Effects of compensatory mutations on evolved strains of antibiotic resistant *Escherichia coli*

Jeffrey Mayo (jmayo@uh.edu) and Tim F. Copper
Biology and Biochemistry, University of Houston, USA

Background

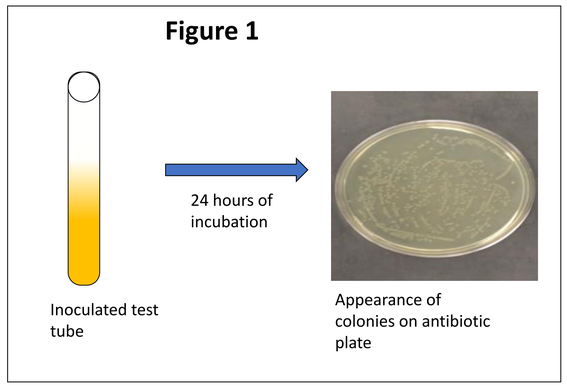
Pathogens that are exposed to antibiotics acquire mutations that confer resistance. In the absence of antibiotics resistant mutants have a lower fitness relative to their sensitive ancestral strain. Fitness costs associated with resistance are often ameliorated through the acquisition of compensatory mutations. To test how compensatory mutation depend on resistance mutations I selected for strains resistant to the common antibiotics trimethoprim and sulfamethoxazole and tracked the evolution of compensation.

Methods

Selecting for antibiotic resistance

Resistant strains were acquired by selection on agar plates containing trimethoprim, sulfamethoxazole or a combination of both antibiotics. Single clones from each plate were isolated after 24 hours of incubation (figure 1)

Figure 1



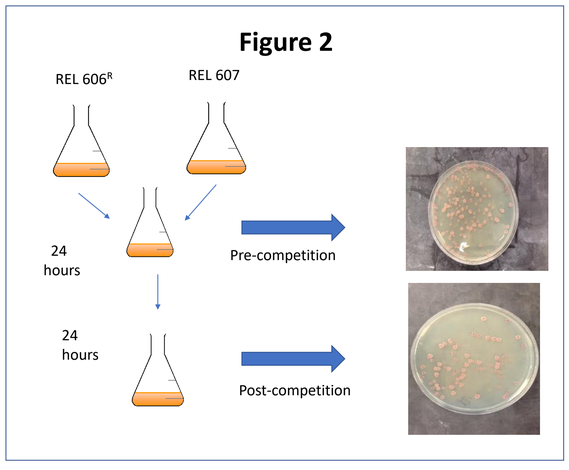
Evolving mutants

I used a 96 well block containing glucose minimum media to evolve the four selected mutants. Serial transfers were carried out for 300 generations to allow for compensation in each population. Once evolved each mutant population was taken and stared at -80° for later use in competition assays

Measuring relative fitness

Relative fitness of each population was determined by measuring the ratio of evolved to ancestral populations following a period of competitive growth. This step was also conducted for populations of resistant mutants not allowed to compensate for their acquired mutation. (figure 2)

Figure 2



Results

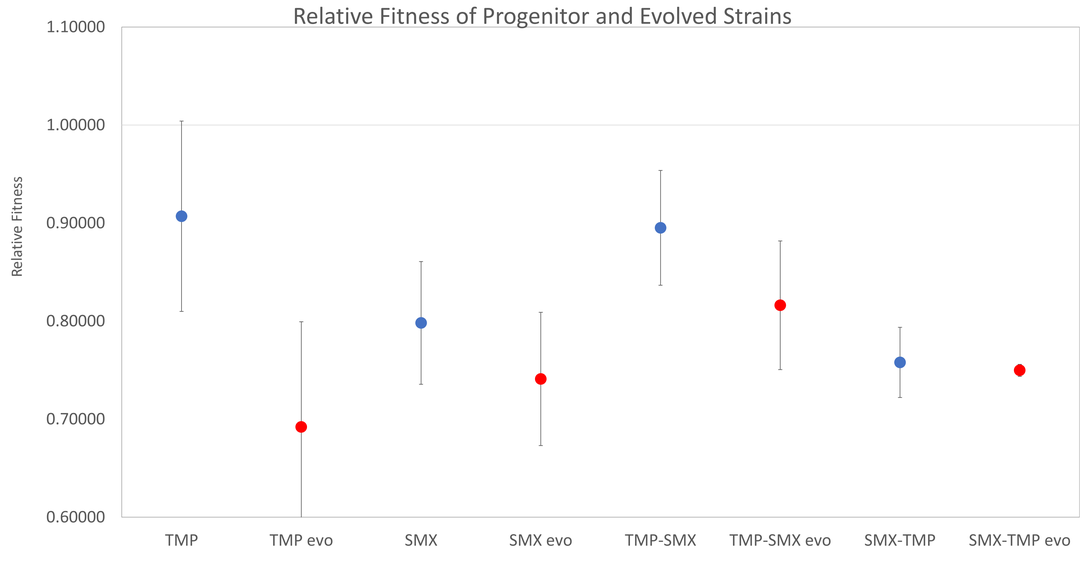


Figure 3. Relative Fitness of Progenitor and Evolved Strains. Data points represent relative fitness of each strain to the 607 ancestor. Note that the difference between SMX-TMP (sulfamethoxazole-trimethoprim) and TMP-SMX (trimethoprim-sulfamethoxazole) is the order at which each resistance conferring mutation was acquired.

Fitness competitions

Evolved and non evolved resistant strains appear to have no significant difference in relative fitness in comparison with the ancestor strain. The exception to this is trimethoprim mutants. This data suggests that evolved strains colonized an ecological niche that doesn't confer a higher fitness (figure 3).

Epistatic interactions.

Positive epistasis for fitness is observed in populations that first acquired resistance to trimethoprim then sulfamethoxazole. This data suggest that the two loci involved interacts in a way the increases fitness greater than the sum of the two parts.

Conclusion

When observing the effects of compensatory mutations on evolved strains their isn't a significant difference in relative fitness of evolved vs progenitor strains. Data does suggest that the order at which resistant mutations are acquired has an effect on how these mutations interact. Future work will be done to show a more direct comparison in fitness between the two mutants.

Acknowledgements: Members of Cooper lab for their thoughtful discussions and NSF for funding