Commensal Bacterial Colonization of the Intestine

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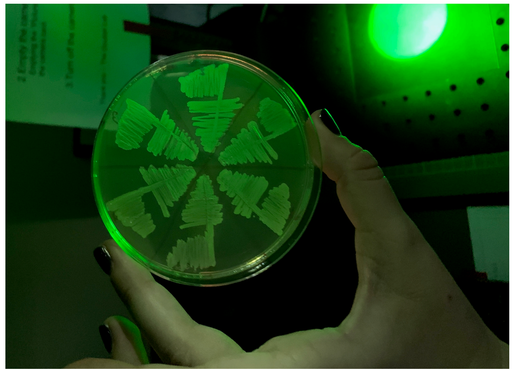
Introduction

- Although scientists have long known about the symbiotic relationship between humans and the commensal bacteria found in our gastrointestinal tracts, researchers in the field are still uncertain as to how these microbes colonize and thrive.
- This research investigated the potential mechanisms through which commensal bacterial strains can colonize, such as protease secretion, expression of adhesion proteins, and swimming motility.

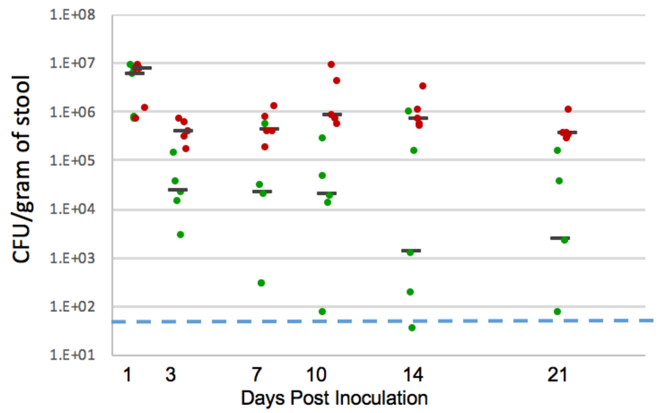
Methods

- All the colonization experiments utilized some form of commensal mouse *Escherichia coli* strain known as MP1 which eliminates the use of antibiotics that could lead to possible GI disruption.
- MP1 strains were marked with either GFP or mCherry to assist with visualization.
- Each mouse was inoculated with 100 uL containing 10^10 bacteria.
- To determine the *E. coli* CFU count, 3-4 fecal pellets were collected at days 0, 1, 3, 7, and then every seven days. The fresh feces samples were weighed and resuspended in PBS as a slurry to reach a final concentration of roughly 0.25 g of feces per 1 ml PBS. Culture samples were then plated onto LB agar, and incubated overnight at 37°C.

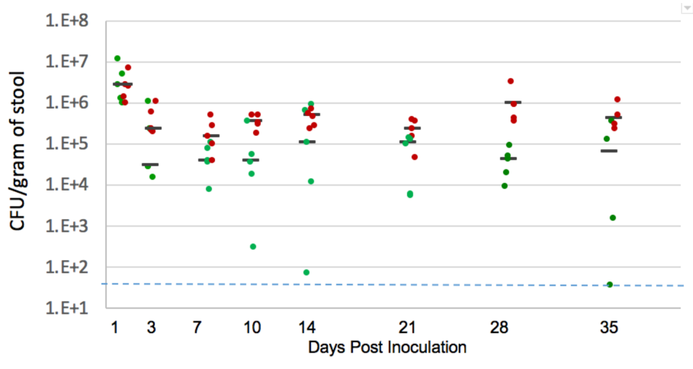


Results

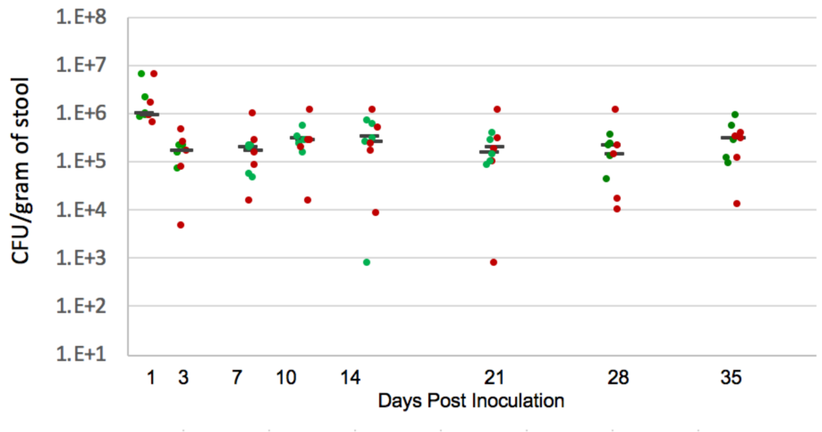
- We first chose to investigate PicU, a serine protease that can be secreted by the bacterium. PicU has been shown to break down substances in the intestines including mucin and pepsin.
- As soon as three days post-inoculation, the PicU KO strain (green) counts dropped significantly when compared to the WT strain (red).



- Another promising secreted protease in bacteria is SsIE (Secreted and surface associated lipoprotein). This metalloprotease has also demonstrated mucinase abilities.
- The double mutant (green) contained both a PicU and SsIE KO, yet displayed similar properties as the previously discussed single PicU KO when compared to the WT (red).



- We then chose to investigate two additional proteins simultaneously: flagellin (FliC), the primary component of bacterial flagella; MAM^{HS}, a multivalent adhesion molecule that assists with bacterial attachment.
- In this experiment, the double mutant (green) did not show significant variation from the WT (red).



Conclusions and Future Studies

- Our results demonstrate that PicU KO strains do not colonize in the mouse intestine as well as WT does. This suggests that PicU may play an important role in bacterial colonization, such as mucin degradation.
- We hypothesize that the MP1 strain has several redundant mechanisms for colonization. A future study could involve deletions in several genes, such as a quadruple FliC, MAM, PicU and SsIE KO strain.
- There are many other potential proteins involved with colonization. Therefore, a large-scale genetic screen can provide many new leads for individual studies.

Acknowledgments

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