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Persister Cell Control Mechanisms in Uropathogenic Escherichia coli

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Persisters are a subpopulation of bacteria that demonstrate high tolerance to antibiotics, but revert to sensitivity after antibiotics are removed. The mechanism for induction of the persister cell state and antibiotic tolerance is not completely understood but likely occurs through the establishment of dormancy. Some of the suggested mechanisms for persister cell formation in Escherichia coli include: toxin–antitoxin systems, starvation, gene regulation by (ppg)pGpp, and stochastic formation. In our study we examine the mechanisms behind persistence in the E. coli strain CFT073, a opportunistic isolate, which forms elevated levels of persisters compared to the laboratory strain MG1655. However, CFT073 lacks many of the type II toxin–antitoxin pairs associated with modulating persister cell formation. In addition, global stress response is impaired in the CFT073 isolate used in these studies, since it contains a five base pair insertion in the rpoS gene, which encodes RpoS, the master regulator of the global stress response. This insertion results in the expression of truncated RpoS. We compared several CFT073 strains mutated at the rpoS locus for the ability to form persister cells, as well as a strain deleted for lon, a protease important for modulating toxin–antitoxin function. To identify additional regulators of persister cell formation in CFT073, we performed mismatch mutagenesis to isolate mutant strains, and screened mutants for persister cell formation. As many pathogenic bacterial species, including CFT073, cause recalcitrant infections attributable to persister cell activity, these studies will identify additional mechanisms underlying the development of bacterial persistence in these organisms.

2. **Persister Cells**

- Persister cells are dormant cells that are able to survive antibiotic attack and repopulate after removal of antibiotics.
- Persister cells result from (Maisonneuve & Gerdes, 2014):
  - Stochastic formation
  - Environmental changes (lack of nutrients)
  - Cellular interactions (quorum sensing and biofilm formation)
- Persister cell formation levels in WT E. coli are approximately 10^-10 to 10^-14 of population in 24 h (Keren et al., 2004).
- Regulators of persister cell formation in E. coli are thought to include (Maisonneuve & Gerdes, 2014):
  - (ppGpp)
  - Toxic–antitoxin systems
  - Stress response pathways
  - Cellular interactions (quorum sensing and biofilm formation)

3. **Persister cell growth patterns**

- Persister cells form stochastically during growth of E. coli and form in greater numbers upon cell stress.
- If antibiotic resistance was acquired through chromosomal or extrachromosomal changes, cells would be expected to survive repeated treatment with antibiotics.

4. **Stress Response Pathway**

- During stress conditions RpoS regulates the expression of stress response genes.
- RpoS is actively degraded by ClpXP during logarithmic growth, but accumulates during stationary phase.
- RpoS transcription is also regulated by toxin–antitoxin systems, which are further controlled by Lon degradation (Wang et al., 2013).

5. **RpoS (αp) in CFT073 contains a five base pair insertion that truncates RpoS**

- A five base pair insertion in the CFT073 rpoS includes a STOP codon resulting in a truncated RpoS.
- The resulting N-terminal fragment is 15 kDa.
- A secondary start codon lies downstream of the inserted STOP codon, which would result in a 20 kDa protein fragment, if made.

6. **CFT073 isolate does not synthesize full-length RpoS during logarithmic or stationary phase**

- Full-length RpoS is not expressed in CFT073 during logarithmic or stationary phase growth.
- RpoS is synthesized in MG1655 and actively degraded.

7. **CFT073 grown in minimal media with ampicillin forms persister cells**

- CFT073 cells were grown in M9 minimal media in the presence and absence of ampicillin. CFUs were determined at various intervals.
- CFT073 produces 10 to 100 fold higher numbers of persister cells compared to these conditions compared to other E. coli strains.

8. **CFT073 contains 3 out of 12 type II toxin–antitoxin (TA) systems found in MG1655**

- Type II toxin–antitoxin systems are attributed to an toxin protein that can bind and inhibit the action of the protein toxin (Maisonneuve & Gerdes, 2014).
- Overexpression of type II toxins results in reduced cell growth and dormancy, excess type II toxins result in increased persistence.
- Single in frame insertion deletions were made using λ red recombineering to address the importance of toxin–antitoxin systems in CFT073 persistence (Datsenko & Wanner, 2000).

9. **ΔhipBA displays reduced persister cell formation in the presence of ampicillin**

- HipBA: Histidine kinases.
- Antitoxin: HipR
- Mutant strains of ΔhipBA displayed high frequency persister formation (Kawano et al., 2009).
- HipBA may aid in mediating cell death in stationary phase.
- Overexpression of HipA inhibits protein, RNA, and DNA synthesis (Koch & Hill, 2006).

10. **ΔyeF/M-yeoB displays reduced persister cell formation in the presence of ampicillin**

- Toxin: YeoB
- Antitoxin: YeF/M
- Shares homology with Aye–Txe system from Enterococcus faecium, which selectively kills bacteria missing a plasmid transfer (Christensen et al., 2004).

11. **Δrpf/v-yhaV displays reduced persister cell formation in the presence of ampicillin**

- Toxin: YhaV
- Antitoxin: Pfr
- Pfr has a swapped–hairpin barrel structure (Schmidt et al., 2007).
- YhaV cleaves mRNA at ribosomal site and is a member of the RfeI super family (Maisonneuve et al., 2011).

12. **Δlon displays reduced persister cell formation in the presence of ampicillin**

- Lon protease regulates toxin–antitoxin systems by proteolysis (Maisonneuve et al., 2013).
- Deletion of lon from MG1655 causes a 10-fold decrease in persistence (Maisonneuve 2011).
- We also observed a 10-fold decrease in ampicillin tolerance of CFT073 Δlon, compared to CFT073 after 24 h with ampicillin.

13. **Comparison of persistence of CFT073 strains after 4 h of growth in ampicillin**

- There is reduction in persister cell formation in all tested strains when compared with WT CFT073.

14. **Live/Dead stain at 6 h confirms viable cells persisting**

- Live/Dead stain shows viable CFT073 cells after 6 h in ampicillin and minimal media.
- Approximately 4% of CFT073 cells at 6 h as dead as ampicillin, while remaining population represents persister cells.
- Preliminary results indicated that ΔhipBA and Δlon cultures contained higher percentage of dead cells (12.1% and 14.3%, respectively).

15. **Conclusions and future directions**

- Our isolate of CFT073 does not synthesize full-length RpoS, suggesting that it is not required for persistence.
- Type II toxin–antitoxin systems seem to play a subtle role in mediating persistence in CFT073 grown in minimal media.
- We plan to restore full-length rpoS in CFT073 and measure persister cell formation.
- We will compare various antibiotics for the ability to induce persistence and construct double and triple toxin–antitoxin deletions to abolish persistence.

16. **References**


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