Exploring the linkage between behavior changes and the gut microbiome and metabolome in C57BL/6 mice

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Overview

- The intestinal microbiome consists of a enormous bacterial community that resides primarily in the gut and lives in a symbiotic interplay with the host.
- The gut-brain axis, which is a bidirectional neurohumoral inter-communication system, integrates the host gut and brain activities.
- Metabolomics study in altered gut microbiome systems can assist to identify specific metabolites which can influence on brain activities might results in following host behavior changes.

Introduction

- While the interactions between the microbiome and host are essential for determining health and disease states, a detailed understanding of the inter-connected metabolisms of individual microbes and the host is limited.
- This is due to the chemical complexity and inhomogeneity of food sources, the biological variability of hosts, and variations in environmental exposures.
- To begin to address this, we utilized conventional, germ-free (GF), and GF mice inoculated with specific bacteria to understand the complex link between the microbiome, metabolome and behavior changes.
- We performed MS based metabolomics analyses in the mouse studies to measure changes in the fecal, blood, and brain metabolomes.

Methods

Mouse preparation

- Mice were maintained in normal conventional facility, or germfree specific facility in Lawrence Berkeley National Laboratory (LBNL).

C57BL/6

Conventional
Germfree
Germfree-Lactobacillus inoculated
(reuteri, brevis, plantarum)

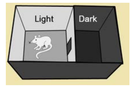
Gnotobiotic mouse facility (LBNL)

Sample collection and processing

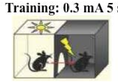
- Blood, brain issue, and fecal samples were collected from three different types of mice based on the status of mouse gut microbiome.
- Samples of fecal, plasma, and brain homogenate were extracted with cold-methanol (-20°C) and the mixture of extracted metabolites was dried and stored at -70°C until GC-MS analysis.
- Brain tissue samples were cyro-sectioned for the FISH imaging.

Memory test

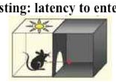
- All the mice in the study were subjected to a test to see their memory function has been affected.
- Mice are nocturnal, and want to move into a dark room. Measuring the latency time to enter the dark room after their first education of electric foot-shock training.



Training: 0.3 mA 5 sec

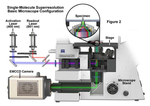


Testing: latency to enter



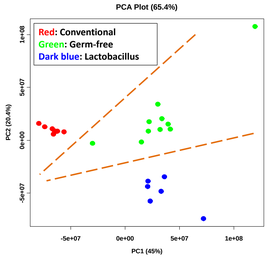
Instrumental analyses

- Extracted metabolites were analyzed by Agilent GC-MSD system and collected data were processed against PNNL metabolomics database contains the information of more than 850 metabolites.
- Sequencing machine was used to confirm germfree status and inoculation of lactobacillus in mice.
- Fluorescent microscope was deployed to view spatial visualizations of metabolites in brain tissue.



Results

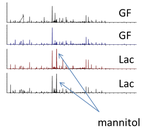
Fecal metabolome profiles of three conditioned mice

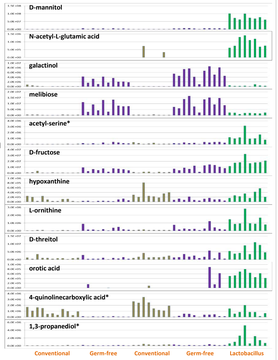


Metabolome profiles from three different types of fecal samples showed clear separation in a principal component analysis (left).

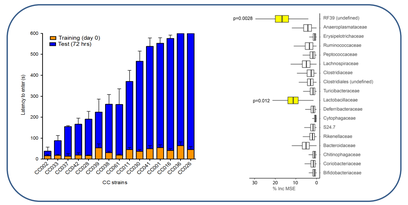
Microbiome contributes metabolome profile significantly and selected metabolites are shown in the right.

Very strong accumulation of mannitol was observed in *Lactobacillus reuteri* inoculated.



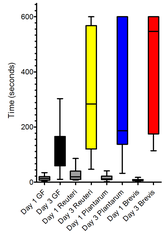


Memory test

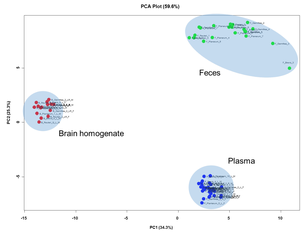


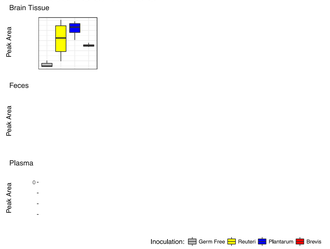
Memory test in conventional mice showed significant variations based on inbred genetic lines (left).

Memory test in germfree and *Lactobacillus* inoculated mice showed significant improvements between them (right).



Metabolome analysis on Lactobacillus inoculated mice



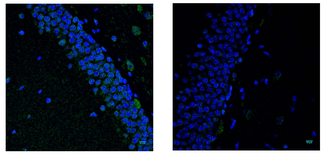


Metabolome profiles from three different sample types over germfree and three Lactobacillus inoculated mice showed interesting data to discuss for further gut-brain axis interpretation.

GABA imaging in brain tissue

*GABA: gamma aminobutyric acid (the main inhibitory neurotransmitter in brain)

Lactobacillus reuteri Germ-free



hippocampus in mice

Blue = nuclei (DAPI)
Green = GABA (fluorescent antibodies)

Treatment	Total cells	GABA cells	%
GF Control male (C2NO)	2876	142	4.9
GF Control male (C2R)	3064	259	8.5
GF Control female (C2LL)	3310	236	7.1
GF control female (C2R)	2125	122	5.7
Reuteri male (NO)	4296	586	13.6
Reuteri male (R)	2254	167	7.4
Reuteri female (C2R)	2823	411	14.6
Reuteri female (C2NO)	1386	326	23.5
Plantarum male (C10L)	1986	331	16.7
Plantarum male (C1R)	1790	281	15.7
Plantarum female(C5LR)	1549	366	23.6

The number of hippocampal cells expressing GABA was significantly higher in Lactobacillus inoculated mice compared with germ-free mice.

Conclusions

- Metabolome profiles were very distinctive in mouse fecal samples from conventional, germfree and germfree-Lactobacillus inoculated.
- Based on the memory test, some Lactobacillus species in germfree mouse gut improve host memory over the control mice.
- Metabolome profiles from three different biological samples showed interesting patterns which need deep interpretations for possible gut-brain axis communication.
- Lactobacillus inoculation on germfree mouse increases GABA accumulation in hippocampus.
- What are the underlying signals between Lactobacillus and the brain? Can this be applied to improve memory in humans?

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References

1. The interplay between the intestinal microbiota and the brain. Collins et al. *Nature Reviews of Microbiology* (2012) 10, 735-742
2. Hippocampal synaptic plasticity, spatial memory and anxiety. Bannerman et al. *Nature Reviews of Neuroscience* (2014) 15, 181-192
3. Influence of early life exposure, host genetics and diet on the mouse gut microbiome and metabolome. Snijders, Langley, and Kim et al. *Nature Microbiology* (2016) 2,16221

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