Biocide Resistance in E.coli

The ideal lab in an Evolutionary Biology class would be one where we demonstrate natural selection in response to a selection pressure. This is easy enough to do with bacteria (see Lenski and Travisano 1994, Travisano and Lenski 1995, 1996, or any of the publications listed on Richard Lenski’s website: http://myxo.css.msu.edu/Publications.html). However, what are less well studied in the classroom are the costs of evolution. In the case of bacteria, there is a demonstrated cost of antibiotic resistance (Lenski 1998).

The selective agent for this lab will be 2,4,4’-Trichloro-2’-hydroxydiphenyl ether, better known as Triclosan (and marketed as Irgasan DP300). Triclosan is classed as a biocide (a substance toxic to cells in general) or antimicrobial agent (a substance toxic to bacteria, fungi, and protistans) because it kills or inhibits the growth of a wide range spectrum of microbes. It is not considered an antibiotic (a microbial product that kills or inhibits the growth of susceptible microbes) because of its origin and its broad spectrum of toxicity.

Bacteria and fungi are well-known for evolving resistance to antibiotics (Levy 1998), but are thought to be less likely to evolve resistance to biocides because these compounds often act by different mechanisms. Indeed, the multiple mechanisms by which Triclosan could or did kill bacteria suggested little risk for the evolution of resistance. However, targets of Triclosan activity were discovered which are associated with mechanisms of antibiotic resistance in bacteria (Schweizer 2001) indicating that Triclosan may act more like an antibiotic than a true biocide (McMurray, et al. 1998; Levy et al. 1998).

The goal of this lab is to conduct a series of selection events on a population of *E. coli* for six generations, then challenge the selected population to competition with its near ancestral line. The selective agent will be a solution of Irgasan applied to a population of Dr. Thompson’s *E. coli* that express GFP. After 6 rounds of selection, you will test the competitiveness of the selected GFP expressing *E. coli* against a near ancestral line – BFP expressing *E. coli*. The synthesis of the fluorescing proteins differs by one amino acid, tyrosine in GFP and tryptophan in BFP.
1) Create a lawn of \textit{E. coli} on each of three petri dishes (containing typtic soy agar).
2) Place one treatment paper disk in each petri dish (1 for Triclosan, 1 for EtOH, 1 for ddH2O)
3) Seal petri dish with Parafilm. Label the dish with
   - the treatment
   - the date
   - your name
4) Place in growth chamber (35°C for 24 hours)
   
   - After 24 hours
5) Measure the width of the zone of inhibition (make and record 2 measurements report the average)
6) Isolate the most resistant \textit{E. coli} by rubbing a sterile cotton swab across the inner margin of the zone of inhibition. If no zone of inhibition is apparent, swab bacteria from an area adjacent to the paper disk. If isolated bacterial colonies are visible within the zone of inhibition, swab those.
7) Transfer the bacteria collected from the petri dish to a culture tube containing sterile tryptic soy broth by swirling the cotton-tipped swab in the broth. Cap the tube and label with:
   - the treatment
   - the date
   - your name
8) Place in growth chamber (35°C for 24 hours)
9) Repeat 5 more times
Literature Cited

The following articles should be freely available through Schmidt Library electronic journal collection or through internet searching. Exceptions are as noted.


