NanoKorea 2018

Graves, J.L., Ewunkem, J., Rhinehardt, K., Van Beveren, E., Yang, B., Almuhaysh, F., Hammood, S., Boyd, S., and Thomas, M., Han, J., and Harrison, S.H.

Iron-related fitness epistasis is antagonistic to the evolution of silver resistance in *Escherichia coli*.

Fitness epistasis (or genetic background effect) can have powerful influences on the evolutionary trajectory of populations. Here we examine the influence of a prior resistance to ionic iron (Fe^{2+}/Fe^{3+}) on the capacity for *E. coli* to evolve silver resistance. Five replicate populations of Fe^{2+}-resistant and Fe^{3+}-resistant *E. coli* were cultured for > 80 days in 23 μg/L of ionic silver. This low concentration was chosen because both Fe^{2+}/Fe^{3+} showed diminished resistance to Ag^{+} relative to both Ag^{+}-selected and controls (grown in standard DMB medium w/o Fe or Ag). After 80 days of selection in Ag^{+}, no increase in Ag^{+}-resistance was observed in either the $AgFe^{2+}$ (silver selection w/Fe^{2+} background) or $AgFe^{3+}$ (silver selection w/Fe^{3+} background) replicates. Furthermore, the observed correlated resistances to traditional antibiotics (e.g. ampicillin, rifampicin) as well as to gallium, displayed in the iron-resistant ancestors was lost. Whole genome sequencing of the $AgFe^{2+}$- and $AgFe^{3+}$-selected replicates demonstrated that the iron/metal resistant mutations observed in the Fe^{2+}/Fe^{3+}-resistant ancestors (e.g. *fecA*, *rho*, *fur*, *murC*, *dnaK*, *tolC*, and *nusA*) were all lost. Also no anti-silver mutations were observed (e.g. *cusS*, *ompR*) in the AgFe replicates. These results suggest that the AgFe replicates were undergoing reverse selection relative to Fe^{2+}/Fe^{3+}-resistance, but could not be cultured at concentrations of Ag^{+} required for selection to drive Ag^{+}-resistance. Early replicates cultured at concentrations (~ 75—100 μg/L) that allowed rapid evolution of Ag^{+} resistance consistently went extinct. We suggest that this is evidence that Fe^{2+}/Fe^{3+} genetic background is antagonistic to the evolution of silver resistance. Knowledge of this antagonism may provide new insights into the design and composition of nanostructured surfaces that provide sustainable antimicrobial features.