Escherichia coli resistance against 'living antibiotic' *Bdellovibrio bacteriovorus*

Authors:

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Abstract

The rise of antimicrobial resistance (AMR) in bacterial pathogens requires investigation of alternative treatments like predatory bacterium *Bdellovibrio bacteriovorus*. This predator kills and feeds on many AMR Gram-negative pathogens of the WHO priority pathogens list as prey (Fig. 1). *B. bacteriovorus* has potential as a 'living antibiotic' as it is non-pathogenic, has low cytotoxicity and elicits only a minimal immune response. To use this predator and to better understand its impact on microbial populations, it is essential to determine whether a bacterial prey could develop resistance to *B. bacteriovorus.* To address if and how fast bacterial prey resistance arises and determine its potential genetic determinants, we performed an experimental prey evolution study. We exposed *Escherichia coli* as prey intermittently to predator *B. bacteriovorus* in alternate cycles, involving a predation phase where prey was exposed to different predation pressures, followed by a recovery phase, where surviving prey was grown in absence of the predator. After the experimental prey evolution, we compared the growth of evolved prey lineages versus the ancestor. We found evidence for media adaptation under no-, partial resistance under low-, and strong resistance under high-predation pressure respectively. We found that lineages which evolved without predation pressure were susceptible to predation, suggesting that resistance adaptations are specific to predation and not media. When comparing the growth rate of evolved prey lines to ancestor in the absence of predator, we observed a trade-off between predation resistance and fitness. To investigate the genetic basis of resistance, we sequenced the genomes of evolved prey lineages and compared them to the ancestor prey. We identified resistance mutations primarily associated with the outer membrane. *E. coli* gene knockout mutants confirmed that a small subset of genes was mainly responsible for the resistant phenotypes. Altogether our study reveals predation

resistance is associated with outer membrane changes in prey bacteria. While further studies are required to investigate predation resistance development of clinical AMR pathogen strains in differentiated cell culture, our study represents a basis for the development of an alternative, sustainable AMR treatment.

A preprint of our study is available at:

https://www.biorxiv.org/content/10.1101/2024.09.27.615459v2

Fig.1:

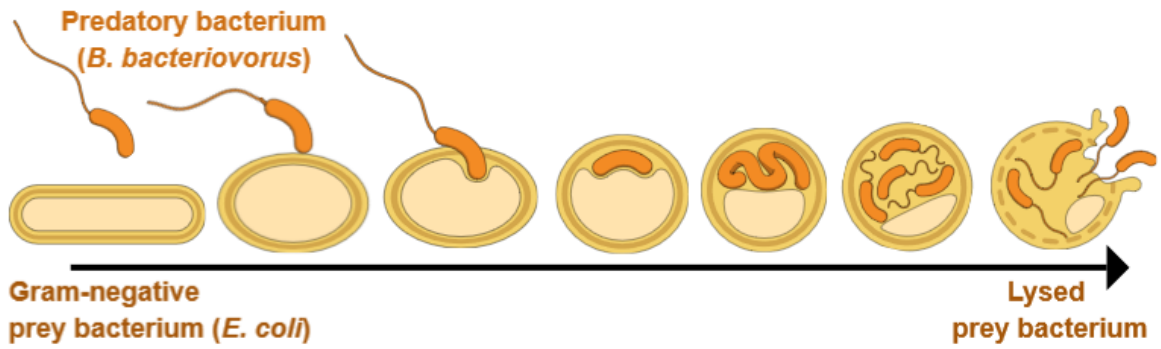

Fig.1: legend:

Fig. 1: Predatory bacterium *Bdellovibrio bacteriovorus* kills, feeds and replicates in the Gram-negative bacterium as prey, which can be an AMR pathogen. In our evolution study model organism *Escherichia coli* was used as prey.