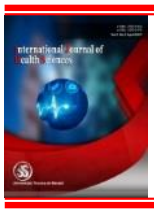

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Gram Positive Bacteria and Their Distributions According Anatomical Site in Oral Cavity and Effects on Oral Health

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Abstract

A healthy adult's body is thought to contain ten times more microorganisms than human cells. These include a wide variety of bacteria, fungi, viruses, and archaea. The Human Microbiome Project (HMP), recently launched by the National Institutes of Health, emphasizes how critical it is to characterize human microbiota in order to comprehend health and illness. An imbalance in the usual microbiota or alterations in colonization sites may be the cause of the illness. A wide variety of infectious disorders are frequently intimately associated with oral bacteria, as the mouth is home to a complex, diverse, and prolific microbial population. Because they negatively impact people individually, in groups, and throughout society, oral disorders are a significant public health problem.

Contents

Abstract 180
1 Introduction 181
2 Materials and Methods 181
3 Results and Discussions 184
4 Conclusion 190
Acknowledgments 190
References 191
Biography of Authors 193

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1 Introduction

A healthy adult's body is thought to contain ten times more microorganisms than human cells. These include a wide variety of bacteria, fungi, viruses, and archaea. The Human Microbiome Project (HMP), recently launched by the National Institutes of Health, emphasizes how critical it is to characterize human microbiota in order to comprehend health and illness. An imbalance in the usual microbiota or alterations in colonization sites may be the cause of the illness. A wide variety of infectious disorders are frequently intimately associated with oral bacteria, as the mouth is home to a complex, diverse, and prolific microbial population. Because they negatively impact people individually, in groups, and throughout society, oral disorders are a significant public health problem (Peng et al., 2021; Peng et al., 2020).

The Global Burden of Disease Study (GBD) has released statistics showing that over 3.5 billion individuals globally have experienced untreated dental conditions, primarily in the form of tooth loss, caries, periodontitis, and edentulism.. It is also commonly recognized that chronic systemic problems like diabetes and cardiovascular disease are significantly correlated with dental diseases like periodontitis and that the development and management of these disorders are negatively impacted by poor oral health. A notable degree of site specificity is shown by cavities and other dental diseases, such as chronic periodontitis (Barr et al., 1957Petersen & Round, 2014). The human mouth cavity is a great body location to study mechanisms underlying spatial patterns in the human microbiota because of this, among other reasons. Additional justifications encompass the practicability of doing comprehensive geographical sampling, the abundance of distinct microbial environments, such as keratinized vs non-keratinized soft tissues and soft versus hard tissues, and elements of host physiology that provide environmental gradients (Peng et al., 2020; Hsiao et al., 2013).

It has recently been demonstrated that Scardovia wiggsiae is a significant etiological agent of caries in early childhood (Peng et al., 2020; Downes et al., 2011; Wang et al., 2017; Collado et al., 2016). Most studies on oral microbiota concentrate on bacterial species linked to social disorders, such as inflammatory periodontal diseases (from genera Actinobacillus, Porphyromonas, and Campylobacter) and caries (e.g., streptococci: S. mitis, S. oralis, and S. salivarius). Changes in the masticatory system and disturbances to the delicate balance of the oral microbiome can alter oral ecology and microbial composition. This can facilitate the infiltration and establishment of various foreign species in the mouth, potentially harmful ones included. Notably, bacteria within a polysaccharide matrix adhering to human tissues as biofilms are less vulnerable to the human immune response, antibiotics, and antiseptics compared to free-floating bacteria outside of biofilms (Tuominen & Rautava, 2021). Consequently, infections associated with biofilms can pose challenges in treatment.

2 Materials and Methods

Microflora in the oral cavity

The human body has one of the most varied oral microbiomes. The mouth harbors over 700 discovered species, with new sequencing methods promising further discoveries. Its unique anatomy includes hard structures (teeth), exocrine gland tissue (main and minor salivary glands), and mucosal surfaces (tongue, buccal mucosa, gingiva, and palate), each hosting distinct microbial communities. Recent research has extensively explored the link between oral microbiota and systemic illnesses. Furthermore, studies have associated oral microbiome composition with the onset of cancer (Tuominen & Rautava, 2021).

Individuals with periodontitis, a prevalent advanced gingival disease resulting from bacterial dysbiosis, are at a 2-4 times greater risk of developing any type of cancer in comparison to those in good health. It has been demonstrated that certain oral taxa, particularly Porphyromonas gingivalis and Fusobacterium nucleatum, have the ability to cause cancer through a number of distinct pathways. They have the ability to cause chronic inflammation, stimulate cell division, block apoptosis, encourage cellular invasion, and directly create carcinogens. Potentially cancerous lesions in the oral cavity are already a sign of these microbial alterations. There is a complicated causal link between microbiome and cancer. Precise research on the influence of

certain bacteria on the development of cancer in people is challenging. Clarifying the relationships between the bacterial microbiota of the oral cavity and cancer is the main goal of this review. We compile research on the state of our understanding of the role bacteria play in the development of cancer and the underlying processes (Wang et al., 2017; Tuominen & Rautava, 2021).

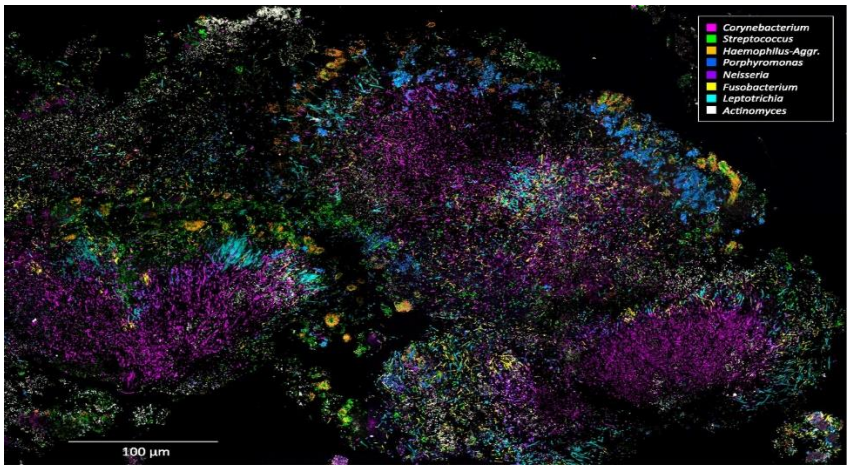

Figure 1. Bacterial diversity in dental plaque by scanning microscope

The Anatomy of the Oral Cavity

A lip mucosa is where the oral cavity starts. The pharynx is responsible for the soft palate, whereas the oral cavity is responsible for the hard palate. Similar to this, the pharynx is located behind the vallate and foliate papillae at the base of the tongue, which is movable (oral) and a component of the oral cavity. When compared to other human body locations, the anatomy of the mouth cavity is unique. Hard tissue, or teeth that poke through the mucosa covering much of the oral cavity, is a distinctive characteristic. Teeth offer non-shedding surfaces that are ideal for the creation of unique bacterial biofilms. In contrast, mucosal surfaces are constantly renewing and older epithelial layers are pealing off the surface, making persistent bacterial colonization more difficult.

Oral mucosal surfaces can be broadly classified as masticatory or non-masticatory mucosa, depending on their anatomical position. The upper surface of the tongue, the hard palate, and the gingiva around the teeth are home to the masticatory mucosa, also known as keratinized stratified squamous epithelium. Moreover, taste buds called lingual papillae are implanted in the tongue's upper surface. The mucosa in the oral cavity is referred to as non-masticatory mucosa or stratified squamous non-keratinized epithelium at the floor of the mouth, buccal, and labial locations, respectively, everywhere else. Non-neoplastic hyperkeratinization can cause the non-keratinized epithelium to become keratinized in continuous trauma instances (Koray & Tosun, 2019; Deo & Deshmukh, 2018; Costalonga & Herzberg, 2014). In the oral cavity, teeth are hard, calcified structures that are in close contact with the mucosa. There are no other structures like this in the human body. Infants are born toothless, and by the time they are six months old, their first primary (deciduous) teeth begin to sprout. Beginning at age seven, the shift to permanent teeth (wisdom teeth) lasts into the early twenties. The gingival sulcus, which lies between the teeth and the mucosal gingiva, is a crucial anatomical location for the production of bacterial biofilms, or plaque (Koliarakis et al., 2019; Könönen et al., 2019). The production of saliva is a key component of oral cavity health. The major and minor salivary glands create saliva. The human body contains two primary openings for salivary glands: one in the buccal mucosa, known as the Stensen duct, and another located on the floor of the mouth, specifically at the sublingual caruncles. On a daily basis, approximately one to two liters of saliva are produced and swallowed (Könönen et al., 2019). Saliva consists predominantly of water, electrolytes, mucus, antibacterial substances such as IgA, and enzymes crucial for food digestion and bacterial control (van't Hof et al., 2014; Marsh et al., 2016). Saliva plays a crucial role in maintaining dental health. Conditions like dental caries, gingivitis, and periodontitis are more prevalent in the absence of adequate saliva due to increased oral bacterial activity (Marsh et al., 2016).

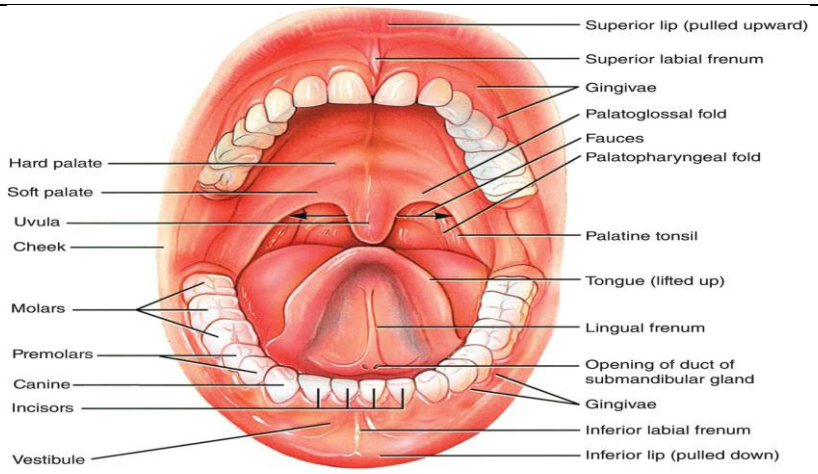

Figure 2. Diagram illustrating the anatomy and main structures of the oral cavity

Dental Caries

Dental caries, the main cause of tooth loss and oral discomfort, is one of the most prevalent disorders that may be prevented. It is a serious oral illness affecting the general population that makes it difficult for people of all ages to attain and preserve dental health. Oral sickness is still a global problem, according to the World Health Organization, despite the fact that oral health has greatly improved in many countries. Poor oral health may have a major influence on general health and quality of life, and the World Health Organization (WHO) has connected certain dental illnesses to chronic diseases (Dinesh, 2024). Dental caries is the term used to describe the localized breakdown of dental hard tissues that are vulnerable to acidic byproducts resulting from the fermentation of carbohydrates in food by bacteria. The majority of patients have a sluggish progression of this chronic condition (Mallya & Mallya, 2020).

It is brought on by an ecological imbalance in the ratio of tooth minerals to oral biofilms, or plaque. The plaque's pH varies due to microbial activity in the biofilm. This is caused by the creation of bacterial acid as well as the salivary and dental structure's buffering effects. As a result, there is a dynamic balance between the tooth surface and its surroundings. Enamel, dentine, or cementum get demineralized when the pH drops below a certain point, but they regain their mineral content when the pH rises. Pain and difficulties eating, chewing, smiling, and speaking due to missing, stained, or broken teeth are some of the symptoms of caries. Numerous facultatively and obligately anaerobic bacteria from the genera Actinomyces, Bifidobacterium, Eubacterium, Lactobacillus, Parvimonas, and Rothia make up the varied microbial population associated with caries (Peng et al., 2021; Dinesh, 2024). Other types of bacteria may also be the cause (Tuominen & Rautava, 2021; Koray & Tosun, 2019; Deo & Deshmukh, 2018), such as those belonging to the streptococci mitis, anginosus, and salivarius groups, Propionibacterium, Enterococcus faecalis, Scardovi, Prevotella, Selenomonas, Dialister, Fusobacterium, Pseudoramibacter, Veillonella, Atopobium, Granulicatella, Leptotrichia, and Thiomonas. Porphyromonas, Prevotella, and Bacteroides species are common on mucosal surfaces and can be found in very high quantities in tonsillar crypts, gingival fissures, and dental plaque (Mallya & Mallya, 2020).

Periodontal Disease

Bacterial imbalance is the cause of periodontitis, an advanced inflammatory gingival disease that can finally result in tooth loss (Hajishengallis, 2014; Michaud et al., 2018). Gingival bleeding occurs first as a reaction to inflammation caused by bacterial biofilm deposition (plaque) along the margins of the gingival surfaces of the teeth. Periodontitis is categorized as a chronic infection because it occurs over years as a result of dental plaque accumulation that creates periodontal pockets and destroys tissue (Michaud et al., 2017). Untreated chronic illness raises CRP levels in the blood and perpetuates low-level inflammation (Paraskevas et al., 2008; Ebersole et al., 1997). A considerable number of individuals (between 50 and 70%) are thought to exhibit

some clinical indications of periodontitis, which is a quite prevalent dental illness (Eke et al.,2012). In healthy human oral cavities, known periodontal pathogens such P. gingivalis, Tannerella forsythia, and Treponema denticola are typically not seen (Aas et al., 2005). A distinct group of so-called periodontal pathogens is really presented in the literature. Aggregatibacter actinomycete mcomitans, Fusobacterium nucleatum, Parvimonas micra, Campylobacter rectus, P. gingivalis, T. forsythia, T. denticola, Prevotella intermedia, P. nigrescens, and P. gingivalis make up this unit (Wade, 2013; Wang et al., 2016; Carrillo-de-Albornoz et al., 2010).

However, given that these species are occasionally found in healthy people, it is now clear that dysbiosis and the relative abundance of pathogenic species serve as the primary initiators of illness (Abusleme et al., 2013; Diaz et al., 2016; Hajishengallis et al., 2011). Moreover, a variety of microorganisms function in concert rather than just one. Even in those who have never smoked, people with periodontitis are two to five times more likely to develop any kind of cancer than healthy controls (Michaud et al., 2018; Corbella et al., 2018). In particular, the connection with OSCC appears to be stable (Bundgaard et al., 1995). Consequently, a higher incidence of OSCC has been associated with a greater number of missing teeth, which is an indication of periodontitis (Michaud et al., 2017).

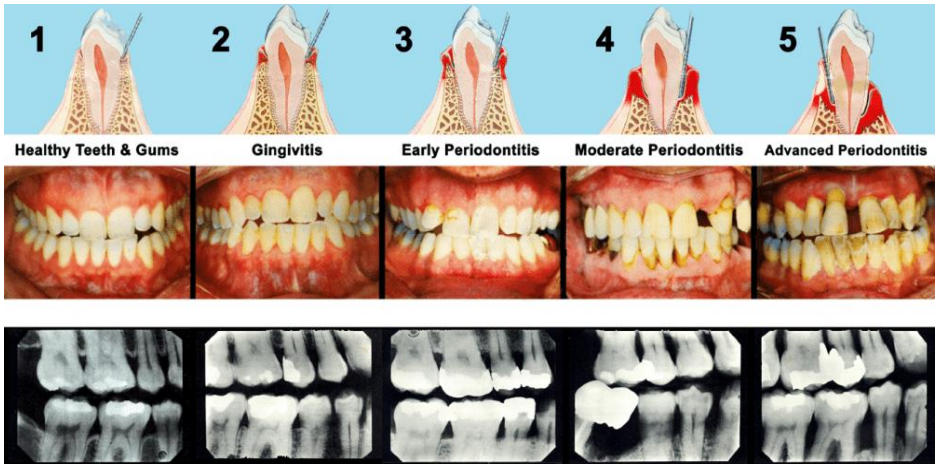

Figure 3. Stages of periodontal disease

3 Results and Discussions

Supragingival Microbes

Gram-Positive Bacteria

1. Actinomyces
   Anaerobic bacteria that are often discovered in oral samples are gram-positive bacilli called actinomyces. Actinomycetes were initially classified as "other microorganisms" or thought to be fungus when they were found. Actinomycetes are considered prokaryotic creatures since they share many general traits with bacteria, as demonstrated by a number of recent investigations. According to Bergey's Manual of Determinative Bacteriology (1984), Actinomyces are classified as gram-positive irregular bacilli. Common representatives within the Actinomyces genus in oral microbiology include Actinomyces naeslundii, Actinomyces odontolyticus, and Actinomyces viscosus. Actinomyces bovis serves as the type species in this genus (Peng et al., 2021; Peng et al., 2020; Zawadzki et al., 2016). Actinomyces are prevalent bacteria in dental plaque and are natural components of the oral flora, found in saliva, dental calculus, and human dental plaque. Actinomyces naeslundii, A. odontolyticus, and A. viscosus are commonly present in these environments. Actinomyces mai primarily colonizes the gingival sulcus. Clinical samples from conditions such as gingivitis, periodontitis, pulp periapical

infection, and pericoronitis can be used to identify A. naeslundii and A. mai, A. viscosus is believed to be a cariogenic bacterium (Mahdi et al., 2016).

2. Bifidobacterium

   A genus of bacteria known as Bifidobacterium exists in a variety of forms. These bacteria are gram-positive, non-sporulating, non-motile, and have drawn attention due to their physiological value to the host organism. Bifidobacterium bifidum, Bifidobacterium infantis, Bifidobacterium adolescentis, and Bifidobacterium longum are species that are significant human gut bacteria. Bifidobacterium dentium, Bifidobacterium breve, Bifidobacterium inopinatum, and Bifidobacterium denticolenu are the most common species of bacteria that have been isolated from the oral cavity. The bacterial cells are irregular, slender, and have pointy ends. Additionally, they resemble lengthy, multibranched cells as well as spoon-shaped, slightly branching cells. A cell can be organized as a single cell, a chain, a polymer, a V, or a palisade (Peng et al., 2021; Peng et al., 2020) Dentium Bifidobacterium B. dentium was first identified as B. appendicitis after it was separated from pus specimens. Similar bacteria were later found in adult dental cavities, feces, and the vagina; they were subsequently designated Actinomyces eriksonii or classified as B. adolescentis. Later studies reveal that these bacteria comprise a separate branch on the evolutionary tree; in the 1970s, the species was given the name B. dentium. (Peng et al., 2021; Karim et al., 2009). Breve Bifidobacterium Anaerobic, gram-positive, irregular bacillus is B. breve. comparable to B. dentium, it has comparable culture properties.B. breve does not ferment starch or l-arabinose, however, it can ferment d-ribose, lactose, and raffinose. B. breve grows as spherical, smooth, transparent, gray-white, sticky, and soft colonies on agar plates (Peng et al., 2021; Karim et al., 2009).

3. Lactobacillus

   A class of gram-positive, anaerobic, microaerobic bacilli without spore production is known as lactobacillus. This species of bacteria is a typical component of the oral cavity and digestive system flora in humans. Since they have been found in samples from decaying oral cavities, this genus is cariogenic. Lactobacillus acidophilus, Lactobacillus salivavius, Lactobacillus plantarum, Lactobacillus fermentum, Lactobacillus brevis, and Lactobacillus casei are frequent species found in the oral cavity. (Peng et al., 2021; Peng et al., 2020; Preshaw & Bissett, 2019).

   a) Lactobacillus acidophilus

      These are regular-shaped, gram-positive bacteria that thrive in anaerobic environments. At 45 °C, cells develop well; at 15 °C, they do not. The two usually used media are Rogosa agar, which is a selective medium, and BHI blood agar. Bacteria that need homo fermentation are called acidophilus. Human lips, human vaginas, and the gastrointestinal tracts of both humans and animals are the primary sites from which L. acidophilus is isolated. A small percentage of newborns' lips may be isolated to produce L. acidophilus. The quantity of bacteria in the children's mouths rapidly declines with age, until at two years old, very little L. acidophilus is seen. Dental plaque is the primary location where Lactobacillus acidophilus colonizes the mouth; saliva, the tongue, and the gingival sulcus are comparatively uncommon sites. L. acidophilus is thought to be connected to the emergence of dental caries as it is frequently seen in material from deep cavities (Peng et al., 2021; Peng et al., 2020; Preshaw & Bissett, 2019).

   b) L. casei

      Four subspecies were first recognized: Lactobacillus casei subsp. paraplantarum (now called Lactobacillus paracasei subsp. paracasei), Lactobacillus casei subsp. rhamnosas (now called L. rhamnosus), and Lactocasei subsp. toleons (now called Lactobacillus paracasei subsp. tolerans). L. casei subsp. alactosus, formerly known as L. paracasei subsp. paracasei, is a recently recognized subspecies. The cells show gram-positive staining. Cultures thrive in anaerobic environments. The two most often used media are Rogosa agar and BHI blood agar; the latter is a selective medium. The human mouth, vagina, and gut are where L. casei primarily colonizes. It is also possible to find the bacteria in milk and other dairy products. Dental plaque is the primary site of colonization in the mouth. It is thought to be a pathogen of dental caries since it is frequently detected in material from deep cavities (Peng et al., 2021; Peng et al., 2020; Preshaw & Bissett, 2019).

c) Lactobacillus fermentum

Gram-positive L. fermentum bacteria Both Rogosa agar, which is a selective medium, and BHI blood agar are frequently used to cultivate this species. L. fermentum is thought to be connected to the emergence of oral infectious disorders including dental caries and root canal infections since it may be found in the human mouth as well as in yeast, dairy products, sourdough, and fermented vegetables (Peng et al., 2021; Peng et al., 2020; Preshaw & Bissett, 2019).

4. Rothia

Rothia are gram-positive facultative anaerobic bacilli that do not produce spores. R. dentocariose is the type species of the Rothia genus.

a) Rothia dentocariose

A gram-positive bacillus called R. dentocariose doesn't make spores. The bacteria's cells might be filamentous, spherical, or pleiomorphic (like Corynebacterium diphtheria). The stain of cells is gram-negative. After being cultured in solid medium, cells resemble virtually filamentous structures, whereas in broth media, they resemble spheres. After being cultured in solid medium, cells resemble virtually filamentous structures, whereas in broth media, they resemble spheres. Facultative anaerobes are what these bacteria are. They develop effectively in an aerobic environment. When R. dentocariose is injected into PYG broth, the primary acid product is lactic acid; propionic acid is not produced. Acid can be produced by R. dentocariose by the fermentation of glucose, maltose, sucrose, trehalose, fructose, and salicylate. Saliva and subgingival plaque are the primary sites of colonization. They have no known connection to oral infections and are nonpathogenic components of the human oral microbiota. It is an opportunistic pathogen that has been found in clinically infected specimens and endocarditis samples. Under aerobic circumstances, new colonies can grow to a diameter of 1 mm after being inoculated. (Peng et al., 2021; Peng et al., 2020)

5. Staphylococcus

Gram-positive cocci that are part of the Micrococcus family are referred to be Staphylococcus genus members. The creatures are dispersed across the surroundings. Three species, Staphylococcus aureus, S. epidermidis, and S. saprophyticus, were first identified from clinical samples. In the early 1980s, the Staphylococcus genus was divided into subgroups of pathogenic and nonpathogenic species as a consequence of the examination of biochemical processes (such as mannitol fermentation) and cellular components (such as the availability of coagulase) (Peng et al., 2021; Peng et al., 2020).

Based on nucleic acid analysis and cell wall composition, the Staphylococcus genus is classified into four groups and 19 species in Bergey's Manual of Systematic Bacteriology. The Staphylococcus bacteria are classified as gram-positive, flagellar, spherical, nonmotile cocci that can be arranged into single, pair, tetrad, and cluster forms. Except for S. saccharolylicus, a facultative anaerobic bacterium, all staphylococci are anaerobic bacteria. The ideal temperature range for Staphylococcus growth is 18°C to 40°C. The majority of Staphylococcus species are capable of growing on medium with 10% NaCl. S. aureus is the type species of Staphylococcus (Peng et al., 2021; Peng et al., 2020).

a) Staphylococcus epidermidis

S. epidermidis is Gram-positive bacteria.. S. aureus and S. epidermidis grow under identical culture conditions; however, S. epidermidis grows more slowly in media containing 10% NaCl. S. epidermidis mainly colonizes human skin and is linked to infections obtained in hospitals, which is dangerous. It is hypothesized that the organisms are associated with angular stomatitis, pericoronitis, acute and chronic pulpitis, and periodontitis. They are frequently discovered in dental plaque and saliva. Colonies of S. epidermidis are complete, round, raised, glossy, and gray. It measures around 2.5 mm in diameter. They frequently do not develop hemolytic zones. Colonies made of mucus-producing strains are translucent and sticky.

6. Streptococcus

The most prevalent gram-positive facultative anaerobic cocci in the oral cavity are those belonging to the Streptococcus genus. Because the bacteria of this species constantly organize themselves into chains, the name Streptococcus was given. Based on their capacity to cause hemolysis, Streptococcus members are classified into three groups in clinical bacteriology: α-hemolytic Streptococcus (also called Streptococcus viridans), β-hemolytic Streptococcus, and γ-hemolytic Streptococcus (also called

nonhemolytic Streptococcus). Streptococcus salivarius, streptococcus sanguinis, streptococcus mutans, streptococcus sobrinus, streptococcus oralis, streptococcus mitis, and streptococcus gordonii are the most common species in the oral cavity (Peng et al., 2021; Peng et al., 2020; Okahashi et al., 2022).

a) Streptococcus salivarius

A coccus S. salivarius is gram-positive. The majority of S. salivarius strains are members of the Lancefield group. K. Salicin, inulin, glucose, sucrose, maltose, raffinose, trehalsorbitol, xylose, and arabinose can all be fermented by S. salivarius. While arginine cannot be hydrolyzed by most strains, esculin and urea can.

In the meanwhile, glucose can be converted by most strains into acetoin. Features of colonization. S. salivarius is mostly isolated from the oral cavities of both people and animals. It is a typical member of the microbial communities that inhabit the tongue and saliva. Additionally, endocarditis patients' feces and blood samples include S. salivarius. Studies on gnotobiotic animals have demonstrated the cariogenicity of S. salivarius. The smoothness or roughness of S. salivarius colonies is determined by their capacity to synthesis extracellular polysaccharides. (Peng et al., 2021; Peng et al., 2020; Okahashi et al., 2022).

b) Streptococcus sanguinis

A coccus S. sanguinis is gram-positive. Most S. sanguinis strains are members of the Lancefield group. H. Since S. sanguinis is a facultative anaerobe, cultures should be maintained in an atmosphere with 5–10% carbon dioxide. At 37°C, S. sanguinis develops swiftly. Bacterial cultures need complex medium that is high in nutrients. Features of colonization. The primary component of dental plaque, S. sanguinis, can only be isolated from oral cavities where teeth have erupted. Because it can produce PABA, this organism is thought to aid in the colonization and reproduction of S. mutans. Due to its capacity to produce H2O2, S. sanguinis is recognized as a crucial probiotic in the oral environment and is linked to good periodontal tissues. The majority of strains exhibit an α-hemolytic zone surrounding their colonies when cultured in an aerobic environment, but a small number of bacteria display a β-hemolytic zone. The several strains that comprise S. sanguinis. (Peng et al., 2021; Peng et al., 2020; Okahashi et al., 2022).

c) Streptococcus gordonii

In the past, S. gordonii was categorized as S. sanguinis serotype II. But S. gordonii does not have IgA1 protease. This coccus is gram-positive.Glycerol, teichoic acid, and rhamnose make up the majority of the cell wall's constituents, while Lys–Ala peptidoglycans are prevalent. There is 40–43% GC content in the genome. The anaerobe S. gordonii is facultative. On blood agar, zones of α- or γ-hemolysis can be visible, while on chocolate agar, a green hemolytic zone can be recognized. There are three biotypes in this species, and none of them produce catalase. Features of colonization. The oral cavity and pharynx are the primary habitats of S. gordonii. (Peng et al., 2021; Peng et al., 2020; Okahashi et al., 2022).

d) Streptococcus mutans

Mutans streptococci are Streptococcus mutans, Streptococcus sobrinus, Streptococcus rattus, Streptococcus ferus, Streptococcus cricetus, and Streptococcus macacae combined. These bacteria were previously associated with S. mutans serotypes a, b, c, d, e, f, g, or h. Although S. mutans is a facultative anaerobe, the ideal atmosphere for its growth should be either anaerobic or include only 5–10% carbon dioxide and a low amount of oxygen. At 37°C, S. mutans thrives rapidly; many strains may even grow at 45°C.

Features of colonization. The surface of teeth is where S. mutans is primarily isolated. It produces a range of extracellular polysaccharides from sucrose, including as fructan and glucan that are both water-soluble and non-water-soluble. These polysaccharides are important virulence factors in the development of dental caries because they encourage bacterial colonization. S. mutans has long been recognized as one of the primary oral pathogens because of its ability to form adhesives, formation of acid, tolerance to acid, and synthesis of water-soluble glucan. It also has a role in other secondary infections such endocarditis and bacteremia. After 48 hours of anaerobic incubation, S. mutans colonies formed on blood agar are either uniform and smooth or uneven, hard, and sticky. (Okahashi et al., 2022).

e) Streptococcus sobrinus
Because of its strong resemblance to S. mutans, S. sobrinus was first categorized as serotypes d and g of S. mutans. After S. mutans, research indicates that S. sobrinus has the second-highest rate of cariogenicity. Similar growing conditions are employed for S. sobrinus cultures as they are for S. mutans cultures and their colony. Features of colonization. The surface of human teeth is where S. sobrinus mostly colonizes. On sucrose agar plates, S. sobrinus may produce rough, stacked colonies that are roughly 1 mm in diameter. It is possible to see liquid-like glucan products above or around these colonies. (Peng et al., 2020)

f) Streptococcus oralis
Gram-positive, spherical, and organized in brief chains are the characteristics of S. oralis cells. Furthermore, S. oralis cells lack a capsule, are nonmotile, and do not produce spores. S. oralis does not make catalases, yet it is capable of reducing tetrathionate. S. oralis is really comparatively biochemically inert. This species is a frequent part of the oral microflora and is mostly isolated from the human oral cavity. Clinically, streptococci may be categorized into three main groups: α-hemolytic, β-hemolytic, and γ-hemolytic (Peng et al., 2020).

g) α-hemolytic Streptococcus
All Streptococcus species that produce a grass-green hemolytic zone surrounding their colonies on blood agar plates are classified as α-hemolytic streptococci. Streptococcus vestibularis, S. mitis, and S. sanguis are among the species that fall under this group. α-hemolytic cells are spherical, nonmotile, and gram positive, just as other streptococci. (Peng et al., 2020; Okahashi et al., 2022).

h) β-hemolytic Streptococcus
All streptococcal species that are capable of forming a β-hemolytic zone, such as S. pyogenes and S. agalactiae, are classified as β-hemolytic species. The most pathogenic streptococci are betahemolytic streptococci, sometimes referred to as pyogenic hemolytic streptococci. These are the agents that cause a number of infectious disorders of the mouth, such as acute tonsillitis, periodontal abscess, and phlegmon in the craniofacial area. (Peng et al., 2020; Okahashi et al., 2022).

Sub gingival Microbes

1. Enterococcus
Enterococci are members of Lancefield group D and are facultative gram-positive cocci. The most prevalent species in the Enterococcus genus is Enterococci faecalis, often known as Streptococcus faecalis. Because of its high detection rate in infected root canals, it has been the subject of much research in recent years.
   a) Enterococcus faecalis
   Gram-positive, oval, and non-motile E. faecalis cells. The majority of cells are organized into short chains or pairs. Most carbohydrates can be fermented by E. faecalis. Lactic acid is the primary acid generated during the fermentation of glucose. Ammonia can also be produced by E. faecalis hydrolyzing arginine. (Peng et al., 2021; Peng et al., 2020; Kryvtsova & Kostenko, 2020; Yodgorova et al., 2023)
2. Eubacterium
A genus of purely anaerobic, gram-positive, nonsporulating bacilli is called Eubacterium. The genus's name is still up for debate. Eubacterium alactolyticum, Eubacterium saburreum, Eubacterium lentum, Eubacterium limosum, Eubacterium nodatum, Eubacterium brachy, Eubacterium timidum, Eubacterium saphenus, and Eubacterium minutum are currently known species of bacteria that have been found in the oral cavity that are members of this genus. Rod-shaped cells can be polymorphous or homogenous. Spores are not generated. Although cells are gram-positive, older cultures and cultures that have developed acid in the culture media will not respond well to Gram staining. The sole state of eubacteria is anaerobic. The stringent anaerobic requirements of this bacteria make cell culture challenging, and certain strains are limited to growing on prereduced media.
The majority of oral cavity Eubacteria are comparatively biochemically inert. Eubacteria are mostly found in saliva and plaque, where they are a part of the normal oral microbiome. In the oral cavity, Eubacterium lentum and E. limosum are found. Potential periodontal infections include the newly

discovered species Eubacterium nodatum, E. brachy, E. timidum, E. saphenus, and E. minutum, which were identified from the subgingival plaque of individuals suffering from periodontitis (Preshaw & Bissett, 2019; Sivapathasundharam & Gururaj, 2020; Kryvtsova & Kostenko, 2020).

a) Peptostreptococcus
The most prevalent gram-positive anaerobic coccus in the human oral cavity and in clinical settings is called proteostreptococcus. The two species in this genus that are met most frequently are Peptostreptococcus micros and Pepto streptococcus anaerobius.

b) Propionibacterium
Gram-positive, polymorphic bacilli of the genus Propionibacterium do not sporulate. The genus Propionibacteria may be classified into two groups: the sores and blisters group (P. acnes) which is found on the skin, including inside the oral and intestinal systems, and the dairy group (also known as typical propionibacteria) which is found in dairy products, cheese, and green fodder.

Oral Mucosal Microbes

1. Staphylococcus
   a) Staphylococcus aureus
   S. aureus. Is gram positive bacteria , Although S. aureus is a facultative anaerobe, it may also thrive in aerobic environments. Following incubation on semisolid thioglycollate medium, colonies thicken, homogeneous, and develop quickly. Human illnesses such face furuncles and carbuncles, loose connective tissue inflammation, and tumor postoperative wound infections have been linked to S. aureus. On standard agar plates, S. aureus can create golden colors. (Peng et al., 2020; Sivapathasundharam & Gururaj, 2020)
2. Streptococcus
   a) Streptococcus mitis
   Gram-positive S. mitis cells have an elliptical or spherical shape with a diameter of around 0.6–0.8 µm. The cultivation of broth allows them to create lengthy chains. A notable α-hemolytic response is discernible when S. mitis is cultivated on blood agar plates.On sucrose agar plates, S. mitis develops tiny colonies like shattered glass, with relatively few strains able to form the more common sticky colonies. Human waste, sputum, saliva, and oral mucosa can all contain S. mitis. It belongs to the oral microbiota and is a frequent oral Streptococcus. (Peng et al., 2020; Okahashi et al., 2022)
   b) Streptococcus pyogenes
   S. pyogenes is a gram-positive, often spherical member of Lancefield group A. On the other hand, aged cultures' cells could be oval. Furthermore, the majority of S. pyogenes cells in broth culture form long chains, while they can also be grouped in short, medium, or long chains. Clinical isolate cells are often organized in pairs. Since S. pyogenes is a facultative anaerobe, 37°C is the ideal growing temperature. For S. pyogenes to flourish, it needs a medium that is high in nutrients, and serum and blood can both help. S. pyogenes primarily colonizes the upper respiratory tract, the hypopharynx, and dental plaque. Blood, inflammatory fluids, or skin lesions can all be used to isolate clinical samples. Additionally, pulpitis, infection following exelcymosis, and loose connective tissue inflammation in the maxillofacial area can also be associated with S. pyogenes.
   c) Streptococcus pneumoniae
   Spherical or elliptic in shape, S. pneumoniae cells are typically seen in pairs. Occasionally, they are discovered as single cells or in small chains. This species' typical cells are lanceted and grouped in pairs. Capsule staining allows for the observation of the extracellular capsule. Actually, S. pneumoniae's species-specific antigen and virulence factor is this capsular polysaccharide.S. pneumoniae may be isolated from clinical samples of amygdalitis, pneumonia, meningitis, and otitis media. It primarily dwells in the upper respiratory tracts of healthy people or animals.
   d) Streptococcus vestibularis
   Because this species was initially isolated from the vestibule of the human oral cavity, it was given the name S. vestibularis. Gram-positive, spherical, and organized in chains, S. vestibularis cells. The vestibule or mucosa of the human oral cavity is the primary source of S. vestibularis isolation (Tortora & Derrickson, 2018).

Mycoplasma

Mycoplasma does not require a cell wall to survive on their own. They can be separated from healthy human and animal respiratory mucosa and are the tiniest prokaryotic microorganisms. MRSA, M. oralis, M. salivalis, and M. nominis may all be isolated from the oral cavity (Pinto et al., 2008; Pfefferbaum et al., 2005; Lestari et al., 2016). The tiniest prokaryotic microbial cells are called mycoplasma. Mycoplasma may grow on PPLO agar with 10–20% horse serum and beef heart infusion, however they have high nutritional requirements. There are two similarities between L type and mycoplasma bacteria: (1) they both have pleomorphic cells without a cell wall; and (2) they may both pass through an antimicrobial filter. Typically, mycoplasma invade the oral cavity's tartar, bacterial biofilm, or throat. A frequent cause of acute respiratory infections is Mycoplasma pneumoniae. The location and toxicity of Mycoplasma in the oral cavity remain unknown, despite earlier research showing that it can be distinguished from clinical specimens of periodontitis, gingivitis, and root canal infections (Rice, 2006; Berger-Bächi, 2002; Rasmussen et al., 1966; Kilian & Schiott, 1975). Mycoplasma colonies typically have an omelet-like form (Peng et al., 2021).

4 Conclusion

The human body has one of the most varied oral microbiomes. The mouth harbors over 700 discovered species, with new sequencing methods promising further discoveries. Its unique anatomy includes hard structures (teeth), exocrine gland tissue (main and minor salivary glands), and mucosal surfaces (tongue, buccal mucosa, gingiva, and palate), each hosting distinct microbial communities. Recent research has extensively explored the link between oral microbiota and systemic illnesses. Furthermore, studies have associated oral microbiome composition with the onset of cancer.

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