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Critical Analysis of Changes in Mouth Microflora after Different Types of Food Eating Habits

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Abstract: *In recent decades, a body of literature examining the relationships between oral health and general health has rapidly developed. However, the biological mechanisms involved in explaining such relationships have not been fully described. Recent evidence has suggested that these relationships could be partially explained by the composition and interaction of the microbiome/microbiota between local and systemic body sites. For instance, it has been suggested that intestinal microbiota could have effects on non-communicable diseases, such as diabetes or cardiovascular diseases. The objective of this study is to explore current evidence of the link between oral and systemic diseases, to discuss whether oral microbiome/microbiota could represent an unexplored biological pathway partially explaining those relationships. A non-systematic review of the literature was carried out using keyword searches in PubMed from February to May 2019. The ultimate goal was to present recent scientific evidence to update the general knowledge on this topic to professionals in dentistry. This review is divided in two parts for journal publication; however, it is intended to be used as one piece. In this first part, we will summarize the conceptual background of oral microbiome/microbiota, we will describe the main methods used in microbiology to characterize oral organisms, and will present the main composition of bacteria in oral microbiome/microbiota.*

Keywords: Microflora, Microbiota, food habits, communicable diseases

I. INTRODUCTION

Oral microbiome is defined as the collective genome of microorganisms that reside in the oral cavity. After the 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. The teeth, tongue, cheeks, gingival sulcus, tonsils, hard palate and soft palate provide a rich environment in which microorganisms can flourish.The surfaces of the oral cavity are coated with a plethora of bacteria, the proverbial bacterial biofilm.

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.[1] The number of microbes present in our bodies is almost the same or even more as compared to that of our cells.[2]Oral microbiome, oral microbiota or oral microflora refers to the microorganisms found in the human oral cavity.[3] Oral microbiome was first identified by the Dutchman Antony van Leeuwenhoek who first identified oral microbiome using a microscope constructed by him.[4] He was called the father of microbiology and a pioneer who discovered both protists and bacteria.[5] In 1674, he observed his own dental plaque and reported "little living animalcules prettily moving."[6]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 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

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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.[7] The teeth, tongue, cheeks, gingival sulcus, tonsils, hard palate and soft palate provide a rich environment in which microorganisms can flourish.[8] The surfaces of the oral cavity are coated with a plethora of bacteria, the proverbial bacterial biofilm.[9]

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. [10]

1.1 Development of the Oral Microbiome

The womb of the fetus is usually sterile.[11,12,13] However, recent studies have reported intrauterine environment colonization, specifically the amniotic fluid, by oral microorganisms, in up to 70% of the pregnant women.[14] 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.[12]

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.[11,12,13]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 1st 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.[6]Bacteria form multigeneric communities by adhering not only to oral surfaces, but also to each other. Their composition and stability is influenced by specific partner relationships.[15] 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.[16]

1.2 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.[17]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.[18]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.[8,19]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.[20]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.[7]

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.[21]

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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.[9] The 12 phlya are *Firmicutes, Fusobacteria, Proteobacteria, Actinobacteria, Bacteroidetes, Chlamydiae, Chloroflexi, Spirochaetes, SR1, Synergistetes, Saccharibacteria* (TM7) and *Gracilibacteria* (GN02).[22]
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.[23]
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.[21]
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.[24]

The principal bacterial genera found in the healthy oral cavity are as follows:[12]
Gram Positive:
Cocci – *Abiotrophia, Peptostreptococcus, Streptococcus, Stomatococcus*
Rods – *Actinomyces, Bifidobacterium, Corynebacterium, Eubacterium, Lactobacillus, Propionibacterium, Pseudoramibacter, Rothia.*

Gram Negative:
Cocci – *Moraxella, Neisseria, Veillonella*
Rods – *Campylobacter, Capnocytophaga, Desulfobacter, Desulfovibrio, Eikenella, Fusobacterium, Hemophilus, Leptotrichia, Prevotella, Selemonas, Simonsiella, Treponema, Wolinella.*

1.3 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.[28]
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 neighbours with which they commonly interact is necessary for mechanistic understanding of the key players.[29]
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.[1]

II. MATERIAL AND METHODS

Materials	Quantity	Methods
Sugar cane	1tsp	Gram staining
Nuts	1tsp	Spreading
Lemon	1tsp	Streaking
Bread	1tsp	
Sterile nutrient media	4	
Sterile swab	4	

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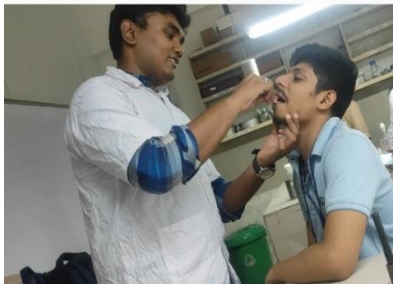
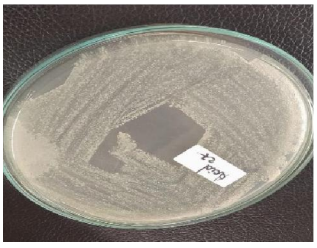
Dyes	crystal violet, Methyl red, Alcohol, Safranin	
Microscope	1	

Suspensions of all the three four food samples were prepared using saline. Nutrient Agar plates was used for the isolation of microorganisms. Isolation of air mouth micro flora was done by using isolation method. For this Nutrient Agar plates and was used. For preparation of nutrient agar, 28gms of nutrient agar was added to 1l distilled water and the medium was sterilized at 120°c and 15 lbs pressure. 20 ml of sterilized NA was poured into sterile petri plates and medium was allowed to cool till solidified. Mouth micro flora Samples were collected within the microbiology lab of TSDC.

		
Sample Collected after Eating of foods which is Basic in nature	Sample Collected after Eating of foods which is acidicin nature	Sample Collected after Eating of foods which is neutralin nature

Photoplate 1: Sample Collection

III. OBSERVATION AND RESULTS

		
Petri plate with growth of Microorganism after Eating of foods which is Basic in nature	Petri plate with growth of Microorganism after Eating of foods which is acidicin nature	Petri plate with growth of Microorganism after Eating of foods which is neutralin nature

Photoplat .2: Observation in Petriplate

Observation: Cultural Characteristics

Parameters	**Observation**
Shape	Rod shaped
Size	1-3 mm
Elevation	Convex
Surface	Smooth (fresh isolation)
Colour	Greyish white
Structure	Translucent –Opaque
Emulsifiability	Smooth form – Easily emulsifiable;

Table 1: Observation on Petriplate

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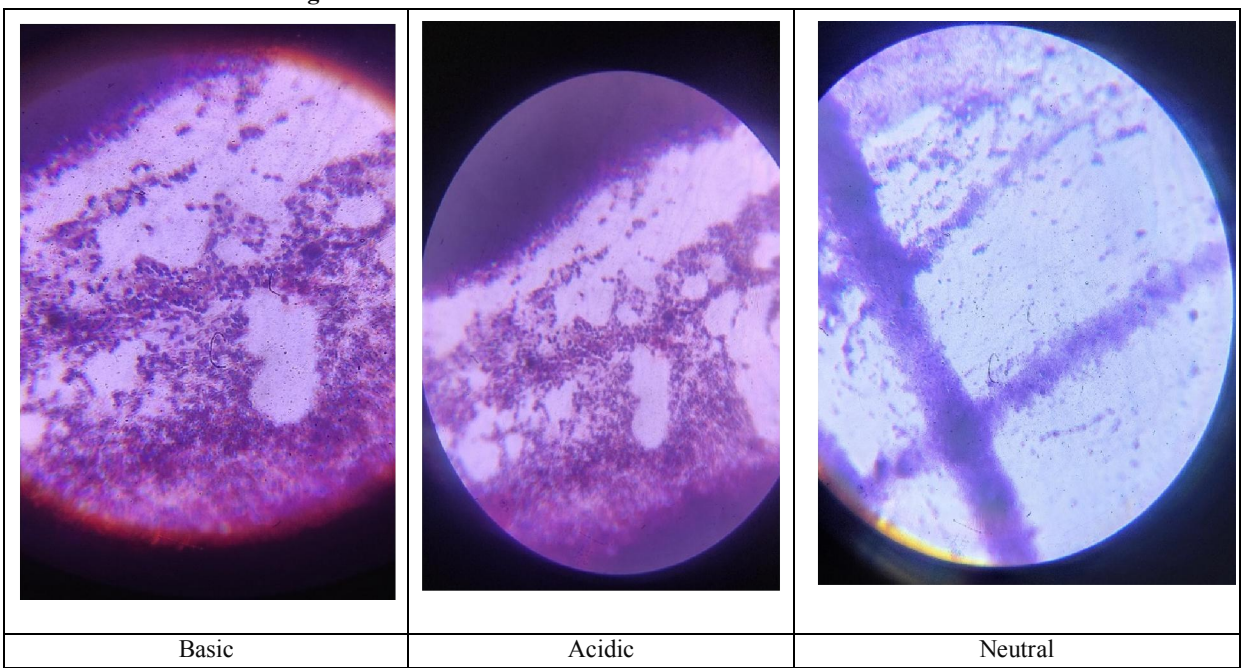
Results after Gram Staining



Basic	Acidic	Neutral

Photoplate 3: Microscopic Observation of Sample

IV. DISCUSSION

Bacteria that can grow in acidic condition: Acid ithiobacillus ferrooxidans, Pediococcus acidilactic, Burkholderia multivorans, Acetobacter aceti etc.

Bacteria that can grow in basic condition: -Bacillus, Pseudomonas, Streptomyces, and Synechocystis

Bacteria that can grow under neutral condition: -Escherichia coli, staphylococci, and Salmonella spp.

V. 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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