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A high-throughput platform to quantify bacterial defense against protist predation

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Background

- Protists are major predators of bacteria and thus a selection pressure towards the evolution of defense mechanisms, many of which are known[1] (Figure 1)
- "Microbial dark matter" (unannotated genes) may play roles in natural settings, such as interactions and predation
- No quantitative high-throughput assays exist to screen large strain libraries for predation defense
- Screening a large number of isolates may reveal new predation defense mechanisms that contribute to microbial ecosystem shaping

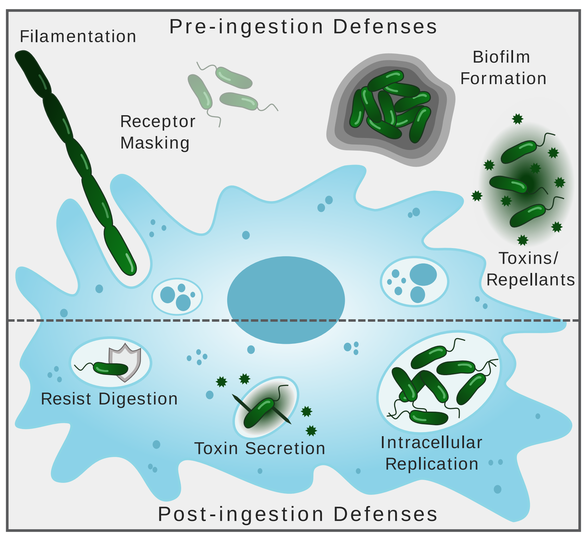


Figure 1. Prototypical bacterial defenses against protist predation

Aims

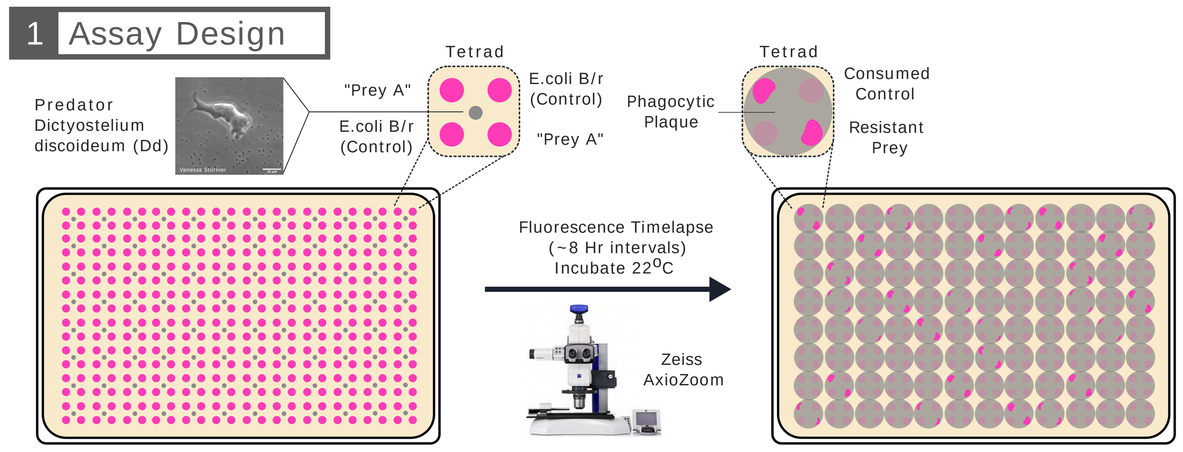
1. Develop a fluorescence plate-based assay to screen for bacterial defense against Dictyostelium discoideum predation

2. Screen a global library of Escherichia coli isolates for predation defense

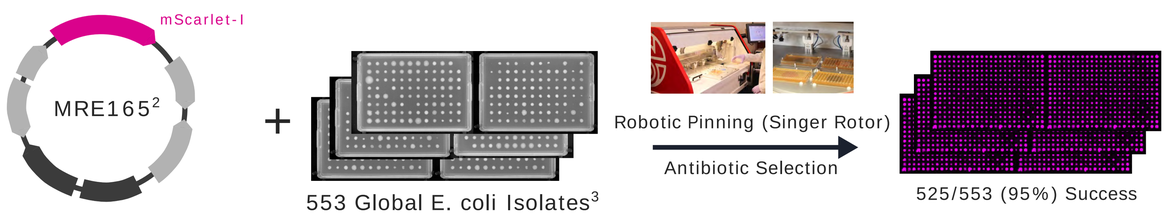
3. Validate the assay with gain and loss of function genetic screens

Methods

1 Assay Design



2 Reporter Conjugation



3 Quantification

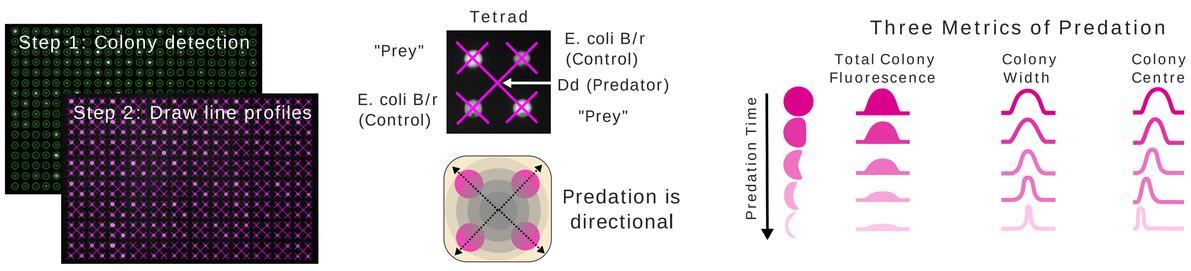


Figure 2. Protist predation assay design and quantification. "Tetrads" with duplicates of control and test prey strains are analyzed separately. The predator and prey are robotically pinned on SM/5 agar plates. D. discoideum spreads on the agar surface and consumes fluorescently labelled bacteria, the signals of which are recorded and processed in three ways to get rates of predation.

Results

Total Colony Fluorescence Measurement Validly Identifies Resistant Isolates

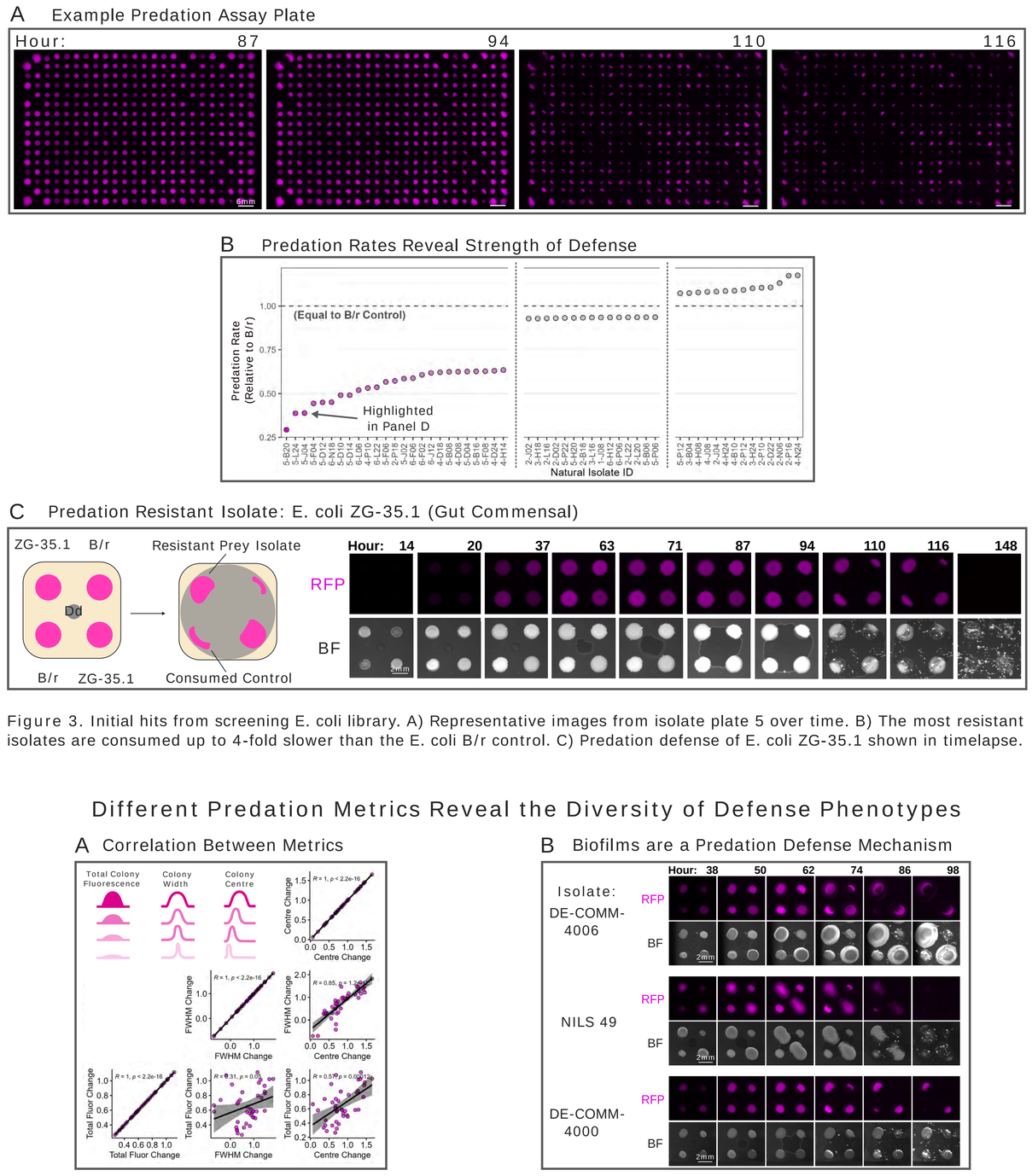


Figure 3. Initial hits from screening E. coli library. A) Representative images from isolate plate 5 over time. B) The most resistant isolates are consumed up to 4-fold slower than the E. coli B/r control. C) Predation defense of E. coli ZG-35.1 shown in timelapse.

Different Predation Metrics Reveal the Diversity of Defense Phenotypes

Figure 4. Diverse defense strategies exist in the E. coli isolates. A) All vs all correlation suggests differences in how each metric scores defense. B) Some defense phenotypes are related to biofilm formation.

Key Outcomes

- Developed and validated a high-throughout quantitative assay to screen for bacterial isolates with defense against protist predation
- A spectrum in strength and diversity of predation defense phenotypes was observed in a global collection of 553 E. coli isolates
- This assay will be useful for any bacteria-protist pairs that can grow on an agar surface

Outlook

- Gain- and loss-of-function genetic screens to identify predation defense genes
- Examine effect of defense genes on single cell predator-prey interactions
- Screen libraries of soil bacterial isolates for resistant isolates

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We're curious to try other protist-bacteria combinations. If you have bacterial strain libraries or a model protist, and you'd like to find resistance mechanisms, reach out to us!

References

1. Jousset A. 2012. Environ. Microbiol. 14(8): fiac057. DOI: 10.1093/femsec/fiac057

2. Schlechter et al. 2018. Front. Microbiol. 9: 3052 DOI: 10.3389/fmicb.2018.03052

3. Galardini et al. 2017. eLife. 6: e31035. DOI: 10.7554/eLife.31035

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