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Research letter

Does salivary microbiome reflect the functional activity profile of oral microbiota?

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Abstract: *Rationale* — *Human* oral cavity is a diverse habitat, consisting of many locations with its microbiotas. It was proven that bacteria detected in saliva could be the indicators of disease and be useful for diagnosis, monitoring, and overall assessment of the patient health. As pilot research of microbial communities associated with polycystic ovary syndrome (PCOS), biomaterials were collected from two patients (saliva, the contents of gingival pockets) for sequencing the amplicon libraries V4-V6 of variable regions of the 16S rRNA gene. Our study aimed at comparing the taxonomic and functional profiles of different locations in the oral cavity.
Material and Methods — DNA samples were sequenced using Illumina MiSeq; the qiime2-2020.2 and PICRUSt2 v2.4.1 software packages were used to process the sequencing results of the amplicon libraries.
Results — We demonstrated that the salivary microbiome had a greater taxonomic diversity, compared with the microbiome of the periodontal pockets. Regarding the ratios of different taxonomic group abundances, the species ratio in the saliva community significantly differed from the ratio in periodontal pockets. The microbiota of the oral cavity was classified as a producing community, since many different biosynthetic pathways were predicted. Similar functional features were identified for microbial communities in other locations.
Conclusions — Different locations in the biotope of the oral cavity have varying species richness of their communities and specific taxonomic composition. However, the microbiotas of different microniches perform similar metabolic functions. This finding allows considering the analysis of saliva microbiota sufficiently representative tool for characterization of the entire oral microbiome.

Keywords: oral microbiome, PCOS, 16S rRNA gene, protein function prediction.

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Introduction

Human microbiome is a set of genomes of all commensal microorganisms (bacteria, archaea, fungi, and viruses) and fragments of their nucleic acids circulating in the blood and urine. The Human Microbiome Project [1,2], initiated by the National Institute of Health, was designed to characterize the microbiota of various anatomical regions in healthy adults, including their oral cavity. The human oral cavity is diverse: it consists of teeth, gingival grooves, tongue, hard and soft palates, cheek mucosa, and tonsils. Each location has its specific microbiota. Saliva is a liquid fraction that is in contact with all surfaces of the oral cavity. It was proven that bacteria detected in saliva could be the markers of diseases and, as a result, could be useful for diagnostics, monitoring, and general assessment of the patient health [3]. The discovery of various biomarkers (including those of microbial nature), based on saliva, would provide unique opportunities for assessing the health status, since collecting saliva -based biomaterial is a non-invasive, fast and safe procedure; moreover, the samples are easily transported and stored [4].

The taxonomic structure of a community and its functionality represent different albeit complementary views of the microbiome. However, these two aspects of the microbiome organization are not independent. The representativity of genes in the metagenome is a derivative of the community member genomes and relative abundance of each member in the community [5]. High-throughput sequencing has made significant advances in our understanding of microbial ecology and is now widely used in the fields ranging from personalized medicine to bioenergetics. Complete information about the scope of the functional activity in the community can be obtained via processing the data of the whole genome sequencing (WGS) of that community. However, this technique does not work well if there is contamination of the host DNA (e.g., biopsy), or a community with a low biomass is examined. The most optimal approach in such cases would be the use of 16S rRNA gene fragment sequencing. This approach does not presume direct functional annotation; however, several tools allow predicting the available activity of a community via comparing the fragments of 16S rRNA genes with the database of reference genomes (for example, PICRUSt2 software: *Phylogenetic Investigation of Communities by Reconstruction of Unobserved States*) [6-10].

Polycystic ovary syndrome (PCOS) is a female endocrine disease of unclear origin, characterized by hyperandrogenism, oligoovulation or anovulation, and ovarian cysts [11-13]. The relationship between disease and microbiome dysbiosis has been shown in model studies performed on rats [14].

The pilot study was conducted to characterize the taxonomic diversity and predict the functional diversity of microbial

communities associated with PCOS in women. The samples were collected from two patients as saliva and periodontal pocket content for the metasequencing of V4-V6 variable regions in the 16S rRNA gene.

The objective of our study aimed at comparing the taxonomic and functional profiles of different locations in the oral cavity.

Material and Methods

DNA samples were sequenced using the Illumina MiSeq platform. To process the results of amplicon libraries metasequencing, the qiime2-2020.2 software package was employed [15]. The PICRUSt2 v2.4.1 software package was used to predict the functional potential of the microbial community [16]. To process the primary data of amplicon libraries and conduct metagenomic analysis, we used the shared research facility (SRF), *Irkutsk Supercomputer Center of the Siberian Branch of the Russian Academy of Sciences* (ISCC). The amplicon metasequencing data were deposited at the National Center for Biotechnology Information (NCBI) as *BioProject PRJNA778444*.

Results

As a result of sequencing, a total of 925,297 paired reads were obtained from 14 different locations in the oral cavity (*Table* 1). It should be noted that for five libraries (1_11, 1_16, 1_26, 1_31, 2_31), the number of reads per sample was 5,051±2,561; and after quality control and data trimming, this value decreased to 2,033±1,035. For all remaining libraries, the number of reads per sample remained equal to 81,822±2,090 (after trimming: 36,932±1,529). To prevent misinterpretation of biological data, samples with insufficient sequencing depth were excluded from further analysis. The total number of amplicon sequence variants (ASVs) was 2,054, with 236±15 ASVs per an individual sample.

To assess the similarity of microbiome composition, a Venn diagram was constructed based on the prevalence of detected ASVs (*Figure* 1). Only seven ASVs were detected in all locations of the oral cavity. There was a significant difference in ASV abundance between the microbiome of saliva and gingival pockets.

Table 1. Sequencing summary statistics

Patient	*Location**	*Sample name*	*Number of reads*	*Number of reads after quality check*	*ASV*
1	11	1_t11	743	279	-
	16	1_t16	939	382	-
	26	1_t26	962	387	-
	31	1_t31	11,927	4,843	-
	36	1_t36	78,467	34,851	237
	46	1_t46	81,944	37,391	224
	saliva	1_saliva_1	81,856	35,836	158
	saliva	1_saliva_2	97,897	48,217	233
2	11	2_t11	71,297	30,532	334
	16	2_t16	84,056	36,367	194
	26	2_t26	82,422	34,358	314
	31	2_t31	10,684	4,274	-
	36	2_t36	79,902	34,806	230
	46	2_t46	80.903	35,547	243
	saliva	2_saliva_1	74.031	33,715	188
	saliva	2_saliva_2	87.267	44,642	246

* The numbers indicate the specific number of each gingival pocket sensu FDI notation.

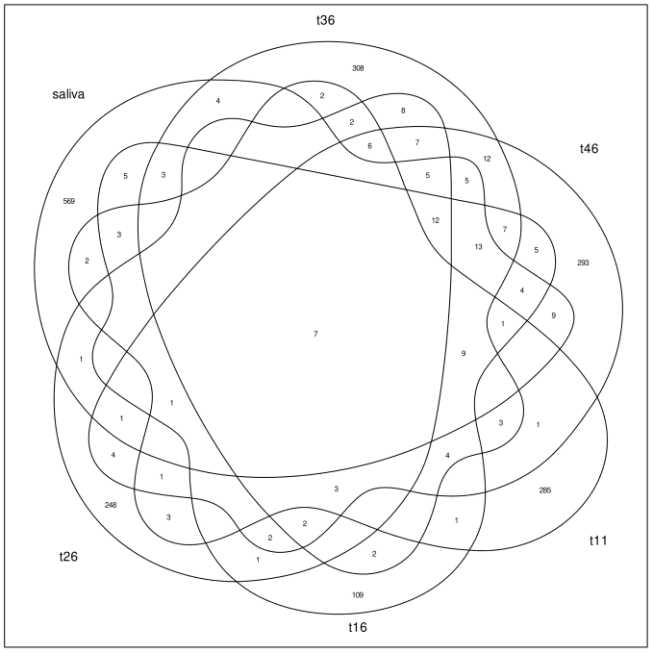

Figure 1. Common and unique amplicon sequence variants in different biotopes of the oral cavity.

Accordingly, the salivary microbiome had a larger number of unique ASVs, which was indicative of a more diverse microbial community, compared with the gingival pockets.

Ten phyla, 23 classes, 31 orders, 47 families, and 87 genera of bacteria were identified in the studied biotopes. The dominant phyla were *Firmicutes* (average relative abundance 37.7%), *Bacteroidetes* (27.5%), and *Proteobacteria* (20.7%). The following genera dominated the microbiome: *Prevotella* (16.2%), *Streptococcus* (16.2%), *Haemophilus* (14.1%), and *Veillonella* (10.8%) (*Figure* 2).

The species ratio in the saliva community differed from the ratio in periodontal pockets. Saliva was characterized by higher proportions of *Prevotella* (30-50%) and *Veillonella* (15-30%), whereas teeth were characterized by prevailing *Streptococcus* (3-30%), *Haemophilus* (5-30%), and *Porphyromonas* (8-10%). The genus *Prevotella* was represented by 33 species. The most numerous species were *P. melaninogenica* (opportunistic), *P. oris, P. pallens*, and one unidentified phylotype, identified only at the generic level. The genus *Streptococcus* was represented by a single phylotype not assigned to any known species. The genus *Haemophilus* was represented by the only species, *H. parainfluenzae.* Among the representatives of the genus *Veillonella*, *V. dispar* was the dominant species. However, there were several more species: *V. atypica, V. parvula*, and a phylotype not identified to the species level. The genus *Porphyromonas* was represented by the species *P. pasteri*, the *Porphyromonas* sp. HMT 278, and an unidentified phylotype.

Consequently, in terms of the taxonomic group ratio, the saliva community differed significantly from the communities of the gingival pockets.

For studied biotopes, using the PICRUSt2 program, a prediction of the microbiota functional profile was obtained.

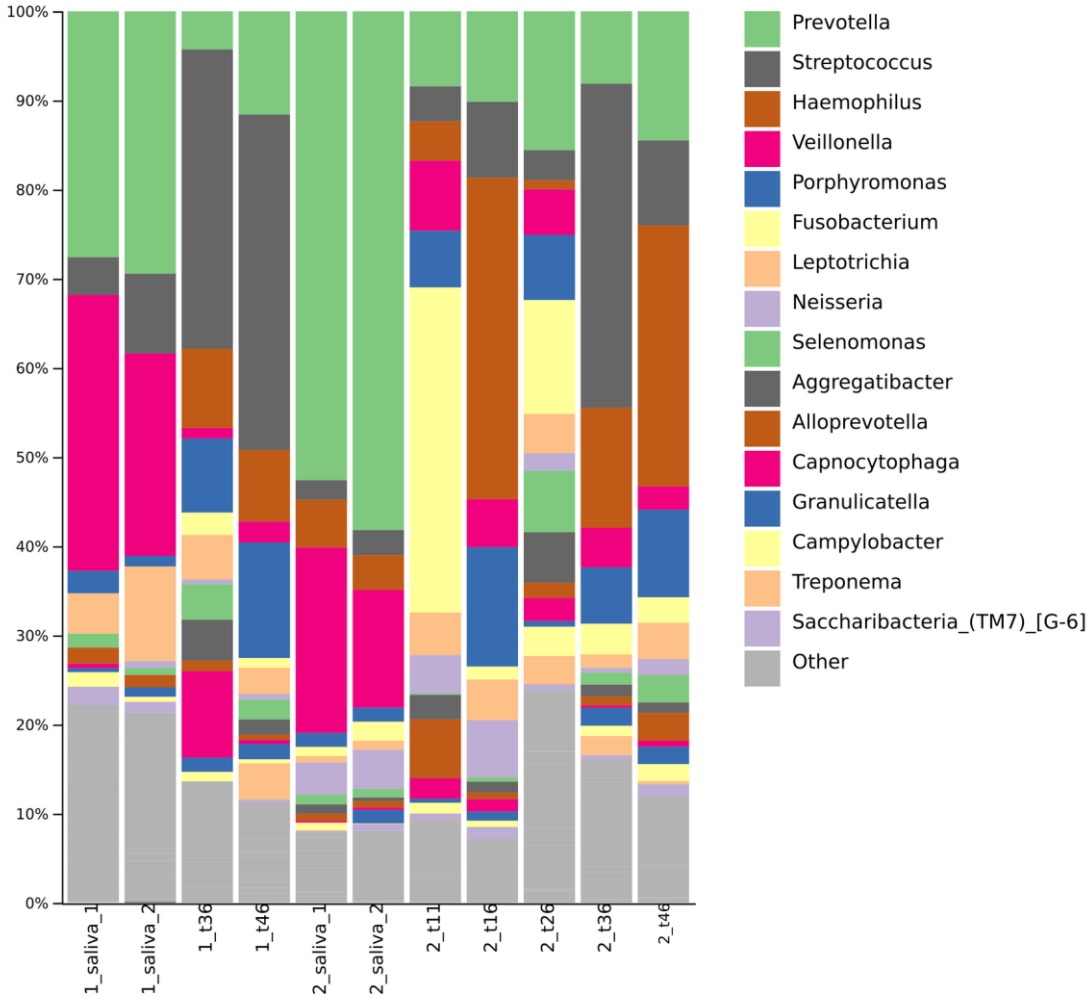

Figure 2. Taxonomic composition of microbiota in different locations of the oral cavity.

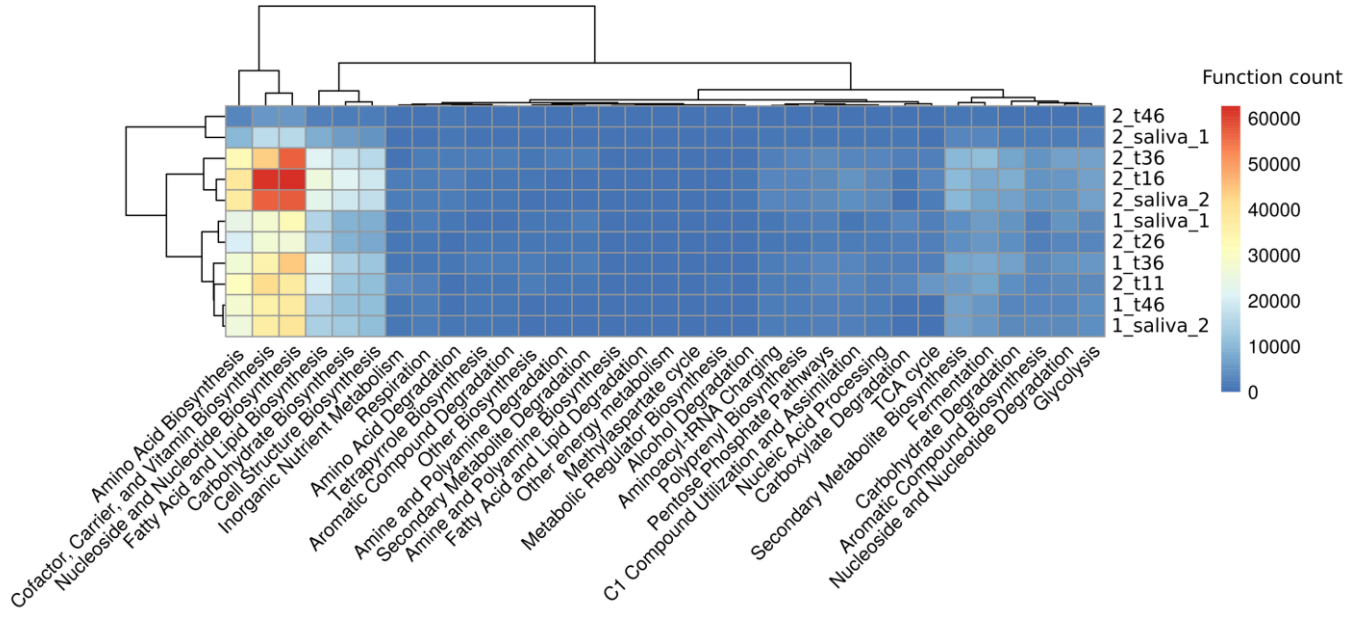

Figure 3. Heatmap of relative abundances of predicted metabolic pathways.

For the microbial community of the entire oral cavity, a high potential for biosynthetic pathways was predicted (81.7%). On average, the most common functions were: biosynthesis of nucleotides and nucleosides (20.3%); biosynthesis of cofactors, transporters and vitamins (18.9%); and biosynthesis of amino acids (13.5%) (*Figure* 3). The pathways for de novo biosynthesis of adenosine nucleotides (PWY-7229), utilization of pyrimidine nitrogenous bases (PWY-7208), gondoate biosynthesis (PWY-7663), and de novo biosynthesis of adenosine-deoxyribonucleotides (PWY-7220, PWY-7222) accounted for over 1% of all predicted metabolic pathways.

All but two locations exhibited similar predicted function profiles, implying a similar metabolic potential of the microbiota. Compared with the rest of the locations, saliva location and gingival pocket location No. 46 sensu FDI notation in the second patient had a low number of predicted metabolic pathways. Less intensive metabolism was predicted for these biotopes.

Hence, the microbial community of the oral cavity is a producing community since a large abundance of biosynthetic pathways was predicted for it. Despite different taxonomic profiles, similar functional features were identified for microbial communities from other locations, suggesting that representatives of different taxonomic groups use identical metabolic pathways.

Discussion

The studied salivary microbiome was characterized by a higher diversity at the ASV level than the microbiome of the periodontal pockets. Moreover, the ratios of the numbers of dominant genera in these two niches were different as well.

The presence of a core microbiome suggests some level of community stability. Major taxa make up a large proportion of the sequences, demonstrating that the most abundant organisms are stable in terms of taxonomic composition (but probably not quantitively). In our research, the core microbiome was represented by *Prevotella, Veillonella, Streptococcus, Haemophilus,* and *Porphyromonas*. The results of previous studies indicated a relatively stable structure of bacterial communities in the oral cavity with the following predominant phyla: *Firmicutes*, *Bacteroidetes*, Proteobacteria, *Fusobacteria*, *Actinobacteria*, and *Saccharibacteria*. Disorders of their balance may lead to diseases in the oral cavity [17]. The genera *Streptococcus, Haemophilus, Veillonella*, etc., are early colonizing bacteria. In a new environment, they can quickly adapt via regulating the expression of specific genes and providing new adhesion receptors for the bacteria colonizing at later stages: e.g., for the representatives of the genera *Fusobacterium, Prevotella, Porphyromonas*, and others. With an increase in the number of bacteria in the oral cavity, the concentration of signaling molecules that can activate the expression of related genes, such as virulence factors and mucopolysaccharides, increases [18].

The study by Hall *et al*. [19] demonstrated that bacterial communities of the oral cavity, inhabiting supragingival plaque, plaque of the tongue, and saliva, are different from each other. Taxonomic profiles of saliva and tongue communities are more similar to each other than profiles of saliva and supragingival plaque communities. Teeth represent a hard surface that promotes the development of biofilms different from those on the surfaces of the oral mucosa. Besides, supragingival plaque is regularly exposed to external influences during oral hygiene procedures. The similarity of the communities among saliva and plaque from the surface of the tongue is explained by the fact that the dorsum of the tongue is a large surface area containing biomass with a high content of biofilms. Tongue undergoes bacterial exfoliation and desquamation of cells, whereas saliva, being liquid, is in contact with the entire surface of the oral cavity and contains fragments of exfoliated biofilms [19-21].

The studied microbial community can be considered biosynthetic since, among the predicted metabolic pathways, the pathways responsible for the biosynthesis of nucleotides and secondary metabolites prevail, which is indicative of the productive capacity of the community.

For the studied locations of the biotope in the oral cavity, different taxonomic profiles of microorganisms were observed; however, the functional profiles were overall similar. The species composition of the microbial community is rarely static: it often fluctuates in one way or another. Changes in taxonomic profiles result in differences in functional profiles, ultimately changing the overall functionality of the community. However, the magnitude of such functional changes strongly depends on how genes are distributed in the genomes of community members. This gene distribution is determined by taxonomic composition. It would, for example, differ markedly between the communities of organisms with similar genomic content, and communities of organisms with genomes encoding relatively different sets of genes. Taken together, such observations show that the functional resistance of a community to taxonomic perturbations can vary widely across different communities with different species compositions [5].

PICRUSt, predicting the metabolic potential of the microbiota based on the concurrence of fragments of the 16S rRNA gene with a database of complete annotated bacterial genomes, is a powerful tool for analyzing the microbiome. However, this approach has its drawbacks: it is extrapolation-based approach, hence two main points should be considered: a) the libraries must have sufficient sequencing depth; b) the prediction of random bacterial genomes yields high correlations merely because certain gene families are common or, on the contrary, rare in all bacterial taxa.

Conclusion

Different locations of the oral cavity have varying species richness of their communities, and each of these communities is characterized by specific taxonomic composition. Despite that, the microbiota of different locations performs similar metabolic functions. This finding allows considering the analysis of salivary microbiome sufficiently representative tool for the functional characterization of the microbiome in the entire oral cavity.

Conflict of interest

The authors declare no conflicts of interest.

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