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A cell-based approach to characterize antimicrobial compounds through kinetic dose response

Craig R. MacNair, Jonathan M. Stokes, Shawn French, Cullen L. Myers, Kali R. Iyer, Eric D. Brown *

Michael G. DeGroote Institute for Infectious Disease Research, Department of Biochemistry and Biomedical Sciences, McMaster University, Hamilton, Ontario L8N 3Z5, Canada

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ABSTRACT

The rapid spread of antibiotic resistance has created a pressing need for the development of novel drug screening platforms. Herein, we report on the use of cell-based kinetic dose response curves for small molecule characterization in antibiotic discovery efforts. Kinetically monitoring bacterial growth at sub-inhibitory concentrations of antimicrobial small molecules generates unique dose response profiles. We show that clustering of profiles by growth characteristics can classify antibiotics by mechanism of action. Furthermore, changes in growth kinetics have the potential to offer insight into the mechanistic action of novel molecules and can be used to predict off-target effects generated through structure–activity relationship studies. Kinetic dose response also allows for detection of unstable compounds early in the lead development process. We propose that this kinetic approach is a rapid and cost-effective means to gather critical information on antimicrobial small molecules during the hit selection and lead development pipeline.

1. Introduction

Widespread antibiotic resistance poses a significant concern for the medical community and threatens the return of a pre-antibiotic era.[1,2] Financially hamstrung by the acute nature of bacterial infections and countless large-scale development failures, the pharmaceutical industry continues to distance themselves from antimicrobial research.[3] Therefore, the renaissance of antibiotic discovery must be orchestrated on budgets a fraction of those available during past campaigns. The enigma of bacterial permeability has resulted in the overwhelming failure of target based enzymatic screening efforts, forcing a shift towards innovative modifications of traditional whole-cell screening.[4,5] Target enriched whole-cell screening platforms circumvent the need to overcome permeability challenges. However, from the often overwhelming pools of actives, challenges arise in selecting a limited number of lead compounds with high potential to succeed in downstream development. Small molecules must be rapidly assessed for mechanism of action, influence of chemical structure on biological activity, and chemical integrity.[6,7] In all, it is difficult to overstate the importance of early stage characterization of active compounds to inform lead selection. Currently available in silico and in vitro tools can assist in early stage small molecule characterization.[8–10] Unfortunately, available techniques have historically fallen short in predicting downstream success.[11] Therefore, enhancing our ability to rapidly characterize growth inhibitory small molecules and identify those of highest drug potential is essential in optimizing antibiotic discovery efforts.

Cell-based dose response has remained a fundamental assay in antimicrobial discovery since its inception. Traditional dose response exposes bacteria to varying antimicrobial compound concentrations and observes growth at a single time-point to determine compound potency. Modification of traditional dose response has created additional layers to this classic technique, uncovering its ability to identify compound bacteriostatic or bactericidal activity[12] and assist in therapeutic dose determination.[13] Herein, we utilize the unique kinetic profiles generated in *Escherichia coli* during exposure to functionally diverse antimicrobial compounds at sub-inhibitory concentrations. We explore dose-dependent phenotypes with kinetic resolution to predict compound target, assist in structure–activity relationship studies, and assess compound stability.

2. Results and discussion

2.1. Characterizing antimicrobials using kinetic dose response profiling

During growth under antibiotic stress, changes in growth kinetics (Fig. S1) are observed as concentrations approach the minimum inhibitory concentration (MIC) of the compound under

* Corresponding author.
E-mail address: ebrown@mcmaster.ca (E.D. Brown).

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investigation. These include changes in the duration of each growth phase, division rate, and maximum culture density. Generation of *E. coli* kinetic growth profiles during exposure to a range of antibiotics demonstrates conserved growth characteristics within classes of cell wall, DNA replication, and protein translation targeting drugs (Fig. 1). For example, cell wall targeting beta-lactam and cephalosporin antibiotics display a characteristic 'pre-lytic peak' in which cultures appear to enter exponential growth successfully, but quickly lyse thereafter. These steep pre-lytic peaks can be attributed to the sensitivity to cell wall targeting antibiotics during active growth[14–16] and allow for rapid identification of cell wall biogenesis inhibitors. Translation targeting antibiotics cluster into 50S and 30S ribosomal subunit inhibitors. 30S targeting aminoglycoside antibiotics display growth kinetics with increased lag time, reduced replication rate and decreased maximum optical density. A shallow pre-lytic peak is observed after reaching maximum cell density and is likely the result of toxic aberrant protein accumulation through mistranslation.[17,18] 50S subunit-inhibitor profiles contain similar increases in lag time and reduction of peak optical density, however, as bacteriostatic inhibitors, no cell lysis or pre-lytic peaks are detected. Antibiotics targeting DNA replication show increased lag phase duration with nominal impact on additional kinetic growth factors; a phenotype for future investigation. Characteristics observed in dose response profiles may provide additional insight towards further understanding the complexities and intricacies of antibiotic action.

While similarities in kinetic profiles are visually evident within classes of known antibiotics, we employed a linear discriminant analysis (LDA) approach to classify variations in kinetic growth curve features. Briefly, a local regression (LOESS) model was fit to each growth curve followed by calculation of first and second derivatives, which provide information on biomass accumulation rate (log phase) and maximum rate of cell division respectively. Concentrations chosen for analysis of each treatment were in relation to MIC, as half-, quarter-, and eighth-MIC. A total of 10 distinct features were extracted from each curve to generate a fingerprint of the antimicrobial agent. The most influential features included final optical density (600 nm), duration of lag phase, maximum growth rate, acceleration into exponential growth phase, deceleration into stationary phase, and time of stationary entry. These data for each concentration were compiled and used in an LDA to rapidly cluster drugs providing similar kinetic profiles. The first two linear discriminants account for the majority (92.5%) of variance between samples, and visualization of this reveals distinct clustering of antibiotics by targeted cellular process (Fig. 1). Future expansion of available kinetic dose response datasets will enhance this tool for application in elucidating the mechanism of action for novel antimicrobial compounds identified during screening efforts.

2.2. Predicting off-target activity during structure–activity relationship efforts

Potential antimicrobial leads are subject to intensive medicinal chemistry efforts to optimize potency and pharmacological properties. These resource intensive efforts have a direct impact on late-stage success and timelines in drug development.[6] Therefore, it is imperative to understand the impact of structural features on biological activity during early stages of the discovery process. Structure–activity relationship (SAR) studies systematically substitute functional groups on parent molecules to understand essentiality for biological activity. In vitro enzyme inhibition by analogues of a parent molecule provides insight into compound specificity, but often fails in predicting whole cell activity and cannot rule out the potential for off-target activity. Thus the impact on whole-cell bacterial growth inhibition during SAR campaigns is critical in directing downstream compound optimization. Nevertheless, changes in chemical structure often result in an increase of compound potency due to the emergence of off-target activities. Since antibiotics generate unique kinetic dose response profiles dependent on the mechanism of action (Fig. 1), we hypoth-

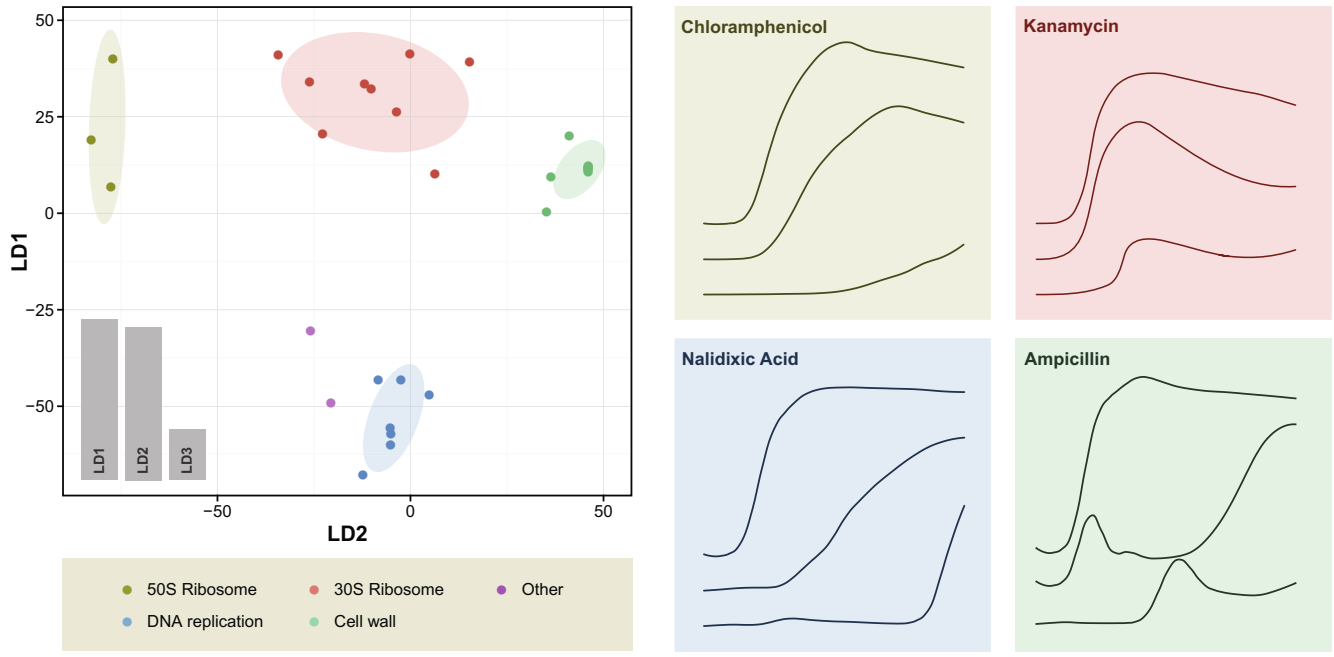

Figure 1. Linear discriminant analysis (LDA) of growth curve features across various antibiotic classes. Using the antibiotic targets as groupings, full treatment separations are evident based on the growth curve features (shown in Fig. S1). Shaded regions represent 95% confidence. Shown to the right are representative curves from each drug class, at half-, quarter-, and eighth-MIC drug concentrations. Inlaid are bar plots showing the proportion of variances described by each discriminant, the sums of which are 1. LD1 and LD2 combine for 92.5% of overall variances, and as such only a 2D plot is necessary for full separations. Shown on the right are several examples of growth curves from different classes of antibiotic. These curves represent culture density over time, and offer a glimpse into the subtle and unique differences that each drug class has on bacterial fitness.