Proteomic Analysis of Translocating Bacterial Taxa Reveals Novel Therapeutic Target

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BACKGROUND

- Microbial translocation is commonly observed in people living with HIV and is a significant contributor to chronic 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 a known bias for translocation across the intestinal epithelium, currently known bacterial taxa profiles derived from 16S sequencing may not represent the full repertoire of systemic antigenicity.
- To fully characterize translocating bacterial populations we isolated systemically translocating bacteria from chronically SIV-infected rhesus macaques (RM) and identified these bacteria subsequent to culture.

METHODS

- Liver, mesenteric lymph node, and spleen samples were collected during necropsy from twenty chronically SIV- or SHIV-infected RM. Tissue samples were homogenized, plated, and cultured under aerobic and anaerobic conditions, with subsequent purification and identification using MALDI-TOF and/or 16S rDNA sequencing.
- Select bacterial isolates (8 translocator species and 4 non-translocator species) were later expanded in liquid culture and identified by proteome analysis by tandem mass spectrometry.
- Bacteria grown in liquid culture were then used to inoculate 96-well plates with or without decitabine. These plates were then incubated in a microplate reader at 37°C with 100rpm with OD_{600} readings taken every 5-10 minutes.
- Area under the curve (AUC) was calculated for growth curves then normalized against the control to calculate a ratio to determine the inhibitory effect of decitabine, with values <1 indicating an inhibitory effect (ratio = AUC of decitabine concentration:Control). Bacterial species were then separated by translocator status (Translocator vs Non-Translocator) and compared using Welch's t test for normally distributed data or Mann-Whitney test for data that was not normally distributed.

ISOLATION RESULTS

Thirty-Six Relevant Species Isolated in SIV+ Animals

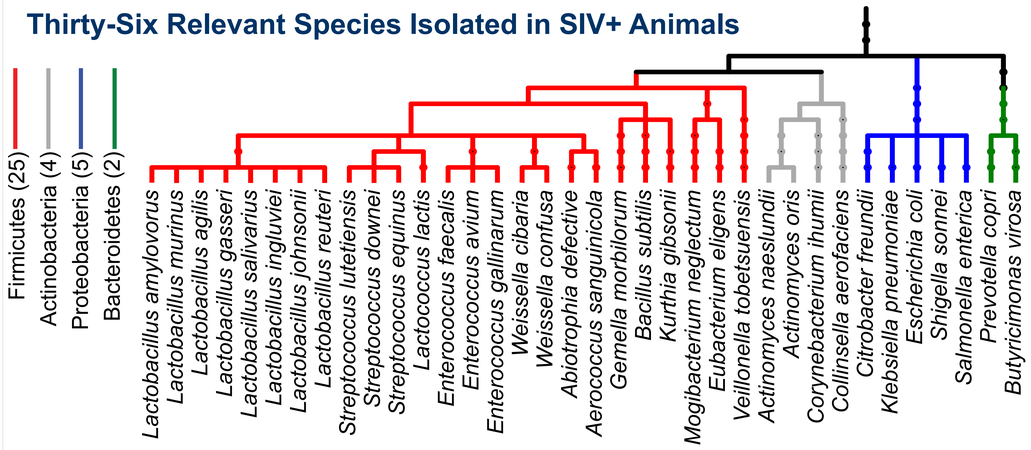


Figure 1. Phylogenetic distribution of identified isolates. Phylogenetic trees displaying identified isolates by 16S rDNA sequence relatedness, identified to the species level. Of particular interest are the species of *Lactobacillus salivarius, Streptococcus equinus,* and *Escherichia coli,* which were found to be translocating in six, five, and four animals, respectively.

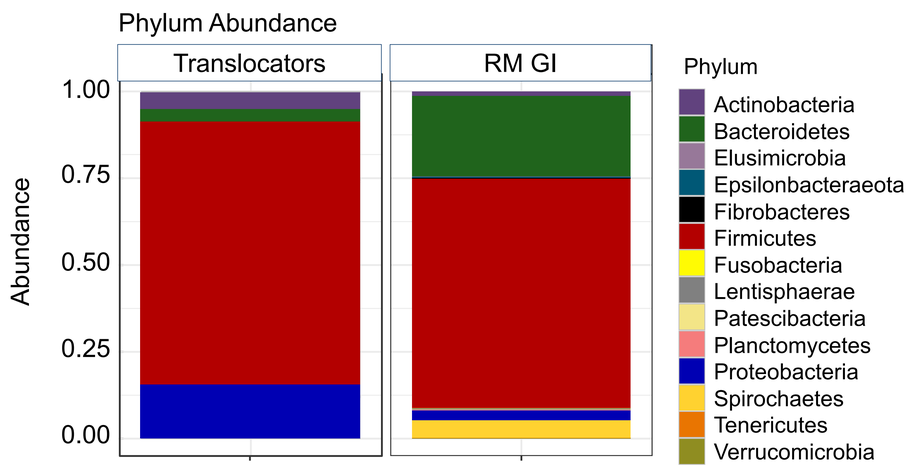


Figure 2. Distribution of Translocating Taxa Compared to Representative GI Microbiome. Larger proportions of Actinobacteria and Proteobacteria and smaller proportions of Bacteroidetes are observed among translocating taxa than would be expected based on normal abundances in the GI microbiome of our RM.

PROTEOME RESULTS

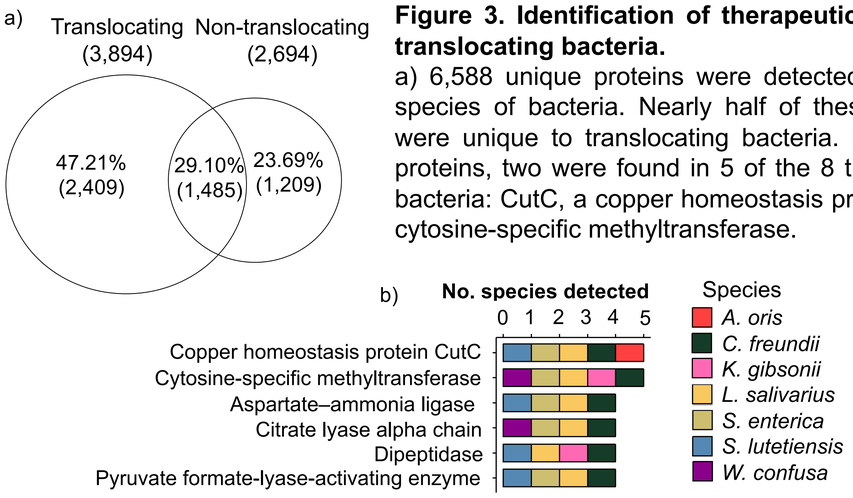


Figure 3. Identification of therapeutic targets in translocating bacteria. a) 6,588 unique proteins were detected across 13 species of bacteria. Nearly half of these, 47.21%, were unique to translocating bacteria. b) Of those proteins, two were found in 5 of the 8 translocating bacteria: CutC, a copper homeostasis protein, and a cytosine-specific methyltransferase.

GROWTH INHIBITION RESULTS

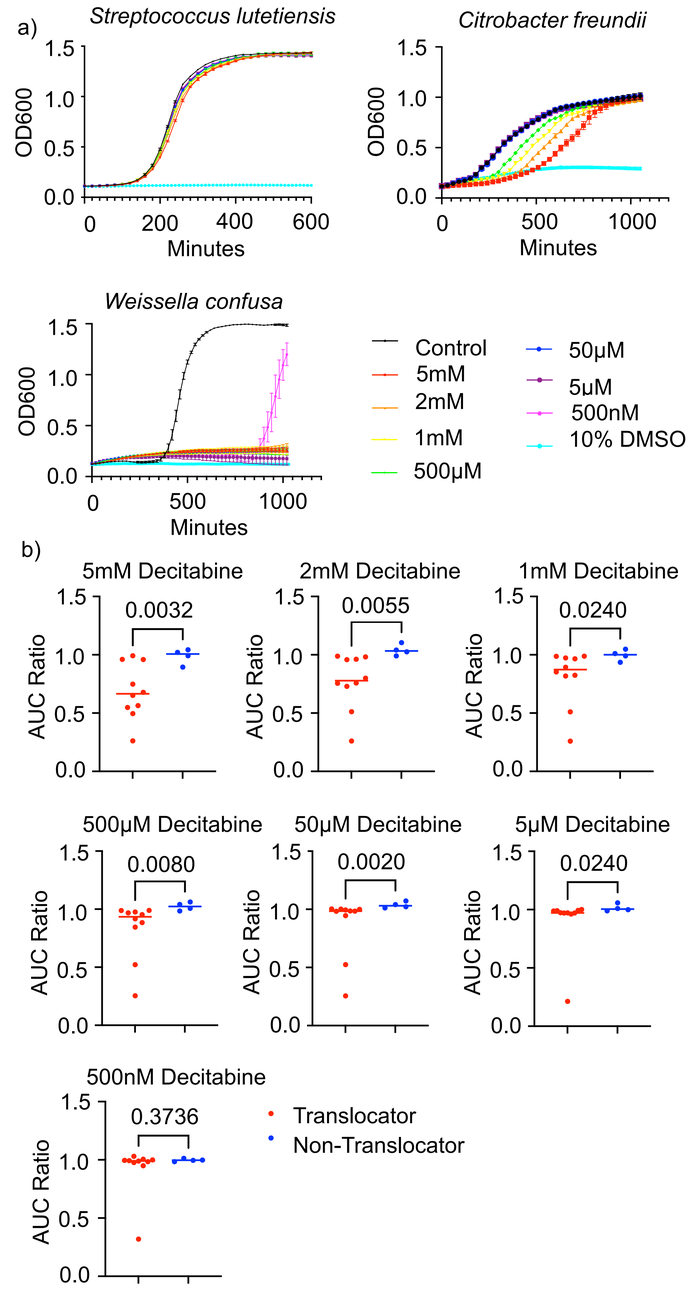


Figure 4. Inhibition of cytosine-specific methyltransferases significantly impairs growth in translocating bacteria. a) Representative growth curves for the three main types of responses to decitabine treatment among tested bacterial species. b) AUC comparisons for each decitabine concentration that bacteria were grown in. Decitabine treatment was significantly more inhibitory of growth for translocator bacteria as compared to non-translocator species at all but the lowest tested concentration of decitabine.

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CONCLUSIONS AND FUTURE DIRECTIONS

- **Translocating bacteria are uniquely enriched for a cytosine-specific DNA methyltransferase and are sensitive to inhibition of this methyltransferase *in vitro*.**
- **This methyltransferase may be a prophylactic therapeutic target in the treatment of microbial translocation which occurs in people living with HIV.**
- We will grow bacterial isolates in the presence of decitabine and extract RNA to perform Total-RNAseq in order to identify cellular functions dysregulated by decitabine, as these cellular functions may be critical for in-host translocation or survival of translocating bacterial taxa.
- We will treat SIV-infected Rhesus macaques with decitabine and sample longitudinally to determine if treatment improves markers of microbial translocation.