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Persister Cell Control Mechanisms in Uropathogenic Escherichia coli

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Persister Cell Control Mechanisms in Uropathogenic *Escherichia coli*

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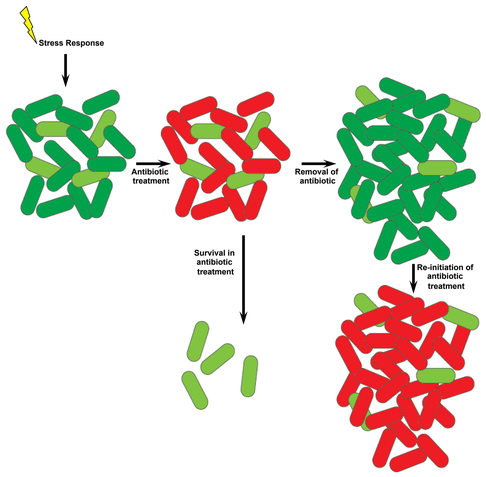
1. Abstract

Persister cells 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 (p)ppGpp, and stochastic formation. In our study we examine the mechanisms behind persistence in the *E. coli* strain CFT073, a uropathogenic 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 minitransposon 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

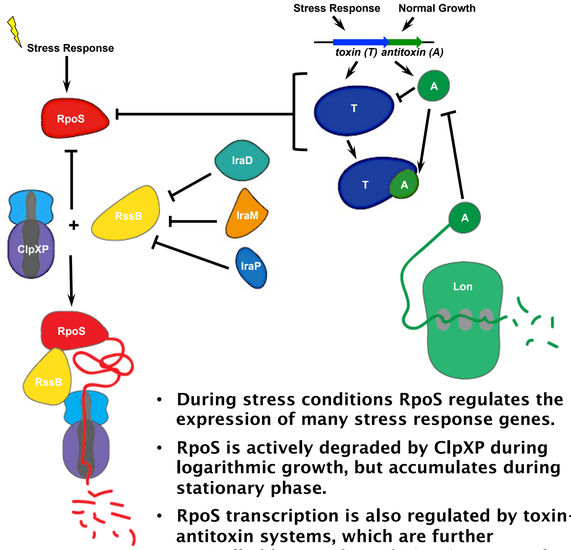
- Persister cells are dormant cells that are able to survive antibiotic attack and repopulate after removal of antibiotics.
- Persister cells result from (Maisonneuve & Gerdes, 2014):
  - Stochastic formation
  - Environmental changes (lack of nutrients)
  - Cellular interactions (quorum sensing and biofilm formation)
- Persister cell formation levels in WT *E. coli* are approximately 10^{-6} to 10^{-4} of the population in LB media (Keren, et al., 2004).
- Regulators of persister cell formation in *E. coli* are thought to include (Maisonneuve & Gerdes, 2014):
  - (p)ppGpp
  - Toxin-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 many 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., 2011).

5. RpoS (σ^S) 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.

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MG1655  ATGAGTCAGAATACGCTGAAAGTTCATGATTTAAATGAAGATGCGGAATTTGATGAGAAC
CFT073  ATGAGTCAGAATACGCTGAAAGTTCATGATTTAAATGAAGATGCGGAATTTGATGAGAAC

MG1655  GGAGTTGAGGTTTTTGACGAAAAGGCCTTAGTAGAACAGGAACCCAGTGATAACGATTTG
CFT073  GGAGTTGAGGTTTTTGACGAAAAGGCCTTAGTAGAAAGGAACCCAGTGATAACGATTTG

MG1655  GCCGAAGAGGAACTGTTATCGCAGGGAGCCACACAGCGTGTGTTGGACGCGACTCAGCTT
CFT073  GCCGAAGAGGAACTGTTATCGCAGGGAGCCACACAGCGTGTGCTGGACGCGACTCAGCTT

MG1655  TACCTTGGTGAGATTGGTTATTCACCACTGTTAACGGCCGAAGAAGAAGTTTATTTTGCG
CFT073  TACCTTGGTGAGATTGGTTATTCACCACTGTTAACGGCCGAAGAAGAAGTTTATTTTGCG

MG1655  CGTCGCGCACTGCGTGGAGATGTCGCCTCTCGCCGCCGGATGATCGAGAGTAACTTGCGT
CFT073  CGTCGCGCACTGCGTGGAGATGTTGCCTCTCGCCGCCGGATGATCGAGAGTAACTTGCGT

MG1655  CTGGTGGTAAAAATTGCCCGCCGTTATGGCAATCGTGGTCTGGCGTTGCTGGACCTTATC
CFT073  CTGGTGGTAAAAATTGCCCGCCGTTATGGCAATCGTGGTCTGGCGTTGCTGGACCTGATC

MG1655  GAAGAGGGCAACCTGGGGCTGATCCGCGCGGTAGAG------AAGTTTGACCCGGAACGTG
CFT073  GAAGAGGGCAACCTGGGGCTGATCCGCGCGGTAGAGCAGAAGTTTGACCCGGAACGTG

MG1655  GTTTCCGCTTCTCAACATACGCAACCTGGTGGATTCGCCAGACGATTGAACGGGCGATTA
CFT073  GTTTCCGCTTCTCAACATACGCAACCTGGTGGATTCGCCAGACGATCGAACGGGCGATTA

MG1655  TCAACCAAACCCGTACTATTCGTTTGCCGATTCACATCGTAAAGGAGCTGAACGTTTACC
CFT073  TCAACCAAACCCGTACTATTCGTTTGCCGATTCACATCGTAAAGGAGCTGAACGTTTACC

MG1655  TGCGAACCGCACGTGAGTTGTCCCATAAGCTGGACCATGAACCAAGTGCGGAAGAGATCG
CFT073  TGCGAACCGCACGTGAGTTGTCCCATAAGCTGGACCACGAACCAAGTGCGGAAGAGATCG

MG1655  CAGAGCAACTGGATAAGCCAGTTGATGACGTCAGCCGTATGCTTCGTCTTAACGAGCGCA
CFT073  CAGAGCAACTGGATAAGCCAGTTGATGACGTCAGCCGTATGCTTCGTCTTAACGAGCGCA

MG1655  TTACCTCGGTAGACACCCCGCTGGGTGGTGATTCCGAAAAGCGTTGCTGGACATCCTGG
CFT073  TTACCTCGGTAGACACCCCGCTGGGTGGTGATTCCGAAAAGCGTTGCTGGACATCCTGG

MG1655  CCGATGAAAAAGAGAACGGTCCGGAAGATACCACGCAAGATGACGATATGAAGCAGAGCA
CFT073  CCGATGAAAAAGAGAATGGTCCGGAAGATACCACGCAAGATGACGATATGAAGCAGAGCA

MG1655  TCGTCAAATGGCTGTTCGAGCTGAACGCCAAACAGCGTGAAGTGCTGGCACGTCGATTCG
CFT073  TCGTCAAATGGCTGTTCGAGCTGAACGCCAAACAGCGTGAAGTACTGGCACGTCGATTCG

MG1655  GTTTGCTGGGGTACGAAGCGGCAACACTGGAAGATGTAGGTCGTGAAATTGGCCTCACCC
CFT073  GTTTGCTGGGGTACGAAGCGGCAACACTGGAAGATGTAGGTCGTGAAATTGGCCTCACCC

MG1655  GTGAACGTGTTCGCCAGATTCAGGTTGAAGGCCTGCGCCGTTTGCGCGAAATCCTGCAAA
CFT073  GTGAACGTGTTCGCCAGATTCAGGTTGAAGGCCTGCGCCGTTTGCGCGAAATCCTGCAAA

MG1655  CGCAGGGGCTGAATATCGAAGCGCTGTTCCGCGAGTAA
CFT073  CGCAGGGGCTGAATATCGAAGCGCTGTTCCGTGAGTAA
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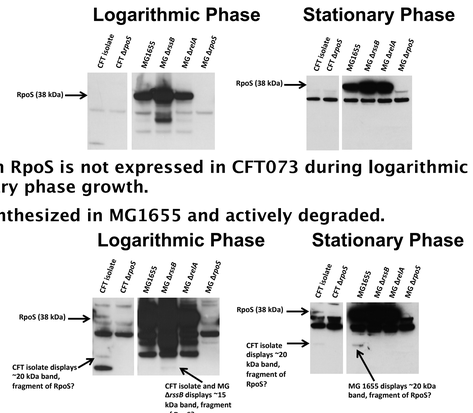
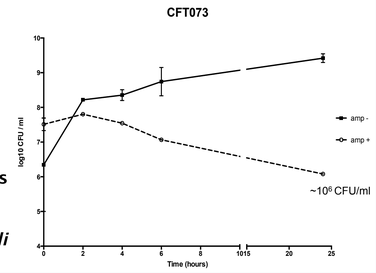
6. CFT073 isolate does not synthesize full-length RpoS during logarithmic or stationary phase



- Full-length RpoS is not expressed in CFT073 during logarithmic or stationary phase growth.
- RpoS is synthesized in MG1655 and actively degraded.
- RpoS fragments are detected in CFT073, but not in CFT *ΔrpoS* during logarithmic and stationary phase.
- RpoS fragments are also detected in MG1655.
- CFT073 does not have active RpoS and must have alternate ways of regulating stress response.

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 under these conditions compared to other *E. coli* strains.



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

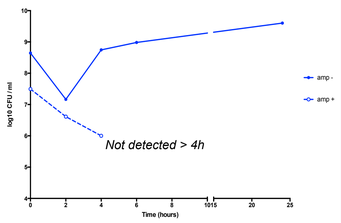
- Type II toxin-antitoxin systems utilize an antitoxin protein that can bind and inhibit the action of the protein toxin (Maisonneuve & Gerdes, 2014).
- Overexpression of type II toxins results in reduced cell growth and dormancy; excess type II toxins result in increased persistence.
- Single in frame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

Type II TA Systems	MG1655	CFT073
dinJ-yafQ	+	-
yafNO	+	-
hicAB	+	-
hipBA	**+**	**+**
relBE	+	-
yefM-yoeB	**+**	**+**
rnlAB	+	-
mazEF	+	-
ygiUT (mqsRT)	+	-
ygiNM	+	-
prlF-yhaV	**+**	**+**
chpB	+	-

Hayes, et al., *Critical Reviews in Biochemistry and Molecular Biology*, 2011

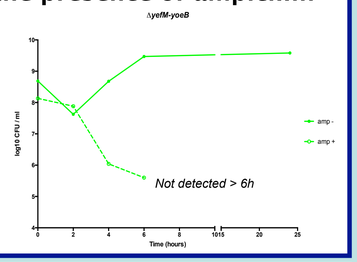
9. *ΔhipBA* displays reduced persister cell formation in the presence of ampicillin

- Toxin: HipA
- Antitoxin: HipB
- Mutant strains of *hip* 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 (Korch & Hill, 2006).



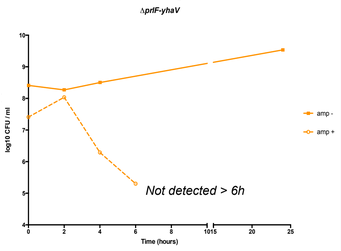
10. *ΔyefM-yoeB* displays reduced persister cell formation in the presence of ampicillin

- Toxin: YoeB
- Antitoxin: YefM
- Shares homology with Axe-Txe system from *Enterococcus faecium*, which selectively kills bacteria missing a plasmid transfer (Christensen, et al., 2004).



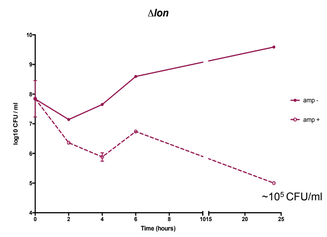
11. *ΔprlF-yhaV* displays reduced persister cell formation in the presence of ampicillin

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



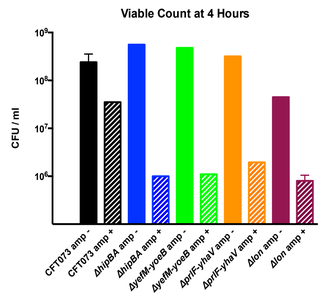
12. *Δlon* displays reduced persister cell formation in the presence of ampicillin

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



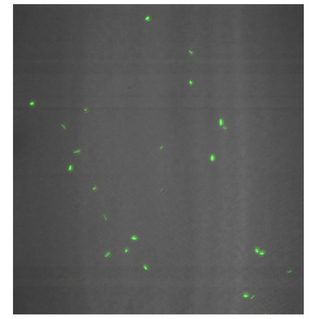
13. Comparison of persistence of CFT073 strains after 4 h of growth in ampicillin

- 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 4.0% of CFT073 cells at 6 h stain as dead cells 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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