Mechanisms of Stress Avoidance and Survival in Vegetative *Clostridioides difficile*

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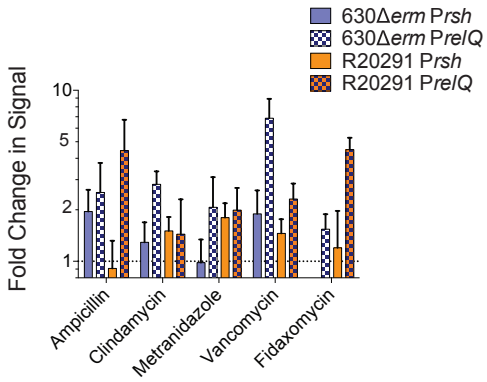
Vegetative *C. difficile* is highly resilient to antibiotic and immune stress

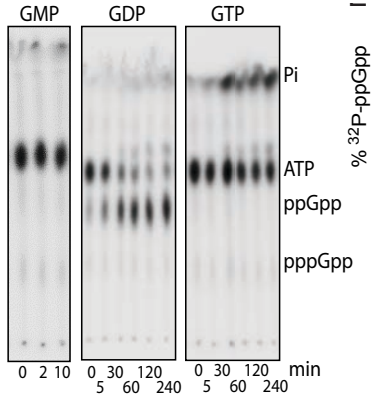
- *C. difficile* encodes (p)ppGpp metabolizing enzymes necessary for the bacterial stringent response (SR), which are induced by antibiotic stress and starvation
  - nutrient abundance reduces flagellar swimming velocity and stimulates biofilm formation
  - we are investigating whether the SR regulates motility and/or biofilm in *C. difficile*
- Unlike other Gram positive (p)ppGpp synthetases, RSH_{Cd} cannot utilize GTP as a substrate, but is remarkably promiscuous in its use of cationic cofactors
  - This may be a strategy to evade immune-mediated metal scarcity ('nutritional immunity')
- The RSH inhibitor Relacin or RNA silencing of the *rsh* gene sensitize *C. difficile* to metranidazole

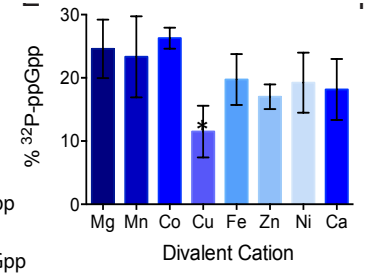
Antibiotic stress induces (p)ppGpp synthetase genes



Fold-change in activity of a fluorescent transcriptional reporter after sublethal antibiotic stress is drug- and strain-specific.

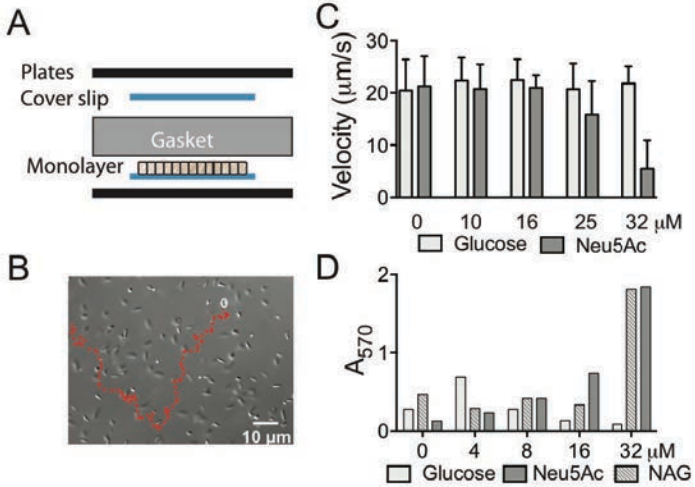
RSH uses GDP exclusively but utilizes diverse metal ion cofactors





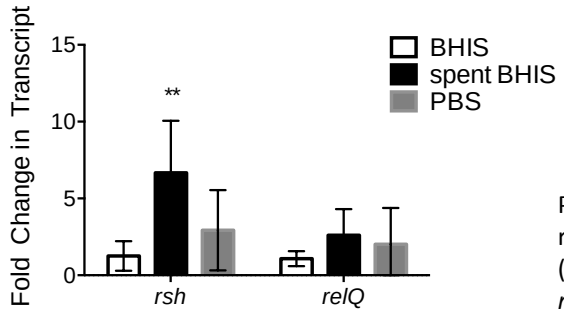
Only Cu^{2+} significantly reduces activity compared to Mg^{2+}.
* $p < 0.05$ by unpaired t test

Nutrients affect motility and biofilm formation



(A) Rose chamber for anaerobic live-cell microscopy. (B) Single-cell motility analysis reveals tumble frequency and swimming velocity. (C) Glucose and mucus component N-acetylneuraminic acid slow swimming. (D) Neu5A and mucus component N-acetylglucosamine stimulate biofilm formation.

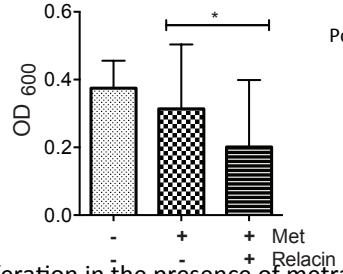
Nutrient deprivation induces SR genes



Fold-change in SR gene transcript levels measured by qRT-PCR after exponentially growing *C. difficile* 630Δ*erm* is incubated in fresh media, spent media, or PBS for 30 min. Spent media depleted in nutrients and enriched in waste metabolites induces *rsh* transcription; PBS devoid of nutrients does not have a statistically significant effect. ** $p < 0.01$ by one-way ANOVA.

RSH inhibition or *rsh* silencing reduce *C. difficile* proliferation under antibiotic stress

Pokhrel *et al*. JBact 2020.



Proliferation in the presence of metranidazole is reduced by co-incubation with RSH inhibitor Relacin (above) or inducting of silencing RNA complementary to *rsh* (below).