Identifying Molecules with Antibiotic Activity Against *E. coli*

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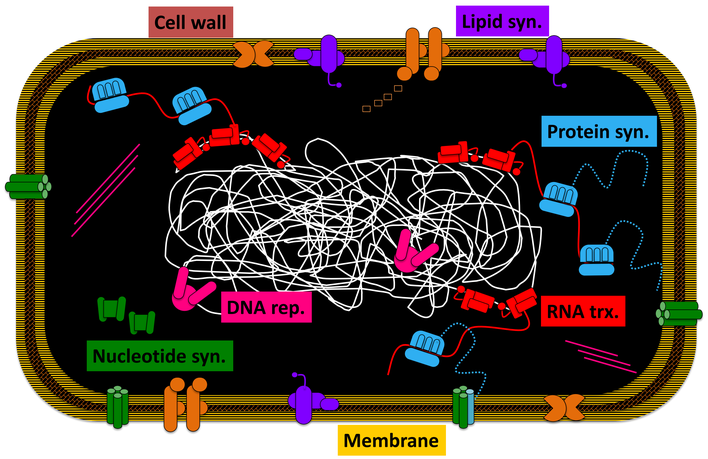
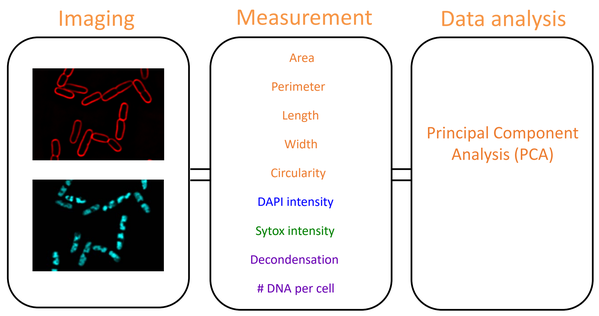
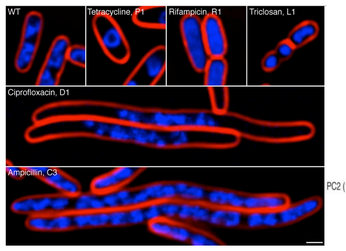
The emergence of multi-drug resistant bacteria and the decline in the number of new antibiotics coming to market poses a major global health threat. There is an urgent need to discover antibiotics that act by novel mechanisms of action (MOA). We have developed a rapid and versatile platform for identifying drugs that act by novel MOAs called Bacterial Cytological Profiling (BCP). BCP utilizes fluorescence microscopy to observe changes in cytological parameters of bacteria exposed to lethal concentrations of antibiotics. Antibiotics that hit targets in different pathways generate different cytological profiles (Nonejuie *et al.*, 2013). We screened 384 molecules for antimicrobial activity against a crippled laboratory strain of *E. coli* that lacks its multi-drug efflux pump. We found that 24 of these molecules (6.2 %) killed this strain, with minimal inhibitory concentrations ranging from 6.2 to 25 µg/ml. BCP revealed that many targeted the cell envelope (wall or membrane), one permeabilized the cells extensively, and one interfered either with phospholipid biosynthesis or the energetics of the cell.

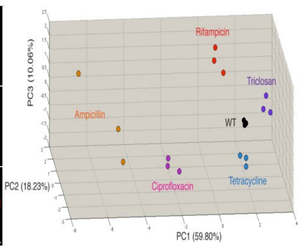
Bacterial Cytological Profiling

- BCP uses fluorescence microscopy to observe changes in cytological parameters of bacteria exposed to lethal concentrations of antibiotics







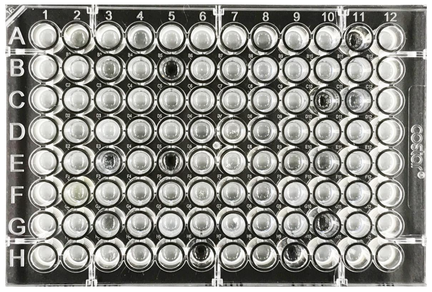


FM 4-64
DAPI
SYTOX green

Using BCP in an antibiotic screen

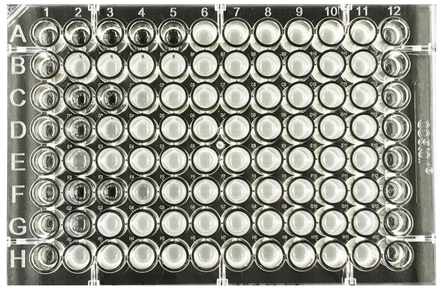
- We screened 384 compounds from a ChemBridge library of 10,000 molecules.
- We first screened the compounds against a strain of *E.coli* that has a mutation in the *tolC* efflux pump. This strain is hypersensitive to antibiotics.
- We identified compounds that killed this strain at a concentration of 50µg/ml.
- We then tested each compound to determine its potency by measuring the minimal concentration necessary to kill growth of an *E.coli* culture.
- We also tested the compounds to determine if they are active against a wild type strain of *E.coli*.
- Finally, mechanism of action of the compounds was determined using BCP.

A screening plate showing "hits" as wells without cell growth



Determining the MIC

MIC (µM)	# of hits
6.25	1
12.5	2
25	8

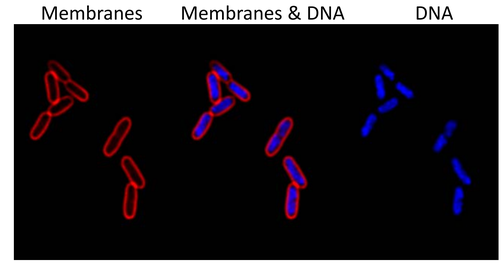


- Compounds were diluted in bacteriological broth (LB)
- *E.coli* was added to the diluted antibiotics
- The minimal concentration necessary to kill growth was observed

We thank the Academic Connections Program and Dr. Elizabeth Komives for overseeing the Research Scholars Program.

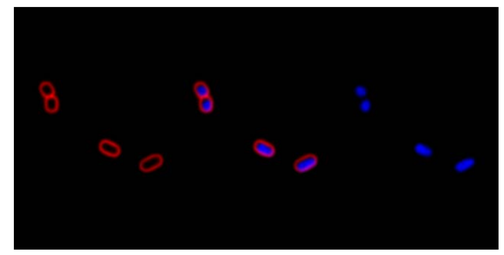
Using BCP to identify the Mechanism of action

- We found many compounds in the library that killed *E.coli* but whose MOA is unknown
- Using BCP we demonstrated that these compounds affect a variety of processes, including cell wall biogenesis, translation, lipid biogenesis and transcription.

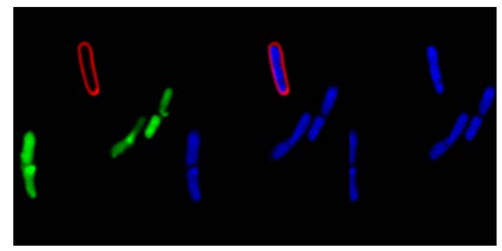


Control untreated *E. coli* cells

Red= membranes
Blue= DNA
Green= Membrane damage



E. coli treated with compound F3 forms tiny cells



E. coli treated with compound B9 have damaged cell membranes and stain green

Conclusions

- We identified 24 compounds that kill *E.coli tolC*
- We determined the MIC of 11 compounds.
- We identified the mechanism of action of three compounds

References

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