Exploring Microbial Diversity in Dental Calculus Through Metagenomic Approaches

M. Uzun, A. Fedorenko

March 28, 2024

Abstract

Metagenomics offers a window into the diverse microbial communities across ecosystems, employing approaches such as 16S rRNA sequencing and shotgun sequencing. In this study, we utilized both techniques to explore the microbial landscape of dental calculus, focusing on an individual labeled G12. Leveraging advanced sequencing technologies and bioinformatic analyses, we uncovered prevalent taxa and intriguing genetic patterns within these microbial populations. Our findings shed light on the taxonomic diversity, functional potential, and evolutionary dynamics of microbial communities inhabiting dental calculus, providing insights into oral health and disease.

1 Introduction

Metagenomics, a powerful tool in microbiology, offers insights into the intricate microbial communities residing in diverse ecosystems [1]. Within metagenomics, two prominent approaches, namely 16S ribosomal RNA (rRNA) sequencing and shotgun sequencing, provide invaluable avenues for understanding microbial diversity and functionality [2]. Through 16S rRNA sequencing, researchers delve into the taxonomic composition of microbial communities, focusing on specific genetic regions like the V5 region [3]. Meanwhile, shotgun sequencing enables a comprehensive analysis of entire microbial genomes present in a sample [4]. In this study, we utilized sequencing data from the V5 region of 16S rRNA and whole metagenome shotgun sequencing to explore the microbial landscape of dental calculus. Leveraging cutting-edge sequencing technologies, we analyzed data obtained from individual G12, shedding light on the composition and genetic makeup of its microbial inhabitants. Through bioinformatic analyses and the use of specialized tools, we unearthed intriguing patterns within the microbial communities inhabiting dental calculus. Our findings not only elucidate the taxonomic diversity but also provide insights into the functional capabilities and evolutionary dynamics of these microbial populations.

2 Materials and Methods

2.1 Data Availability

In this study sequencing data of V5 region from 16S ribosomal RNA was used. Data was obtained by Roche GS Junior (454) technology and was downloaded from here.

Also, in this study data of the whole metagenome shotgun sequencing of dental calculus of individual G12 was used, reads were assembled into contigs. Assembled data can be downloaded here.

2.2 Used Tools

The analysis of V5 region data was conducted with DADA2-based [5] script, and SILVA taxonomy database, downloaded from link.

The analysis of the metagenome shotgun sequencing was conducted with several tools. Firstly, Kraken2 v2.1.3 [6] was used to classify reads and to understand the composition of provided samples. Calculated results for the G12 sample were downloaded from here and here. Results obtained from Kraken were visualized online using Pavian [7]. The obtained results were also aligned to *Tannerella forsythia* 92A2 for gene evolution analysis.

3 Results

The analysis of sequencing data of the V5 region of 16S rRNA revealed the predominance of representatives from the phyla *Acidobacteria*, *Actinobacteria*, *Bacteroidetes*, *Firmicutes*, *Planctomycetes*, and *Proteobacteria* in the samples. Additionally, it was found that in two samples, B61 and G12, bacterial genes belonging to the "red complex" genera *Tannerella*, *Treponema*, and *Porphyromonas* were observed. In G12 sample we also found that the dominant phyla were *Bacteroidetes*, *Firmicutes*, and *Proteobacteria*. Metagenomic sequencing data analysis for individual G12 demonstrated the predominance of representatives from the phyla *Actinobacteria*, *Bacteroidetes*, *Firmicutes*, *Proteobacteria*, *Ca.* Saccharibacteria, and *Fusobacteria* (Fig. 1). Alignment of contigs from this metagenome to the reference genome of *Tannerella forsythia* identified 181 genes that were absent in the ancient sample.

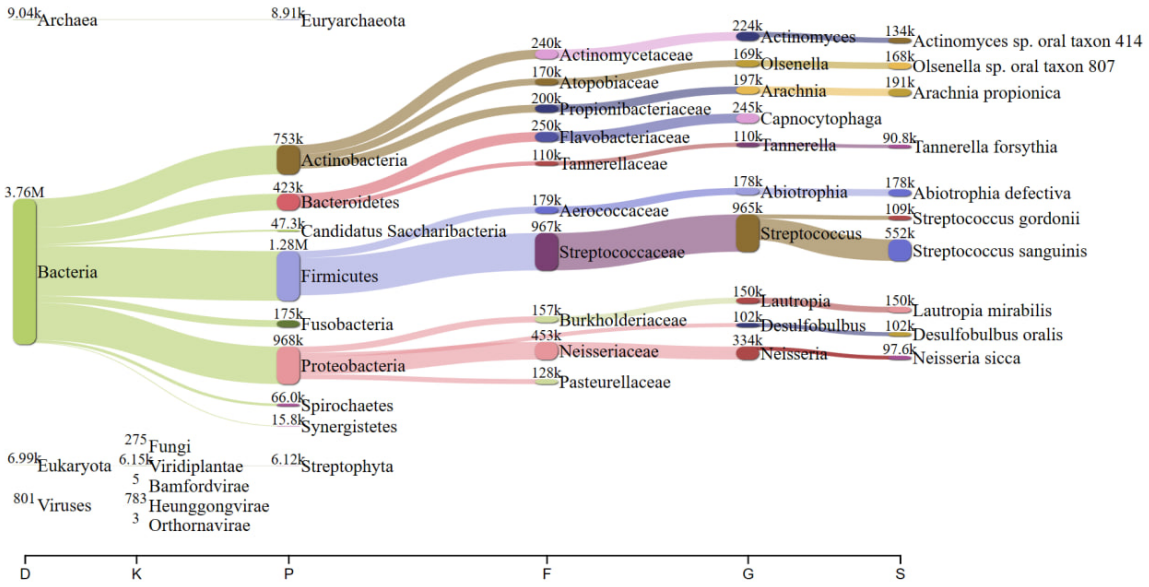

Figure 1: Metagenomic sequencing data analysis for individual G12

4 Discussion

The comparative analysis of sequencing results from the V5 region of 16S rRNA and metagenomic sequencing revealed a high similarity in the taxonomic composition of the presented samples. In both cases, representatives from the phyla *Actinobacteria*, *Bacteroidetes*, *Firmicutes*, and *Proteobacteria* predominated. For G12 sample, the dominant phyla for 16S rRNA sequencing and for metagenomic sequencing mostly were the same. Overall, bacteria from these phyla are well-known dominants in most ecosystems [8–10], and this analysis was no exception. The difference in a few phyla between the obtained results may be attributed to the utilization of different approaches for classifying the obtained sequences – employing the SILVA database and Kraken, which could affect the accuracy and depth of taxonomic assignment.

Furthermore, in two samples, B61 and G12, genes of bacteria from the "red complex," a group associated with severe forms of periodontal disease [11], were observed. Subsequent alignment of contigs from the assembled metagenome to the reference genome of *Tannerella forsythia* revealed genes that were absent in ancient bacteria of this species. Presumably, these genes emerged as a result of the evolution of this bacterium. Analysis of these genes in the NCBI GenBank revealed the presence of numerous IS elements, conjugal transfer proteins, conjugative transposon proteins, as well as tetracycline resistance protein, radical SAM peptide maturase, and proteins with undefined functions. Based on this data, hypotheses regarding the mechanisms of gene evolution in this bacterium can be formulated.

1. Mobile elements and horizontal gene transfer. Certain genes, such as transposases and proteins associated with conjugative transfer, suggest the potential for horizontal gene transfer between bacteria. This process may play a key role in acquiring new genetic material and properties.

2. Defense and adaptation systems. The presence of genes such as antirestriction and antibiotic resistance may indicate bacterial adaptation to external stress conditions, such as the presence of antibiotics or the immune systems of other organisms.

3. Novel functional properties. Some hypothetical proteins may perform new functions or have specialized roles in modern bacteria, which could have been advantageous for their survival in changing environmental conditions.

References

[1] Zhang, L. et al. Advances in Metagenomics and Its Application in Environmental Microorganisms. *Front. Microbiol.* 12, 1–15 (2021).

[2] Durazzi, F. et al. Comparison between 16S rRNA and shotgun sequencing data for the taxonomic characterization of the gut microbiota. *Sci. Rep.* 11, 1–10 (2021).

[3] Abellan-Schneyder, I. et al. Primer, Pipelines, Parameters: Issues in 16S rRNA Gene Sequencing. *mSphere* 6, (2021).

[4] Pérez-Cobas, A. E., Gomez-Valero, L. Buchrieser, C. Metagenomic approaches in microbial ecology: An update on whole-genome and marker gene sequencing analyses. *Microb. Genomics* 6, 1–22 (2020).

[5] Callahan, B. J. et al. DADA2: High-resolution sample inference from Illumina amplicon data. *Nat. Methods* 13, 581–583 (2016).

[6] Wood, D. E., Lu, J. Langmead, B. Improved metagenomic analysis with Kraken 2. *Genome Biol.* 15, (2019).

[7] Breitwieser, F. P. Salzberg, S. L. Pavian: Interactive analysis of metagenomics data for microbiome studies and pathogen identification. *Bioinformatics* 36, 1303–1304 (2020).

[8] Delgado-Baquerizo, M. et al. A global atlas of the dominant Bacteria Found in Soil. *Science (80-.).* 325, 320–325 (2018).

[9] Liu, A. C., Chou, C. Y., Chen, L. L. Kuo, C. H. Bacterial community dynamics in a swine wastewater anaerobic reactor revealed by 16S rDNA sequence analysis. *J. Biotechnol.* 194, 124–131 (2015).

[10] Rinninella, E. et al. What is the healthy gut microbiota composition? A changing ecosystem across age, environment, diet, and diseases. *Microorganisms* 7, (2019).

[11] Mahendra, J. et al. Red-Complex Bacterial Levels in Pregnant Women With Preeclampsia and Chronic Periodontitis. *Int. Dent. J.* 73, 503–510 (2023).