**The Effect of Gastrointestinal *L. plantarum* on Sleep Latency in *D. melanogaster***

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On average, the human gut harbors ~100 trillion microorganisms, and disruption in gut microbiota can result in a litany of physiological and cognitive diseases [1]. The interaction between the gut microbiome, gut, central nervous system, endocrine system, and immune system is referred to as the gut-brain axis [2]. The microbiome interacts with the gut-brain axis through the metabolites of the microorganisms. Recent technological developments have revealed the possibility that the interaction of the microbiome metabolites and the gut-brain axis may be responsible for cognitive and behavioral regulation in the host organism [3]. This claim is bolstered by recent findings that dysbiosis in the gut microbiome is correlated with neurodegenerative diseases such as Parkinson’s and Alzhiemers [3].

*Lactobacillus plantarrum (L. plantarum)* is a probiotic with antioxidant and anti-inflammatory properties that can be found in the gastrointestinal tract of *Drosophila melanogaster*, and humans. Interestingly, *L. plantarum* can secrete multiple neurotransmitters (GABA, Dopamine, Serotonin (5-HT), Glutamate, Histamine and Acetylcholine) which may interact with the gut-brain axis of the host. Preliminary data from our lab shows that young adult flies (with intact microbiome) treated with *L. plantarum* have reduced sleep latency - the time required for the host to fall asleep.

This summer I will be working on a research project to determine if the host sleep latency phenotype observed is due to the presence of *L. plantarum* in the gut. To do this we will generate germ-free (GF) adult female flies using antibiotics. Then these GF flies will be fed either with 5% sucrose (mock treated) or with *L. plantarum* in 5% sucrose*. .* These flies will be placed on DAM2 activity monitors located in a controlled environment to track their movements and sleep patterns for a week. We will analyze the locomotion and sleep data using MATLAB. We expect to observe an increase in sleep latency in the group of GF flies fed mono-associated with *L. plantarum.* This experiment will allow us to solely measure the effect of *L. plantarum* on sleep latency and not on the interaction between *L.plantarum* and the intact gut microbiome.

Next we want to explore the possibility that the effect of *L. plantarum* on sleep latency is through a GABA-specific mechanism. GABA receptor agonists (e.g. benzodiazepines) which enhance GABAergic transmission have been recognized for their sedative properties and ability to induce sleep. Our tentative hypothesis is that *L. plantarum*-derived GABA is detected via receptors found in the intestinal epithelial cells. Therefore, I will work on determining if any of the three described GABA receptors (i.e., *Rdl, Lcch3, Grd)* are expressed in any intestinal epithelial cells and if this is dependent on the presence of *L. plantarum.*  To do this we will be using the UAS/GAL4 system with its two components: the upstream activating sequence (UAS) and the GAL4 transcription factor. The UAS is a DNA sequence that serves as a binding site for GAL4, while GAL4 is a yeast transcription factor. This system will allow me to express a Red Fluorescent Protein (RFP) in a receptor-specific manner. For that I need to generate a cross to obtain progeny that carries the transgenes UAS-RFP and another transgene that expresses Gal4 under the promoter/enhancer of my GABA receptor of interest (i.e., *Lcch3-Gal4, Rdl-Gal4 or Grd-Gal4*). If *L. plantarum* is responsible for sleep latency changes and for the presence of GABA receptors in the intestine, we would like to knock down the receptor to see if this will negate the effect of *L. plantarum* on the host organisms sleep latency.

Finally, I have never independently worked in a lab environment before; previously I was given instructions on all of my tasks. I am prepared to grapple with learning how to trust myself when performing laboratory tasks. I plan to overcome this challenge by doing lots of lab work - I think I will naturally become more comfortable with trusting myself on independent tasks.

**Works Cited**

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