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Isolation of Translocating Bacteria in Progressive SIV Infection of Rhesus Macaques

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BACKGROUND

- Microbial translocation is commonly observed in HIV-infected humans and is a significant contributor to inflammation.
- In SIV-infected Asian macaques, translocation has been demonstrated to occur across the gastrointestinal barrier; however, bacterial taxa translocating across the intestinal epithelium are not representative of the gut microbiota, with Proteobacteria appearing to preferentially translocate.
- Although there is biased translocation across the intestinal epithelium, this may not represent the full repertoire of systemic antigenicity because no one has categorized the repertoire of systemically competent bacteria.
- To fully characterize translocating bacterial populations, we isolated systemically translocating bacteria from chronically SIV-infected macaques and identified them subsequent to live culture.

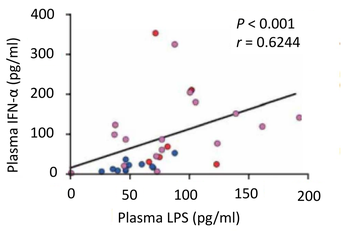


Figure 1. Microbial translocation as a contributor to immune activation
Positive correlation between LPS and IFN-α plasma levels (determined by Limulus Amebocyte assay and ELISA, respectively), in cohorts of subjects chronically infected with HIV and individuals who have progressed to AIDS, as determined by Spearman's rank-correlation[1].

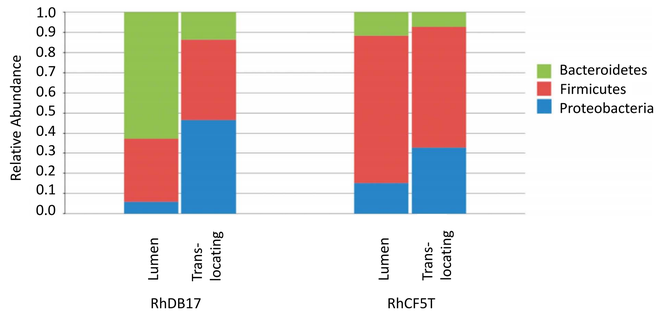


Figure 2. Evidence for preferential translocation of Proteobacteria from the colon
Relative abundance of Bacteroidetes, Firmicutes, and Proteobacteria in the lumen and sites of translocation in the colonic lamina propria of SIV-infected rhesus macaques as determined by 16S qPCR[2].

EXPERIMENTAL GOAL

To isolate bacteria that are preferentially translocating to other bodily sites in rhesus macaques (*Macaca mulatta*) infected with SIV. Identify and preserve these isolates for future experiments.

METHODS

- Liver, mesenteric lymph node (MLN), and spleen samples were collected aerobically and anaerobically during necropsy from twenty chronically SIV- or SHIV-infected rhesus macaques, four of which were treated with Vancomycin, and one SIV-negative rhesus macaque.
- Swabs were used on the chest/abdomen of necropsied macaques to sample the skin microbiome as a control for potential contamination.
- Tissue samples were mechanically homogenized and plated on: a) Brain Heart Infusion (BHI), b) Eosin Methylene Blue (EMB), c) Trypticase Soy Agar (TSA)+Tween 80, and d) TSA+5% Sheep's Blood media under aerobic conditions, and e) Brucella Blood and f) CDC Blood media under anaerobic conditions.
- Isolates were grown at 37ºC for 1-7 days, depending on observed density. Colonies were streaked for pure cultures and identified using MALDI-TOF and 16S rDNA sequencing.
- Isolates found to be present in both the tissue and skin microbiomes of the same animal were considered likely contaminants and thus excluded from our analyses.
- Relevance of a species to the study was determined by whether or not the isolate was a normal resident of the digestive tract based on current literature, with our focus being on species that are intestinal residents (other species have been isolated, but excluded based on this criteria, such as *Mycobacterium smegmatis, Propionibacterium namnetense, Aerococcus urinaeequi,* and *Corynebacterium massiliense*, which are associated with other bodily sites).
- Shannon α-diversity was calculated for a) SIV+, b) SIV+ Vancomycin-treated, and c) SIV+ or SHIV+ animals (no Vancomycin).

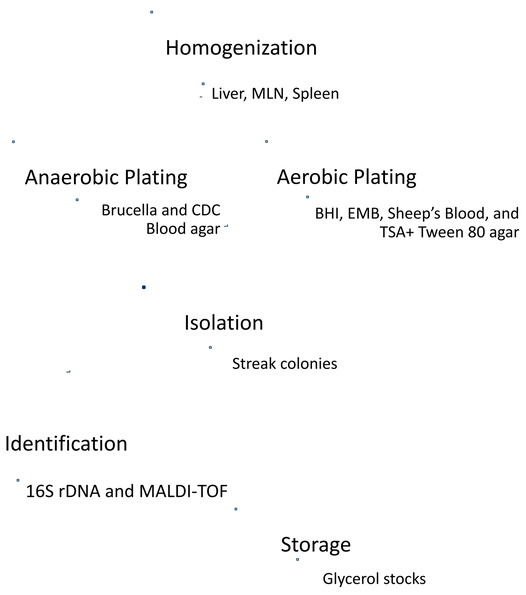


Figure 3. Schematic setup for isolation protocol.
Tissues are homogenized, then spread plated. Colonies are streaked from spread plates for pure cultures, which are used for identification and long term storage preparation.

RESULTS

Thirty-Six Relevant Species Isolated and Identified in SIV+ Animals, Nine Species in SIV-

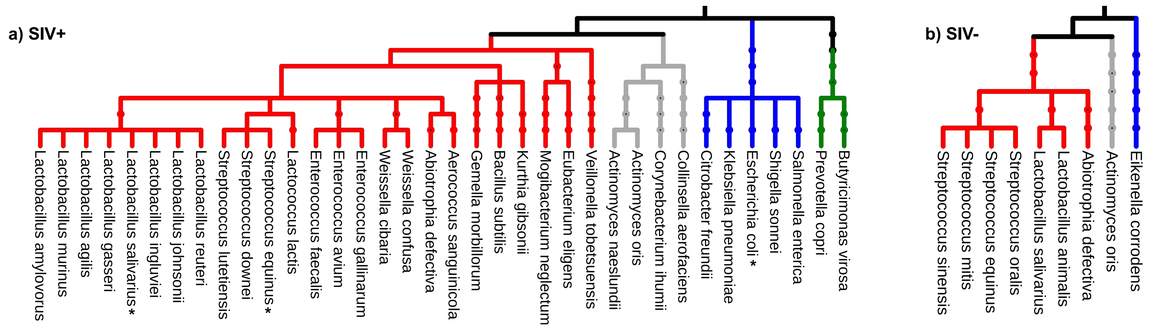


Figure 4. Phylogenetic distribution of identified isolates.
Phylogenetic trees displaying identified isolates by 16S rDNA sequence relatedness, identified to the species level. a) Branches are colored by Phylum of the included bacteria: Bacteroidetes in green (2/36 isolates), Proteobacteria in blue (5/36), Actinobacteria in grey (4/36), and Firmicutes in red (25/36). Of particular interest are the species of *Lactobacillus salivarius, Streptococcus equinus,* and *Escherichia coli,* which have been found to be translocating in six, five, and four animals, respectively. b) For isolates from the SIV- macaque: 1/9 were Proteobacteria, 1/9 Actinobacteria, and 7/9 Firmicutes.

Majority of Translocating Bacteria are from the Phylum Firmicutes

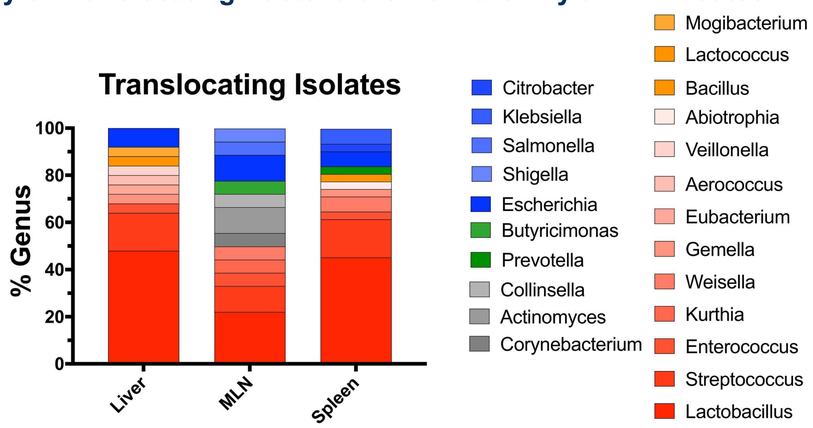


Figure 5. Genera abundance of relevant species isolated.
Distribution plot of relative abundances of isolated taxa by tissue source, color-coded at the genus level. For tallying taxa, each species was given one point per instance of isolation per animal. Genera from Bacteroidetes are in shades of green, Proteobacteria in shades of blue, Actinobacteria in shades of grey, and Firmicutes in shades of red and orange. Surprisingly, species from the Firmicutes phylum make up the majority of isolated, systemically translocating bacteria. Although our cohort exhibited comparable microbial translocation irrespective of antibiotic treatment, when animals were stratified by infected animals treated with Vancomycin or untreated, α-diversity between tissue sites was near significantly reduced in the Vancomycin group as compared to the untreated group (two-way paired t-test, p=0.0739). Higher levels of Proteobacteria were translocating in Vancomycin-treated animals.

Conclusions and Future Directions

•Although bacterial taxa have been shown to translocate across the gastrointestinal epithelium, translocating taxa do not mirror the composition of the gastrointestinal microbiome, suggesting that other bodily sites contribute to translocating taxa or that translocating bacterial taxa from this system are enriched for motile taxa or are functionally altered. Our focus is on the latter.

•In an effort to identify proteins that may contribute to the ability of specific taxa to translocate, we have prepared bacterial isolates for comparative proteomic analyses.

•In order to confirm localization of bacteria in tissues, we are planning on performing fluorescence in situ hybridization targeting 16S rRNA on preserved tissues.

•We intend to grow these isolates in the presence of sorted immune cell populations from SIV-uninfected and -infected macaques in order to measure microbial growth and motility to determine how infected host immunity contributes to biased translocation.

ACKNOWLEDGEMENTS

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