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Identifying a healthy oral microbiome through metagenomics

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Abstract

We present the results of an exploratory study of the bacterial communities from the human oral cavity showing the advantages of pyrosequencing complex samples. Over 1.6 million reads from the metagenomes of eight dental plaque samples were taxonomically assigned through a binning procedure. We performed clustering analysis to discern if there were associations between non-caries and caries conditions in the community composition. Our results show a given bacterial consortium associated with cariogenic and non-cariogenic conditions, in agreement with the existence of a healthy oral microbiome and giving support to the idea of dental caries being a polymicrobial disease. The data are coherent with those previously reported in the literature by 16S rRNA amplification, thus giving the chance to link gene functions with taxonomy in further studies involving larger sample numbers.

Keywords: Binning, dental caries, metagenomics, probiotic, pyrosequencing
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Introduction

Unlike most infectious diseases where a single causing agent can be found responsible for the infection, oral diseases appear to be the outcome of multiple microorganisms. In periodontitis, for instance, at least three bacterial organisms have been found to be directly associated with the development of the disease [1]. Similarly, the complexity of the microbial community in the oral cavity has hampered the identification of a single aetiological agent for dental caries. It has been demonstrated that *Streptococcus sobrinus* and above all *S. mutans* are acidogenic and play an important role in caries initiation [2]. However, the use of molecular techniques like PCR amplification and cloning of the 16S rRNA gene have revealed that a high proportion of samples from cavities do not contain *mutans* streptococci, whereas other acid-producing bacteria are present [3]. These include *Lactobacillus*, *Actinomyces* or *Bifidobacterium*. Recent molecular work has confirmed these results and expanded the list of potential cariogenic species to *Veillonella*, *Propionibacterium* and *Atopobium*, among others [4], most of which are poorly characterized species.

Dental caries, microbiome and pyrosequencing

Dental caries is probably better understood as a polymicrobial disease [5] where the interaction and synergistic effect of multiple species should be taken into account for future strategies of diagnosis, prevention and treatment. Given that a large portion of oral bacteria cannot be cultured by current laboratory techniques, the introduction of molecular approaches has provided a significant improvement in our understanding of oral microbiota. However, PCR amplification and cloning still have significant biases that do not allow microbial diversity to be fully studied, as many species or DNA segments cannot be detected. Thus, a metagenomic approach by which the total DNA from a microbial community is obtained obviating the need for culture or PCR amplification has been proposed as a promising strategy to study the full genetic pool of the human microbiome in health and disease [6]. In addition, the extraordinary increase in sequencing output and the reduction of the associated cost

provided by next generation sequencing has been applied to the study of gut microbiota, providing a more complete picture of human-associated bacterial communities [7].

We used a 454 GLX Titanium pyrosequencing approach to obtain over 800 Mbp of DNA sequence from supragingival dental plaque samples from eight individuals who varied in oral health status. Two of them (healthy controls, with no caries) were volunteers who had never suffered from dental caries in their lives and another four samples were from individuals with one, four, eight and 15 cavities at the moment of sampling. In addition, two samples were taken from individual cavities in order to give a first glimpse of the diversity at these diseased sites. Over 2 million pyrosequencing reads of 425 bp average length were analysed by phymmBL [8], a binning method that combines the assignment of sequences by homology and by nucleotide composition using hidden Markov models, thus allowing taxonomic binning and prediction for each single read. All the available complete whole genome sequencing as well as reference genomes for the Human Microbiome Project and the Human Oral Database were used to build a local database to predict taxonomic affiliation. Filtering the reads under 200 bp, we managed to taxonomically assign over 1.6 million reads to the 1150 genomes analysed, with an estimated accuracy at the class level over 75% [8].

When a correspondence analysis was performed with the assigned reads, samples with bad oral health tended to cluster together (Fig. 1). As can be observed in the figure, the principal component separated the two healthy samples and the sample from the individual with a single cavity from the other five samples with dental plaque of individuals with more than four cavities and from the two samples within cavities. Whereas the dental plaque samples from individuals of bad oral health clustered tightly at the positive values of the main axis, the three samples from healthy individuals occupied different positions at the secondary axis. Taken together, the results show hints of a specific microbiota associated with the presence of dental caries, and there appear to be several combinations of bacteria under good oral health, a finding that should be confirmed with larger

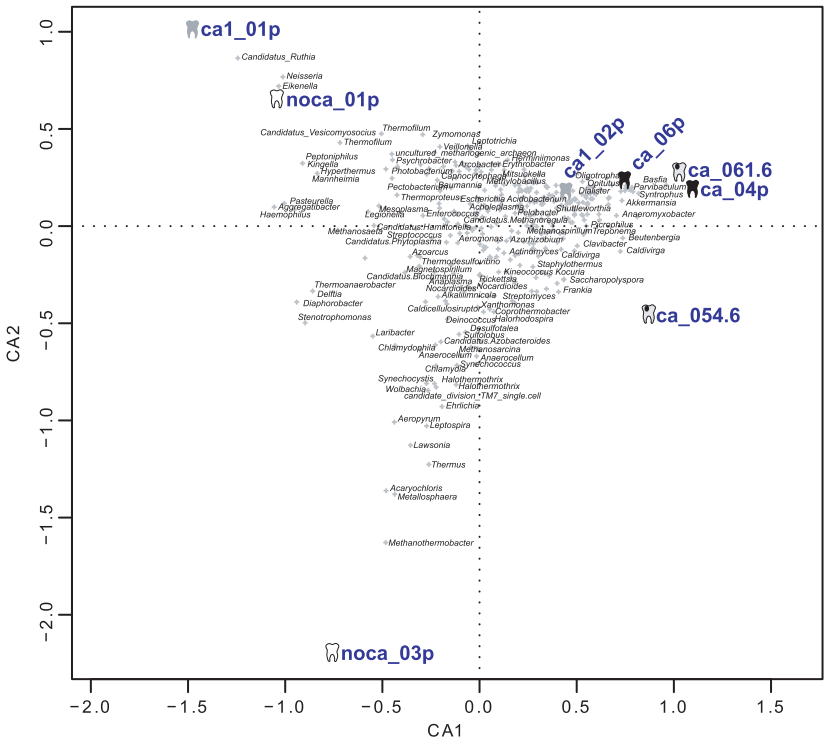

FIG. 1. Correspondence analysis of the bacterial diversity in eight oral samples based on the taxonomic assignment of 1.6 million pyrosequencing reads by the binning PhymmBL approach. The first axis successfully separates healthy from diseased individuals. Around the healthy samples some bacterial genera are suggested to be potentially associated with absence of caries. The samples are represented with symbols according to health status: individuals that have never suffered from dental caries are marked with white teeth symbols (samples noca-01p and noca-03p); individuals with one cavity (sample ca1-01p) and four cavities (sample ca1-02p) are marked with grey teeth symbols; individuals with eight and 15 cavities (samples ca-06p and ca-04p, respectively) are marked with black teeth symbols; samples from individual cavities are marked with a black spot within a white tooth and correspond to teeth 1.6 (sample ca-06_1.6) and 4.6 (sample ca-05_4.6), following WHO nomenclature.