Dissecting corpses (Saint Petersburg addition)

Polina Guseva, Valeria Rubinova

March 31, 2023

Abstract

Sequencing samples from a habitat, metagenomics could describe the composition and functional diversity of communities living there. In this research, dental calculus as intact fossils of oral microbiome is analyzed from medieval skeletons along with their teeth roots. The periodontal damage correlates with the presence of the red complex of specific bacteria. In comparison to this preserved genome of *Tannerella forsythia*, new transposons and genes associated with antibiotic resistance are found in the modern reference.

Introduction

Metagenomics is a powerful tool for studying microbial communities in various environmental samples, such as soil, water, or human dental calculus [1], [2]. One of the main biological motivations for metagenomics analysis is to identify and understand the diversity and function of the microorganisms within a given community [3]. These microorganisms play essential roles in various biological processes and their remains could help us unveil the history [4].

Metagenomics analysis can provide valuable information for understanding microbial diversity and its functions [5] and there are two main approaches to researching it [6]. The 16S rRNA approach targets a conserved region of the bacterial genome and is useful for identifying and comparing bacterial taxa within a community [7]. This approach provides information on the community's taxonomic composition but does not give information on the functional potential of the microorganisms. Shotgun sequencing, on the other hand, involves sequencing all DNA in a given sample, and provides information on both the taxonomic composition and functional potential of the community [8]. This approach allows for identifying genes and pathways present in the community, which can provide insights into the metabolic processes and interactions within the community.

In this work, we explore the diversity and functions of the oral microbiome from medieval people. For that, we analyzed 16S amplicon and shotgun sequencing data of dental calculus which is a well-preserved graveyard of ancient DNA.

Methods

The samples of dental calculus caused by periodontitis and control from teeth roots (BioProject: PRJNA216965 [9]) are collected from the four skeletons (G12, B17, B61, and B78) excavated from the medieval site (approximately AD 950-1200) of Dalheim, Germany. Sequencing of V5 16S ribosomal RNA is perfumed using Roche GS Junior (454) and the shotgun is by a single Illumina HiSeq 2000 lane.

For 16S RNA amplicon sequencing, single-end reads with Phred33 encoding are analyzed by QIIME2 (version 2023.2.0) [10]. After importing necessary files (`qiime tools import`), the presence of any QIIME artefact is checked with `qiime tools validate`. Further, (`qiime demux`) is used for checking the distribution of sequence qualities and the number of sequences obtained per sample. The barcodes (m=35) and chimeric sequences (n=140) are cleaned by `qiime dada2 denoise-single`. To assess the performance, statistics (`qiime metadata tabulate`) and visual summary (`qiime feature-table`) are created. Continuing the subsequent analysis, feature IDs are mapped to sequences (`qiime feature-table tabulate-seqs`). After this, the representative sequences are compared with the taxonomy database (`qiime feature-classifier`) by Naive Bayes classifiers and visualised (`qiime metadata tabulate`). The taxonomic composition could be also viewed with interactive plots (`qiime taxa barplot`).

In the second step, the MetaPhlAn tool (version 4.0.6) [11], [12] analyzes shotgun sequencing data of only the affected G12 skeleton. Thus, the sequencing reads are aligned to the microbiota database by the bowtie2 aligner algorithms via `metaphlan` and at the end, organism abundances are calculated. Additionally, a comparison with the Human Microbiome Project [13] is conducted.

To get more solid alignment, the contigs are aligned to the reference *Tannerella forsythia* genome (GenBank ID #11045) by bwa (version 0.7.17) [14] with the Burrow-Wheeler Aligner (`bwa mem`) along with a suite of programs Samtools (version 1.7-1) [15] (`samtools view`). To detect new regions that appeared in the modern reference, the alignments transformed from the BAM extension to into the BED extension (`bedtools bamtobed`) are intersected by BEDTools (version 2.27.1) [16] (`bedtools intersect -v`).

Results

There are 4 samples of dental calculus as one per each skeleton and 5 controls from teeth roots with the G12 individual being sampled twice (tab.1). In general, the coverage of forward reads per each sample site and individual is more than 4k sequences (tab.1) for 16S RNA amplicons. Whereas, the quality of the first 180 bases is higher than 30 on average (fig.1).

Table 1: Per-sample sequence counts

Sample ID	Forward sequence count
S10-V5-Q-B61-calc	5957
S16S17-V5-K1-G12-root	5788
S20S21-V5-M-B61-root	5516
S8-V5-O-G12-calc	5362
S16S17-V5-K2-G12-root	5272
S18S19-V5-L-B17-root	4955
S22S23-V5-N-B78-root	4695
S14-V5-P-B17-calc	4491
S15-V5-R-B78-calc	4212

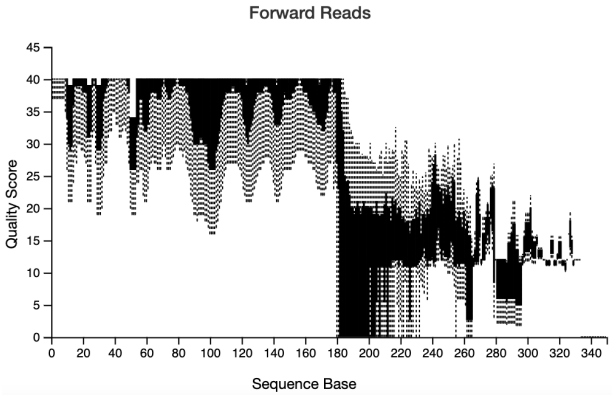

Figure 1: Quality plot

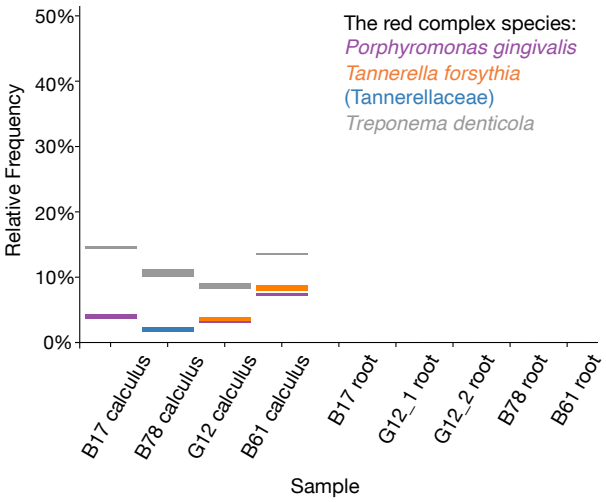

Figure 2: Barplot showing the taxonomic composition of our samples with members of the red complex (*Porphyromonas sp.*, *Tannerella sp.*, *Treponema sp.*)

There are three bacterial species called "the red complex" that are usually found together in periodontal pockets and cause dental calculus [17]. The three members of the red complex are:

- *Porphyromonas gingivalis*,
- *Tannerella forsythia*,
- *Treponema denticola*.

Their presence is confirmed in the dental calculus but none on the teeth roots (fig.2). The calculus samples from the G12 and B61 individuals contain all 3 members of the red complex. Whereas, the B17 skeleton got only *P. gingivalis* and *T. denticola* and B78 had *T. denticola* too and additionally someone from the Tannerellaceae family.

As for newly obtained genes by *Tannerella forsythia*, there are more than 400 new unique functions according to the Gene Ontology Terms. Most of them are related to the transposase activity (fig.3).

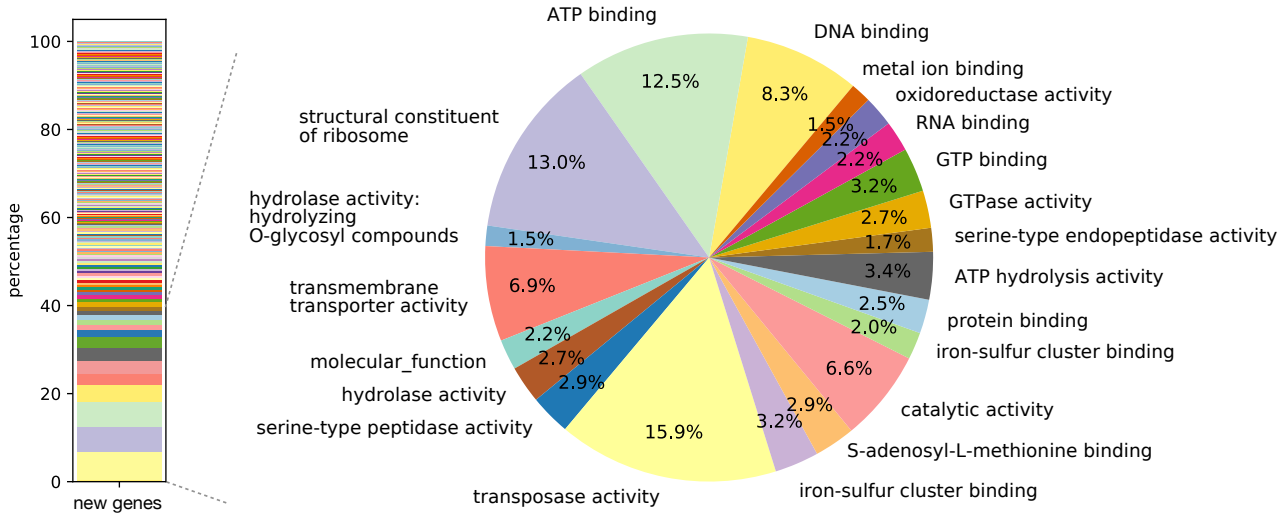

Figure 3: New genes obtained throughout centuries. The pie chart represents the most frequent ones

Discussion

Despite having hundreds of species in oral microbiome [18], only consortiums of specific organisms could cause the severe manifestation of periodontal disease [19]. In our case, the polymicrobial coalition called the red complex [17] is found in the skeletons G12 and B61 (fig.2). Therefore, these individuals were potentially prone to the disease which is consistent with the results of the original study [20]. The possible mechanisms of pathogen evolution are forming biofilms and working together in a complex network of interactions and division of labour [21] that facilitate their survival and growth.

There might be several reasons behind the difference in microbiome content in different samples. These could include variations in host genetics, environmental factors, diet, age, social status, and other factors [22]. For further information, the multiple aspects of lifestyle should be investigated.

Comparing the set of genes from medieval samples and the reference sequenced nowadays, specific clusters of adaptations are obtained in the last centuries (fig.3). Apart from transposases that normally appear and disappear everywhere in nature [23], the number of new functions is associated with antibiotic resistance. The main mechanisms of resistance mechanisms are 1) alternation of a target molecule, 2) destruction of a drug, and 3) decreasing its concentration by pumping out and/or lowering permeability [24]. Here we can see modifications related to changes in 1) the epitope (a structural constituent of ribosomes), 2) the degradation or neutralisation of antibiotics (various hydrolase activities, protein binding, serine-type (endo)peptidase activity), and 3) membrane permeability (transmembrane transporter activity). Probably that happened due to the appearance and the wide application of all kinds of antibiotics in the last century.

References

[1] J. Handelsman, "Metagenomics: application of genomics to uncultured microorganisms," *Microbiology and molecular biology reviews*, vol. 68, no. 4, pp. 669–685, 2004. doi:10.1128/MMBR.68.4.669-685.2004.

[2] C. Warinner, C. Speller, and M. J. Collins, "A new era in palaeomicrobiology: prospects for ancient dental calculus as a long-term record of the human oral microbiome," *Philosophical Transactions of the Royal Society B: Biological Sciences*, vol. 370, no. 1660, p. 20130376, 2015. doi:10.1098/rstb.2013.0376.

[3] L. W. Mendes, L. P. P. Braga, A. A. Navarrete, D. G. de Souza, G. G. Silva, and S. M. Tsai, "Using metagenomics to connect microbial community biodiversity and functions," *Current issues in molecular biology*, vol. 24, no. 1, pp. 103–118, 2017. doi:10.21775/cimb.024.103.

[4] D. Birnbaum, F. Coulier, M.-J. Pébusque, and P. Pontarotti, ""paleogenomics": looking in the past to the future," *Journal of Experimental Zoology*, vol. 288, no. 1, pp. 21–22, 2000. doi:10.1002/(SICI)1097-010X(20000415)288:1<21::AID-JEZ2>3.0.CO;2-Q.

[5] M.-E. Guazzaroni, A. Beloqui, P. N. Golyshin, and M. Ferrer, "Metagenomics as a new technological tool to gain scientific knowledge," *World Journal of Microbiology and Biotechnology*, vol. 25, pp. 945–954, 2009. doi:10.1007/s11274-009-9971-z.

[6] I. Laudadio, V. Fulci, F. Palone, L. Stronati, S. Cucchiara, and C. Carissimi, "Quantitative assessment of shotgun metagenomics and 16S rDNA amplicon sequencing in the study of human gut microbiome," *OMICS: A Journal of Integrative Biology*, vol. 22, no. 4, pp. 248–254, 2018. doi:10.1089/omi.2018.0013.

[7] A. Kamble, S. Sawant, H. Singh, *et al.*, "16S ribosomal RNA gene-based metagenomics: A review," *Biomedical Research Journal*, vol. 7, no. 1, p. 5, 2020. doi:10.4103/BMRJ.BMRJ_4_20.

[8] C. Quince, A. W. Walker, J. T. Simpson, N. J. Loman, and N. Segata, "Shotgun metagenomics, from sampling to analysis," *Nature biotechnology*, vol. 35, no. 9, pp. 833–844, 2017. doi:10.1038/nbt.3935.

[9] C. Warinner, "Human dental calculus LC-MS/MS." doi:10.6019/PXD000412, Oct 2017.

[10] E. Bolyen, J. R. Rideout, M. R. Dillon, N. A. Bokulich, C. C. Abnet, G. A. Al-Ghalith, H. Alexander, E. J. Alm, M. Arumugam, F. Asnicar, *et al.*, "Reproducible, interactive, scalable and extensible microbiome data science using QIIME 2," *Nature biotechnology*, vol. 37, no. 8, pp. 852–857, 2019. doi:10.1038/s41587-019-0209-9.

[11] D. T. Truong, A. Tett, E. Pasolli, C. Huttenhower, and N. Segata, "Microbial strain-level population structure and genetic diversity from metagenomes," *Genome research*, vol. 27, no. 4, pp. 626–638, 2017. doi:10.1101/gr.216242.116.

[12] A. Blanco-Miguez, F. Beghini, F. Cumbo, L. J. McIver, K. N. Thompson, M. Zolfo, P. Manghi, L. Dubois, K. D. Huang, A. M. Thomas, *et al.*, "Extending and improving metagenomic taxonomic profiling with uncharacterized species with MetaPhlAn 4," *bioRxiv*, pp. 2022–08, 2022. doi:10.1038/s41587-023-01688-w.

[13] P. J. Turnbaugh, R. E. Ley, M. Hamady, C. M. Fraser-Liggett, R. Knight, and J. I. Gordon, "The human microbiome project," *Nature*, vol. 449, no. 7164, pp. 804–810, 2007.

[14] H. Li and R. Durbin, "Fast and accurate short read alignment with Burrows–Wheeler transform," *bioinformatics*, vol. 25, no. 14, pp. 1754–1760, 2009. doi:10.1093/bioinformatics/btp324.

[15] P. Danecek, J. K. Bonfield, J. Liddle, J. Marshall, V. Ohan, M. O. Pollard, A. Whitwham, T. Keane, S. A. McCarthy, R. M. Davies, *et al.*, "Twelve years of SAMtools and BCFtools," *Gigascience*, vol. 10, no. 2, p. giab008, 2021. doi:10.1093/gigascience/giab008.

[16] A. R. Quinlan and I. M. Hall, "BEDTools: a flexible suite of utilities for comparing genomic features," *Bioinformatics*, vol. 26, no. 6, pp. 841–842, 2010. doi:10.1093/bioinformatics/btq033.

[17] S. Socransky, A. Haffajee, M. Cugini, C. Smith, and R. Kent Jr, "Microbial complexes in subgingival plaque," *Journal of clinical periodontology*, vol. 25, no. 2, pp. 134–144, 1998. doi:10.1111/j.1600-051X.1998.tb02419.x.

[18] J. F. Siqueira and I. N. Rôças, "The oral microbiota: general overview, taxonomy, and nucleic acid techniques," *Oral Biology: Molecular Techniques and Applications*, pp. 55–69, 2010. doi:10.1007/978-1-60761-820-1_5.

[19] R. Mohanty, S. J. Asopa, M. D. Joseph, B. Singh, J. P. Rajguru, K. Saidath, and U. Sharma, "Red complex: Polymicrobial conglomerate in oral flora: A review," *Journal of family medicine and primary care*, vol. 8, no. 11, p. 3480, 2019. doi:10.4103/jfmpc.jfmpc_759_19.

[20] C. Warinner, J. F. M. Rodrigues, R. Vyas, C. Trachsel, N. Shved, J. Grossmann, A. Radini, Y. Hancock, R. Y. Tito, S. Fiddyment, *et al.*, "Pathogens and host immunity in the ancient human oral cavity," *Nature genetics*, vol. 46, no. 4, pp. 336–344, 2014. doi:10.1038/ng.2906.

[21] A. Smith, *The Wealth of Nations*, vol. 11937. W. Strahan and T. Cadell, London, 1776.

[22] A. Renson, H. E. Jones, F. Beghini, N. Segata, C. P. Zolnik, M. Usyk, T. U. Moody, L. Thorpe, R. Burk, L. Waldron, *et al.*, "Sociodemographic variation in the oral microbiome," *Annals of epidemiology*, vol. 35, pp. 73–80, 2019. doi:10.1016/j.annepidem.2019.03.006.

[23] M. Correa, E. Lerat, E. Birmelé, F. Samson, B. Bouillon, K. Normand, and C. Rizzon, "The transposable element environment of human genes differs according to their duplication status and essentiality," *Genome Biology and Evolution*, vol. 13, no. 5, p. evab062, 2021. doi:10.1093/gbe/evab062.

[24] B. Alberts, *Molecular biology of the cell*. WW Norton & Company, 6 ed., 2017.