

Microbial Complexes in Subgingival Plaque: A Bacterial and Eukaryotic Metataxonomic Study

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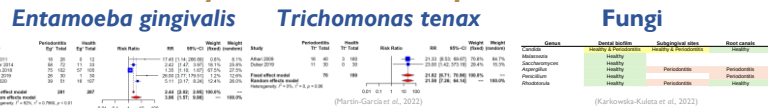
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Introduction

Eukaryotic microbes in periodontitis



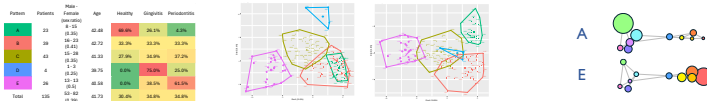
Some eukaryotic microbes are more prevalent in periodontitis than in health
E. gingivalis presents pathogenic traits (Bao et al., 2021; Rosenfeld et al., 2025)

Microbiota patterns and bacterial complexes

Patterns are linked to health states (k-clustering)

Patterns are individualized and not aligned

Abundance of bacterial complexes varies with patterns



Bacterial metataxonomics revealed 5 microbiota patterns defined by 10 bacterial complexes (Santi-Rocca et al., JCP, in press, Jaccard Prize finalist)

⇒ How are eukaryotes related to patterns, complexes, and health states?

Methods

Eukaryotic sequencing

- Same samples as bacterial sequencing (Stage II-III Grade B)
- Universal eukaryotic primers designed for this study (18S rRNA)

Bioinformatics

- Taxonomic assignment with our PRONEX tool
 - Probabilistic and non-exclusive
 - Results as "Unambiguous Taxon Groups" (UTGs)

Presented here

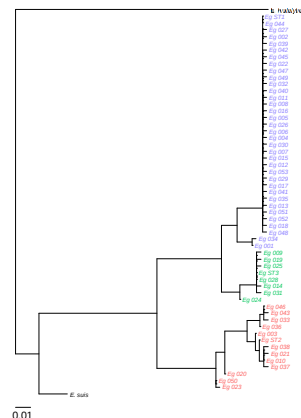
- Main observations (first 6 UTGs)
- Preliminary results: 41 samples out of 135

Composition in UTGs

- Entamoeba gingivalis**
 - Prevalence: 100% of samples
 - Abundance: 64.4% of reads
 - 53 amplicon sequence variants
 - Different subtypes (>0.05 substitution/site)
- Trichomonas tenax**
 - Prevalence: 75.6%
 - Abundance: 33.1%
 - 44 ASVs
 - Low genetic variability (<0.01 subst./site)
- Candida spp.**
 - Prevalence: 19.5%
 - Abundance: 1.6%
 - 4 ASVs
 - Low genetic variability
- Penicillium spp.**
 - Prevalence: 14.6%
 - Abundance: 0.26%
- Rhodotorula spp.**
 - Prevalence: 51.2%
 - Abundance: 0.20%
- Malassezia restricta**
 - Prevalence: 70.7%
 - Abundance: 0.17%

⇒ 6 main UTGs account for 99.7% of reads

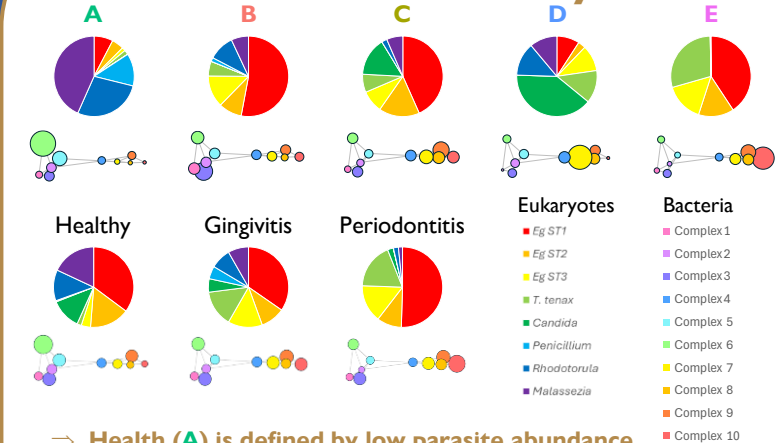
Entamoeba gingivalis diversity



- Phylogenetic tree from multiple sequence alignment (CLUSTALW)
- Subtypes clustered according to their distance (<0.05 substitution / site)
- Reference sequences:
 - ST1: Kikuta et al., 1996
 - ST2: Garcia et al., 2018
 - ST3: Köhler et al., 2023
- Outgroups:
 - E. suis* and *E. histolytica*
- Prevalence:
 - ST1: 100% of samples
 - ST2: 58.5%
 - ST3: 65.9%

⇒ 3 subtypes are detected, consistent with the literature

Abundance of eukaryotes



- ⇒ Health (A) is defined by low parasite abundance
- ⇒ Pattern D exhibits high fungus abundance (64.1% of reads)
- ⇒ Other patterns are dominated by parasites (75.8-99.7%)
- ⇒ Pattern E shows high flagellate content (29.1%)

Conclusion & Discussion

- ⇒ Eukaryotes are ubiquitous in periodontal sulci/pockets
 - ⇒ Parasites are prevalent and abundant
 - ⇒ *Candida* infections are linked to specific microbiota patterns and bacterial complexes
 - ⇒ *Malassezia* and *Rhodotorula* genera may be commensal fungi
- ⇒ Microbial patterns are linked to bacterial complexes and to eukaryotes, suggesting their specific interactions

Perspectives

- Relative quantification between eukaryotes and bacteria
- Detection of viruses and archaea in these samples
- Test with peri-implantitis and other periodontitis grades/stages