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The Predation Resistance of Squashed E. coli

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Changes in *E. coli* Susceptibility to Predation by Predatory Bacteria

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Introduction

Bdellovibrio are predatory bacteria that invade and digest Gram-negative bacteria, such as *E. coli*. *Bdellovibrio* strain HD100 efficiently kills *E. coli* ML35, but *Bdellovibrio* NC01 is less successful at killing this prey. We observed the *E. coli* prey population recover after 24 hours in the presence of NC01 and adopt an altered colony morphology, which we describe as “squashed”. These “squashed” *E. coli* are less susceptible to predation by NC01, but the mechanism underlying this predator-specific change in susceptibility is not known.

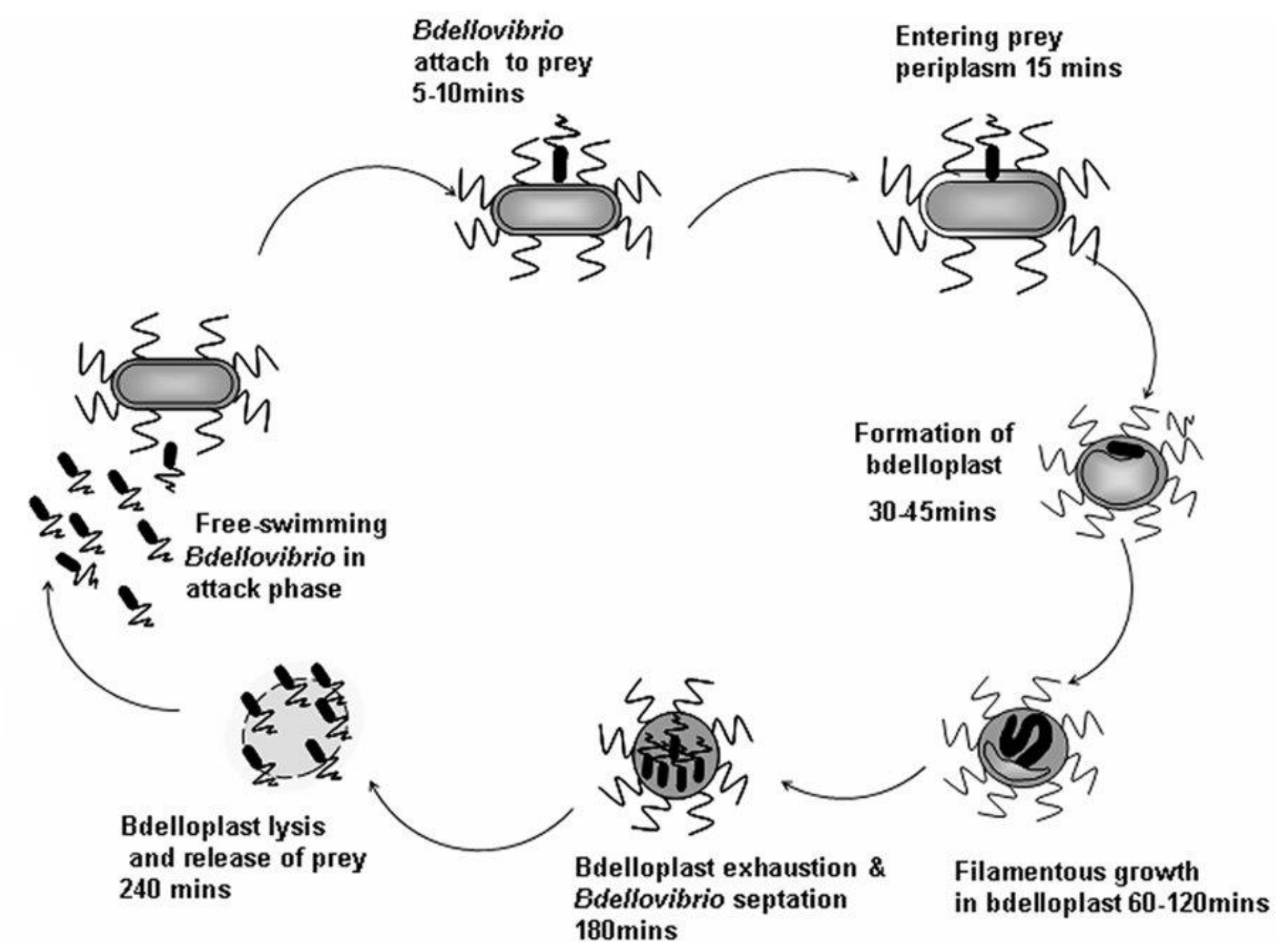


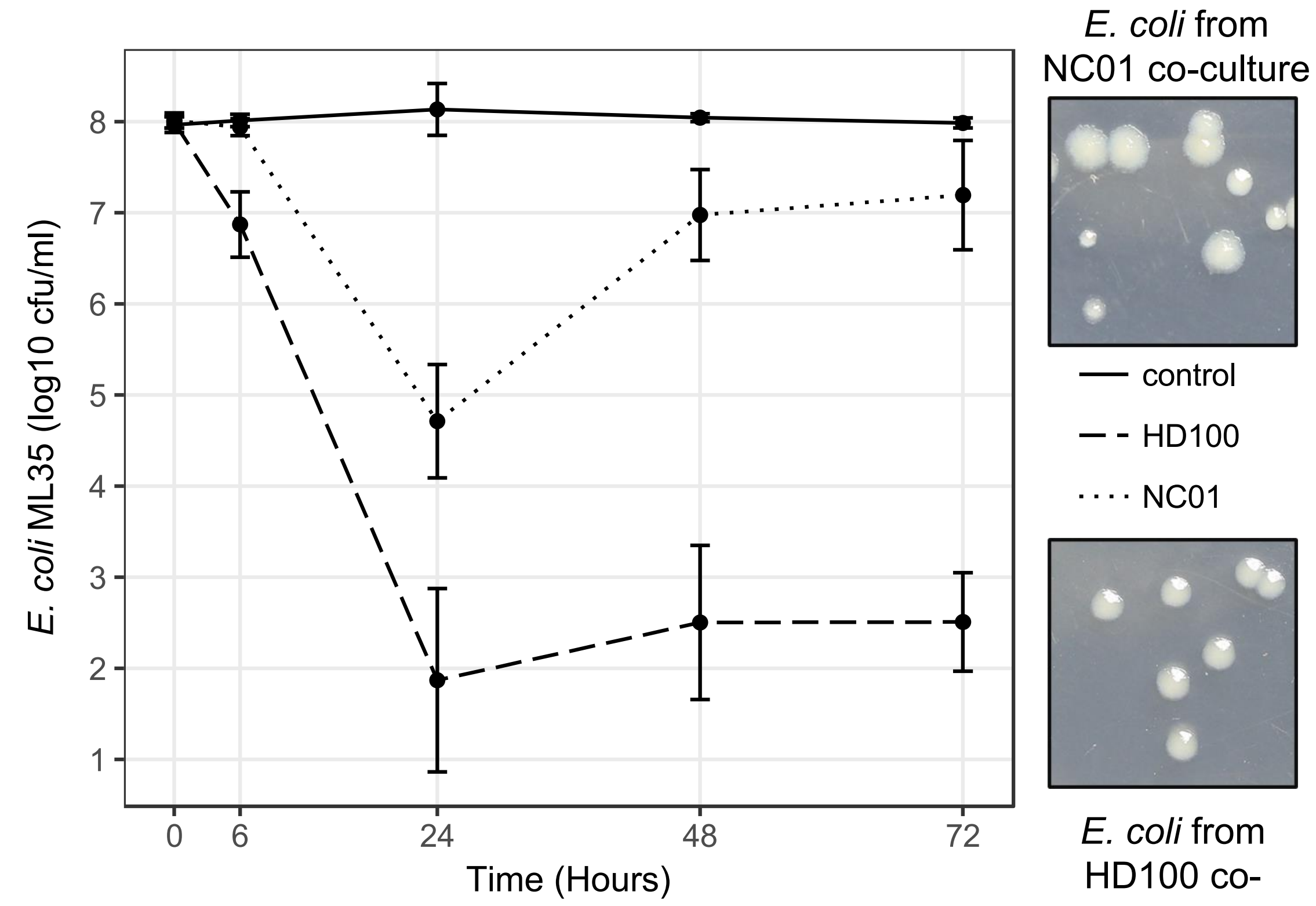
Figure from Lambert et al. 2010

E. coli replicate lines

Run	Name	Phenotype
	ML35	Ancestor Strain
1	0130	Squashed
	0131	Regular
2	0132	Squashed
	0133	Regular
3	0134	Squashed
	0135	Regular

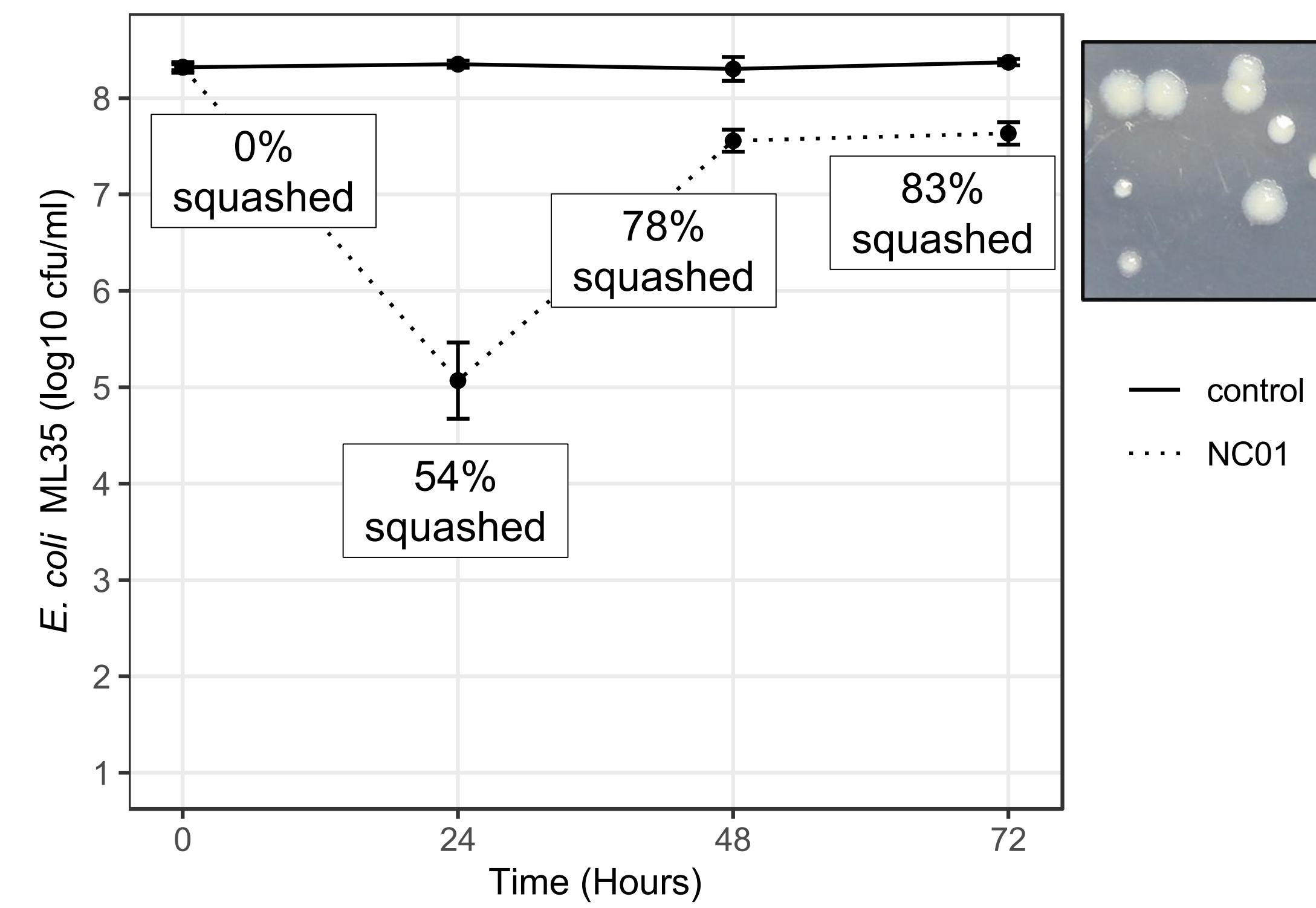
To make stocks, we picked single colonies from spread plates of 48 h co-cultures with NC01, performed two rounds of streaking, and then inoculated an overnight culture.

What is the outcome of the different predatory bacteria strains on *E. coli*?



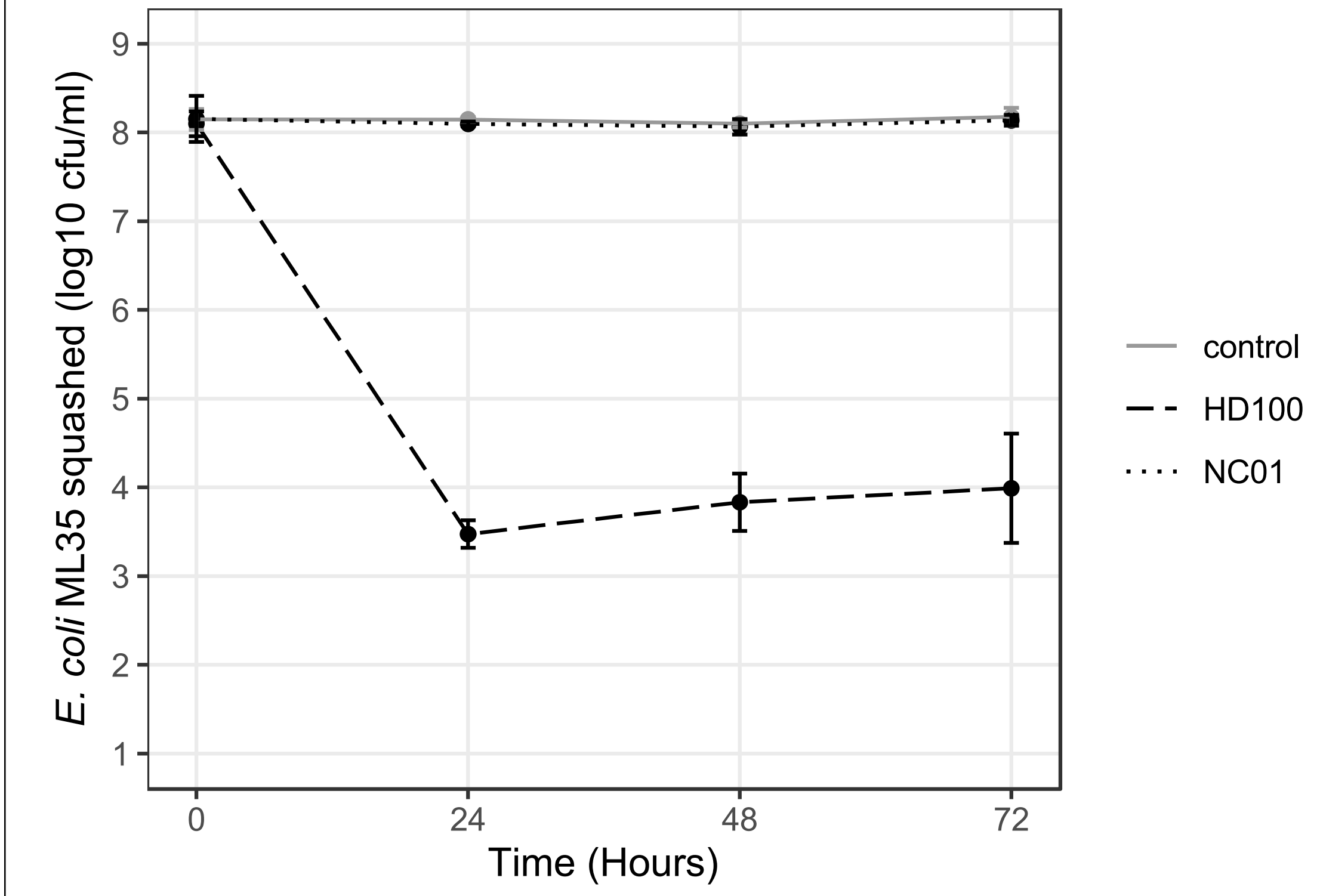
HD100 dramatically reduces the *E. coli* prey population, whereas NC01 causes a smaller reduction, after which the prey population recovers. We observed the appearance of a “squashed” colony morphology when *E. coli* was co-cultured with NC01, but not HD100.

Is the squashed phenotype correlated with *E. coli* population recovery?



As the *E. coli* prey population recovers over 72 hours of co-culture with NC01, the proportion of squashed colonies increases.

Are squashed *E. coli* less susceptible to predation?



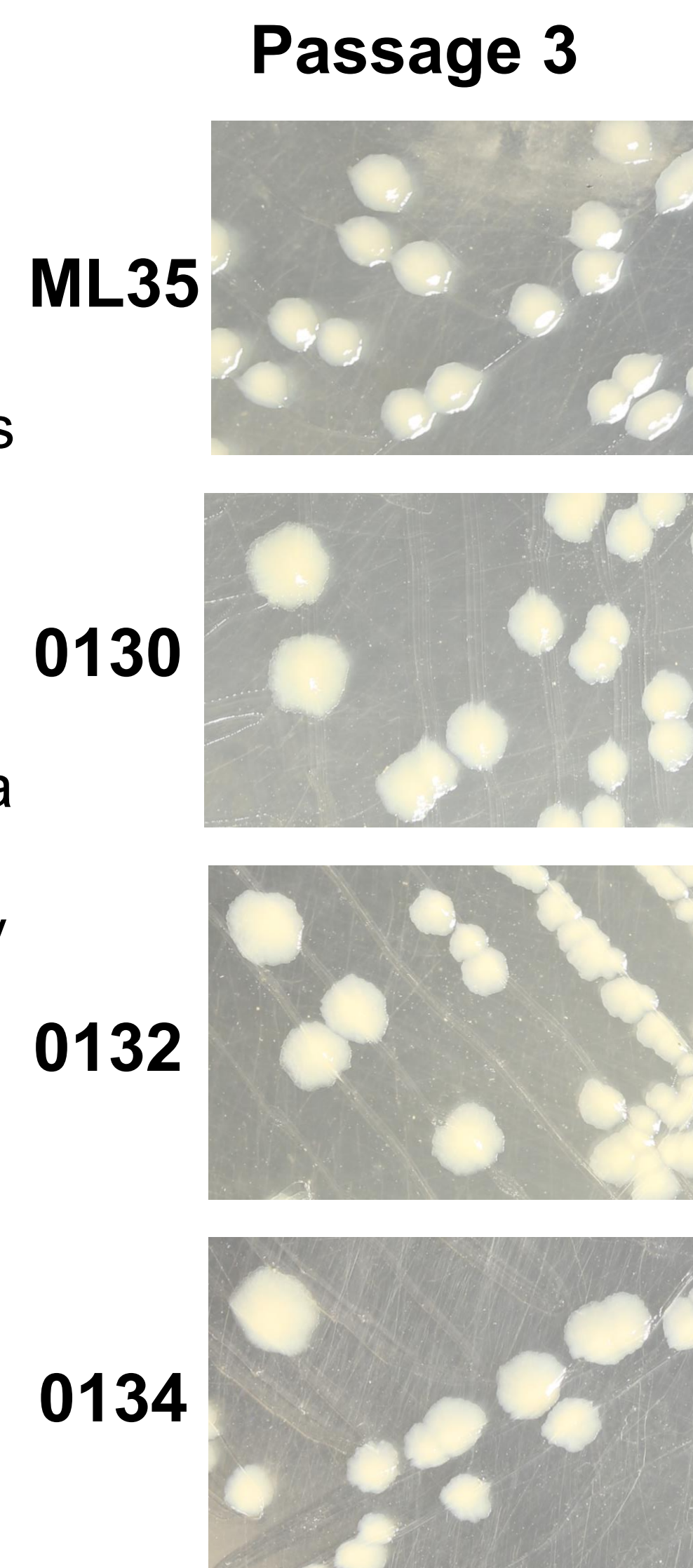
When co-cultured with HD100, squashed *E. coli* are killed with similar efficiency as the original strain. However, NC01 are unable to kill squashed *E. coli*, suggesting a predator-specific defense mechanism.

Does the squashed phenotype persist in the absence of predatory bacteria?

“Squashed” *E. coli* (0130, 0132, 0134) retains its altered colony morphology after multiple periods of growth on TSA plates without *Bdellovibrio*.

This suggests that a heritable genetic change may underly *E. coli*'s change in susceptibility to predation by NC01.

Each image is representative of entire plate.



Is there a common mutation across all three squashed lines of *E. coli*?

Squashed *E. coli*

Name	Mean Coverage	% Mapped Reads
0130	263.8	90.9%
0132	237.6	94.0%
0134	215.5	93.8%

Regular *E. coli*

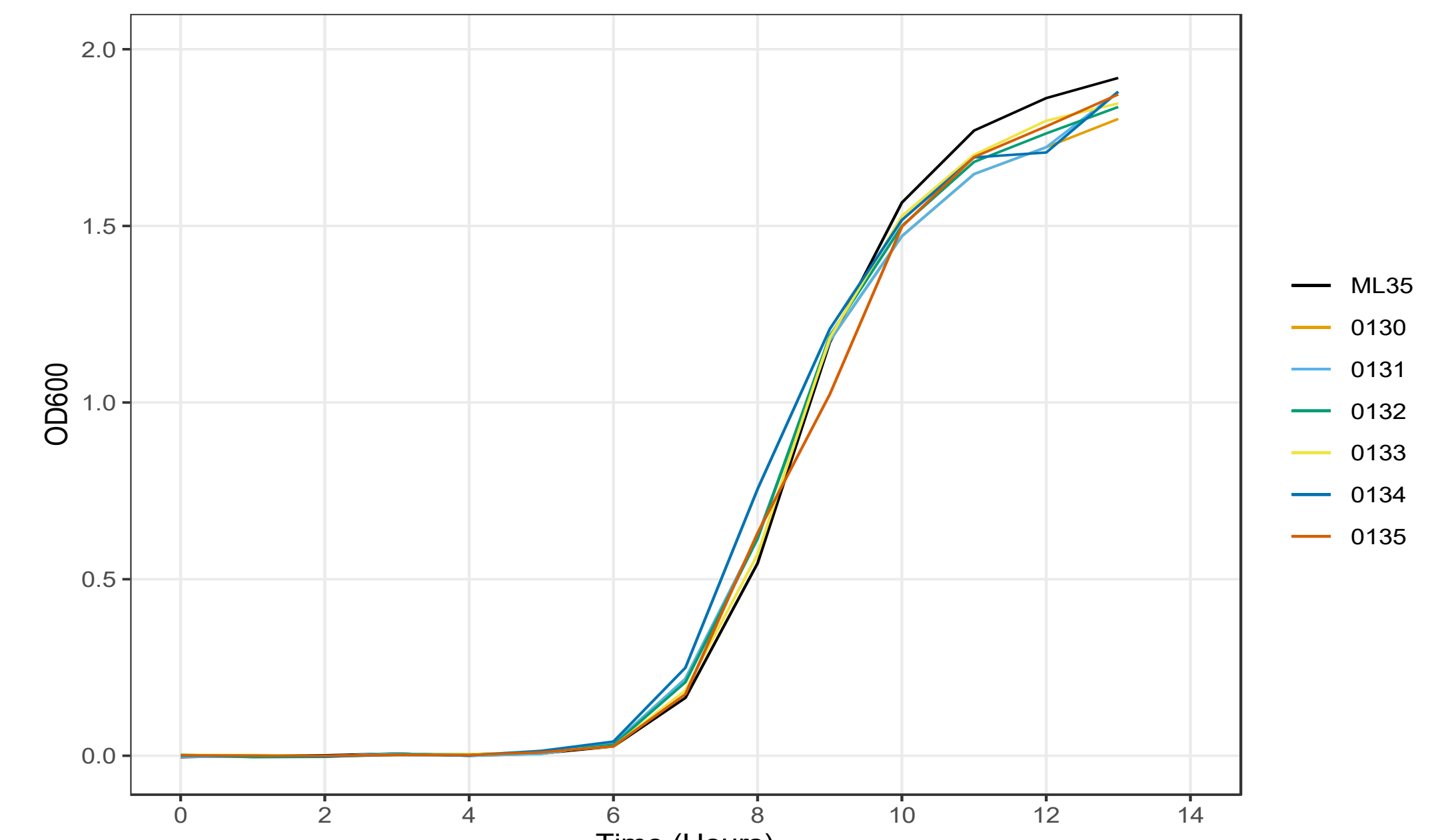
Name	Mean Coverage	% Mapped Reads
0131	227.3	91.5%
0133	239.8	93.3%
0135	214.1	94.8%

To determine if a common mutation was shared among the three squashed lines of *E. coli*, we compared the genomes of each squashed and regular line from a replicate pair to the original *E. coli* ML35.

The analysis did not identify any shared mutations exclusively among the squashed lines and not found in the regular lines. These data indicate that the cause of the squashed colony morphology and reduced susceptibility to NC01 predation is not a simple mutation.

Moving forward, we are analyzing the sequencing data for larger structural variants.

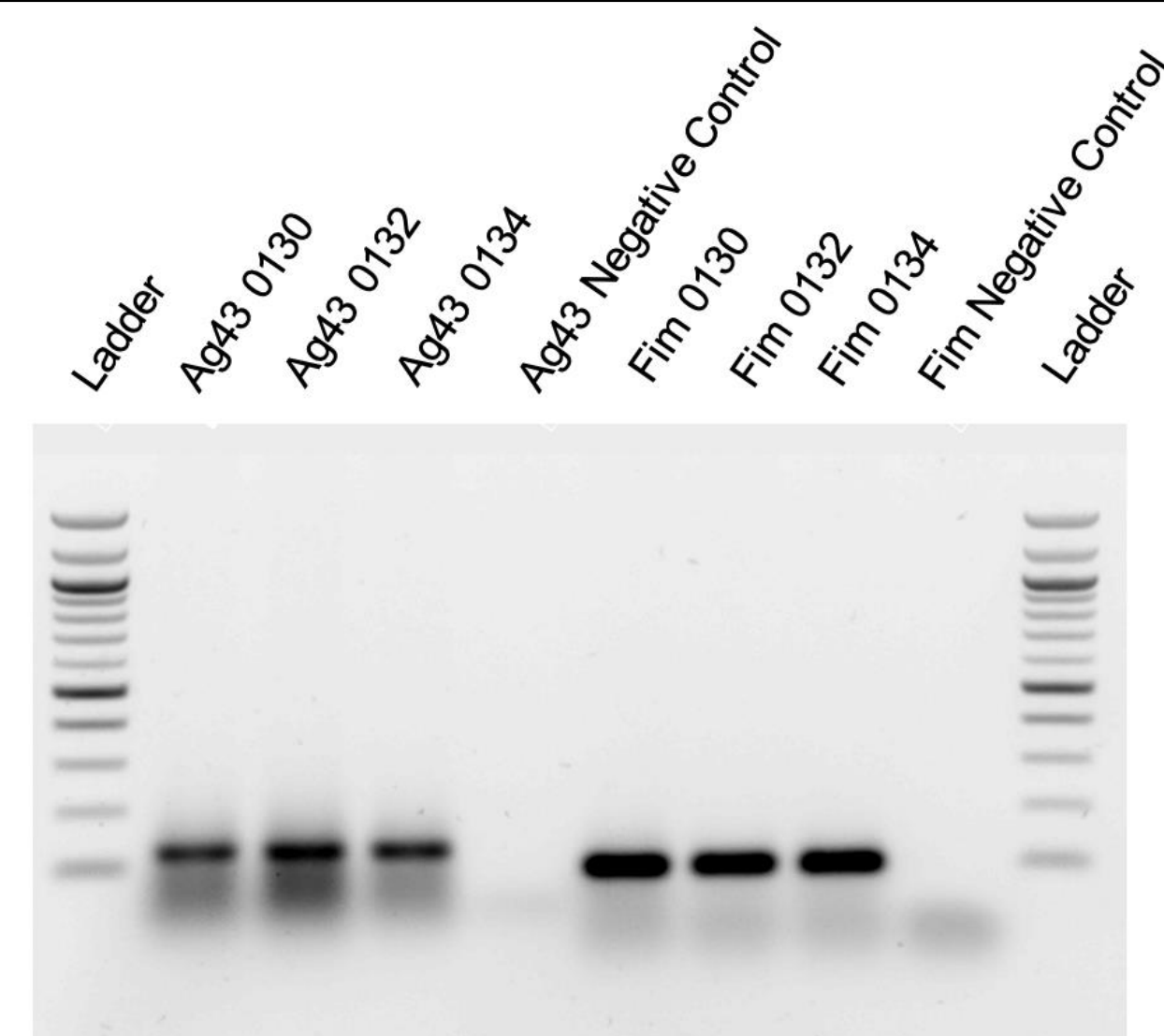
Does the squashed phenotype change growth rate?



We did not detect any substantial differences in growth rate among the original strain of *E. coli*, the three “squashed” lines, and the three regular lines. (Preliminary data from one replicate.)

Are there changes in squashed *E. coli* gene expression?

- We suspect that the mechanism underlying *E. coli*'s morphology change and reduced susceptibility to predation is due to an altered surface protein such as called Antigen 43 or Type 1 Fimbriae.
- To determine if Antigen 43 or Type 1 Fimbriae is responsible, we will examine gene expression using PCR and qPCR.
- We verified primers to target Ag43 and Fim using PCR and gel electrophoresis and plan to begin quantifying gene expression with qPCR.



Acknowledgments

We thank Karla Martinez and Molly Oser for previous work on these projects. This research was supported by an Institutional Development Award (IDeA) from the National Institute of General Medical Sciences of the National Institutes of Health under grant number P20GM103430 and by a Providence College Summer Undergraduate Research Grant. This material is based upon work conducted at a Rhode Island NSF EPSCoR research facility, the Genomics and Sequencing Center, supported in part by the National Science Foundation EPSCoR cooperative agreement number EPS-1004057. We are grateful to Janet Atoyan at the Genomics and Sequencing Center at the University of Rhode Island

References

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Conclusions

- Our initial observation of *E. coli* prey population recovery during co-culture with NC01 led to further examination of the squashed *E. coli* colony morphology.
- This phenotype was correlated with population recovery.
- Co-culturing squashed *E. coli* with NC01 and HD100 further supports that the squashed phenotype is related to decreased susceptibility to predation.
- The mechanisms underlying this phenotype is unknown.

Defining the mechanisms governing changes in bacterial susceptibility to predation is important to understanding predator-prey interactions and informing development of *Bdellovibrio* as a clinical therapy against antibiotic-resistant bacterial pathogens.