Mechanisms of Action and Resistance of the Natural Product Antibiotic Tropodithietic Acid

Maxwell Z. Wilson[1], Mohammad Seyedsayamdost[2] and Zemer Gitai[1]

***Short Abstract* — When natural product antibiotics are discovered, it is straightforward to characterize their structures and the range of their activities. However, determining the mechanisms of action and resistance for antibiotics remains challenging. Here we harness quantitative approaches to study the natural product antibiotic tropodithietic acid (TDA) and determine how TDA kills cells and how cells develop resistance to it. Using bacterial cytological profiling we find that TDA causes cell death by collapsing the proton motive force like the electroneutral proton antiporter nigericin. We also identified genes necessary and sufficient for conferring resistance to TDA. Together our studies characterize the mode of action of an antibiotic that is important in marine ecosystems and establish a rapid and quantitative roadmap for characterizing other new antibiotics in the future**.

***Keywords* — Natural Product, Antibiotic, Antibiotic Resistance, Drug Targets, Bacterial Cytological Profiling.**

While almost all recently discovered natural products are tested for their activity profiles and assessed for their chemical structures, few are understood in their biological contexts on the molecular level. One such antibiotic, tropodithietic acid (TDA), is produced by a phylogenetically diverse set of marine bacteria including multiple members of the genera *Reugeria* and *Roseobacter*(*1, 2*). One *Roseobacter*, *Phaeobacter gallaciencis,* upregulates production of TDA at high cell densities (*3*) and in the presence of TDA itself (*4*).

TDA is chemically unique. It consists of a seven membered tropolone ring that undergoes a transition between two valence tautomers requiring a relatively low activation energy (*5*). Thus, in its physiological environment TDA can be considered to be two molecules in one.

Using fluorescence microscopy and calcein green indicators for both eukaryotic and prokaryotice cell death we first showed that TDA possesses cross-domain antimicrobial activity. We then used high-speed, time-lapse microscopy to examine the flagellum motor speed of immobilized *E. coli,* demonstrating that TDA targets the Proton Motive Force (PMF). To support this hypothesis we performed a metabolomics analysis of *E. coli*'s nucleotide levels before and after drug treatment. Nucleotide triphosphate levels relative to untreated cells decreased with time post-TDA-treatment, indicating that the treated cells struggle to re-generate triphosphorylated molecules.

To understand how TDA targets the PMF, we compared TDA-treated *E. coli* with bacteria treated with a series of drugs that each collapse the PMF in unique ways. In order to compare the 'dead states' of antibiotic treated cells we adapted an imaging-based method for identifying the cellular pathway(s) targeted by small-molecule antibiotics, known as Bacterial Cytological Profiling (BCP) (*6*). BCP uses quantitative imaging of a series of morphological and cytological die staining metrics to cluster bacterial cells in similar states. BCP revealed TDA works via a mechanism similar to the well-characterized electroneutral proton antiporter, nigericin.

Finally, we sought to understand how the *Phaeobacter gallaeciensis* cells that synthesize TDA are, themselves, resistant to its effects. We identified a set of 3 genes that were predicted to be co-regulated with the biosynthesis of TDA. Deleting these genes confirmed that they are necessary for TDA resistance and their heterologous expression in *E. coli* confirmed that they are sufficient to confer TDA resistance. Out of the initial 3 genes, we further characterized *tdaR3*, which alone can confer TDA resistance to *E. coli*. TdaR3 is highly homologous to the glutathione degradation gene *chaC* conserved from *E. coli* to humans. We are currently investigating the mechanism by which TdaR3 confers TDA resistance and the implications of this mechanism for marine ecology.

Acknowledgements: This work was funded by NSF.

[1]Department of Molecular Biology, Princeton University, Princeton NJ. E-mail: mzwilson@princeton.edu

[2]Department of Chemistry, Princeton University, Princeton NJ.

References

[1] L. Gram, J. Melchiorsen, J. B. Bruhn (2009) Antibacterial Activity of Marine Culturable Bacteria Collected from a Global Sampling of Ocean Surface Waters and Surface Swabs of Marine Organisms, *Mar Biotechnol* **12**, 439–451

[2] T. Brinkhoff *et al.* (2004) Antibiotic Production by a Roseobacter Clade-Affiliated Species from the German Wadden Sea and Its Antagonistic Effects on Indigenous Isolates, *Applied and Environmental Microbiology* **70**, 2560–2565

[3] M. Berger, A. Neumann, S. Schulz, M. Simon, T. Brinkhoff (2011) Tropodithietic Acid Production in Phaeobacter gallaeciensis Is Regulated by N-Acyl Homoserine Lactone-Mediated Quorum Sensing, *Journal of Bacteriology* **193**, 6576–6585

[4] H. Geng, R. Belas (2010) Expression of Tropodithietic Acid Biosynthesis Is Controlled by a Novel Autoinducer, *Journal of Bacteriology* **192**, 4377–4387

[5] E. M. Greer, D. Aebisher, A. Greer, R. Bentley (2008) Computational Studies of the Tropone Natural Products, Thiotropocin, Tropodithietic Acid, and Troposulfenin. Significance of Thiocarbonyl−Enol Tautomerism, *J. Org. Chem.* **73**, 280–283

[6] P. Nonejuie, M. Burkart, K. Pogliano, J. Pogliano (2013) Bacterial cytological profiling rapidly identifies the cellular pathways targeted by antibacterial molecules, *Proc Natl Acad Sci USA* **110**, 16169–16174