**Name(s)**

**June Bernstein; Samarth Kadaba; Vincent Leong**

**Project Number**

**S1503**

**Project Title**

# The Effects of a Neurotoxin on the Gut Microbiome of Eisenia feita

**Abstract**

**Objectives**

D-limonene, a known neurotoxin to the worm Eisenia fetida, can be used to model neurodegenerative diseases in annelids. When exposed to acute d-limonene, the worms exhibit physical symptoms such as intense writhing and mucus secretion. These underlying side effects hint that the neurological toll d-limonene has on Eisenia fetida and may be indicative of alterations in their gut microbiome. This report presents an approach to monitor the change in the bacterial population of a worm s gut microbiome when the organism is exposed to a neurological stress. The gut microbiome was measured using the worm fecal matter. The feces of intoxicated worms and stock worms were collected and compared. Fecal solutions were grown in order to observe 1) the amount of relative CFU (colony-forming units), 2) the number of different colony morphologies, and 3) the differences in visible concentrations of gram positive and gram negative bacteria in each fecal sample. The goal in analyzing quantitative and compositional changes in the gut microbiome is to draw conclusions about changes in the human microbiome in response to the onset of neurodegenerative diseases such as Parkinson's or Alzheimer's disease. This novel research in the gut-brain axis holds implications for the future where treatments for these human conditions may use the gut microbiome to diagnose or treat symptoms.

**Methods**

Preparation of the Worms

Eisenia fetida worms were obtained from Island Seed and Feed, Santa Barbara. The worms were split into two groups of 10 worms (Groups A and B). Each sample group was isolated from nutrients and starved for 24 hours before being exposed to their respective conditions. Following the starvation period, we weighed and fed 6.000g of soul to Group A and B (Mettler Toledo New Classic MF MS303S). Introduction of the Neurotoxin

Group A was withheld from nutrients for 0.5 hours (control group). Group B absorbed d-limonene vapor for 0.5 hours in a 50 mL container. We added 2.5Lof d-limonene to the filter paper, resulting in a roughly 42.1 ppm concentration in the container. Then, both groups were sterilized using Kimwipes and DI water.

Collection of the Feces

The worms were allowed to excrete for 24 hours and then removed from the container. The feces that remained were collected using an inoculating loop. Approximately 0.001g of feces (Mettler Toledo New Classic MF MS303S) from each group were placed in 7.0mL of LB medium (1 g:700mL). Each sample was duplicated, producing four stock solutions (A1, A2, and B1, B2).

**Summary Statement**

This project models human nuerodegenerative diseases through observation and analsysis of the fecal bacteria in earthworms to establish the prescnece of compositional and quantitative changes in gut microbiome of a stressed organism.

**Help Received**

I would like to thank Baoqing Zhou and Mary McElroy for the assistance in the laboratory. I would also like to thank UC Santa Barbara, Dr. Lina Kim, Ms. Lisa Stamper, Mr. Ben Lopez, Ms. Jen Smith, Summer Discovery, and the Science & Engineering Research Academy for funding our project and providing a