Identifying Chemical Compounds Targeting Persister Cell's Related Mechanisms in Bacteria

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BACKGROUND

- Bacterial persisters are rare, phenotypic variants that are temporarily tolerant to high concentrations of antibiotics.
- Persister cells are an important health concern because they underlie the proclivity of recurrent infections to relapse, and they can serve as a reservoir from which drug resistance mutants can emerge (1-4).
- Recurrent infections account for 65% of hospital-treated infections (5-6), and in the U.S. alone it is estimated that they are attributed to half a million deaths, and cost the healthcare system approximately $94 billion per year (7).
- It is desirable to discover novel compounds that can potentially serve as adjuvants to enhance the killing of persisters.

OBJECTIVES

- We approach the problem of persister through inhibiting the SOS response of bacteria under stress.
- When antibiotics are introduced into the bacterial culture, the bacterial cells' DNA is damaged (8). Bacterial cells respond to this damage by inducing the SOS response genes, such as *recA,* a gene essential for the repair mechanisms of DNA (Figure 1).

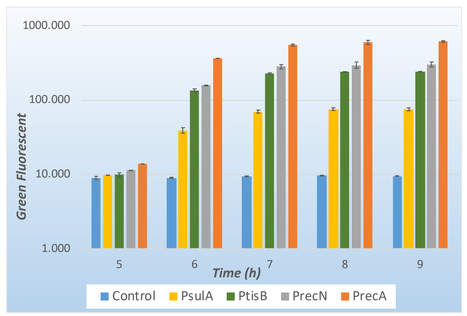

Figure 1. SOS response in *E. coli* cells after ofloxacin treatment. Cells harboring plasmids with green fluorescent protein (GFP) under the control of indicated promoters were treated with ofloxacin in early stationary phase (t = 5h). GFP levels were monitored using a plate reader. *Control: Empty vector*

- Inhibition of the SOS response, or impairment of the DNA repair mechanisms has been found to decrease persister levels (9).
- Discovering medicinally relevant chemical compounds that target DNA repair mechanisms so as to permanently damage the cells' DNA can significantly reduce persister levels.

APPROACH & PROCEDURE

- The chemical screening assays were performed in 15 different 96-well Phenotype MicroArrays plates (PM) (Biolog, Inc.) with E. coli cells carrying an expression system (plasmid) that has green fluorescent protein gene under the control of *recA* promoter (P_{recA}).

Chemical Screening Assay

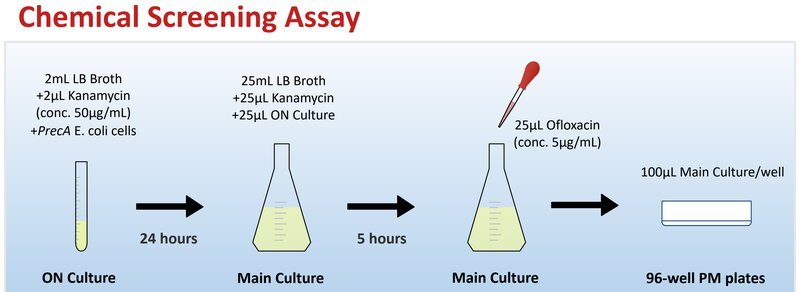

Figure 2. Chemical screening assay procedure

- The GFP expression of each well from PM plates was monitored every 2 hours for 4 hours.
- Bacterial culture under the effect of ofloxacin started to induce SOS response, which in turn increased the GFP expression.
- The wells that maintain constant GFP expression after the introduction of ofloxacin are the wells that contain chemical compounds that target the cells' DNA repair mechanisms.

Testing Persister Levels

- 20 hours after culture's treatment, the cell cultures from the wells containing the chemical hits were washed and plated on agar plates to determine the persister levels.

Controlled Experiments

- Two different controlled experiments were performed:
  1. Without the presence of the chemicals, monitoring the GFP expression and persister levels of a) cell cultures treated with ofloxacin, and b) cell cultures not treated with ofloxacin, under the same culturing time and condition.
  2. In the presence of the chemical hits from a different set of PM plates, monitoring the persister levels of cell cultures not treated with ofloxacin to determine the independent effects of these chemicals on the bacterial cultures.

RESULTS & DISCUSSION

Controlled Experiment Results

	t = 0h			t = 2h			t = 4h			CFU/well @t=0h			CFU/well @t=20h		
Rep.	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3
No OFX	13.00	13.26	12.92	13.35	13.45	13.31	14.70	14.73	14.43	5.40E+07	5.90E+07	7.00E+07	5.70E+07	6.60E+07	5.60E+07
OFX	14.35	14.40	14.39	57.82	65.31	57.76	143.1	144.7	146.8	1.90E+06	2.10E+06	2.50E+06	1.20E+05	2.10E+04	1.50E+05

Figure 3. GFP expression and persister levels from controlled experiment

- Cell cultures not treated with ofloxacin maintained the GFP expressions over time since there was no SOS response being induced (Figure 3).
- Cell cultures treated with ofloxacin induced SOS response, resulted in increased GFP expressions.
- After 20 hours of treatment with ofloxacin, the survival fraction of E. coli is about 2-2.5 persisters/1000 cells.

PM Plates Results

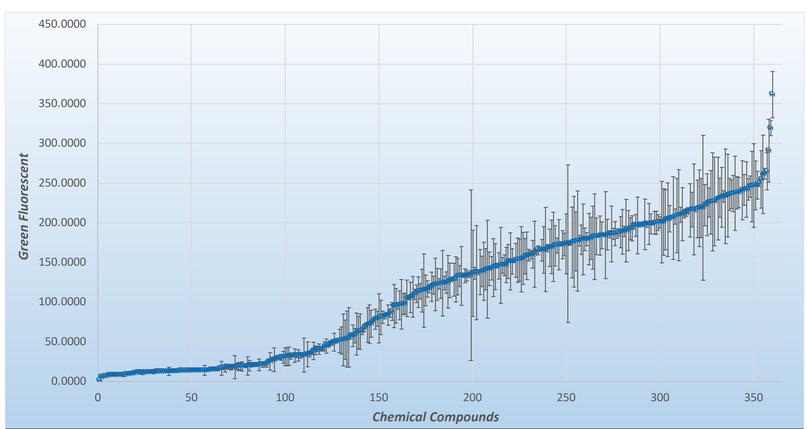

Figure 4. GFP expression of cell cultures after 4 hours of treatment with 360 different chemical compounds from PM plates. Data are average values ± standard errors from two biological replicates.

- After 4 hours of culturing, the wells from PM plates that had GFP expression lower than 50 were considered to be our hits.
- Although the results from the wells with high GFP expressions fluctuated greatly between the two replicates, the GFP expressions of the wells containing our chemical hits produced considerably consistent results (Figure 4).

Persister Levels Results

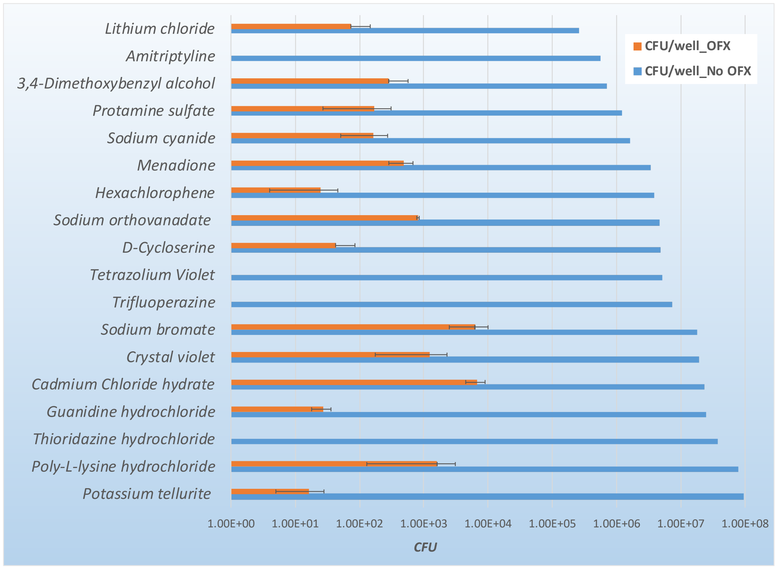

Figure 5. Chemical compounds that inhibited the SOS response, resulted in significantly reduced persister levels but did not independently kill the cells

- These chemical compounds will be further tested at various concentrations to identify the compounds that result in reproducible and nearly complete inhibition of the cells' DNA repair mechanisms.

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