Dual Transcriptome Analysis of Host-bacterial Interactions in Peri-implant Health and Disease

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Introduction

The prevalence of peri-implantitis (10% of implants in 20% of patients) and rates of recurrence following treatment have been steadily increasing in the US. Although several patient-level variables have been known to increase risk of developing peri-implantitis, they do not fully predict the outcomes of multiple implants in each individual [1]. To effectively treat peri-implantitis, it is important to evaluate the site-specific predictors and also to understand the mechanisms of etiopathogenesis for this disease. The goal of the present investigation was to identify microbiological and immuno-inflammatory biomarkers of this disease using a case-control cross sectional analysis.

Aims

- Investigate mechanisms involved in the etiopathogenesis of peri-implantitis
- Identify potential biomarkers of disease in the host transcriptome and the microbial metatranscriptome
- Elucidate key interactions between the host and the microbiome in both health and disease

Methods

Subject Recruitment: Dentate adults with multiple single, non-splinted implants in function for at least 1 year were identified from those treated at the graduate periodontics clinics of The Ohio State University. Exclusion criteria included diabetes, current or past smoking, current pregnancy, HIV, use of immunosuppressant medications, bisphosphonates or steroids, antibiotic therapy or oral prophylactic procedures within the last 3 months, need for antibiotic coverage before dental treatment and less than 20 teeth present in the dentition. **Sample Collection**: Five subjects, each with at least one healthy implant and one with peri-implantitis, were selected. Soft tissue biopsies were collected for host transcriptome sequencing, and paper points were used to collect microbial biomass from the peri-implant sulcus for metatranscriptome sequencing. **Sequencing**: Total RNA was isolated and mRNA enriched for sequencing on the Illumina HiSeq platform (150bp PE). **Data Analysis**: Kallisto was used to align sequences to the human transcriptome (GRCh38). Differential expression analysis was performed using the Bioconductor package *limma*. Microbial transcripts were quality filtered (SolexaQA++), screened for human DNA (Bowtie 2), and aligned against the Human Oral Microbiome Database (HOMD) using DIAMOND. Aligned sequences were annotated to the KEGG database using Megan 6. Kraken was used, along with our custom tool kraken-biom, for taxonomic identification. Analysis and visualization of the distribution of Operational Taxonomic Units (OTUs) was performed using QIIME and PhyloToAST.

Acknowledgements

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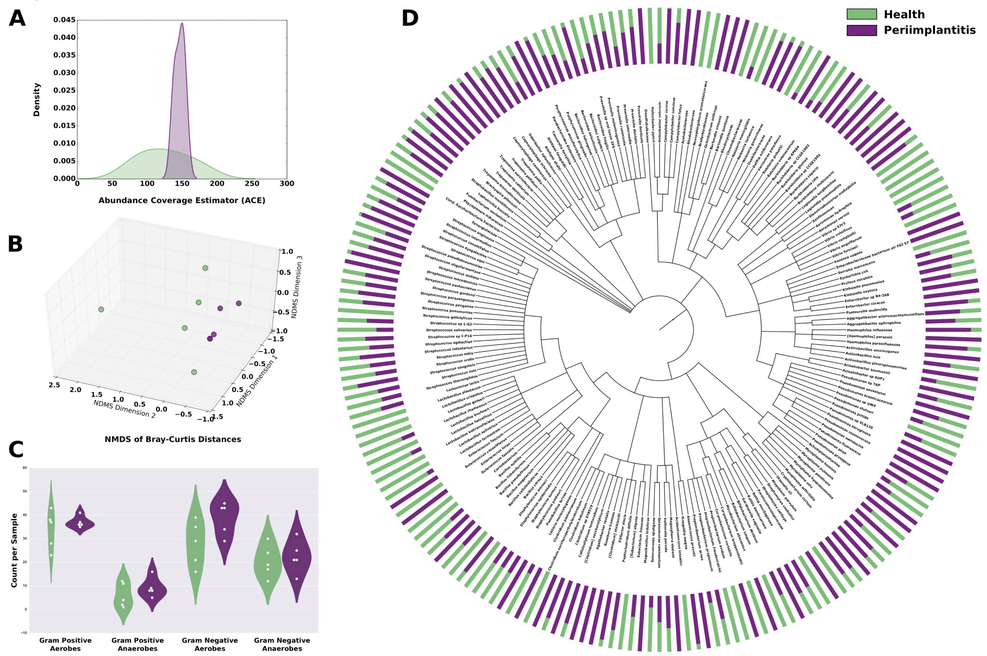
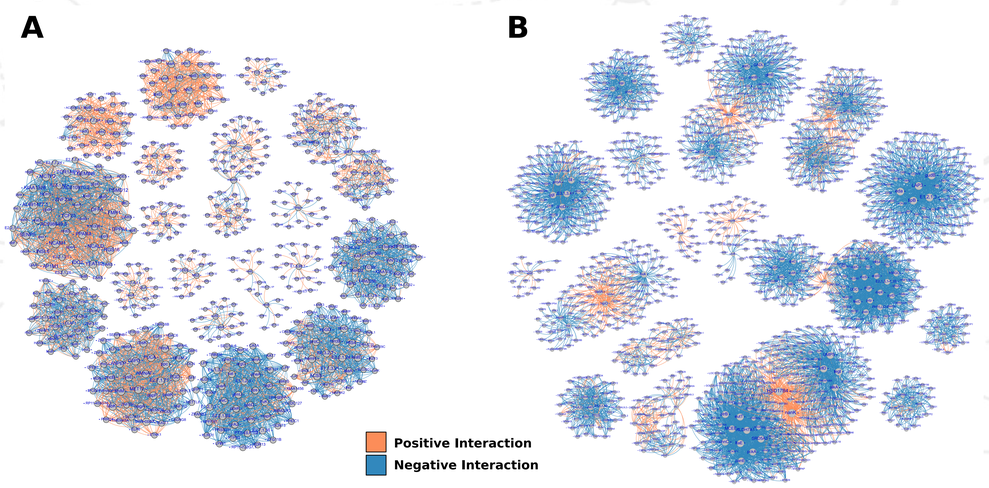
Results

Figure 1



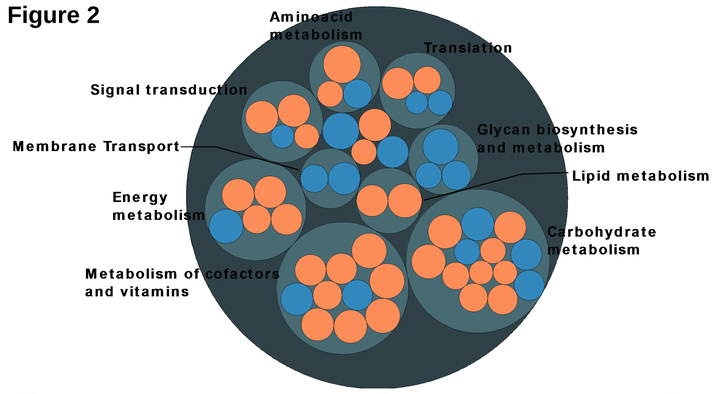
A total of 258 species level operational taxonomic units (s-OTUs) in 71 genera were identified. **A** Alpha diversity was significantly different between health and disease ($p = 0.03$, Levene test). **B** NMDS plot of Bray Curtis dissimilarity, indicating a narrower landscape of variation in disease. **C** Per-sample distribution of microbial species by gram stain and oxygen requirement. **D** A phylogenetic tree displaying the mean relative abundance of each s-OTU in health and disease as stacked bars. Healthy implants were enriched for *Lactobacillus*, *Ralstonia*, *Streptococci* and *Pseudomonas*. Peri-implantitis samples exhibited higher abundances of *Treponema*, *Capnocytophaga*, *Prevotella*, *Campylobacter*, *Neisseria*, *Burkholderia*, *Aggregatibacter*, *Haemophilus*, *Rothia*, and *Filifactor* genera.

Figure 4



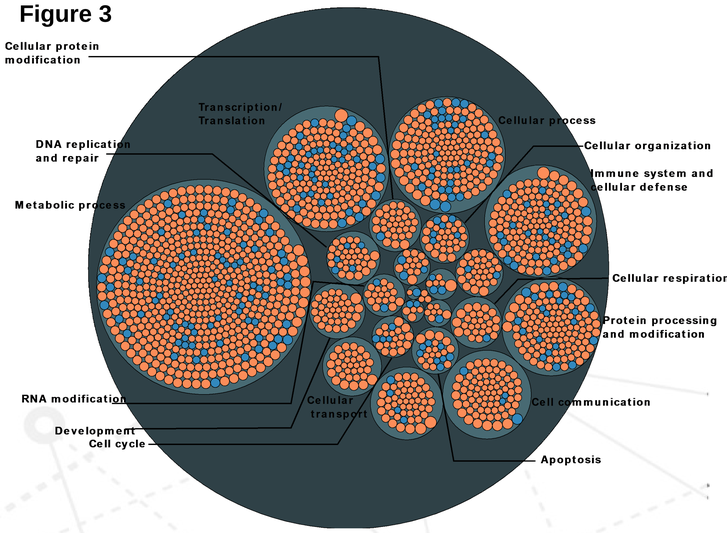
Host-bacterial gene interaction networks in health (**A**) and disease (**B**). Positive/synergistic interactions (orange) and negative/inhibitory interactions (blue). Significant interactions were discovered in disease between microbial genes involved in iron acquisition, adhesion, and secretion systems, and host genes responsible for innate and adaptive immune responses, zinc finger proteins, cell communication, membrane and cellular transport, and apoptosis.

Figure 2



32 microbial genes were up-regulated and 18 were down-regulated in disease. Three-fourths of the significantly differentially expressed genes encoded for metabolism; including carbohydrates, lipid metabolism, glycan biosynthesis, and energy metabolism. The remaining genes encoded for translation, signal transduction, and membrane transport.

Figure 3



Across all host samples, 1821 genes, contributing to 24 broad functional categories, were significantly differentially expressed between the groups. 1585 genes were up-regulated and 236 were down-regulated in the peri-implantitis samples. This circle plot visualizes gene fold-change (circle size) of significant differences in transcription; orange denoting up-regulated genes and blue representing down-regulated genes.

Conclusions

Site-specific factors supersede subject-level factors as predictors of peri-implantitis. Similarly, host-bacterial interactomes, rather than microbial or host factors alone, provide greater scope for biomarker discovery.

Bibliography

[1] Zitzmann, Nicola U., and Tord Berglundh. "Definition and prevalence of peri-implant diseases." Journal of clinical periodontology 35.s8 (2008): 286-291.