FATHOM ON HUMAN ORAL MICROBIOME

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ABSTRACT

Microorganisms that have unique traits and play important roles in both health and disease can flourish in the human mouth because of its favorable environment. As science progresses, there is mounting evidence that the oral microbiome is important in conditions other than oral diseases. The oral cavity's ease of monitoring and manipulation of the microbiome opens up new possibilities for microbiome-based diagnostics and therapeutics.

Keywords: *Oral microbiome, Oral artificial substitutes, Oral hygiene, Oral biofilm, Probiotics,*

BACKGROUND

Term "oral microbiome, “oralmicro biota ","oral micro flora" refers to microbial population lives in the human oral cavity. Oral microbiome was first discovered by Dutchman Antony van Leeuwenhoek [1] did so using a microscope he had built. His observations of his own dental plaque in 1683, which he described as "tiny live animalcules," served as the foundation for the earliest written accounts of the existence of numerous microbes. Joshua Lederberg, a 1958 Nobel Prize winner, is credited with coining the term "microbiome" [2] [3]. In the mouth, there is 700 different types of bacteria, fungus, viruses, and protozoan’s[4]. The oral cavity is a perfect complicated environment for the growth of complex many microorganisms because it has a variety of microbial habitats, including the teeth, buccal mucosa, soft and hard palate, and tongue. Any alteration in the organisms' ordinarily harmonious connection with the host could result in mouth infections [5].

TYPES OF ORAL MICROBIOME

A core microbiome and a variable microbiome can be used to categorise the human microbiome. All humans have a core microbiome that is made up of the predominate species that live at various locations throughout the body in a healthy environment. The unique lifestyle, phenotypic, and genotypic determinants, as well as the individual's own unique lifestyle, have all contributed to the evolution of the varied microbiome. Although people have comparable micro biota at different locations on their bodies, there are some distinctions between species and strains of bacteria that can make individual's microbiome as unique to them as their fingerprint [6].

HOW AND WHEN ORAL MICROBIOME DEVELOP?

From birth, the oral micro biota is acquired mostly through vertical transmission from mother to the child. From the first meal onward, the mouth is regularly injected with microbes, and the process of acquiring resident oral micro flora starts [2] [7]. The tongue&cheek, palate, tooth surfaces, and gingival crevice are just a few of the diverse settings found in the oral cavity that are home to various bacteria. Host factors including tooth eruption or tooth loss, dental health, and hormonal changes in the host all have an impact on these micro flora [8]. Also, as people age, the types and numbers of oral microbes alter, particularly during the initial stages of dentition development [9]. In a child's

mouth, the emergence of teeth produces new surface for microbial infection and constitutes a significant ecological event. The oral microbial environment is significantly altered when primary teeth are replaced by an adult dentition [7]. In the retrospective study by PaweB J. Zawadzki, et al. to explore the micro flora. The existence of diverse microorganisms from distinct families, species, and bacterial strains, protozoan's, and fungus was shown by microscopic studies of samples derived from oral mucosal swab, culture, and in vitro tests in the patient groups examined [10] .

BIOFILMS IN ORAL MICROBIOME

Bio films are extracellular microbial aggregations that are highly structured and frequently self-produced. Bacteria in biofilms interact with one another, lead a synergistic lifestyle, and exhibit a variety of distinctive traits that define them apart from free-living cells [11]. A biofilm covering the oral structures made by a large number of bacteria that are impacted by their surface and composition composition The biofilm's self-produced extracellular matrix is mostly made up of extracellular DNA. *S. salivarius* with *E. faecalis* The most significant species in dental caries, biofilm, and endodontic infection are *Fusobacterium nucleatum, Treponema spp*, Body that contains forsythensis, *P. gingivalis, and Aggregatebacter actinomycetem comitans*. Bacteria living in biofilm compete fiercely with one another because they are a sophisticated and diverse civilization. For resources, binding, and the chance to survive, the bacterial species compete. Bacitracin production, the two most frequently utilized competitiveness mechanisms by bacteria are quorum sensing and hydrogen peroxide excretion. Bacteriocins. Certain bacteria in biofilms express bacteriocins, which are specialised or non-specialized proteins that can affect other bacteria. Bacteriocins differ from conventional antibiotic in that they often only affect strains of the generating species or strains that are similar to it [12, 13].

Although fewer than 100 phylotypes are observed in a typical human, the species represented as adherent cells in biofilm communities within the oral cavity have been estimated to represent a varied population of more than 700 phylotypes, including bacterial and archaeal subdomains [14].Commensal organisms make up the vast majority of oral microorganisms. Oral infections and occasionally systemic disorders can be brought on by harmful bacteria. It is difficult to manage disease-cause biofilms that include microorganisms [15].

PREDOMINANT MICROBIAL FLORA IN ORAL CAVITY

Protista	**Fungi**	**Bacteria**	**Viruses**
• Trichomonas. • TenaxEntamoeba. • Gingivalis	• Candida albicans • Candida glabrata • Candida spp	**Gram-positive bacteria strains** • Enterococcus faecalis • Enterococcus faecium Staphylococcus epidermidis Staphylococcus aureus Micrococcus luteus **Gram-negative bacteria strains**Enterobacteriaceae: Escherichia coli Klebsiellaoxytoca Klebsiellapneumoniae Non-Enterobacteriaceae: Acinetobacterbaumannii Pseudomonas aeruginosa	Herpesvirus Retroviruses Coronaviruses, poxviruses, Norovirus, human papillomavirus, Epstein–Barr virus, herpes simplex virus, Hepatitis C virus, and HIV

Adapted references:[16,17,18]

THE TOOLS USE TO DEFINE ORAL MICROFLORA

SAMPLE COLLECTION:

The oral microbiome is receiving a lot of interest, and there are reliable, practical, and efficient sample techniques for isolating oral microorganisms that are mostly found in saliva, the supragingival area, the sub gingivalsub mucosal

region, affected root canal system, and mucosal surface. The collection, transportation, processing, and storage of samples are all parts of the oral microbiome sampling strategy [19].

MICROSCOPY

Microscopic investigations enable count or detection of physically distinct structures, such as various cell shapes or gram-stain responses, and can immediately provide the investigator with a great deal of knowledge about the physical structure of a material. A wide range of stain techniques can be employed to designate certain features of interest in the absence of visually discernible features. For many years, the taxonomic differentiation of bacteria has been based on frequently indefinable characteristics of their cells thanks to the employment of straightforward staining techniques like the gram-stain, acid fast stain, or capsule stain. Similar techniques are still effective today, although they are typically learned through error and trial and only allow for the differentiation of stark structural changes [20].

CULTURE AND MICROSCOPY:

The separation of pure cultures to enable the investigation of individual taxa is a key step in the microbiological analysis process, which is deeply anchored in culture-based techniques. Culture-based analyses are still significant, as well as a pure culture still is necessary before a new tax on can be granted a legitimate taxonomic name, despite the fact that molecular-based approaches are getting more and more sophisticated. The viable count, which is the most often used culture-based metric, is typically determined by serially diluting a bacterial solution and then plating it onto an agar growth medium. For some microbe groups, viable counts are available.

The goal has traditionally been to find and characterise cultivated area bacteria that were connected to specific oral illnesses, guided by Koch's postulates. Culture, however, is not always able to accurately depict the heterogeneity of a oral microbiome. Miller acknowledged his inability to cultivate every bacterium he saw in 1890 [21].

There are still a lot of uncultivated microorganism from the human mouth cavity. Bacteria and viruses from human oral cavity were cultured utilising novel techniques of Isolation of Prior Uncultivated Oral Bacteria, with an emphasis on anaerobic species, by M. V. Sizova et al. In vivo culturing to selectively enhance on organisms actively developing inside the mouth (the "mini-trap" method) is one of these breakthroughs.

To reduce the impact of fast-growing microbes, (ii) single-cell long-term cultivation was used, and (iii) adaptations of traditional enrichment procedures were made utilizing medium which had no sugar, including glucose.maintained tight anaerobic conditions in the majority of their cultivation studies in order to facilitate the growing of obligatory anaerobes.

According to their findings, mini traps enrichment (11%), only one cultivation (3%) and standard plating (1%), had the highest successful recovery rates per cell. The single-cell cultivation approach was used to create the taxonomically richest collection [22]. The separation and preservation in pure culture of 10 strains, including members of what are probably three new microbial taxa, that were earlier only recognized by their molecular fingerprints, is a significant outcome. they concluded that their mix of cutting-edge methods will probably help bridge the divide among wild and domesticated species that originate from human mouths [23]. This DGGE-guided method could lead to the creation of novel medium of various complex microbial populations [24]. Many bacterial species detected in biological specimens can't be grown in culture, which is the fundamental issue with conventional culture and culture-based analytical tools, rendering them unsuitable for research [2].

CULTUREINDEPENDENT:

Before cultivation, many molecular techniques have been employed can physically reduce the quantity and variety of bacteria in mixed samples. With the development of culture-independent technologies, these include filtering techniques like density-gradient centrifuged and elutriation and extinction-dilution in which materials were diluted, preferably down to single cells, before being cultured in isolation [25].

DNA MICROARRAYS:

FeiTeng mechanical lysis-based DNA extraction techniques out performed all other experimental protocols studied when it came to characterising the oral micro biome [26].

Teng F, Darveekaran Nair SS,et al. concluded that orally microbiota structure is mostly influenced by the type of DNA extraction used, whereas the influence of 16S rRNA variable region areas is only marginally significant. Enzymatic

POLYMERASE CHAIN REACTION (PCR)

Inside the human oral cavity, bio films are made up of a vast variety of microbes. By presenting PCR-generated 16S rDNA fragment that migrate at varying distances, indicating the variations in the base-pair, PCR-based denaturing gradients gel electrophoresis (PCR-DGGE) examines microbial diversity [21 , 22]

NEXT-GENERATION SEQUENCING (NGS) TECHNOLOGY

Ya Zhang et al. evaluated 12 studies before reaching their findings. Prior to intervention, the most common genera were Porphyromonas, Treponema, Tannerella, & Prevotella, although Streptococcus and Actinomyces often expanded and were the top genera identified by NGS technology [27]. The NGS technology showed promise in monitoring and analysing the danger of probable laboratory contamination by being able to track the nucleic acids bacterial contamination via various sources in the lab. While analysing data and interpreting results, it is important to take the possibility of contamination from chemicals, residual DNA, and the environment into account. In order to comprehend the complicated oral microbial population in clinical samples, researchers have been able to efficiently gather vast volumes of DNA fingerprint data in a single instrument run thanks to NGS technology. For point-of-care testing, it is essential to develop quick, easy, and sensitive detection methods due to the growing clinical significance of oral infections. The biggest problem right now and a serious worldwide health emergency is the corona virus disease (COVID-19) pandemic.

METAGENOMICS:

A technique for locating bacteria that are impossible to grow is metagenomic analysis. It also uncovers the genomic variety in microbes by applying the power for genomic studies to the full society for microorganisms & their functional structures through a review of metabolic pathway genes. Also, it provides information on using databases on protein coding sequences. Have between 120 gig abases and 1.5 terabases of sequence data each run, met genomics, the genetic analysis of the microbiome, provides sequence alignment analysis for studying microbial diversity, population organisation, and functional activity [28].

THE HUMAN ORAL MICROBIAL IDENTIFICATION MICROARRAY

Actinobacteria, Bacteroidetes, Chlamydiae, Chloroflexi, Euryarchaeota, Firmicutes, Fusobacteria, Proteobacteria, Spirochaetes, [29, 30] and are a few of the more prevalent oral bacterial species that can be detected simultaneously with HOMIM. This analysis of HOM advances knowledge of the oral microbiome in healthy subjects [31].
Standards strains , clinical frequent isolates (containing bacteria and fungi) were identified using molecular approaches in this investigation that involved a partial sequence of 16S rDNA or ITS2. Also, throughout the past three years in our lab, molecular techniques were used to identify isolates that had eluded conventional laboratory testing [32].

Bacterial and fungal isolates :A Laboratory Medicine Centre of Nanfang hospitals, a 2,200-bed tertiary-care facility connected with a university, provided the bacterial and fungal isolates. Infectious diseases caused by bacteria and fungi, including those caused by anaerobic bacteria, aerobes, Mycobacteria, Candida, Aspergillus, and other isolates, are frequently diagnosed at this laboratory using culture-based methods. All fungi were found in deep mycosis isolates. With the help of a MicroScanWalkAway 96 plus System and the BD Phoenix 100 Automatic Microbiological Systems, both manufactured by Siemens Healthcare Diagnostics Inc. in West Sacramento, California, all isolates were phenotyped according to conventional laboratory practises [32].

DNA extraction: Using the TaKaRaMiniBEST Universal Genomic DNA Extraction Kit Ver.5.0 (TaKaRa) and the Lysis Buffer for Microorganism to Direct PCR kit, nucleic acids were extracted in accordance with the manufacturer's instructions (TaKaRa Bio Corp., Tokyo, Japan). Colonies have to be obtained from fresh cultures of bacteria or fungi in Sabouraud's or blood plate media. Moreover, the lysis buffer's colony and hyphae concentrations should be within a certain range (visible turbidity). Direct PCR amplification of previously obtained DNA was performed [32].

Ingredients utilised in PCR amplifications included: 1.25 U of ex Taq DNA polymerase (TaKaRa), 5 L of template, 5 L of 10 PCR buffer, 4 M of each primer stock solution, 4 mM of each dNTP, and 50 L of sterile distilled water. For the amplification, a Mastercycler® PCR System was employed (Eppendorf International, Hamburg, Germany). The

35 cycles of 94°C for 30 s, 55°C for 1 min, and 72°C for 2 min that made up the thermocycling conditions also included a final extension step of 72°C for 5 min. Gel electrophoresis (100 V through a 1.5% agarose gel with 0.5 TBE (Tris-borate-EDTA) running buffer) was used to check the PCR amplification products, which were then stained with ethidium bromide and analysed using the GelDoc XR Gel Documentation System (Bio-Rad, USA.). PCR amplicon sizes were only evaluated at the Beijing Genomics Institute using TaKaRa comparisons of molecular size markers, followed by purification and sequencing (Sanger capillary sequencing) (Shenzhen, China) [32].

WHAT ARE THE ROLE OF ORAL MICROBIOME IN HEALTH

The human body contains a unique microbiome that really is essential for maintaining health . These microbes give significant biological benefit to their host, such as promoting the development of a healthy gut microbiome.controls the saliva's ability to buffer (at high PH) to neutralized oral acids, the kind that lead to tooth erosion.lessens the bacterial acid load that causes tooth decay and cavities by decreasing the quantity of pathogenic acid-producing microbes through competitive exclusion.aids in avoiding gum diseasereduces gum inflammation, starts the digestive process, turns nitrates into nitrites, which is a vital chemical for lowering blood pressure, and prevents plaque prevents halitosis [33,34,35,36].

- Encouraging healthy gastrointestinal and immunological systems.
- Certain bacteria assist to nitrate metabolism, that helps promote healthy blood pressure, as well as digestive and metabolic activities that support a normal metabolism.
- Facilitating the procedure of using saliva to break down meals and convert food's nutrients into energy
- Circulating the mouth with ionic minerals that are carried by saliva
- Supporting tooth remineralization
- Delivering oxygen to the soft tissues and gums
- Shielding us from hazardous environmental germs while preventing diseases
- Preventing inflammation and oxidative stress
- Removing trash from the mouth's outside [37]

HOW TO IMPROVE ORAL HEALTHY MICROBIOME?

Probiotics are "live microorganisms that, when administered in sufficient proportions, impart a the host benefits from," according to the WHO definition. By balancing the oral flora, probiotic strains aid in the management of gingivitis. Acidogenic probiotic bacteria like Lactobacilli, Streptococci, and Bifidobacterium [28] are the most frequently used bacteria for probiotics because they release antimicrobial substances that have an inhibition activity against pathogens through co-aggregation, producers of toxic by-products, and competition for substrates [38]. Studies have looked into using the probiotic genera Lactobacillus and Bifidobacterium, which are often used in formulations for digestive health, to prevent dental caries [39].

The oral microbiome is a term used to describe the bacteria that inhabit the oral cavity. It is, after the gut. When comparing to other bodily parts, they exhibit an amazing variety of expected protein actions. The human microbiome is composed of both the fundamental microbiome and a changeable microbiome. Everyone has the same basic microbiome, but because everyone lives differently and has various physical characteristics, everyone also has a unique variable microbiome. There are two sites inside the oral cavity wherein germs might invade: the hard and soft tissues that make up teeth, in addition to the oral mucosa. Microbes can flourish in the oral cavity.

The oral cavity and the nasopharyngeal regions that are linked with it provide an environment that is perfect for the growth of bacteria. The typical temperature of the mouth cavity is 37 degrees Celsius, and this temperature rarely shifts much. This provides bacteria with a favourable habitat in which to thrive. In addition, the pH of saliva is consistently between 6.5 and 7, which is the ideal range for the majority of bacterial species. It fulfils the role of the a medium for transfer of nutrients to the microorganisms, in addition to maintaining the hydration of the bacteria [40].

SIGNS OF POOR ORAL MICROBIOME:

Oral diseases, including dental cavities and periodontal disease, are among the most common diseases in the world, and they impact practically all age groups and geographic regions [41] The bacteria that live in the mouth can create metabolites, which in turn can have an effect on the progression of a variety of oral disorders. Significant difficulties may be produced by strains of *Staphylococcus aureus* that also take part in the creation of biofilm; biodegradable polymer infections are most commonly caused by this kind of bacterium.

Excessive plaque just on teeth can leave a morning film on your tooth that really is thick, sticky, unpleasant, and off-white. poor breath, Gum recession and bleeding gums delicate teeth oral sores A fungal ailment known as oral candidiasis or oral thrush occurs when the candida fungus (often C. albicans) overgrows in the mouth. Cavities and tooth decay, endodontic infections (root canals), and alveolar osteitis are all examples of gum disease (periodontitis) (dry socket), respiratory infections, heart illness, stroke, tonsillitis, and more [42, 43].

ORAL MICROBIOME FOR SYSTEMIC DISEASES

- **Endocarditis**. Infections of the inner layer of your heart chambers or valves frequently arise when bacteria or even other organisms from another part of your body, such your mouth, travel through your bloodstream and stick to particular spots there (endocardium).
- **Cardiovascular disease.** Although while the connection between oral infections and cardiovascular disease, clogged arteries, and stroke is not fully understood, certain research have suggested a link [8].
- **Pneumonia.** Pneumonia as well as other respiratory illnesses can be brought on by specific oral cavity bacteria that can be drawn into your lungs.
- **Diabetes.** By reducing the body's capacity to fight against infection, diabetes raises a risk of gum disease. Gum disease appears to be more prevalent and severe in people with diabetes [41].
- **HIV/AIDS.** HIV-positive individuals are especially susceptible to a number of oral microbial infections. In persons with HIV/AIDS, oral issues such uncomfortable mucosal sores are frequent[42].
- **Osteoporosis.** This bone-weakening disorder is linked to both peridontal loss and tooth loss. There is a slight possibility that a few osteoporosis drugs could affect the jawbones[32].
- **Alzheimer's disease.** When Alzheimer's disease advances, dental health is shown to deteriorate[43].

Anorexia and bulimia, rheumatoid arthritis, certain malignancies, and Sjogren's syndrome, an immune system ailment that produces dry mouth, are additional conditions that may be connected to oral health.

ORAL FLORA ON ARTIFICIAL SUBSTITUTES

CHANGES IN ORAL FLORA BEFORE AND AFTER DENTURES INSERTION

Because implants are now being used widely to treat edentulism, it is becoming more and more important to study that oral flora of missing teeth people. The study by Saeed Abdul Latteef Abdul-Kareem, B, shows found there was no statistically significant difference in the types of microorganisms during the post-insertion period while the overall number of bacteria reduced. While E. coli, Klebsiella, and Moraxella (Branhamella) have been observed after one

month of wearing dentures, the mouth of the newly edentulous patient had previously harboured Neisseria species, which disappeared after dentures were inserted. Candida and streptococci were two other microorganisms that reduced. Staph aurous, Diphtheroids, Veillonella, and Acinetobactor, on the other hand, were thought to be a part of the typical flora of edentulous patients that remained unaffected by denture use. Even while different germs can colonise dentures, when worn for a brief amount of time and with appropriate oral hygiene, the number of microorganisms in the mouth cavity does not rise[11]. As people aged, so did the isolation of various non-resident oral microorganisms, possible opportunist pathogens, and viability count and proportion of lactobacilli in saliva (yeasts and staphylococci). With age, there were also changes in the ratio of Actinomyces spp. predominance. R. S. Percival et al. examine the level of the inherent and specialised host defences between healthy persons in the various age groups outlined here and patients of the same age who have different disorders. In some people, changes to the host defences' effectiveness may disturb the stability of a native oral microbiota and increase the possibility of colonisation by possibly hazardous species[9]. In addition, it was shown that the oral microbiomes of young men and women differed [12]. Extraction of teeth unavoidably changes the oral microbiota, and edentulous people have significantly lower microbial diversity, albeit some species can recover when wearing dentures[13].
Salivary substitutes can be used continuously and are sometimes beneficial for senior citizens and radiation patients. It's probable that this ecological change will have an impact on the oral flora[44].

Bridges-Porcelain is typically glued to precious metal to create bridges. For added strength, different non-precious metals are occasionally utilised in the base. There are also modern bridges that are fully constructed of a unique, durable porcelain[45].

Understanding the makeup the oral flora and the bacteria that make it up has been studied. The microbes are typically found on the surface tissues of all humans, such as the mouth cavity. These microorganisms come in different numbers and types according on an individual's age, food, and level of personal hygiene [2].

Many systemic illnesses, including bacterial endocarditis, pulmonary pneumonia, paediatric osteomyelitis, premature low birth weight, & cardiovascular disease, are brought on by these oral bacteria [46].

To compete inside the ecosystem, one bacterium uses a lengthy chain of peptides called bacteriocin, which is synthesised by bacterial ribosomes. The oral community produces bacteriocins, which contribute to the diversity and ecological appropriateness of bacteria. Many species of naturally occurring oral cavity occupants create bacteriocin through quorum sensing, which controls the development of oral flora[47].

Gram-positive bacteria make bacteriocin, which is a shorter substance with 60 amino acids but a broad spectrum of activity among the gram positive bacteria. Aiming to define the production of bacteriocin while taking into account the aforementioned information, this current study were created to investigate & compared the bacteria fauna of the both healthy and unhealthy dental samples[48].

CONCLUSION:

Understanding the diversity of the oral microbiome helps improve health and control systemic and oral disorders. The development of more specialised methods for treating related disorders has been substantially hastened by the use of improved technical tools. These technologies have greatly accelerated the ability to detect the oral microbiome in a variety of samples taken from of the oral cavity.

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