Identifying Mechanism of Action of Antibacterial Compounds Using Bacterial Cytological Profiling

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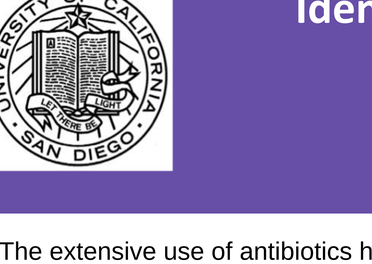
The extensive use of antibiotics has caused many pathogenic bacteria to develop resistance. This calls for not only new antibacterial compounds, but also compounds that have a unique mechanism of action (MOA). Unfortunately, while identifying antibacterial activity is fairly straightforward, determining the MOA of those compounds is not. In order to simplify and accelerate this process, we used bacterial cytological profiling (BCP). Through the use of fluorescence microscopy, BCP aids in the identification of MOA through analysis of cellular phenotypic changes upon treatment with compounds of interest. A series of experiments were performed to identify which compounds could kill or inhibit growth of a sensitized strain of *E. coli* with a deletion of the TolC efflux pump. We screened 576 synthetic compounds from a ChemBridge library to determine whether they would kill this strain as well as their minimal inhibitory concentration (MIC). After being treated with various concentrations of our identified compounds, the bacteria were examined using BCP to identify MOA. Additionally, we isolated bacteria from soil samples collected around the UCSD campus and screened them for the production of antimicrobial compounds.

Library Screening

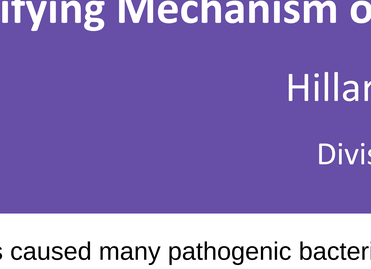
One 96-well plate was prepared by filling each well with 100 µL of LB media. Using a multichannel pipette, 2 µL from each well in the compound plate was pipetted into its corresponding well in the media-filled 96-well plate. Then, 1µL of a 0.05 OD bacterial culture was pipetted into each well. After incubating at 30ºC with shaking for 24 hours, the wells with no bacterial growth were noted for the MIC assays.



We found a total of 52 compounds that killed the *E. coli* at the highest concentration.

MIC

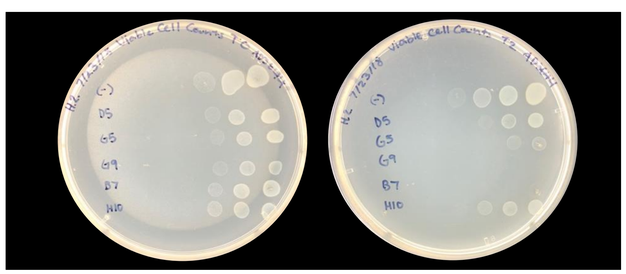
The standard, 96-well, broth microdilution method was used to determine the minimum inhibitory concentration (MIC) of all 8 compounds that killed. The assay was performed in 100µL and serially diluted 2 fold. The highest test concentration for all compounds was 100µg/mL. One microliter of *E.coli* **Δ***tolC* AD3644 was added to all wells except the media control then incubated overnight at 30ºC while shaking.



We found that the MICs of various compounds to range from 50µg/mL to 6.25µg/mL.

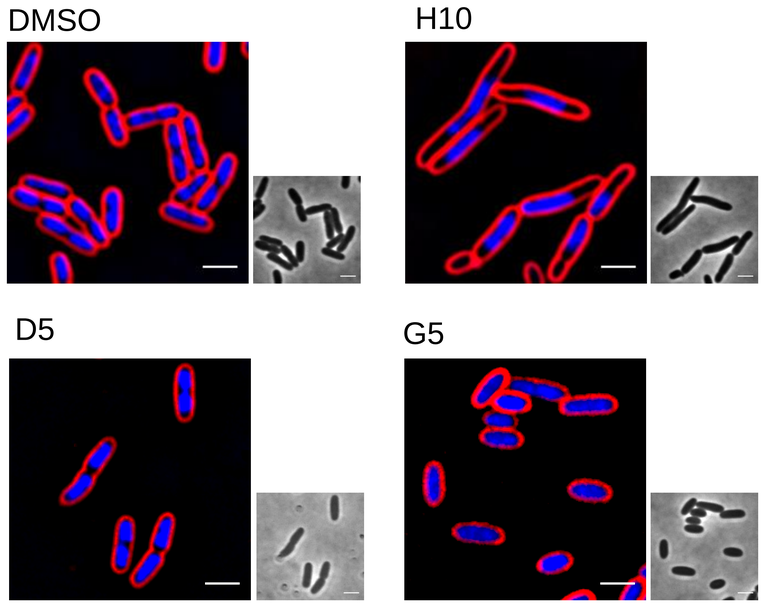
Viable Cell Counts

Viable cell counts identify whether the tested compound kills all bacteria or simply slows their growth. We prepared 2 96-well plates for 4 timepoints, T0, T1, T2, and T4hr. In several test tubes, we added the cells into DMSO, as a control, or the compounds to be tested. In the first column, we pipetted 50 µL of the cell culture from the test tubes. The tubes were then placed back in the incubator at 30ºC with rolling until the next timepoint. After adding T-base to the wells, we serially diluted them at a 1:10 ratio and spotted 5 µL on an LB agar plates. This was repeated for all four timepoints. After the bacteria colonies were grown, we counted visible single colonies per dilution and recorded them in a table.



Fluorescence Microscopy

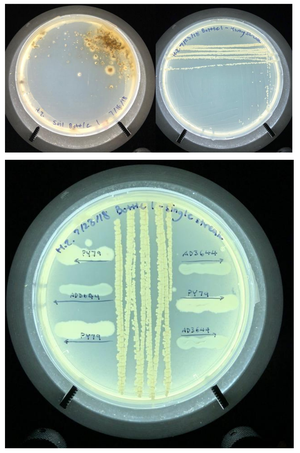
BCP helps identify the MOA, or how the antibiotic hinders the growth of the bacteria. Depending on the MIC of each tested compound, we took either 1x, 2x, or 5x times the MIC to ensure that we could witness the effects of the antibiotics on the cells during microscopy. We mixed three dyes into the bacteria: FM 4-64, a red dye that stains the cell membranes; DAPI, a blue dye that stains DNA; and SYTOX Green, which also stains DNA, but can only enter the cell when the membrane of the cells has been compromised. Therefore, SYTOX Green's presence in the cell is taken as an indicator of cell lysis.



The different phenotypes of the treated bacteria give us clues as to what part of the cell the antibiotic targets. For example, an elongated bacteria with condensed chromosomes implies that the antibiotic inhibits the bacteria's ability to synthesize DNA. Smaller, rounder bacteria may be signs that the antibiotic is a membrane or transcription inhibitor.

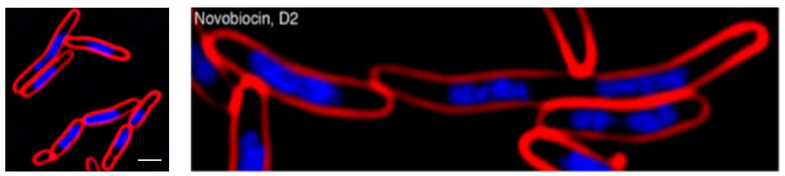
Soil Streaking

We collected soil from the UCSD campus and streaked it out on AIA plates supplemented with rifampicin and cycloheximide to prevent the growth of *Bacillus sp.*, bacteria commonly found in soil, and fungus. Once single colonies were grown, we streaked them again onto an LB plate and an AIA plate without antibiotics and incubated overnight at 30ºC. 10µL of AD3644 and *B. subtilis* PY79 were spotted next to the streaked bacteria and incubated overnight at 30ºC.



Streptomyces was isolated, but under the conditions that we tested in the LB plate and AIA plate, no antibiotics were formed to inhibit the growth of AD3644 and *Bacillus subtilis*.

Analysis and Conclusion



Well H10

Novobiocin is not the same scale as the H10 [1]

52 compounds out of total screened compounds managed to kill the sensitized *E. coli* strain, approximately a 10% success rate. Several compounds had a relatively low MIC of 12.5 µg/mL. Additionally, we were able to identify many of the MOAs of the compounds using BCP, such as DNA and RNA synthesis inhibition. Additionally, we were able to identify whether the compounds of interest were bacteriostatic or bactericidal. We were able to isolate *Streptomyces* from the soil samples. In the future, we would like to identify compounds analogs similar to compound H10 from and E7 (not shown) and screen them for their activity. Compound H10 has similar effects of DNA synthesis inhibition on AD3644 that novobiocin has, as seen in previous publication, so we would like to test H10 on wild strains of *E. coli* and other pathogenic bacteria.

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References

[1] Nonejuie, P., Burkart, M., Pogliano, K. and Pogliano, J. 2013. Bacterial cytological profiling rapidly identifies the cellular pathways targeted by antibacterial molecules. ProcNatlAcadSciUSA **110**:16169-16174.