The Effect of Cross-Feeding in a Spatially-Structured Environment on the Evolution of Antibiotic Resistance in A Synthetic Bacterial Mutualism

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Abstract

Antibiotic resistance is an urgent global public health threat, as resistant bacteria can overcome antibiotic stress and grow despite treatment. In combination with another strategy of antibiotic failure, antibiotic persistence - which describes the survival of a tolerant subpopulation of bacterial cells, development of full resistance mutations may evolve faster. Bacteria tend to live in microbial communities, where they may participate in mutualistic ecological interactions as they exchange essential nutrients in a cross-feeding manner. Specifically in natural microbial communities, bacteria participate in spatially-structured, localized interactions on surfaces. How these microbial ecological factors affect the evolution of antibiotic persistence and resistance is unknown. Here, I studied the evolution of antibiotic persistence and resistance in *Escherichia coli* evolved in monoculture and in a synthetic cross-feeding mutualism with *Salmonella enterica* in structured habitats. After 16 cycles of cyclical exposure to short-term ampicillin treatment and drug-free growth on agar surfaces, evolutionary rate of persistence and subsequent resistance were analyzed. I found that while *E. coli* in the mutualistic coculture begins with high persistence, the monoculture persistence evolves at a faster rate. In contrast, *E. coli* in the spatially-structured mutualism evolves 3-fold higher resistance than the wild-type, while the monoculture counterpart does not. These results warrant further studies regarding the mechanism behind the differential evolution of antibiotic resistance between the mutualistic coculture and the monoculture.

Hypothesis

Escherichia coli grown in cross-feeding mutualism with _Salmonella enterica_ will evolve concurrent antibiotic resistance faster than its monoculture counterpart in a spatially-structured environment.

Methods

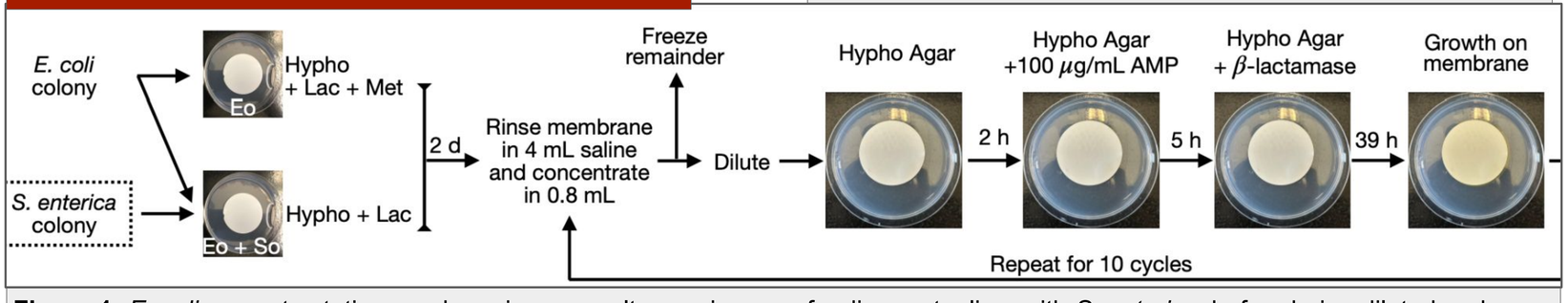

Figure 1: *E. coli* grown to stationary phase in monoculture or in cross-feeding mutualism with *S. enterica,* before being diluted and fixed to a nitrocellulose membrane for 2 day growth on corresponding Hypho agar. Membranes were rinsed in saline, cultured were diluted and fixed onto fresh membranes for a 2 hour growth period. Membranes were transferred to corresponding agar with high concentration of ampicillin (100 ug/mL) for 5 hours, before being moved to media with ampicillin-deactivating beta-lactamase for 39 hour growth period.

Results

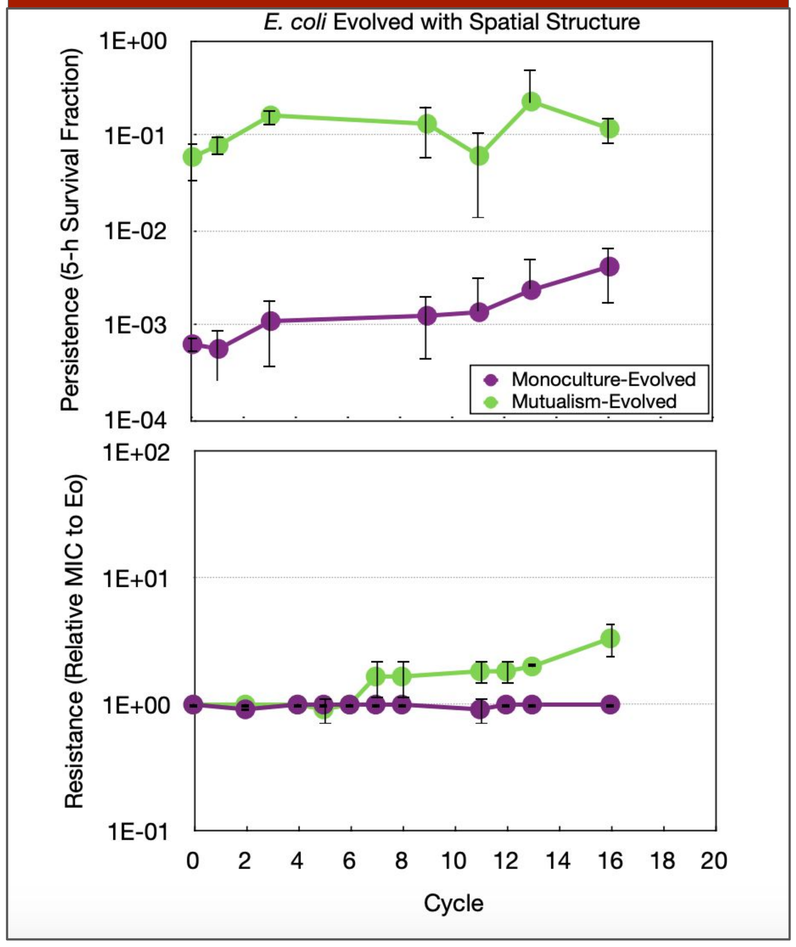

Figure 2: *E. coli* Evolves to be 3-Fold More Resistant to the ancestral strain (Eo) in the spatially-structured mutualistic coculture. While the antibiotic persistence level in the mutualistic *E. coli* begins at higher persistence, its monoculture counterpart evolves at a faster rate.

Conclusion

- Higher persistence in evolved cross-fed populations leads to the accumulation of antibiotic resistance mutations at a faster rate than monoculture counterparts
- With persistence and cross-feeding on spatially structured surfaces both having an increased effect on resistance, the combination have significant clinical implications contributing to the antibiotic resistance public health crisis

Future Directions

- Whole genome sequencing for mutational analysis to better understand the molecular basis for persistence evolution in spatially structured conditions
- Replicate experimental set up with more commonly occurring human gut bacteria to better replicate the clinical implications of antibiotic usage
- More accurately replicate the spatial structure and inconsistent nutrient profile of the human gut
- Replicate experimental set up with new nutrients and species to address further evolutionary questions regarding mutually beneficial relationships as a whole

References

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