2026 NEOMED Summer Research Fellowship Program

1. **Title**: The Effects of *Lactobacillus murinus* Supernatant on Metabolic Dysfunction-Associated Steatotic Liver Disease (MASLD)
PI: Dr. Jessica Ferrell, PhD; Assistant Professor; jfrancl@neomed.edu; x6468
Location: NEOMED, Department of Biomedical Sciences, Laboratory F-205A

2. **Abstract**: Metabolic dysfunction-associated steatotic liver disease (MASLD) is a progressive disease that begins with steatosis and can develop into metabolic dysfunction-associated steatohepatitis (MASH), hepatic cirrhosis, or hepatocellular carcinoma. The involvement of various metabolic and inflammatory pathways in MASLD suggests that exploring a range of interventions targeting different aspects of the disease is necessary. Probiotics, including live bacteria or the bacterial culture supernatant that contain metabolites, have been proposed to have alleviating effects on the progression of MASLD, primarily by improving liver enzymes, gut microbiome modulation, and reducing inflammation and oxidative stress. Growing evidence suggests that bacterial metabolites, in the form of supernatants and other bacterial extracts, have immunomodulatory properties and are considered safer than live probiotics. *Lactobacillus murinus (L. murinus*) is a gram-positive bile acid hydrolase bacterium with the ability to reduce the transport of bacterial products and reduce markers of systemic inflammation in mice. However, the mechanisms by which this occurs, and the metabolites *L. murinus* produces to exert these effects, are largely unknown. Here, we will determine if administering *L. murinus* supernatant can modify the progression of diet-induced MASLD in mice.

3. **Significance**: Our lab previously reported that mice lacking the bile acid receptor TGR5 had increased steatosis, but reduced inflammation and fibrosis compared to wild type counterparts when fed a high fat, high fructose diet. 16s rRNA sequencing of cecal microbial populations indicated TGR5 knockout mice had decreased relative abundance of several bacteria including *Lactobacillus murinus*. *L. murinus* has been reported to reduce hepatic steatosis and promote fatty acid oxidation and gluconeogenesis in obese mice. Its reduced abundance in TGR5 knockout mice might be associated with their steatotic phenotype. Our aims are to determine if *L. murinus* supernatant alleviates diet-induced MASLD in mice, and to investigate the specific mechanisms of action.

4. **Goals and Objectives**: This will determine the effects of *L. murinus* supernatant in attenuating the progression of MASLD-related symptoms in mice fed high-fat diet. The summer student will learn scientific techniques, experimental design, molecular biology and biochemical analyses, and how to interpret, display, and present scientific results.

5. **Research methods**: Male wild-type mice will be fed a high fat diet for 8 weeks and will be simultaneously gavaged twice weekly with supernatant from cultured *L. murinus*.

Tissues will be collected (blood, brain, liver, intestine, colon, muscle, adipose) and analyses will be performed (lipid and bile quantification, tissue histology, gene and protein expression) to ascertain the metabolic effects of *L. murinus* supernatant and to determine MASLD progression.

6. **Data Analysis**: Appropriate statistical tests including t-test, one-way ANOVA, etc., using GraphPad Prism Software will be performed to determine statistical significance ($p<0.05$). Immunoblotting and immunofluorescence quantification are visualized using ImageJ software**.**
7. **Contribution of Findings**: The findings obtained from this research will provide a better understanding of whether *L. murinus* supernatant can alleviate steatosis and systemic inflammation, and the mechanism of action involved. These findings will also further our understanding of untargeted metabolites produced by *L. murinus* supernatant which have not been published to date, and could provide more insight into its safety, efficacy, and strain-difference effects

8. **Student Fellow Training/Mentoring Plan**: The student will complete safety and lab training modules prior to the start date. The training plan for the students encompasses individual and group mentorship from Dr. Ferrell (mentor) and Ph.D. students who will be available to help instruct in the techniques necessary to complete this research. The student will become familiar with the research topic by reading primary and reviewing journal articles. Basic lab techniques will be introduced through one-on-one instruction and will progress to independent work when appropriate. In addition to lab work, the student will be expected to keep records of the experiments and will learn to interpret the data collected. These results will be discussed with the mentor as necessary and during weekly lab meetings. Additionally, lab members participate in biweekly Diabetes, Obesity, and Metabolism Research Focus meetings, which include data and journal article presentations by graduate students, post-docs and staff. The student will attend these meetings and will have the opportunity to present research results at the end of training program. Lastly, the student will prepare and present a poster of their work at the Summer Research Fellow Poster Day. This work will be conducted in the Ferrell lab at NEOMED in F-205A