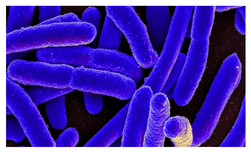
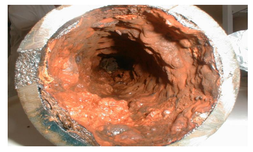
Targeting DNA Repair Mechanisms in Bacterial Persisters

Cristian Oviedo, Maria Bolanos, Sayed Mohiuddin, Adesola Saba, Mehmet Orman

Department of Chemical and Biomolecular Engineering, University of Houston, 77004 TX

Introduction

- Bacterial persisters are responsible for recurrence of bacterial infections such as those that affect patients struggling with lung infections and implanted organs
- Many current antibiotics target growing cell populations but limitations on their ability to target slow growing persister subpopulations has prompted interest in targeting persister metabolic mechanisms via metabolomics
- Recently interest in inhibiting *RecA* enzyme, which catalyzes cell SOS response, used by cells to repair harmful breaks occurring on double strand breaks
- Biofilms are of interest in the medical and industrial fields due to being highly resistant to antimicrobial treatment, leading to recurring infections and financial losses

Objective I

Targeting DNA Repair Mechanism

- One approach to limiting the amount of persisters is by inhibiting the SOS response of bacteria under antibiotic stress
- Fluroquinolones are a class of antibiotic that damages the DNA of bacteria
- 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)

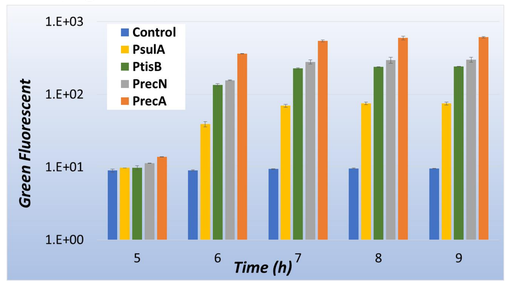


Figure 1. SOS response in *E. coli* cells after ofloxacin treatment. Cells harboring plasmids with green fluorescent protein (GFP) under the control of indicated promoters were treated with ofloxacin in early stationary phase (t = 5h). GFP levels were monitored using a plate reader.
Control: Empty vector

- Inhibition of the SOS response, or impairment of the DNA repair mechanisms has been found to decrease persister levels
- Our objective is to examine the effects of fluroquinolones on bacterial persisters with inhibited *RecA* protein expression, in order to reduce persister levels for applications in the medical and industrial fields

Methods I

Persister Assay

- *Wild type E.coli* and *RecA* cells stock were used to create an overnight culture
- Main culture was treated with ampicillin, ofloxacin, and gentamicin for 6 hours
- Cell suspensions were plated at 1 hour increments

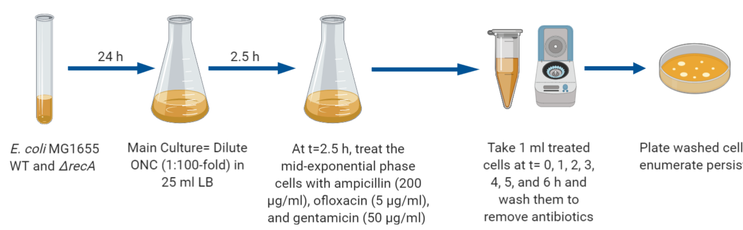
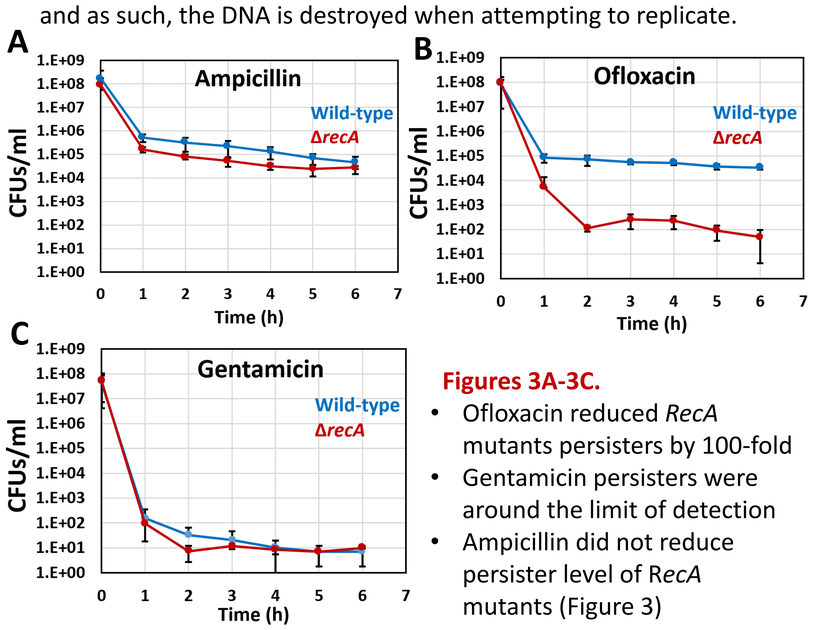


Figure 2. Schematic diagram of persister assay.

Results I

DNA Repair Mechanism

- *RecA* protein degrades the *LexA* protein, which suppresses the SOS response responsible for the DNA repair mechanism in *E.coli*
- Ofloxacin is a fluoroquinolone which prevents DNA from unwinding, and as such, the DNA is destroyed when attempting to replicate.



Figures 3A-3C.

- Ofloxacin reduced *RecA* mutants persisters by 100-fold
- Gentamicin persisters were around the limit of detection
- Ampicillin did not reduce persister level of R*ecA* mutants (Figure 3)

Figure 3. Level of persisters in *E. coli* MG1655 wild-type, and Δ*recA* mutant. Mid-exponential phase cells were treated by (A) Ampicillin, (B) Ofloxacin, and (C) Gentamicin. The data represents 3 biological replicates.

Objective II

Biofilm Persister Distribution

•Quorum sensing is utilized by biofilms to coordinate gene expression, thus regulating physiological activities such as virulence and sporulation
•Biofilms are able to endure much higher concentrations of antibiotic treatment due to a coating of extracellular polymeric substance (EPS) and coordination of gene expression due to quorum sensing
•Our objective is to determine the persister distribution present in *E.coli* biofilms, with the determining factor being color

Methods II

Persister Assay on membranes

- Main Culture was seeded on semi-permeable membrane with agar plate serving as nutrients, and placed in an incubator for a week
- Cells from different regions of the biofilms with different colors were collected and suspended in LB Broth
- Cells were washed in Phosphate-buffered saline (PBS) and treated with antibiotics to measure persister levels (Figure 4)

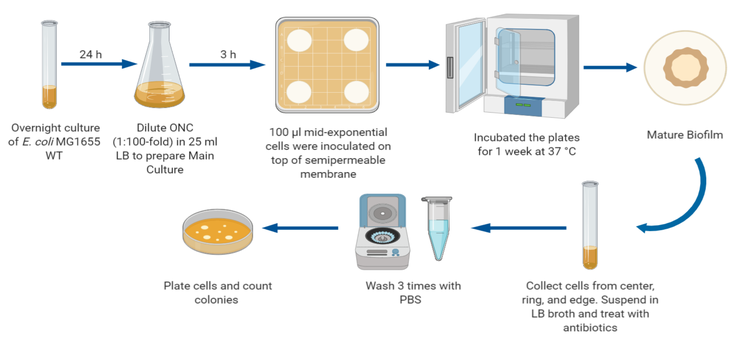


Figure 4. Schematic diagram of persister assay on Biofilm

Results II

Persister Distribution in E.coli

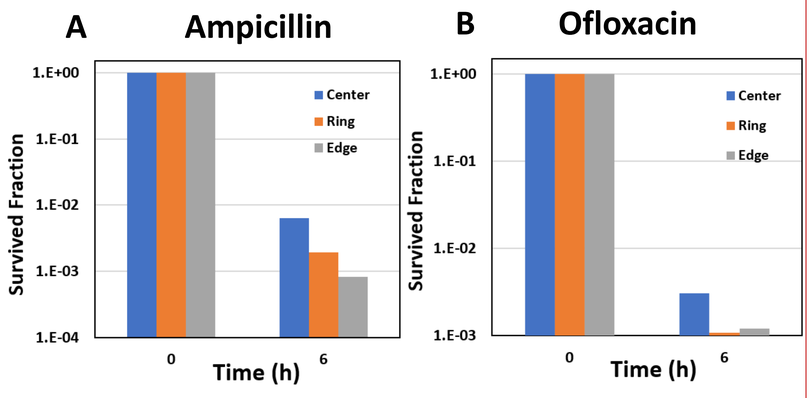


Figure 5. Persister cell levels in the center, ring, and edge regions were not significantly different. This suggests that persisters are randomly distributed within biofilms, despite location and cell density. Biofilm cells were treated by (A) Ampicillin and (B) Ofloxacin.

Figure 6. One-week growth of E. coli Biofilms on semipermeable membranes.

Conclusion

DNA Repair Mechanism

- Bacterial persisters require DNA repair mechanism, specifically *RecA*, during their recovery from fluroquinolone treatment
- The master gene of SOS response, *RecA* is essential for the resuscitation of ofloxacin induced persisters from the transient state
- Impairment of *RecA* protein expression can provide anti-persister therapy for infections treated with fluroquinolones

Biofilm Persister Distribution

- There was no significant difference observed in the level of persisters collected in the center, ring, and edge of the biofilms
- This suggests that persisters are a randomly distributed throughout biofilms despite differences in cell density and region

References

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Acknowledgements

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