Differential Effect of Antibiotics on Microbiomes and the Host Immune Response in the Gut Environment and their Influence on Colonization with *Candida*.

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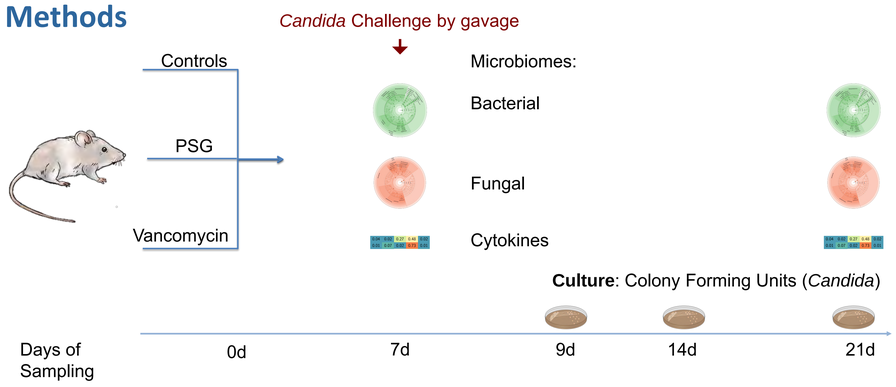
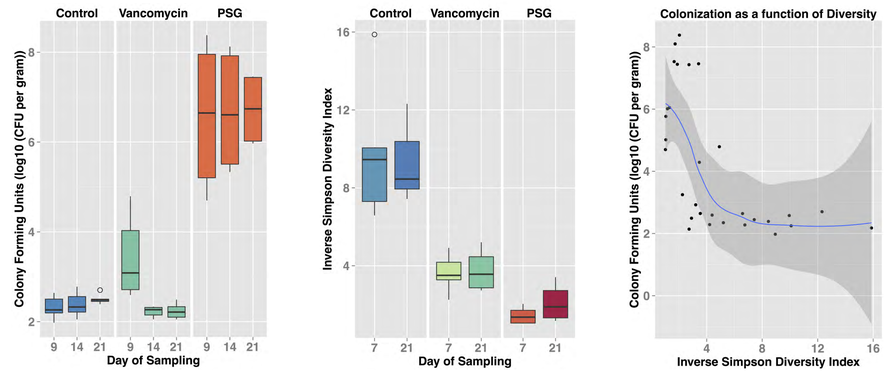
Introduction

- Vancomycin, and combinations of penicillin, streptomycin and gentamicin (PSG) are commonly used antibiotics in clinical settings.
- Given increasing prevalence of immunocompromised states in current patient populations, it is important to examine microbiome and host factors that promote opportunistic fungal pathogenesis in the backdrop of antibiotic therapy.
- Recent work in this area includes studies by the the Huffnagle (U.Mich) and the Blaser (NYC) group.
- In our experiments, we expand the scope of research to include (a) common antibiotics, (b) both the bacterial and fungal microbiomes and (c) relevant host immune response. We use high dimensional statistical modeling to analyze and obtain insights from our multi-faceted data.

Methods





(a) While mice treated with vancomycin had transient GI colonization with *C. albicans*, those treated with PSG showed sustained, high level colonization. (b) Diversity of the fecal microbiome community was the least in PSG treated mice, followed by vancomycin treated mice and control mice. (c) CFU shows a negative correlation with diversity.

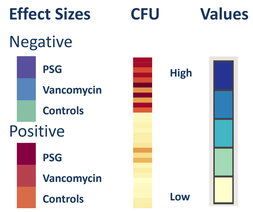
Elastic Net Multivariable Regression Modeling and Stability-based Variable Selection

Model Specifications

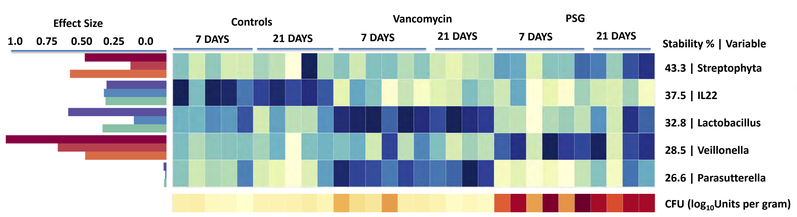
- **Data:** Taxonomic assignment of 16S V3-V4 amplicons using **YAP**: a computationally optimized open-source 16S classification workflow. All analyses presented in this poster is at genus level. Log transformation of proportions and cytokine expression levels for better integration of datasets in models.
- **Model:** Linear regression with Elastic net Regularization and Cross-Validation followed by Stability Selection.
- **Model Outcome:** Colonization : Log_{10} CFU units/g
- **Model Variables:** Microbiome relative abundances and Cytokine Expression levels

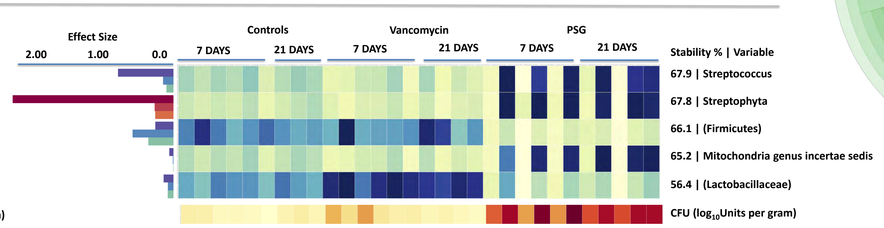
Modeling Strategy

- L1 penalty achieves sparsity in model parameters.
- L2 penalty takes into account correlation between variables.
- Cross validation selects the optimal **L1 (λ_1)** and **L2** weights (λ_2)
- Rank stability of variables based on a model for each λ_1 **(100 models)**
- Select top N/log(p) variables based on frequency of selection across models
- Estimate effect size of variables using standardized regression coefficients
- Effect Sizes with Stability ranks: elegant ways to assess influential variables

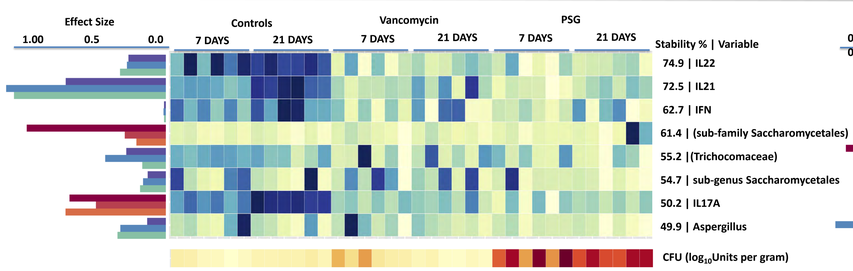


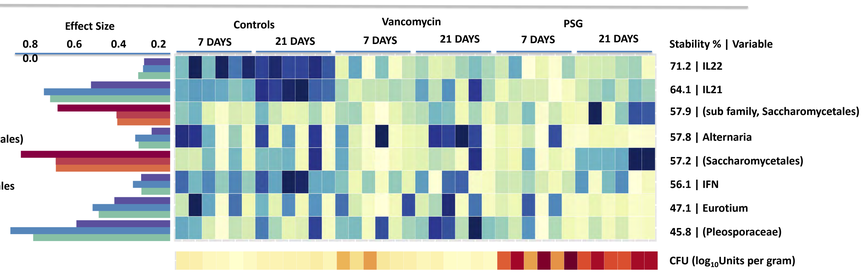
Model 1: Bacterial Microbiome + Cytokines



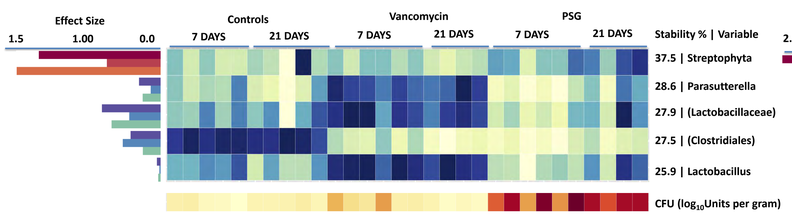


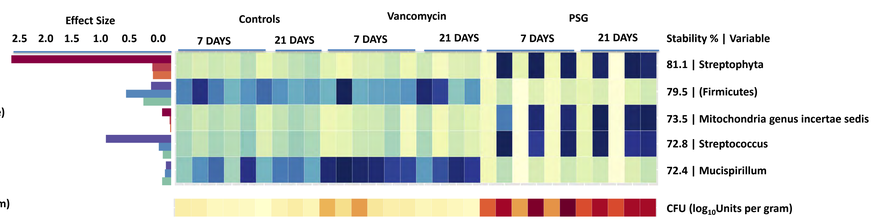
Model 2: Fungal Microbiome + Cytokines



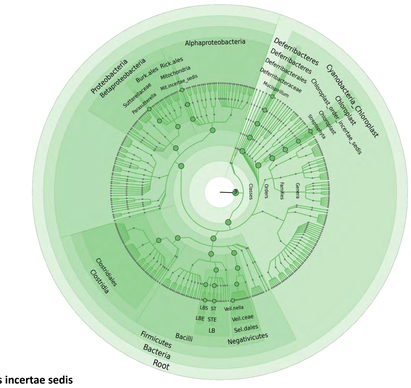


Model 3: Bacterial + Fungal Microbiome + Cytokines

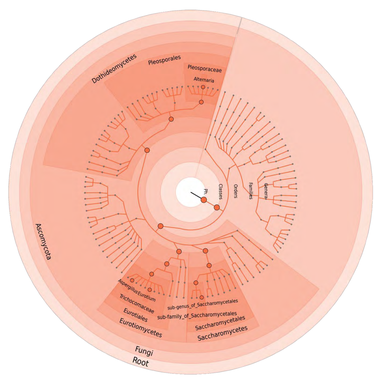




Bacterial Microbiomes



Fungal Microbiomes



Phylogenetic positions of the most stable Bacterial and Fungal community members from the Elastic Net models. Legend for Bacterial Microbiome: *Burk.ales:Burkholderiales, LB: Lactobacillales, LBE: Lactobacillaceae, LBS: Lactobacillus, Rick.ales:Rickettsiales, ST:Streptococcus, STE: Streptococcaceae, Sel.dales: Selenomonadales, Veil.ceae:Veillonellaceae, Veil.nella: Veillonella*

What did we learn?

Study and Significance

- PSG and Vancomycin differ substantially in their effects across the gut environment
- Differences in site-specific microbiome/cytokines possibly contribute to variation in colonization susceptibility
- Presence/absence of bacterial members influence colonization much more compared with either fungal members or the host immune response.
- Interaction networks exist between microbiomes and the host immune response
- Common antibiotic regimens have the potential to be tailored towards desirable clinical outcomes

Methods and Ongoing Challenges.

- Multivariable statistical methods for high dimensional data offer powerful and elegant ways to model and dissect contribution to clinically relevant outcomes.
- Data scaling procedures are crucial for effective modeling across multifaceted measurements
- Misclassification of taxonomies are effectively revealed via modeling. e.g. Candida is misclassified as unclassified members of the sub-family of Saccharomycetales.
- Fungal taxonomies and databases lag behind their bacterial counterparts.

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