Investigating the Effects of Novel Probiotics on Alzheimer's Disease Pathogenesis

Victoria H. Anderson
Leah C. Beauchamp, Lily A. Palumbo, Laura M. Cox
Ann Romney Center for Neurological Diseases, Brigham and Women's Hospital
Harvard Medical School

Background

- The gut microbiome has previously been strongly linked to various neurodegenerative diseases, especially Alzheimer's disease (AD), in both humans and mouse models.
- Probiotics have potential to ameliorate AD, but have not been specifically designed for AD.
- Previous studies have shown that AD mice supplemented with short chain fatty acids (SCFAs), including butyrate, had decreased Aβ deposition and tau hyperphosphorylation (Sun, Wang, et al., 2023).
- Patients with AD have been found to have lower levels of butyrate producing bacteria in their microbiome compared to healthy controls (Wu et al., 2021).
- We selected three bacteria which are high butyrate producers and produce various possibly beneficial and neuroprotective metabolites. These bacteria have also shown promising effects in Parkinson's models in our lab.
- These three bacteria were cultured to create the novel TriAD probiotic, that we investigated in this study.

Study Design and Methods

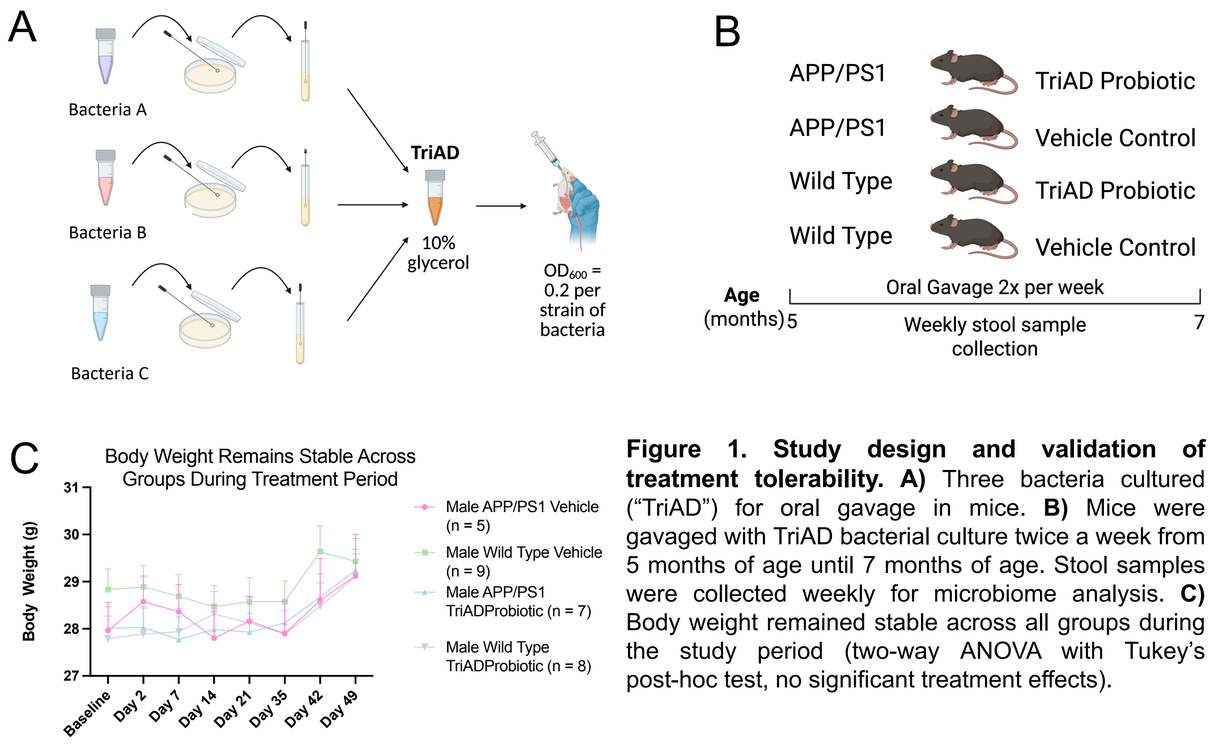

Figure 1. Study design and validation of treatment tolerability. A) Three bacteria cultured ("TriAD") for oral gavage in mice. **B)** Mice were gavaged with TriAD bacterial culture twice a week from 5 months of age until 7 months of age. Stool samples were collected weekly for microbiome analysis. **C)** Body weight remained stable across all groups during the study period (two-way ANOVA with Tukey's post-hoc test, no significant treatment effects).

Metabolomics

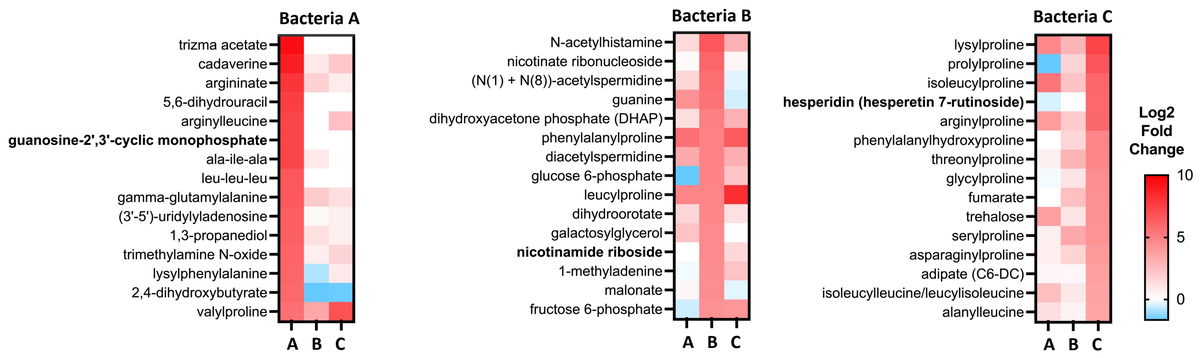

Figure 2. Global metabolite production of TriAD *Bacteria.* Top 15 metabolites produced, as measured by global metabolomics (Metabolon). Data presented as log2 fold change compared to OTEB or BHI media control.

Guanosine protects neural cells against glutamatergic toxicity and therefore can be neuroprotective in neurodegenerative diseases (Lanznaster & Tasca, 2017).

Nicotinamide riboside is a precursor to nicotinamide adenine dinucleotide (NAD+). NAD+ depletion contributes to aging and increased levels could counteract neurodegenerative disease processes (Lautrup et al., 2020)

Hesperidin has anti-inflammatory and anti-oxidant properties. It downregulates TGF-β1 and NF-κB, which are known to be involved in AD progression. Hesperidin has also improved memory and learning in AD mouse models (Hajialyani et al., 2019).

16s rRNA Sequencing

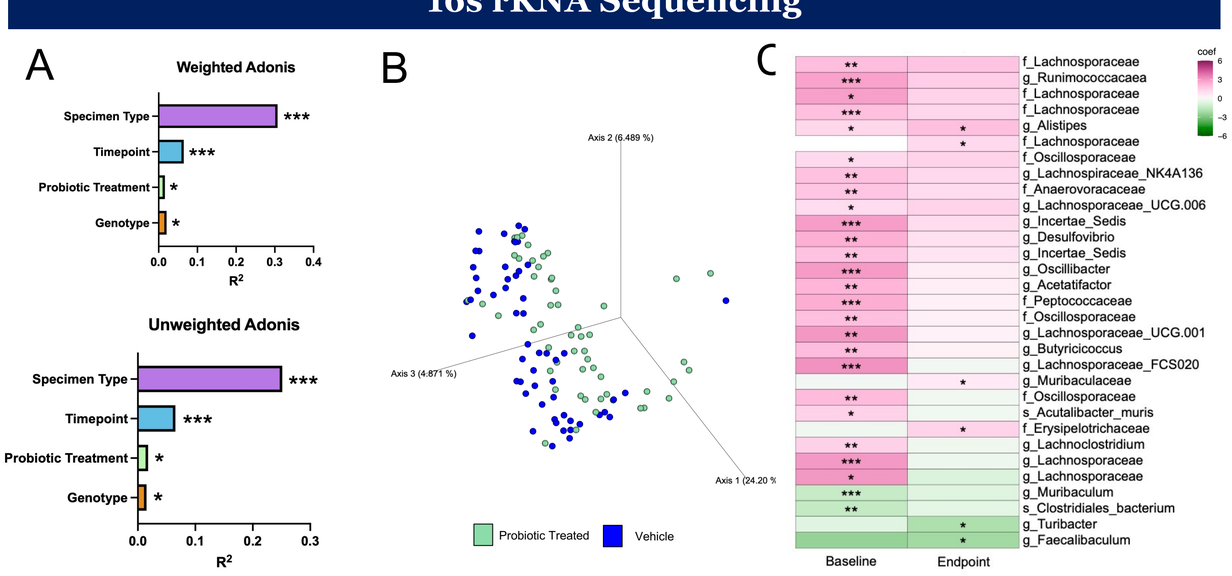

Figure 3. 16s rRNA sequencing of feces, colon, and ileum samples from mice. A) Weighted Adonis test and Unweighted Adonis test for beta diversity comparing proportion of variance (R^2) of specimen type, timepoint, probiotic treatment, and genotype **B)** Jaccard Emperor Plot comparing vehicle vs probiotic treated mice. **C)** Heatmap of microbiome differences in probiotic treated APP/PS1 vs Wild Type mice at baseline and endpoint (day 56). *P<0.05, **P<0.01, ***P<0.001 .

Immunohistochemistry

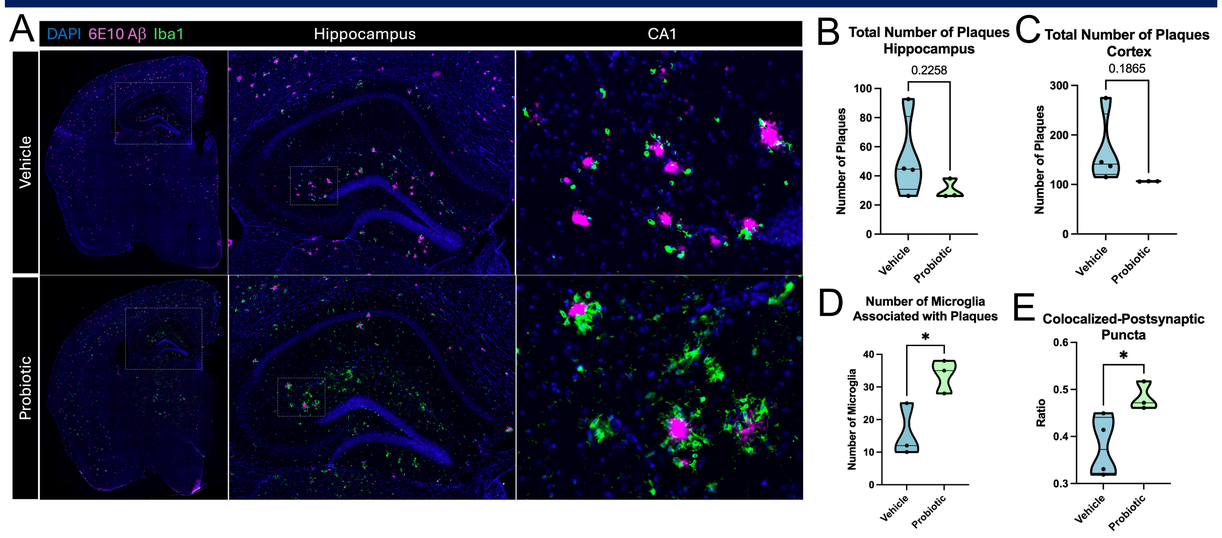

Figure 4. Immunohistochemistry analysis of APP/PS1 hippocampus. A) Representative images of vehicle and probiotic treated mice brains stained with DAPI (blue, all nucleated cells), 6E10 Aβ (pink, Aβ plaques), and Iba1 (green, microglia). **B)** The number of Aβ plaques in the hippocampus of APP/PS1 mice with vehicle and probiotic treatment, **C)** number of Aβ plaques in the cortex of APP/PS1 mice with vehicle and probiotic treatment and **D)** the number of microglia associated with Aβ plaques. **E)** Ratio of colocalized (presynaptic and postsynaptic) puncta and postsynaptic puncta in the hippocampus. Unpaired t- test, *P<0.05

T cells

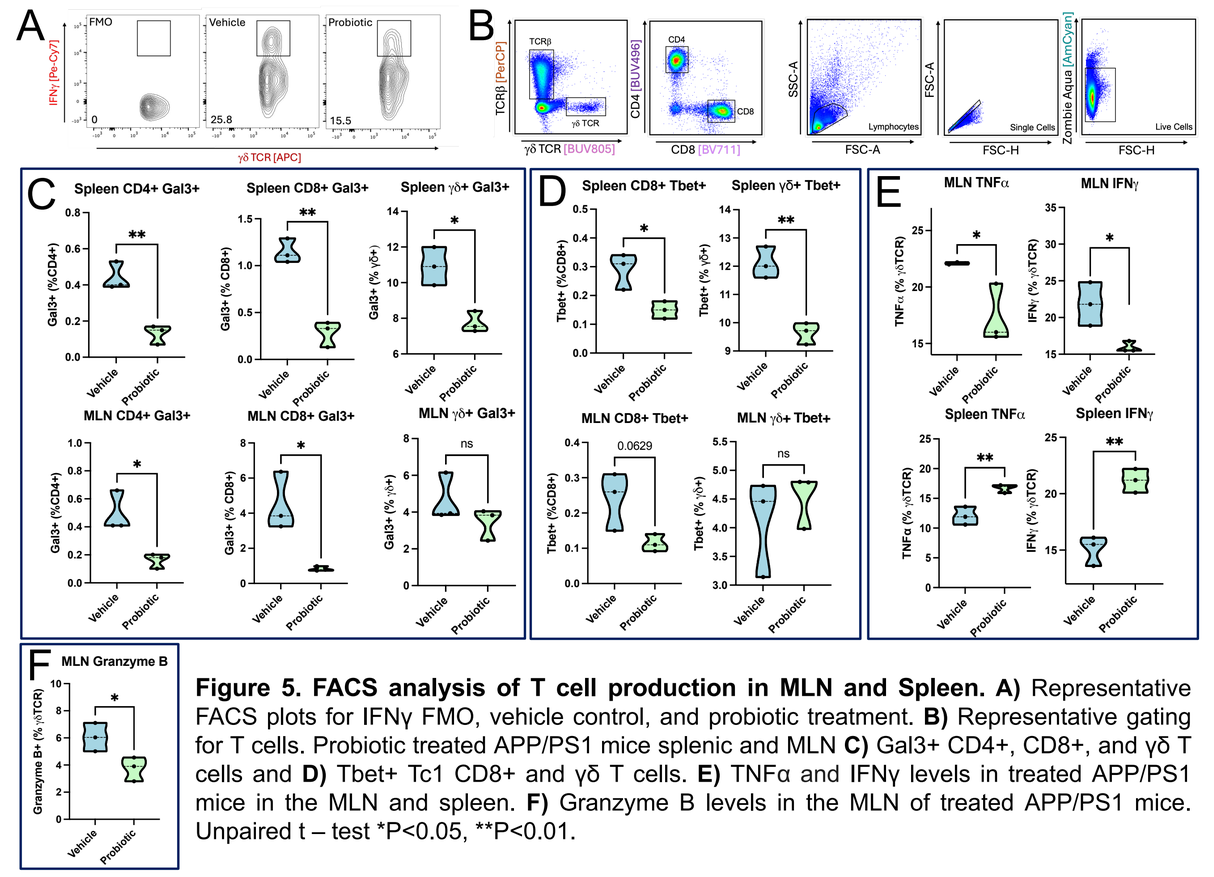

Figure 5. FACS analysis of T cell production in MLN and Spleen. A) Representative FACS plots for IFNγ FMO, vehicle control, and probiotic treatment. **B)** Representative gating for T cells. Probiotic treated APP/PS1 mice splenic and MLN **C)** Gal3+ CD4+, CD8+, and γδ T cells and **D)** Tbet+ Tc1 CD8+ and γδ T cells. **E)** TNFα and IFNγ levels in treated APP/PS1 mice in the MLN and spleen. **F)** Granzyme B levels in the MLN of treated APP/PS1 mice. Unpaired t – test *P<0.05, **P<0.01.

Conclusions

- **AD Pathology:** The TriAD probiotic significantly increased the ratio of colocalized puncta to postsynaptic puncta, indicating altered synaptic dynamics in AD. TriAD also had lower average Aβ plaque counts in the hippocampus and cortex of APP/PS1 mice.
- **T cell Modulation**: TriAD treatment reduced T cell populations of Tbet γδ and CD8+ T cells in the spleen, and reduced Gal3+CD4+ and CD8+ T cells in the spleen and MLN.
- **Cytokine Modulation:** TriAD treatment reduced TNFα, IFNγ, and Granzyme B+ γδ T cells in the MLN of APP/PS1 mice.
- **Microbiome:** TriAD probiotic significantly altered beta diversity in the microbiome of mice. TriAD also shifted the microbiome of APP/PS1 mice towards the microbiome of WT mice over the course of treatment.
- **Metabolites:** These changes may be mediated by neuroprotective metabolites produced by TriAD.
- **Overall:** These findings suggest the TriAD probiotic may have therapeutic potential for Alzheimer's disease by reducing pro-inflammatory activity, especially in the gut, and decreasing hippocampal synaptic loss pathology in this mouse model.

Future Directions

- Examine Aβ plaque morphology in hippocampal tissue
- Profile microglial transcriptomic shifts through bulk RNA sequencing
- Validate findings in female APP/PS1 mice
- Study the effect of TriAD on cognitive outcomes through behavioral tests

Acknowledgements

COXLAB
Cure Alzheimer's FUND
NIH National Institutes of Health
BrightFocus® Foundation Cure in Mind. Cure in Sight.