Comparative Analysis of Opioid-Induced Microbiome Alterations in Rat Small Intestine, Cecum, and Colon

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INTRODUCTION

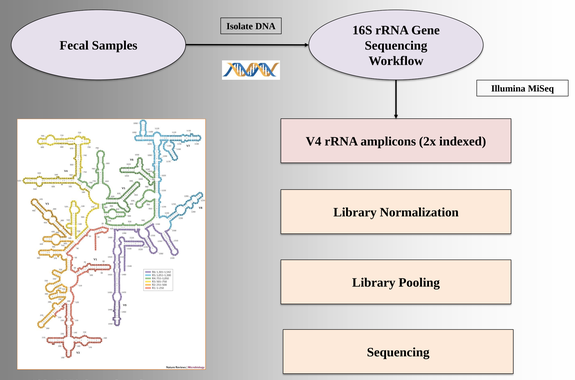
Trillions of bacteria, archaea, fungi, and viruses comprise the animal microbiome and virome. The composition and diversity of these complex communities have profound impacts on the health of their human or animal host. Changes in the composition of these microbiome profiles are multifactorial such as diet, health, or medication. Embedded in these communities might be groupings such as individual bacterial species/strains or consortia that have key roles and could serve as biomarkers for host health and disease. Due to new sequencing techniques and data analysis approaches, the characteristics of healthy (eubiotic) and dysbiotic microbiomes are being discovered (Shreiner et.al 2015).

OBJECTIVES

To develop workflows and bioinformatic approaches to identify microbiome profiles within different gastrointestinal regions after oxycodone administration.

METHODS

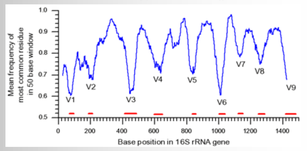
We are using fecal samples to perform 16S ribosomal RNA gene sequencing at the hypervariable region V4 in order to profile and compare microbiota in the small intestine, cecum, and colon. Next-generation sequencing using an Illumina MiSeq system is employed to sequence 16S rRNA amplicons as described by Kozich and coworkers (Kozich et al., 2013). Demultiplexed sample sequence reads are quality controlled and analyzed using the CLC Genomics Workbench (Qiagen) or QIIME 2 (Bolyen et al., 2019). The SILVA ribosomal RNA database is used for sequence read classification (Quast et al., 2013) and dedicated bioinformatics workflows are employed to determine microbial composition, alpha and beta diversities, and differential abundance analyses. All animal procedures used in this study were performed under protocols approved by the OSU-CHS Institutional Animal Care and Use Committee (IACUC).



16S rRNA Secondary Structure

16S rRNA Gene Hypervariable Region V4 Sequencing Strategy

16S rRNA Gene Hypervariable Regions



RESULTS

Biomarker Discovery Workflow

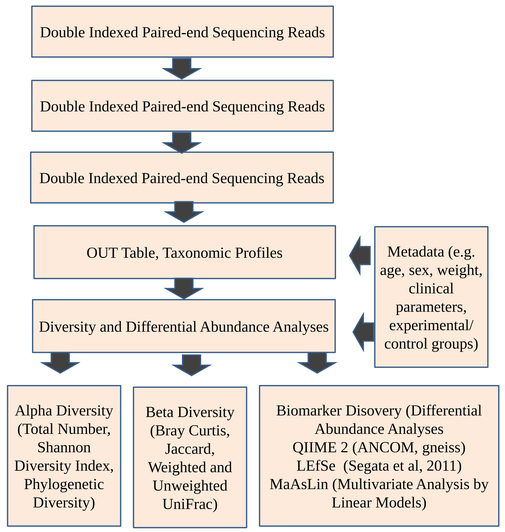


Fig 1: Bioinformatics Workflow. *This flowchart summarizes the bioinformatic approaches used to analyze this data.*

Relative Abundance-Phylum Level

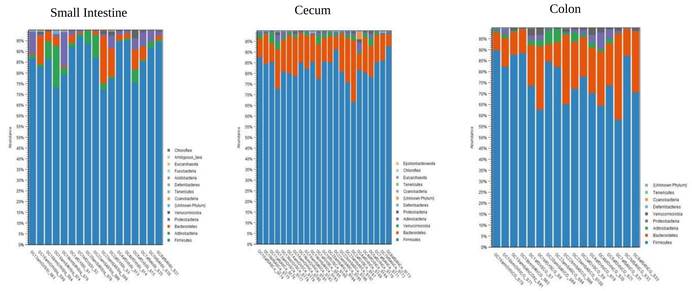


Fig 2: Relative abundance at the phylum level. *Taxonomic classification revealed differences in relative abundance of phyla between the small intestine, cecum, and colon. The phylum Bacteriodetes appears to be less abundant in most small intestinal samples compared to the cecum and colon samples.*

Genus Level Abundance Tables

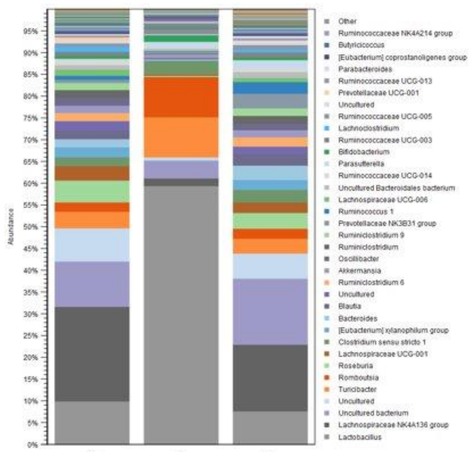


Fig 3: Relative abundance of genera at the three intestinal sites. *The small intestinal microbiota are remarkably different to cecal and colon microbiotas at the genus level*

Alpha Diversity

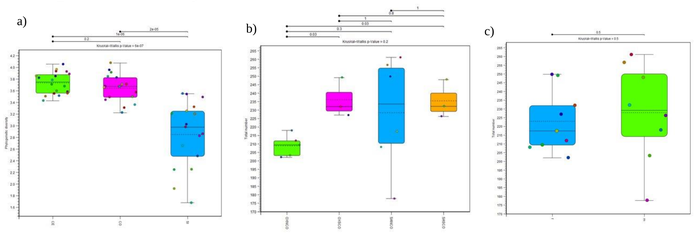


Fig 4: Alpha diversity analysis. *The phylogenetic diversity of microbiota is significantly reduced in the small intestine (a). Differences in the total number of OTU's between the treatment types (saline and oxycodone administration) in the colon microbiota are shown (b). The total number of OTU's in the cecum are not significantly different between male and female rats. This graph illustrates that there are differences between the male and female samples used (c).*

Beta Diversity

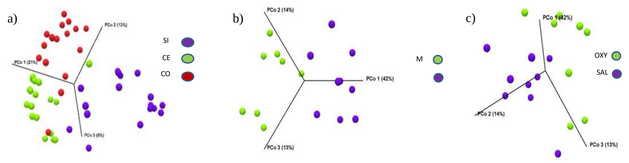


Fig 5: Beta diversity analysis. *The microbiota at the different sites showed differences in beta diversity (a). Sex differences in Bray-Curtis dissimilarity were seen in cecal samples (b). Oxycodone administration affected the microbial composition in cecal samples (Bray-Curtis) OXY, oxycodone, SAL, saline (c).*

LEfSe Analysis

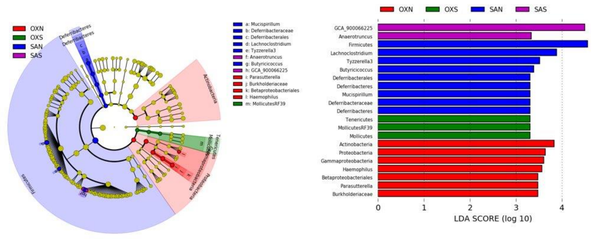
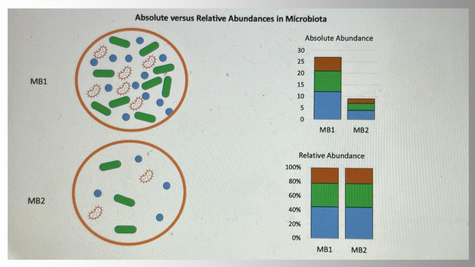


Fig 6: Biomarker discovery. *LEfSe analysis of the rat cecum microbiota in the different treatment groups of oxycodone and saline for first treatment and saline and naloxone for the second treatment. This provides analysis of the bacterial taxa affected by each treatment that could provide potential biomarkers of health and disease in the gut microbiome. OXN, oxycodone-naloxone; OXS, oxycodone-saline; SAN, saline-naloxone; SAS, saline-saline;*

LIMITATIONS

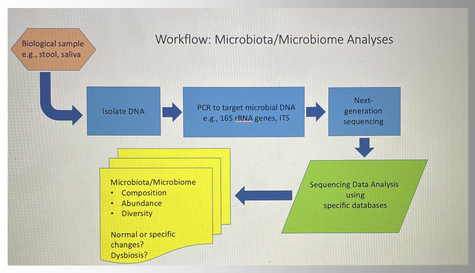
Microbiota profiling using 16S rRNA gene sequencing is limited due to

- 16S rRNA primer biases
- Absolute versus relative abundance
- RNA operon number differences in bacteria and archaea
- Functional profiles can at best only be predicted
- Elucidation of causal relationship require in-depth functional studies of microbiota-host interactions



CONCLUSION

Analysis of animal microbiotas by 16S rRNA gene sequencing has provided invaluable insights into the role of the microbial communities in health and disease. Correlation of these profiles with host metadata will aid in the identification of potential biomarkers of oxycodone use in the different host organ environments that could lead to novel diagnostic targets and preventative or therapeutic interventions. However, these analyses must be reinforced with functional evaluations to elucidate causal relationships.



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