Different Probiotic Strains Alter the Return of Colonization Resistance Against *Clostridioides difficile* After Antibiotics

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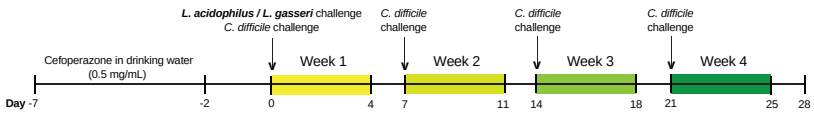
Background

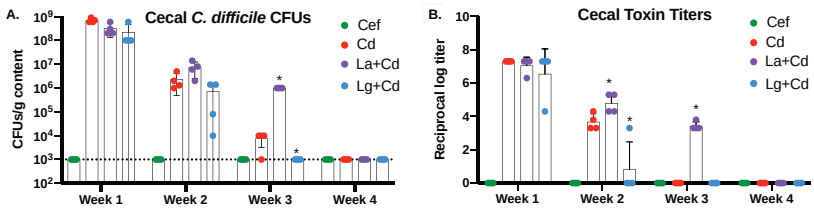
Probiotics are increasingly used worldwide to promote health and reconstitute the gut microbiota after antibiotic treatment. However, their effect on the antibiotic-altered microbiome is poorly understood and recent studies have raised concerns about probiotic efficacy, safety, and the impact on the host. *Clostridioides difficile* is a public health threat and a leading cause of antibiotic-associated diarrhea. Colonization resistance against *C. difficile* often occurs after antibiotics perturb the gut microbiota and metabolome, allowing *C. difficile* to colonize and establish an infection. Probiotic trials for the prevention or treatment of *C. difficile* have had varied success, likely due to the unknown nature of the probiotic's beneficial mechanisms and interaction with the indigenous microbiome. The aim of this study is to determine the mechanisms that drive popular probiotic lactobacilli to accelerate or prolong the return of colonization resistance against *C. difficile* after antibiotics.

Methods



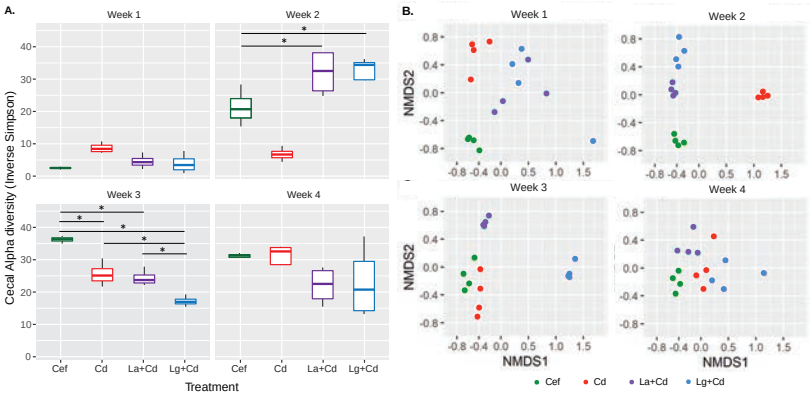
Cefoperazone treated mice were challenged with probiotic strains *Lactobacillus acidophilus* NCFM or *Lactobacillus gasseri* NCK2638 After a one-time probiotic administration, groups of mice (Cd, La+Cd, Lg+Cd) were then challenged with *C. difficile* each consecutive week for four weeks (n=4 mice per group) excluding a healthy control group (Cef) . Mice were monitored for clinical signs of disease, bacterial burden, and toxin activity. Using 16S rRNA sequencing and targeted bile acid metabolomics, we define how different *Lactobacillus* strains are able to alter the return of the gut microbiota and bile acid metabolome after antibiotics.

C. difficile Colonization and Toxin Activity



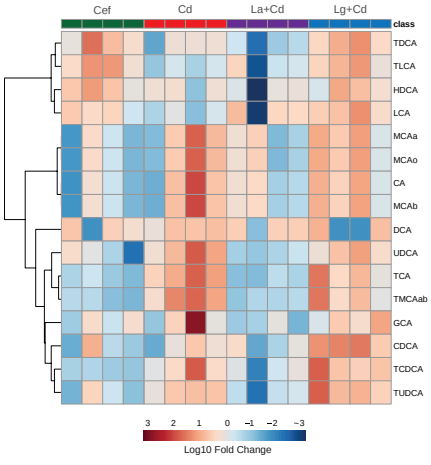
Colonization resistance was restored in cefoperazone treated mice without probiotic administration 4 weeks after antibiotic cessation. *L. gasseri* administration accelerated the return of colonization resistance by 1 week (**A**), whereas *L. acidophilus* increased the *C. difficile* burden and toxin load in the cecum (**B**). "*****" denotes $p < 0.05$ by Mann-Whitney U test.

Probiotics Alter the Antibiotic-Treated Microbiome's Return



Sequencing the V4 region of the 16S rRNA gene and analysis using DADA2 was performed to characterize the taxonomic diversity within cecal snips. α-diversity as measured by the Inverse Simpson Index is typically correlated with colonization resistance, however this association wasn't seen in lactobacilli-treated mice (**A**). "*****" denotes $p < 0.05$ by Kruskal-Wallis test. Nonmetric Multidimensional Scaling ordination based on Bray-Curtis distances was used to visualize β-diversity of the microbiota as the murine microbiota recovers from cefoperazone treatment (**B**). Notably, differences in the Week 3 *L. gasseri*-challenged cecal communities suggest *that L. gasseri* alters the assembly and reconstitution of the microbiota to accelerate the return of colonization resistance. Week 2, 3, and 4 community structures between treatments are significantly different by Permanova Adonis.

Probiotics Generate Unique Cecal Metabolomes



Targeted bile acid analysis was performed on Week 3 cecal contents by UPLC-EDI-MS/MS with the Biocrates® kit containing calibration and internal standards of 20 bile acids. Microbial-derived CDCA and LCA, bile acids known to be inhibitory to *C. difficile* growth and/or toxin activity, are enriched in *L. gasseri*-treated mice compared to *C. difficile* only and *L. acidophilus*-treated mice.

Future Directions

We will continue our microbiome analysis to understand how the relative abundance of microbial taxa changes over time as a result of cefoperazone and probiotic treatment as well as what Lactobacilli factors influence the alteration of the microbiota and metabolome after antibiotics.