi-drug resistance. This implies that areas where environmental conditions are rapidly and unpredictably changing can possibly become hotspots for the evolution of potentially dangerous pathogens.

Evolution of generality does not seem to entail any fitness cost w.r.t. acclimation or long-term evolution under novel environments. Also there was no sign of reverse evolution in 30-40 generations of growth under non-stressful environment. If indeed no fitness costs exist, one would be forced to ask why generalists (and therefore phenomenon like multi-drug resistance and invasiveness) are **not** a lot more common in nature.

## References

- (1) Buckling A et al (2007) Journal of Evolutionary Biology 20: 296-300.
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