Title

Genomic profiling with machine learning approaches to identify mechanisms of action and resistance to antibiotics

Abstract

Identifying targets of antibacterial compounds remains a challenging step in the development of antibiotics. We have developed a two-pronged functional genomics approach to predict mechanism of action that uses mutant fitness data from antibiotic-treated transposon libraries containing both upregulation and inactivation mutants. We treated a *Staphylococcus aureus* transposon library containing 690,000 unique insertions with 32 antibiotics. Upregulation signatures identified from directional biases in insertions revealed known molecular targets and resistance mechanisms for the majority of these. Because single-gene upregulation does not always confer resistance, we used a complementary machine-learning approach to predict the mechanism from inactivation mutant fitness profiles. This approach suggested the cell wall precursor Lipid II as the molecular target of the lysocins, a mechanism we have confirmed. We conclude that docking to membrane-anchored Lipid II precedes the selective bacteriolysis that distinguishes these lytic natural products, showing the utility of our approach for nominating the antibiotic mechanism of action. Using the chemical biology approaches, we also established the mechanism of Lysobactin which is a potent antibacterial natural product with in vivo efficacy against *S. aureus.* We show that lysobactin forms 1:1 complex with Lipid I, Lipid II, and Lipid II(A)(WTA), substrates in the PG and wall teichoic acid (WTA) biosynthetic pathways. Therefore, lysobactin, like ramoplanin and teixobactin, recognizes the reducing end of lipid-linked cell wall precursors.