Evolutionary Genomics of Antibiotic Resistance

Semen Leyn[1,2], Oleg Kursanov[2], Xiaoqung Li[2], Lourdes Mujak[2], Fernando Garcia-Alcalde[3], Martin Ebeling[3], Andrei Osterman[2]

[1]*Institute for Information Transmission Problems RAS (Kharkevich Institute), Moscow, Russia;*

[2]*Sanford Burnham Prebys Medical Discovery Institute, La Jolla, CA, USA;*

[3]*Roche Pharma Research and Early Development, Roche Innovation Center, Basel, Switzerland.*

sleyn@sbpdiscovery.org

Antibiotic discovery needs not only novel compounds with antibacterial activity but also tools to assess and prioritize new drug candidates by the likelihood of acquired drug resistance in target pathogens. To that end, we have developed the approach based on experimental evolution monitored by population deep sequencing followed by systems-level mechanistic analysis. The experimental evolution of drug resistance is performed in a morbidostat, a modification of the chemostat approach enabling a constant selective pressure via gradual (software-controlled) increase in drug concentration. The established workflow was optimized and validated in a model of experimental evolution of resistance to metabolic drug triclosan (TCL) in *E. coli*. The known target of TCL, a popular biocide widely used in consumer products, is enoyl-ACP-reductase FabI, an essential enzyme in bacterial fatty acid biosynthesis. In course of 4x24 hrs consecutive evolutionary cycles in six parallel reactors, we have observed a gradual (up to 20-fold) increase in minimal inhibitory concentration (MIC) at the level of populations and individual selected colonies. The bioinformatics analysis of numerous single nucleotide variants (SNV) appearing and disappearing in course of evolution revealed common aspects of otherwise unique evolutionary trajectories observed in all six reactors. Most importantly, early-stage resistance mechanisms, which emerge at relatively low TCL via recruitment of certain natural stress-response pathways, are ultimately outcompeted by a single most robust mechanism implemented via resistance mutations in the primary drug target, FabI. Notably, all of the 13 detected SNVs in *fabI* gene were mapped to the active site area of the enzyme.