Taking a Closer Look at the Disturbed Human Gut Microbiome: A Study of the Interplay between *E. faecalis* and variant *E. coli M*utants.

Senior Project

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Abstract

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This research project aims to identify the interplay between various *E. coli* strains (isolated from mice) and *E. faecalis* both of which play crucial roles in the human gut microbiome. *E. faecalis* can drive inflammation in the gut microbiome (Langfelder, et al. 2019). The mouse isolated *E. coli* that I am working with is being compared to the results of clinical strains of *E. coli* as the project goes on. We know due to Dr. Bleich's sequencing work that more *E. coli* in the mouse model gut microbiome leads to more *E. faecalis*; but the question is: 'Why?' and 'If there are more *E. faecalis* present will it lead to more *E. coli?*'

We also know from other studies as well as Dr. Bleich's sequencing data, that L-Ornithine production from *E. faecalis*' biofilm can act as signaling molecule to increase expression of siderophores in *E. coli*. when Iron is limited (Keogh, Damien, et al. 2016). We experimented with DTPA (Pentetic Acid, metal chelator for Iron) to identify variations in size and morphology in the bacterial colonies. We know the morphologies can change based on genetic and environmental cues as well (Serra, D. O., et al. 2013) which is the purpose for control variables, to maintain consistency throughout experimentation.

Objective

This work is intended to help understand and aid in the treatment of Inflammatory Bowel Diseases in the human gut microbiome such as Crohn's Disease and Ulcerative Colitis. Both of which cause chronic or returning inflammation in the lining of the GI tract and may result in scarring, obstructions, ulcers, fistulas, malnutrition, blood clots and increased risk of colon cancer. It has been found *that E. faecalis* can trigger IBD and is a very prominent microbe found in those with IBD (Zhou, Youlian, et. al. 2016).Considering *E. coli* can prompt *E. faecalis* growth, the correlation between their growth will be an important factor in determining their interplay.

Genetic Variant Purposes

JA 187 is the *E. faecalis* strain that was tested alongside each of the *E. coli* strains. JA 72 (NC 101) is the 'wild type' *E. coli*, meaning that it has no genetic mutations. The other listed strains below have had one or two different knockout mutations induced within their DNA; each signified by a Delta (Δ) symbol.

Strains	Species	Knockouts
JA 187	E. faecalis	N/A
JA 72 NC 101	E. coli	N/A
JA 183	E. coli	Δ ent B
JA 220	E. coli	Δ irpl
JA 223	E. coli	Δ ent B Δ irpl
JA 85	E. coli	Δ ybtx
JA 77	E. coli	Δ fyua
JA 86	E. coli	Δ ybtx Δ fyua

JA 223 and JA 86 are double mutants, they are unable to express two different genotypic traits.

Knockout	Specification
Δ ent B	Enterobactin Production
Δ irpl	Yersiniabactin Production
Δ ybtx	Inner Membrane Importer of Yersiniabactin: Hypothesized for Zinc
Δ fyua	Outer Membrane Importer of Yersiniabactin: Iron & General Chelator

Enterobactin and Yersiniabactin are siderophores produced and utilized for retrieval of iron, a highly necessary micronutrient for *E. coli* bacteria. Adding DTPA or Pentetic Acid molecularly binds Iron ions and makes the micronutrient unavailable for uptake. L-Ornithine production from *E. faecalis'* biofilm can substitute for Iron nutrients for *E. coli* when Iron is limited (Keogh, Damien, et al. 2016). *E. faecalis* is very resistant to Iron deficient environments because manganese serves as a substitute cofactor essential in many cellular enzymes (2016).

Methods

Proximity Plates

Proximity plates are used to determine the growth rates of different *E. coli* isolates in differing proximities to *E. faecalis*. In this plating method, 1 ul of *E. coli* strain is spotted onto the dots on the right-hand side and 1 ul of *E. faecalis* is plated on each dot on the left-hand side of the agar plate. The proximities of the dots grow nearer to show if the *E. coli* will grow better when in closer proximity to the *E. faecalis*.

Mixed Plates

We use mixed plates with *E. coli* isolates on the right side and *E. faecalis* on the left side as controls, and a 1:1 mixture of the two in the middle to observe mixed bacterial colonization. The purpose is to observe whether or not *E. coli* grows better when mixed with *E. faecalis*.

Normalization

Normalization is a process in which live bacterial cultures are scraped off an agar plate and swirled into 1 ml cuvette tubes. After that, they are run through the spectrometer and the OD (Optical Density) readings are taken, then we calculate the dilution of bacteria into LB broth to make the concentration 500 OD. This ensures each liquid vial of bacteria used for plating has the same quantity of bacteria within it and is a control factor for this experiment.

Drip Plates

Drip Plates are made with a stock of bacteria normalized to 500 OD; firstly, we fill a 96 well plate with 90 ul of LB broth in each well, then add 10 ul of liquid bacterial culture to the first well. Then we continuously dilute it by a factor of ten all the way down the well plate and then 10 ul of each dilution factor is spotted onto an agar plate and allowed to drip down the side. This is to see which dilution factor presents the best single colony growth.

Results

Ultimately the data supported the hypothesis that the L-ornithine production did support the growth of *E. coli* in an iron deficient environment considering a popular trend of increased average *E. coli* colony diameter when in closer proximities to *E. faecalis.* The mixed plates show interesting morphologies when cultures are mixed but the colony diameter sizes have not provided any conclusive results as far as a mutualistic symbiosis between co-culture strains.

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