

# Oral microbiome: Unveiling the fundamentals

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## Abstract

The oral cavity has the second largest and diverse microbiota after the gut harboring over 700 species of bacteria. It nurtures numerous microorganisms which include bacteria, fungi, viruses and protozoa. The mouth with its various niches is an exceptionally complex habitat where microbes colonize the hard surfaces of the teeth and the soft tissues of the oral mucosa. In addition to being the initiation point of digestion, the oral microbiome is crucial in maintaining oral as well as systemic health. Because of the ease of sample collection, it has become the most well-studied microbiome till date. Previously, studying the microbiome was limited to the conventional culture-dependent techniques, but the abundant microflora present in the oral cavity could not be cultured. Hence, studying the microbiome was difficult. The emergence of new genomic technologies including next-generation sequencing and bioinformatics has revealed the complexities of the oral microbiome. It has provided a powerful means of studying the microbiome. Understanding the oral microbiome in health and disease will give further directions to explore the functional and metabolic alterations associated with the diseased states and to identify molecular signatures for drug development and targeted therapies which will ultimately help in rendering personalized and precision medicine. This review article is an attempt to explain the different aspects of the oral microbiome in health.

**Keywords:** 16S rRNA, Human Oral Microbiome Database, microbiome, next-generation sequencing

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## INTRODUCTION

The community of microbial residents in our body is called the microbiome. The term "microbiome" is coined by Joshua Lederberg, a Nobel Prize laureate, to describe the ecological community of symbiotic, commensal and pathogenic microorganisms. These microorganisms literally share our body space.<sup>[1]</sup> The number of microbes present in our bodies is almost the same or even more as compared to that of our cells.<sup>[2]</sup>

Oral microbiome, oral microbiota or oral microflora refers to the microorganisms found in the human oral cavity.<sup>[3]</sup>

Oral microbiome was first identified by the Dutchman Antony van Leeuwenhoek who first identified oral microbiome using a microscope constructed by him.<sup>[4]</sup> He was called the father of microbiology and a pioneer who discovered both protists and bacteria.<sup>[5]</sup> In 1674, he observed his own dental plaque and reported "little living animalcules prettily moving."<sup>[6]</sup>

Genome is the genetic material of an organism. It is the complete set of DNA including all of its genes.

Oral microbiome is defined as the collective genome of microorganisms that reside in the oral cavity. After the

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gut, it is the second largest microbial community in the humans. As compared with other body sites, they exhibit an astounding diversity of predicted protein functions. Human microbiome consists of a core microbiome and a variable microbiome. The core microbiome is common to all the individuals, whereas variable microbiome is unique to individuals depending on the lifestyle and physiological differences. The oral cavity has two types of surfaces on which bacteria can colonize: the hard and the soft tissues of teeth and the oral mucosa, respectively.<sup>[7]</sup> The teeth, tongue, cheeks, gingival sulcus, tonsils, hard palate and soft palate provide a rich environment in which microorganisms can flourish.<sup>[8]</sup> The surfaces of the oral cavity are coated with a plethora of bacteria, the proverbial bacterial biofilm.<sup>[9]</sup>

An ideal environment is provided by the oral cavity and associated nasopharyngeal regions for the growth of microorganisms. The normal temperature of the oral cavity on an average is 37°C without significant changes, which provide bacteria a stable environment to survive. Saliva also has a stable pH of 6.5–7, the favorable pH for most species of bacteria. It keeps the bacteria hydrated and also serves as a medium for the transportation of nutrients to microorganisms.<sup>[10]</sup>

#### DEVELOPMENT OF THE ORAL MICROBIOME

The womb of the fetus is usually sterile.<sup>[11-13]</sup> However, recent studies have reported intrauterine environment colonization, specifically the amniotic fluid, by oral microorganisms, in up to 70% of the pregnant women.<sup>[14]</sup> The baby comes in contact with the microflora of the uterus and vagina of the mother during delivery, and later with the microorganisms of the atmosphere at birth. Usually, the oral cavity of the newborn is sterile in spite of the large possibility of contamination. The mouth is regularly inoculated with microorganisms from the first feeding onward, and the process of resident oral microflora acquisition begins.<sup>[12]</sup>

*Fusobacterium nucleatum* was the most common cultivable microorganism found. Any surface acquires the resident microflora by the successive transmission of microorganisms to the site of potential colonization. Although the main vehicle for transmission is saliva, passive transfer from the mother, from the microorganisms present in water, milk and the environment, also occurs.<sup>[11-13]</sup>

At or shortly after birth, colonization begins. Initial colonizers immediately after birth are called the pioneer species, for example, *Streptococcus salivarius*. The oral cavity

is invaded mainly by aerobes by the 1<sup>st</sup> year and may include *Streptococcus*, *Lactobacillus*, *Actinomyces*, *Neisseria* and *Veillonella*. Once tooth eruption begins, these organisms can colonize on the nonshedding surfaces. More surfaces are established for colonization after eruption of all the teeth. Development of gingival crevices occurs for the colonization of periodontal microbes. Plaque accumulation is seen at different sites on the tooth such as smooth surfaces and pit and fissures, for different microbial colonies to be established. High species diversity and microbial succession develop by this process. With aging when all teeth are lost, the flora becomes similar to that in a child before tooth eruption.<sup>[6]</sup>

Bacteria form multigenic communities by adhering not only to oral surfaces, but also to each other. Their composition and stability is influenced by specific partner relationships.<sup>[15]</sup> The formation and the evolution of communities is influenced by factors such as selective adherence to tooth surfaces or epithelium, specific cell-to-cell binding as a driver of early community composition and interaction between the organisms which leads to changes in the local environment, representing the first step on the road to oral diseases.<sup>[16]</sup>

#### COMPOSITION OF THE ORAL MICROBIOME

A wide range of microorganisms are present in the oral cavity. It is in constant contact with and has been shown to be vulnerable to the effects of the environment.<sup>[17]</sup>

The human microbiome consists of a core microbiome and a variable microbiome. The core microbiome consists of predominant species that exist at different sites of the body under healthy conditions. The variable microbiome has evolved in response to unique lifestyle and genotypic determinants and is exclusive to an individual.<sup>[18]</sup>

The microbial ecology of the oral cavity is complex and is a rich biological setting with distinctive niches, which provide a unique environment for the colonization of the microbes. These niches include the gingival sulcus, the tongue, the cheek, the hard and soft palates, the floor of the mouth, the throat, the saliva and the teeth.<sup>[8,19]</sup>

Different surfaces in the mouth are colonized preferentially by the oral bacteria due to specific adhesins on their surface which bind to complementary receptors on an oral surface.<sup>[20]</sup>

The normal microbiome is formed by bacteria, fungi, viruses, archaea and protozoa. The reports on a normal

microbiome, however, are restricted to the bacteriome, and there are very few reports on the mycobiome–fungal microbiome.<sup>[7]</sup>

Oral cavity is one of the most well-studied microbiomes till date with a total of 392 taxa that have at least one reference genome and the total genomes across the oral cavity approaching 1500.<sup>[21]</sup>

Approximately 700 species of prokaryotes have been identified in it. These species belong to 185 genera and 12 phyla, of which approximately 54% are officially named, 14% are unnamed (but cultivated) and 32% are known only as uncultivated phylotypes.<sup>[9]</sup> The 12 phyla are *Firmicutes*, *Fusobacteria*, *Proteobacteria*, *Actinobacteria*, *Bacteroidetes*, *Chlamydiae*, *Chloroflexi*, *Spirochaetes*, SR1, *Synergistetes*, *Saccharibacteria* (TM7) and *Gracilibacteria* (GN02).<sup>[22]</sup> At the genus level, there is a conserved oral microbial community in healthy mouths. Diversity in the microbiome is individual specific and site specific, despite the similarities. The tongue has numerous papillae with few anaerobic sites and hence harbors a diverse microflora which also includes anaerobes. The areas with low microbial diversity are the buccal and palatal mucosae.<sup>[23]</sup>

Oral microbiome may show large and rapid changes in composition and activity both spatially and temporally and are developmentally dynamic with the host. These multiplex, nonequilibrium dynamics are the result of many factors, such as the temporal frequency of host and diet, the response to the changes in pH, interactions among the bacteria and, on a larger time frame, gene mutations and horizontal gene transfer that extend new properties to the strain.<sup>[21]</sup>

There is a symbiotic relationship between the microorganisms in our oral cavity based on mutual benefits. The commensal populations do not cause harm and maintain a check on the pathogenic species by not allowing them to adhere to the mucosa. The bacteria become pathogenic only after they breach the barrier of the commensals, causing infection and disease.<sup>[24]</sup>

The principal bacterial genera found in the healthy oral cavity are as follows:<sup>[12]</sup>

Gram positive:

1. Cocci – *Abiotrophia*, *Peptostreptococcus*, *Streptococcus*, *Stomatococcus*
2. Rods – *Actinomyces*, *Bifidobacterium*, *Corynebacterium*, *Eubacterium*, *Lactobacillus*, *Propionibacterium*, *Pseudoramibacter*, *Rothia*.

Gram negative:

1. Cocci – *Moraxella*, *Neisseria*, *Veillonella*
2. Rods – *Campylobacter*, *Capnocytophaga*, *Desulfobacter*, *Desulfobivrio*, *Eikenella*, *Fusobacterium*, *Hemophilus*, *Leptotrichia*, *Prevotella*, *Selemonas*, *Simonsiella*, *Treponema*, *Wolinella*.

## NONBACTERIAL MEMBERS OF ORAL CAVITY

The oral cavity contains diverse forms of microbes such as protozoa, fungi and viruses. *Entamoeba gingivalis* and *Trichomonas tenax* are the most commonly found protozoa and are mainly saprophytic. *Candida* species is the most prevalent fungi seen associated with the oral cavity. Ghannoum *et al.* carried out culture-independent studies on twenty healthy hosts and reported 85 fungal genera. The main species observed were those belonging to *Candida*, *Cladosporium*, *Aureobasidium*, *Saccharomycetales*, *Aspergillus*, *Fusarium* and *Cryptococcus*.<sup>[25]</sup>

The oral habitats have the highest alpha diversity in the body showing the highest operational taxonomic unit-level richness, after stool samples. Lower alpha diversity is shown with the skin and vaginal microbiota. The oral sites have the lowest beta diversities where samples from the same sites among individuals (beta diversity) are compared, which signifies that members of the population share relatively similar organisms in oral sites than in other sites of the body.<sup>[26]</sup> Taxonomic diversity within the sample is alpha diversity and that between the samples is beta diversity.<sup>[27]</sup>

## FUNCTIONS OF THE ORAL MICROBIOME

The physiology and ecology of the microbiota become intimately connected with those of the host at both micron scale and host scale. The promotion of health or progression toward disease is critically influenced by the microbiota.<sup>[28]</sup> The oral microbiome usually exists in the form of a biofilm. It plays a crucial role in maintaining oral homeostasis, protecting the oral cavity, and preventing disease development. Knowing the identity of the microbiome and the neighbors with which they commonly interact is necessary for mechanistic understanding of the key players.<sup>[29]</sup>

The microbial communities present in the human body play a role in critical, physiological, metabolic and immunological functions which include digestion of food and nutrition; generation of energy, differentiation and maturation of the host mucosa and its immune system; control of fat storage and metabolic regulation; processing and detoxification of environmental chemicals; barrier function of skin

and mucosa; maintenance of the immune system and the balance between pro-inflammatory and anti-inflammatory processes; promoting microorganisms (colonization resistance) and prevention of invasion and growth of disease.<sup>[1]</sup>

## THE HUMAN MICROBIOME PROJECT

In 2008, the National Institute of Health launched the Human Microbiome Project (HMP) recognizing the importance of studying the human microbiome.<sup>[8]</sup>

Recent developments in bioinformatics have improved the ability to study the human microbiome. These advancements gave rise to an overabundance of genomic and metagenomics studies investigating the role of microbes in different ecosystems.<sup>[30]</sup>

The HMP is a summation of multiple projects that are now being launched, concurrently, in multiple parts of the world including the USA, the European Union and Asia and not a single project.<sup>[31]</sup> Understanding that >99% of microbes from the environment could not be easily cultured, microbial ecologists developed approaches for studying the microorganisms *in situ*, principally by sequencing the 16S ribosomal RNA gene (16S). It is a taxonomic and a phylogenetic marker for the identification of members of microbial communities.<sup>[32]</sup>

Due to the advent of high-throughput DNA sequencing, research on what constitutes the normal oral microbiome has expanded dramatically.<sup>[33]</sup>

Nine sites from the oral cavity were sampled from healthy volunteers in the HMP. These sites were the tongue, dorsum, hard palate, buccal mucosa, keratinized gingiva or gums, palatine tonsils, throat and supra- and subgingival plaque and saliva. K Li Bihan and Methe (2013) studied the HMP database and detected a relatively stable and a small core oral microbiome present in a majority of samples but in low abundance.<sup>[34]</sup>

## HUMAN ORAL MICROBIOME DATABASE

The Human Oral Microbiome Database (HOMD) provides a repository of oral bacterial genome sequences and an in-depth resource consisting of the descriptions of oral bacterial taxa, a 16S rRNA identification tool.<sup>[35]</sup>

It is a unique database which was launched in 2010 by the National Institute of Dental and Craniofacial Research for maintaining the information of oral-derived cultivable and noncultivable isolates.<sup>[36]</sup>

The expanded HOMD (eHOMD) is created with a goal of providing the scientific community with comprehensive curated information on the bacterial species present in the human aerodigestive tract (ADT), which includes the upper digestive and upper respiratory tracts, pharynx, nasal passages, sinuses and esophagus and the oral cavity. Genome sequences for the ADT bacteria determined by different projects like – a part of the HOMD project – the Human Microbiome Project and other sequencing projects are being added to the eHOMD as they become available.<sup>[36]</sup>

The eHOMD contains information of approximately 772 prokaryotic species, where 70% is cultivable and 30% belong to the uncultivable class of microorganisms along with whole-genome sequences of 482 taxa. Out of the 70% culturable species, 57% have already been assigned to their names. The 16S rDNA profiling of the healthy oral cavity categorized the inhabitant bacteria into six broad phyla, namely, *Firmicutes*, *Actinobacteria*, *Proteobacteria*, *Fusobacteria*, *Bacteroidetes* and *Spirochaetes* constituting 96% of the total oral bacteria.<sup>[37]</sup>

## METHODS OF STUDYING THE ORAL MICROBIOME

The traditional methods of identification of microbes included culture methods which evolved from culture-dependent studies of a single species to complex *in vitro* multispecies communities. Culture-independent characterization of the entire microbiota *in vivo*, and analyzing the expression of individual gene to meta-omic analysis, has become possible with technological advancements.<sup>[38]</sup>

In recent years, the largest advance is probably by the development of culture-independent “omics” techniques. These include studying the DNA, RNA, proteins or metabolites of the whole microbial community.<sup>[39]</sup>

The two fields of research that have emerged to detect and identify the presence of microbes in the body and understand the nature of microbiome activity in health and disease are microbiomics and metagenomics.<sup>[18]</sup>

Metagenomics is a set of techniques which detects bacteria that cannot be cultured. It also identifies the genomic diversity of microbes by applying the power of genomic analysis to the entire community of microbes.<sup>[40]</sup>

Metagenomics gives information not only about the kind of organisms present, but also their functional potential through an analysis of metabolic pathway genes. It also provides information on the use of protein-coding sequence databases. It sequences the entire DNA from a given sample.<sup>[41]</sup>



Due to the ease with which samples can be obtained, the oral microbiome is arguably the well-studied human microbiome to date.<sup>[42]</sup>

### Culture and microscopy

The historical methods of identification of bacterial taxa were culture dependent. These included microscopy, biochemical and other phenotypic tests, sugar utilization, growth conditions and antibiotic sensitivity.<sup>[43]</sup> The actual diversity of the oral microbiome cannot be completely revealed by culture-based methods. The endeavors of numerous researchers have now isolated, cultivated, identified, characterized and classified approximately 50% of the estimated 700 bacterial species which are commonly present in the oral cavity.<sup>[20]</sup>

The main difficulty with the conventional culture and culture-based analytical technologies is that many of the bacterial species in biological samples cannot be cultured, thus making these approaches unsuitable for research.<sup>[38]</sup>

### Gel-based techniques

Due to several culture-independent techniques, high-throughput analysis of the microbial communities has been possible. The different techniques used are denaturing gradient gel electrophoresis (DGGE), temperature gradient gel electrophoresis and restriction fragment length polymorphism (RELP).<sup>[43]</sup>

### Polymerase chain reaction-based methods

Conventional polymerase chain reaction (PCR), real-time quantitative PCR, PCR-DGGE, random amplified polymorphic DNA/arbitrarily primed PCR, repetitive element-based PCR, multilocus sequence typing, PCR-RELP and terminal-RELP are the different PCR-based methods available for the identification of microbes.<sup>[20]</sup>

### DNA microarrays

In the scientific community, phylogenetic DNA micro-arrays have been identified as valuable tools for high-throughput, systematic and quantitative analysis of bacterial communities in different microbial ecosystems including the oral microbiota.<sup>[44]</sup>

### 16S rRNA sequencing

The two basic DNA sequencing approaches that are commonly applied to study uncultivated oral microbial communities are 16S rRNA sequence analysis and metagenomics. 16S rRNA sequencing involves sequencing of the conserved 16S rRNA gene, whereas metagenomics involves whole-genome shotgun sequencing (WGS). All the samples of DNA are randomly sheared by a “shotgun”

method. Sequencing is then done by either classical Sanger sequencing or NGS.<sup>[45]</sup> 16S rDNA gene profiling is used in most of the recent studies to assess the organisms present in a sample or if complete profiling of gene content in a given habitat is required, shotgun metagenomics is done.<sup>[46]</sup>

Why 16S rRNA? (1) It is present in almost all bacteria, often exists as a multigene family or operons; (2) 16S rRNA gene function has not changed over time, which suggests that random changes in sequence are a more precise measure of time (evolution) and (3) the 16S rRNA gene (1500 bp) is large enough for informatics purpose.<sup>[47]</sup>

It is a highly conserved gene; hence, using it as a marker is more beneficial than using the whole genome, as in our database, the reference gene is less likely to be different than the gene in bacteria collected from environmental samples.<sup>[48]</sup>

16S rRNA sequencing only determines the presence or abundance of bacterial species. It thus only allows researchers to draw conclusions based on observations. Shotgun metagenomics sequencing will also reveal the associated metabolic pathways.<sup>[49]</sup>

16S rRNA profiling provides the taxonomic composition, whereas metagenomics WGS data can provide not only taxonomy, but also the biological functional profiles for the microbial communities.<sup>[50]</sup>

### Next-generation sequencing platforms

Next-generation sequencing (NGS) techniques have revolutionized the study of microbial diversity in the last decade. This has allowed for large-scale sequencing projects to be completed in a few days or sometimes hours.

The main NGS technologies are as follows:

1. 454 pyrosequencing
2. Applied Biosystems
3. Illumina
4. Pacific Biosciences
5. Oxford Nanopore.

For meaningful interpretations, the NGS analysis requires extensive bio-informatics capabilities involving data quality control, aligning and mapping to good reference genomes, filtering for reads of good quality, removing chimeras and normalizations across samples and populations.<sup>[43]</sup>

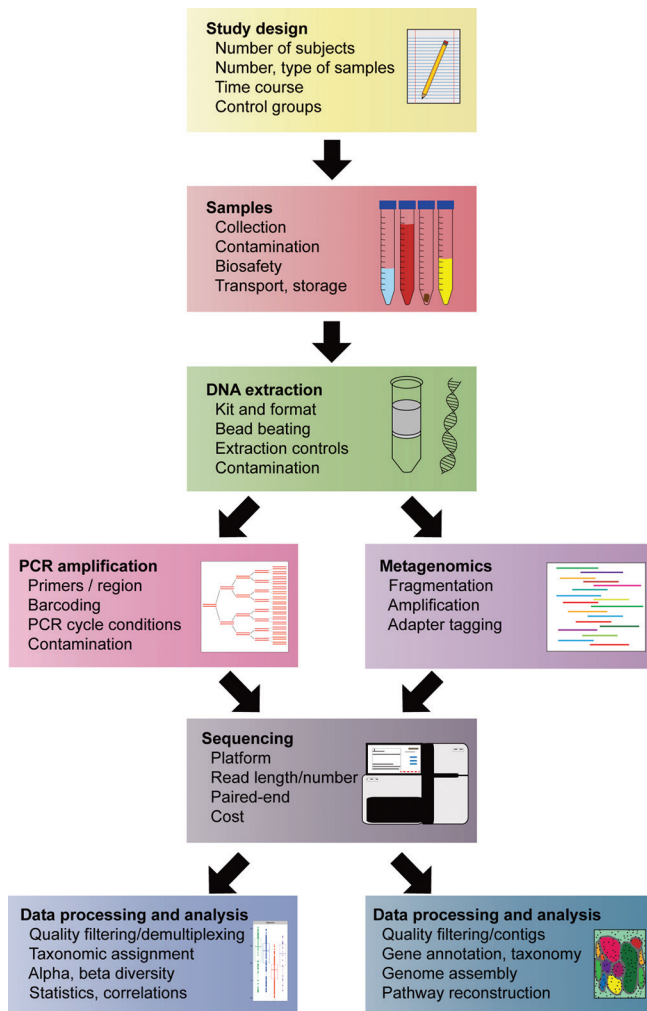
With these tools, it has become possible for researchers for profiling of the microbiomes and metagenomes at unprecedented depths. High throughput and the fact that

specific taxa need not be targeted are the major advantages of NGS.<sup>[51]</sup>

The choices which are made at every step, from study design to analysis, can impact the results regardless of the methodology used to characterize them or the types of microorganisms targeted. Figure 1 shows the steps in conducting a microbiome study.<sup>[53]</sup>

The recommended practices for a microbiome study are as follows:<sup>[52]</sup>

1. Reduce the confounding factors by carefully designing the study
2. Consistency in the application of experimental and analytic methods
3. Good record keeping so that all possible metadata can be used in statistical models



**Figure 1:** General overview of the workflow for 16S rRNA gene-based and metagenomics analysis of microbial communities showing the most important steps and considerations for each stage of the process (Courtesy – Bik EM. The Hoops, Hopes and Hypes of Human Microbiome Research. *Yale J Biol Med* 2016;89:363-373)

4. Matching of the software and the statistical toolkits to the sets of data generated
5. Keep detailed records of the bio-informatics steps of the analysis
6. Deposition of all the data using standard formats in public databases.

## CONCLUSION

The oral microbiome is an exciting and expanding field of research. Oral microbiome is crucial to health as it can cause both oral and systemic diseases. It rests within biofilms throughout the oral cavity and forms an ecosystem that maintains health in a state of equilibrium. However, certain imbalances in this state of equilibrium allow pathogens to manifest and cause disease. Disruption of the oral microbiome leads to dysbiosis. Identifying the microbiome in health is the first step of human microbiome research, after which it is necessary to understand the role of the microbiome in the alteration of functional and metabolic pathways associated with the diseased states.

Microbiome research is currently at a very nascent stage. Lot of research is being done, and data are added continuously. However, the results obtained from various studies are not consistent. This may be due to the techniques used, the standardization methods, sample size etc., Studies with a larger sample size involving different sites in health and disease are required which may develop consistent patterns to generate concrete data. This will further identify different biomarkers and assist in targeted therapies and personalized medicine for better patient management in clinical practice.

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## Conflicts of interest

There are no conflicts of interest.

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