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# The Oral Microbiome, Dental Caries and Probiotics

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# The Oral Microbiome, Dental Caries and Probiotics

BMS 599: Creative Component

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**Abstract**

Over the past 25 years, dental research has begun to focus on investigating the potential of probiotics as an agent to prevent dental caries. Previous efforts to treat cavities have been most successful with preventative measures and then basic reactionary solutions, mainly fillings and extractions. Many hypothesize that probiotics possess the ability to revolutionize caries treatment by providing a maintenance of balance within the oral microbiome. Demineralization and decay are caused by the domination of acidogenic bacteria within the colonizing biofilm and a subsequent build up of their acidic metabolic byproducts. There have been previous efforts to target cariogenic bacteria with vaccines, or their particular virulence factors with small molecular inhibitors yet it is difficult to create a product which will work from individual to individual. Due to the highly variable nature of the oral microbiome, the composition of one person's biofilm can differ from another's based on countless factors. This literature review will compile many recent sources to give a basic understanding of all of the necessary background knowledge and then assess the emerging field of dental probiotics.

**Introduction**

Approximately 3.5 billion years ago the Earth became home to the oldest living organisms, bacteria. During the subsequent emergence and evolution of multicellular organisms, these bacterial species began to inhabit new environmental niches. As higher-level organisms further developed, a beneficial relationship was formed. Coevolution resulted in a complex microbiome, composed of usually more than 1,000 species of bacteria, fungi, archaea and viruses colonizing the membranes of the respiratory, gastrointestinal and genitourinary tracts of mammals and other higher-level organisms. Thus far, the medical industry has focused its efforts primarily on striding to improve the environment housed within the human gut. The microbiome present within the digestive tract acts symbiotically to help with host metabolism, extract energy and nutrients from ingested substrates and regulate host fat storage and hormone levels. Maintenance of these synergistic bacterial relationships helps to promote healthy and efficient digestion.[9] Dental professionals over 25 years ago realized that many oral diseases stem from imbalances within a similar microbial community which resides in the oral cavity. The environment present within the human body is dynamic and constantly shifting in reaction to a variety of internal and external factors. Too large of a disturbance and the beneficial relationship between host and colonizers can be lost to dysbiosis. This balancing act has been a major target of research after the realization that many localized diseases can manifest into systemic health threats.

Dental caries, commonly called cavities, are not often regarded as hazardous since most cases are not life threatening. Surprisingly though, caries are considered the most prevalent human disease, affecting roughly 80-90% of the Earth's population. It is also the single most common childhood disease in the world, with a five times higher prevalence rate than asthma.[16] Within the U.S. more than 50% of the children between ages 5 and 9 have at least one cavity or consequent filling. At 17 years of age, the prevalence rises to about 78%.[7] Efforts worldwide have adopted programs promoting preventive behavior and care, successfully reducing the prevalence of common oral diseases. Yet periodontal diseases and the incidence of caries remain rampant. Interestingly, both stem from the disturbance of the species comprising the oral microbiome.[11] Considering information from the disciplines of anthropology and evolutionary genetics, is it believed that several of the pivotal bacterial players appeared within human dental plaque around the time period in which humans experienced a dietary shift from hunter-gatherers to stable agrarian societies. The acquired dependence upon food containing carbohydrates from domesticated crops not only increased the prevalence of these species of bacteria, but also allowed sparked the retention of new bacterial genes vital for the successful development of carious lesions.[12] The appearance of cavities within 'Post-Agricultural' populations can most likely be attributed to one of the largest driving factors behind oral dysbiosis: the human diet.

Amidst a variety of strategies employed to combat dental caries, perhaps one of the most promising lies within utilization of probiotics. Little previous research has been performed, thereby affording opportunity to either prevent carious formations or heavily supplement pre-existing treatments. Back in 1965 the term probiotic was first used by Lilly and Stillwell to describe "substances secreted by one microorganism which stimulates the growth of another".[2] Applications of the concept was first recorded by the Ukrainian-born Nobel laureate Ilya Metchnikoff. He reported that Bulgarians lived longer lives than other populations and hypothesized that this was due to the consumption of fermented milk, allowing them to obtain a larger number of viable bacteria within their systems.[18] Further work prompted Metchnikoff to propose that we "fight microbe with microbe".[11] This strategy is echoed by the concept of bacteriotherapy or replacement therapy. Instead of allowing harmful pathogens to persist within the internal microbiome, the goal is to insert a harmless microorganism which can outcompete the undesirable bacterial species. This replacement mitigates the harmful process caused by the pathogenic bacteria and theoretically has few other effects within the microbiome.

Modern organizations such as the FAO and the WHO define probiotics as living microorganisms, principally bacteria, that are safe for human consumption and, when ingested in sufficient quantities, have beneficial effects on human health beyond basic nutrition.[3] As mentioned previously, much research has already been completed to manipulate the bacteria present within the human gut to aid with a variety of general processes and avoid diseases. Yet oral probiotic research has just scratched the surface of the symbiotic relationships that could be exploited in order to prevent dental health hazards. Many professionals emphasize the potential importance of these symbiotic species due to the minimal amount of side effects. Besides the many synthetic products that are in current use, there are few naturally occurring compounds that have been studied and approved for use. In the fight against caries it is important to recognize a tool that is suitable for prolonged use. Probiotics have the potential to maintain a high level of activity over an extended period without any negative consequences.[10]

**Background**

In order to understand how probiotics can influence the oral microbiome, it is important to review the mechanism of biofilm formation, the bacterial species involved and the process by which cavities are formed. These three topics will set the stage for a summary of the current probiotic treatments employed.

*Mechanism of Biofilm Formation*

The biofilm present within the oral cavity is a dynamic and highly variable environment, especially between individuals. These communities are three-dimensional structures, composed of many bacterial strains anchored to solid surfaces. This adhesion to tooth enamel or roots is based off interactions with the exopolysaccharide matrix which varies in its make-up as the conditions in the oral cavity change. Variation within the composition of the extracellular polysaccharides consequently effect the properties of the attached microbiome.[10] The microbiome formation process consists of three main steps: initial attachment of a pioneer species, co-adhesion and co-aggregation of other species, and then further propagation of the bacteria as they multiply, separate from the exopolysaccharide matrix and spread throughout the oral cavity. The first critical step, adhesion, is centered around the presence of an acquired pellicle that is formed on the matrix immediately upon cleaning of the hard surfaces utilizing saliva and proteins as the constituents. The pellicle is the major anchor point, allowing bacteria to permanently attach to surfaces via a reaction between bacterial adhesins and proline-rich glycoproteins (PRP) present in the pellicle. As this first pioneer species develops, it will begin to express a biofilm phenotype, prompting the activation of different gene expression. This allows the species to gain characteristics to allow it to survive in a stationary colonization phase. Secretion of extracellular polymeric substances such as polypeptides, carbohydrates and nucleic acids is one of the gained characteristics which preps the initial species for co-aggregation. This cell-to-cell recognition facilitates the formation of a community of species that work in tandem with one another. Communication is fulfilled via quorum sensing, nutrients and genetic components are exchanged via fluid filled channels and the species protect one another by existing in such intimate proximity.[17]

*Bacterial Species Composing Oral Microbiome*

Historically in microbiology classical Koch's theories set forth that one specific microorganism can be found to be responsible for a particular infection. This principle has remained as a pillar of microbiology ideology and has likely influenced the development of research dedicated to defining the main players within the oral microbiome. In the 1920's a sugar fermenting, acidogenic species called *Streptococcus mutans* was isolated from carious lesions. Since this discovery the bacterial species has been regarded by many in classical Koch fashion as the etiological agent responsible for dental caries.[16] Upon further investigation into the properties of *Streptococcus mutans*, it was found that the bacteria had evolved a set of genes that when turned on allow for increased carbohydrate uptake and metabolism. In response to a shifting oral environment, *Streptococcus mutans* can turn on expression of a new set of genes, allowing it to outcompete other species in the microbiome and establish itself as a colonizer on hard dental surfaces.[12] These findings led to the classification of the bacterial species as the main culprit for dental caries, which has been proven to be far from the truth in recent years. According to one study completed in 2015, *S. mutans* accounted for only 0.1% of the community in dental plaque and 0.7 to 1.6% in carious lesions.[16] These results are subject to question though, since they may quantify dead, transient or inactive microorganisms that do not play a role in the disease. Despite results minimizing the presence of the species within the microbiome, most sources assert that S. mutans has potential to be a driving factor of cariogenesis due to its fermenting and acidogenic nature. With the development of new technology such as second-generation sequencing and metagenomic techniques a shift away from classical Koch theories has begun.

The Human Oral Microbiome Database was established in order to investigate whether there is a common set of bacteria that are found in the human mouth. The genera listed in Figure 1 depict the core classified microorganisms as of 2014. *Streptococcus* dominates the genera, with *Veillonella* holding a sizable 9.8% and then the others all ranging from 0.6 to 3.5%. It is important to point out the dichotomy of biofilms present on the surface of teeth, supra and sub-gingival, both of which have unique bacterial compositions. Supragingival dental plaque is predominated by Gram positive bacteria while subgingival plaque on the other hand is composed of Gram negative species. This distinction is important since only the supragingival microbiome is associated with caries, while the subgingival is associated with gingivitis and periodontal disease.[17] Due to the focus on dental caries, the rest of the discussion will be focused solely on the species that comprise the supragingival microbiome.

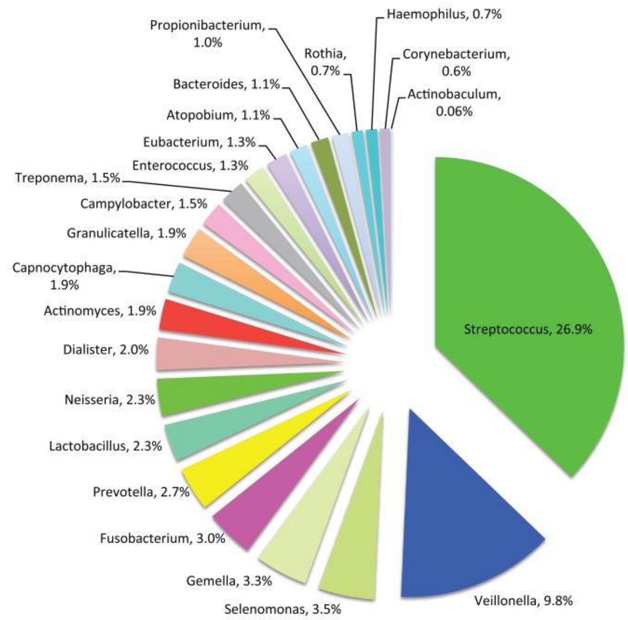


*Figure 1: Chart depicting the microbial components of the oral microbiome. Adapted from Costalonga et al., 2014.[7]*

In 2016, Xiao et al. completed a study that recruited 131 patients with dental caries and 29 healthy individuals. They classified all participants into four groups, No-caries (NC), low-caries (LC), moderate-caries (MC) and high-caries (HC). Then using 16S rDNA amplicon pyrosequencing genetic sequences from patient samples were analyzed. They were able to sort the bacteria via different taxonomic classifications to investigate any potential species that could serve as a biomarker for the progression of caries. Amongst 99 core genera that were classified in the study, the 6 most abundant were *Capnocytophaga* (17.8% of total abundance), *Prevotella* (13.5), *Actinomyces* (13.0%), *Carynebacterium* (8.0%) Within the NC group, they were able to predict that *Cardiobacterium, Corynebacterium, Lachnospiraceae*, *Lachnoanerobaculum,*

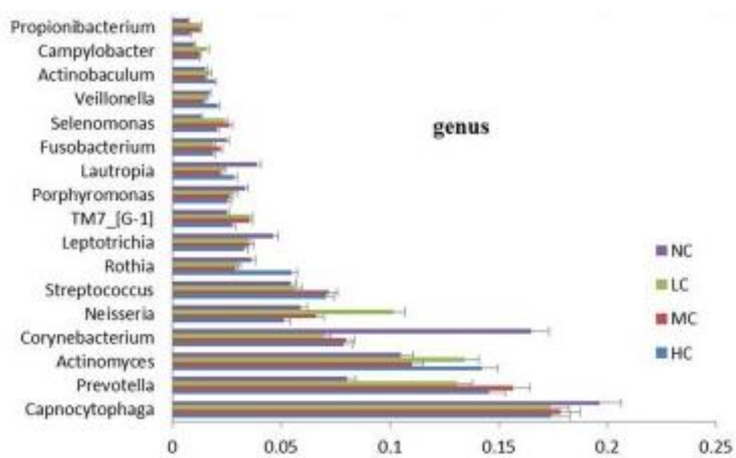


*Figure 2: Bar graph adapted from Xiao et al., depicting the species found in no caries, low caries, medium caries and high caries patients.[19]*

*Aggregatibacter, Eubacterium, GN02, Fusobacterium*, *Clostridiales* and *Tannerella* are all associated with a prevailing healthy microbiome. Additionally, they singled out *Cardiobacterium* and *Corynebacterium* as the two genera that most likely have the most influence within the healthy microbiota. The initial colonizing bacteria were identified as *Streptococcus sanguis*, and then members of the *Actinomyces, Neisseria, Veillonella, Capnocytophaga, Leptotrichia* and *Haemophilus* genera. The potentially cariogenic bacteria were identified as *Atopobium, Cryptobacterium, Lactobacillus, Mogibacterium, Ochrobactrum, Pseudomonas, Rhizobium, Alloprevotella, Bacteroides, Ventipeda, Campylobacter, Megasphaera and Mycoplasma*. Overall the study was able to conclude that the bacterial diversity present within the supragingival microbiome is high with good dental health and then slowly decreases as acidogenic bacteria prevail and cause caries. The group believes that the treatment of dental caries should be focused on restoring the balance of "healthy" bacteria in the oral cavity and preventative strategies directed to modulate intraspecies interactions.[19]

The second relevant study was completed in 2018 by Espinoza et al. who surveyed the supragingival plaque biofilm microbiome in 44 juvenile twin pairs using shotgun sequencing. The taxonomic results of health-related and cariogenic bacteria can be observed within the figures shown. It is interesting to note the differences between the relative abundance of genera between the two studies. For instance, consider Corynebacterium, both regard it as potential healthy microbiome marker yet the study done in 2018 quantifies its presence at a much lower abundance than the 2016 study in relation to the other genera. This more recent study found *Streptococcus* and *Neisseria* to be the most abundant, while Xiao et al. listed the genera as the fifth and sixth most abundant. Espinoza et al. concluded from their data that at the bulk community scale, the healthy individuals had greater diversity than the diseased cohort. Interestingly though they found the trend to be reversed for the 119 total Streptococcus strains. The juveniles with caries possess an overall decrease in bacterial genera diversity due to an increase within the number of *Streptococcus* strains represented. The study also points out that the progression to a carious state is accompanied by an increase in the diversity of sugar compounds available to the

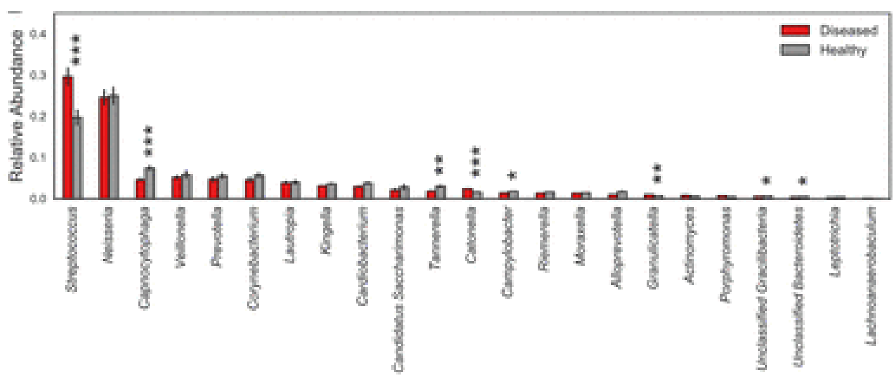


*Figure 3: Adapted from a paper by Espinoza et al., 2018, showing the levels of certain bacterial species in diseased (carious) and healthy individuals.[8]*

bacterial catabolic network. The cause of this increase is unknown but is hypothesized to be related to diet. An important conclusion proposed by Espinoza et al. was that the caries phenotype is a community metabolism disorder. Similar to the 2016 study, the group identified several mechanisms that are heightened within carious states, mainly improving the sugar uptake pathways. These findings directly support hypotheses which point out the shifting microbiome environment that can facilitate the formation of caries.[8]

*Cavity Formation*

Dental caries is a multifactorial disease that is caused by acid demineralization of the tooth enamel.[3] The cariogenic process can be influenced by a variety of factors, such as salivary flow, salivary composition, diet, nutrition, parental education level, transmission and age of acquisition of cariogenic species, oral hygiene practices, fluoride exposure, tooth anatomy and enamel composition. Therefore, there is a wide variation from patient to patient when considering the causes the subsequent bacterial species involved.[1] The acid that contributes to demineralization is a byproduct of metabolism pathways utilized by the bacterial species composing the supragingival biofilm. Organic acids derived from disaccharides such as sucrose are the final metabolic products and main contributors to the overall decrease in environmental pH. As the disease progresses and the acidic pH is maintained, there is a shift in the bacterial species composing the biofilm.[7] As demonstrated by the two studies, certain genera can be classified as health related while others contribute to cariogenesis. This is most likely due to the evolution and acquisition of genes that allow for certain species to establish themselves and outcompete other bacteria when utilizing the available sugars ingested. The prime example of an acidogenic genus being *Streptococcus*, specifically *Streptococcus mutans*.[4] These sets of genes are expressed in response to acidification of the oral cavity or in situations where glucose or sucrose is abundant.[10] As these species take over, they maintain the surrounding environmental pH at a level maximal for their survival. This acidity contributes to the formation of caries, and simultaneously selects for the survival of similar types of bacteria which will further increase acid production and demineralization.[7] The acidogenic bacteria begin to multiply and flourish while the biodiversity of other species declines rapidly.

For many decades, the focus of dental cavity prevention has been on neutralizing the acid that is capable of demineralizing teeth. This approach is flawed since even the natural buffer within the mouth, saliva, cannot reach some sites of acid production. If the biological buffer cannot neutralize the acid, how are externally sourced compounds going to have any effect? The dental plaque possesses chemical and physical properties of a biofilm matrix which inhibits the diffusion of bicarbonate and other therapeutic substances through the bacterial colonies.[4] A more effective method of caries prevention is to limit the amount of acid produced in the first place. This can be significantly impacted by a host of factors previously listed. Consider a community in South America called the Amerindians. They possess a much less diverse oral microbiome than urban individuals due to their decreased exposure to modern diet and multiethnic or multiracial societies. Remote Eskimo tribes were shown to exhibit lower prevalence of dental disease until more modern diets were introduced. Over the past 100 years, the human oral microbiome has become less diverse. As can be see in the two studies described above, a higher phylogenetic diversity is associated with greater ecosystem resilience and health when faced with a complex diet.[7] The ecological plaque hypothesis asserts that specific changes to the local environment allow for cariogenic species to outcompete health-associated bacteria and dominate as cavities are initiated and progress.

**Discussion**

*Previous Efforts Combatting Caries*

So far, most of the focus for reducing the prevalence of caries throughout the world has found success with preventative measures. Urging patients to eat well, practice good oral hygiene habits and not partake in detrimental behaviors such as smoking significantly increases not only oral but also systemic health. Frequent and routine check-ups at a dental office also help reinforce these practices. Some researchers have begun looking into the development of active immunization strategies, basically a caries vaccine. Targeting mainly strains of *Streptococcus* thus far, these vaccines consist of antibodies directed against species specific antigens. Although different genera of bacteria can be targeted, this approach is heavily flawed due to the variability of species comprising patients based on location, environment, age and behavioral patterns. There is a core oral microbiome, but the efficacy of a vaccine against a single species would likely be quite low when attempting to prevent caries due to high species variability. The disease can be initiated and progressed by numerous different acidogenic bacteria as described in the studies previously listed.[16] Other efforts have been made to disrupt and prevent the formation of biofilms through inhibition of the virulence factors, specifically GTF enzymes. GTF stands for glycosyltransferase, which is one of the key tools that allows for members of the *Streptococcus* genus, mainly *S. mutans*, to have enhanced polysaccharide synthesis. An important characteristic that gives the species the ability to act as an early colonizer. When GTF enzymes are inhibited, cariogenic bacteria loose their virulent properties and are kept at bay, not eradicated.[14] Similar to the caries vaccine, the issue of specificity again arises. These treatments are effective only when their targets make up the problematic components of the inflicted individuals oral microbiome. These studies are correct in recognizing the need to simply control and not eradicate the cariogenic species. Comparing Figure 1 to Figures 2 and 3, the “detrimental” bacteria only have negative effects when dominating the biofilm in high concentration. In a healthy individual, these species are still present, just in a controlled state. The cariogenic species are only detrimental when abundant. A maintenance of diversity within the bacteria is the key to preventing oral dysbiosis and disease. Unfortunately, most current caries prevention approaches are too fine-tuned and specifically focused to encompass all the potential imbalances and be effective on a large-scale distribution.

A more broadscale recently developed caries treatment is silver diamine fluoride (SDF). This product combines silver with fluoride and possesses both antimicrobial and remineralization characteristics due to its components.[10] It has been shown that SDF has high promise due to its efficacy and marketability. The drug boasts five main positive qualities: effective control of pain and infection, simplicity of installation, affordability of material, minimal requirements for personnel training and minimal invasiveness. Many call it a “silver-flouride bullet”, able not only halt existing caries but also prevent future developments of the disease.[15] The accessibility and affordability of this product make it a promising solution to dental caries within disadvantaged communities. Unfortunately, there are aesthetic drawbacks since the product stains the tooth surface upon interaction.

*Potential of Probiotics*

Probiotic research within the scope of dental caries aims to identify bacterial species that can be introduced to the oral microbiome to rebalance and keep the acid producing cariogenic species under control. Some researchers even suggest that the harmful bacteria be eradicated and replaced completely with a more docile version.[1] Over the past 15 years, the number of studies focusing on probiotics and oral healthcare has greatly increased. The replacement therapy strategy offers several benefits, mainly decreasing the rate of demineralization, while minimizing negative side effects. Additionally, probiotics are relatively much cheaper to produce that most reactive caries treatments that have been previously described. The mechanisms by which probiotics exert their effects are yet to be fully understood. Hypotheses include modifying gut pH, antagonizing pathogens through production of antimicrobial compounds, competing for binding and receptor sites as well as for available nutrients or growth factors and stimulating immunomodulatory cells.[2] An important factor when considering probiotic agents is the ability of the prospective candidate to be able to establish itself within the oral environment. Whether this is achieved during adhesion via interactions with pellicles or through collaboration with existing species during co-aggregation; a probiotic species must be able to successfully incorporate itself into the biofilm. This integration can be improved by selecting species which possess good adhesion properties, an ability to compete for existing binding sites and inhibitory activity against other species within the oral microbiome.[11] The selection must be well thought-out and thoroughly tested in order to ensure that the desired balance is achieved. Since the oral microbiome is a constantly fluctuating and a highly biodiverse environment, probiotic species must be a perfect fit disrupting primarily the cariogenic species. This could prove to be challenging since caries-free individuals possess about 50% of the same species in their supragingival microbiome as caries-active individuals.[7]

*Most Promising Probiotic Species*

Similar to the classification of the oral microbiome, the species that comprise the successful probiotics thus far have been sourced from a few broad genera of bacteria: *Lactobacillus, Bifidobacterium, Propionibacterium* and *Streptococcus*.[3] The most common belonging to *Lactobacillus* and *Bifidobacterium* which are Gram-positive and lactic acid-producing groups. Studies surrounding these species have been concerned with understanding the particular targets and effect of each probiotic in addition to the most effective method of application.

*Lactobacillus*

From Figure 1, Lactobacillus naturally consists of around 2.3% of a healthy individuals oral microbiome. Maintaining or slightly increasing this abundance has been a major strategy for efforts within oral probiotic studies. The genus is often present naturally in many yoghurts, leading researchers to question whether *Lactobacillus* has always been a component of the microbiome. Some believe that it established itself within the biofilm once the human diet began to regularly include such dairy products. Thus far, particular species within the genus have shown to avoid negative side effects upon increased abundance within the body, contributing to the potential as a probiotic agent. *Lactobacilli* produce hydrogen peroxide and reuterin, which is an antibacterial protein formed from glycerol that is resistant to proteolytic and lipolytic enzymes, both features that inhibit the proliferation of cariogenic species.[10] The bacteria require three main characteristics in order to establish themselves within an environment: a stagnant, retentive niche that is mostly anaerobic, a low pH and access to carbohydrates. The oral cavity, in addition to the gut and the vagina are the only settings within the human body which fulfill these conditions. Within the genus, not all species have been found to have anti-cariogenic properties. Some in fact, have actually been linked to increasing the demineralization process.[5] Others have been regarded as potential caries prophylaxis options due to their ability to compete with species of *Streptococcus*. The low molecular weight bacteriocins produced by species of Lactobacilli inhibit the proliferation of specifically *S. mutans*. The focus on this particular *Streptococcus* member is likely due to its prevalence as a cariogenic agent within the research of the oral microbiome. Since it has been regarded as a major keystone pathogen, countless probiotic agents have been assessed according to their ability to decrease *S. mutans* abundance.[11]

### *Bifidobacterium*

In comparison to Lactobacillus, there is not nearly as much literature investigating the mechanism of action or effects of *Bifidobacterium* as a probiotic. Even though in many studies both are listed as the main focus for oral probiotic perspectives, the majority of the focus is launched into species of the Lactobacillus genus. *Bifidobacterium* has largely been researched as a probiotic option to help improve gut microbiome heath. Within this context, the genus has been shown to have several positive effects. A few strains have been shown to exert a broad spectrum of antimicrobial properties via production of antimicrobial compounds that inhibit growth of pathogens. These effects do not directly influence acid production. Other strains have been shown to prevent infection by out-competing pathogenic species for binding sites on epithelial cells. Showing potential to be incorporated into biofilms at the adhesion phase. The last positive effect *Bifidobacterium* has been shown to possess within the gut is stimulating the immune system through non-specific modes of action. This produces an increased immune response to a wide number of invading antigens.[13] The studies and media fueled craze surrounding gut probiotics have fully invested into *Bifidobacterium*, yet the application to the oral cavity has yet to be explored. There have been a few studies assessing its ability to inhibit *S. mutans* proliferation via delivery in yoghurt and in combination with *Lactobacilli* species, but none have returned satisfactory results. Additionally, some *Bifidobacterium* species may be cariogenic in nature, meaning only certain strains will have a probiotic effect.

### Other Species

There are a variety of other species that have been used in preliminary probiotic studies, sometimes in combination with *Lactobacilli* or *Bifidobacterium*. Of note are several species of *Streptococcus*. Similar to the two genera previously described, species within this genus are not all classified as either cariogenic or healthy. In one study, *Streptococcus thermophilus* was investigated in tangent with *Lactobacillus lactis* strains. This treatment was shown to adhere to saliva-coated hydroxyapatite in a manner competitive with *Streptococcus sobrinus* which is regarded as a close relative to *Streptococcus Mutans*.[6] Other efforts with cariogenic *Streptococcus* species have attempted to genetically modify the virulence factors causing acid accumulation. Since *Streptococcus mutans* is extremely effective pathogen in the early formation of carious lesions, it could be more effective to simply modify a healthier version than try to find a bacterial species that can completely replace its function. One particular study was able to completely remove the gene encoding lactate dehydrogenase, completely inhibiting the bacteria's ability to produce lactic acid. There are many other minor species that have been researched, but none have shown as much promise as *Lactobacilli* or *Bifidobacterium* strains.

**Conclusions**

Considering the past 25 years of effort to incorporate probiotics into the fight against dental caries, it is clear that there is still a lot of unexplored potential. With the recent realization that *Streptococcus mutans* is not the universal causative agent of demineralization, the fact that the oral microbiome is a heavily variable and a multifactorial environment is becoming increasingly evident. Classification of the species comprising the microbial community is helpful, but only if it considers the differences between individuals. These differences can group people based on location, culture, diet, or even age. All variables contributing to the fact that every single person likely has a unique oral bacterial biodiversity, similar to a fingerprint. There are major bacterial players in the process leading to demineralization, but they are not necessarily constant from mouth to mouth. Similar to the variety of bacterial species associated with a healthy microbiome. An in depth understanding of the breadth and complexity of the oral biofilm is necessary when designing a probiotic strategy to maintain balance.

There are a few main points of attack to be considered with the probiotic strategy. The main approach has been to decide upon a single species of bacteria to be introduced into the oral cavity in order to mitigate the acid production. Unfortunately, one species might not have the same effect in the microbiome of a toddler compared to an adult. It could be beneficial instead to take a multispecies approach. Instead of introducing a single strain, adding 3-5 species at the same time could provide better chances of interaction and mitigation of cariogenic species. This could hypothetically make a set probiotic treatment more marketable and effective across a wider variety of patients. Additionally, it could be easier to guarantee the maintenance of balance with several different "healthy" species added into the community. Increasing the oral biodiversity and hopefully keeping the acidogenic bacteria in check. Another aspect of probiotic strategy to consider is whether it is more advantageous to focus on reducing the total number of acidogenic species or targeting the mechanisms by which the acid is produced. If the bacterial composition is so variable and the possible combinations of cariogenic species so numerous then is it really possible to find an agent that will balance the biodiversity? Instead, a possible strategy would be to focus on the mechanisms by which problematic metabolic outputs are created. This approach could potentially be applied widely to acidogenic bacteria, regardless of which specific species they belong to. Future research could be better spent intercepting functions related to disease initiation and progression no matter which cariogenic player is at fault. This approach could also be a more efficient manner of detecting carious states. Measuring the pH or amount of fermentation happening in the mouth could be a better indicator instead of focusing on the species present.[16]

Finally, it is important to keep in mind that a continuous supply of probiotic organisms might be necessary in order to maintain a healthy and balanced biodiversity. Depending in the delivery route, this could prove to be a large obstacle, since patients may not adhere to recommended daily routines. Perhaps different species of probiotics will remain established within the mouth longer than others? Could a more permanent substance similar to SDF be combined with probiotic agents? While facing these questions it is important that the dental industry keep in mind that the introduction of novel bacterial species can present health hazards. From temporary diarrhea to the interspecies transfer of antibiotic resistance genes, there are many possible outcomes that should be carefully monitored while conducting probiotic research. *Lactobacilli* and *Bifidobacterium* both have shown promise within previous studies, yet further research is needed in order to tell if probiotics can be utilized as a method of dental caries prevention.

**References**


1. Banas, Jeffrey A., and David R. Drake. “Are the Mutans Streptococci Still Considered Relevant to Understanding the Microbial Etiology of Dental Caries?” *BMC Oral Health* 18, no. 1 (December 2018). https://doi.org/10.1186/s12903-018-0595-2.

2. Bhushan, Jagat, and Sanjay Chachra. “Probiotics – Their Role in Prevention of Dental Caries,” n.d., 5.

3. Bonifait, Laetitia. “Probiotics for Oral Health: Myth or Reality?” 75, no. 8 (2009): 6.

4. Bowen, W. H. “Dental Caries – Not Just Holes in Teeth! A Perspective.” *Molecular Oral Microbiology* 31, no. 3 (2016): 228–33. https://doi.org/10.1111/omi.12132.

5. Caufield, P.W., C.N. Schön, P. Saraithong, Y. Li, and S. Argimón. “Oral Lactobacilli and Dental Caries: A Model for Niche Adaptation in Humans.” *Journal of Dental Research* 94, no. 9_suppl (September 2015): 110S-118S. https://doi.org/10.1177/0022034515576052.

6. Comelli, Elena Maria, Bernhard Guggenheim, Francesca Stingele, and Jean-Richard Neeser. “Selection of Dairy Bacterial Strains as Probiotics for Oral Health.” *European Journal of Oral Sciences* 110, no. 3 (June 2002): 218–24.

7. Costalonga, Massimo, and Mark C. Herzberg. “The Oral Microbiome and the Immunobiology of Periodontal Disease and Caries.” *Immunology Letters* 162, no. 2 (December 2014): 22–38. https://doi.org/10.1016/j.imlet.2014.08.017.

8. Espinoza, Josh L., Derek M. Harkins, Manolito Torralba, Andres Gomez, Sarah K. Highlander, Marcus B. Jones, Pamela Leong, et al. “Supragingival Plaque Microbiome Ecology and Functional Potential in the Context of Health and Disease.” *MBio* 9, no. 6 (December 21, 2018): e01631-18. https://doi.org/10.1128/mBio.01631-18.

9. Kilian, Mogens. “The Oral Microbiome – Friend or Foe?” *European Journal of Oral Sciences* 126, no. S1 (2018): 5–12. https://doi.org/10.1111/eos.12527.

10. Krzyściak, W., A. Jurczak, D. Kościelniak, B. Bystrowska, and A. Skalniak. “The Virulence of Streptococcus Mutans and the Ability to Form Biofilms.” *European Journal of Clinical Microbiology & Infectious Diseases* 33, no. 4 (April 1, 2014): 499–515. https://doi.org/10.1007/s10096-013-1993-7.

11. Meurman, Jukka H., and Iva V. Stamatova. “Probiotics: Evidence of Oral Health Implications.” *Folia Medica* 60, no. 1 (March 1, 2018): 21–29. https://doi.org/10.1515/folmed-2017-0080.

12. Moye, Zachary D., Lin Zeng, and Robert A. Burne. “Fueling the Caries Process: Carbohydrate Metabolism and Gene Regulation by Streptococcus Mutans.” *Journal of Oral Microbiology* 6, no. 1 (January 1, 2014): 24878. https://doi.org/10.3402/jom.v6.24878.

13. Picard, C, J Fioramonti, A Francois, T Robinson, F Neant, and C Matuchansky. “Review Article: Bifidobacteria as Probiotic Agents – Physiological Effects and Clinical Benefits.” Database. Alimentary Pharmacology and Therapeutics, April 21, 2019. https://onlinelibrary.wiley.com/doi/epdf/10.1111/j.1365-2036.2005.02615.x.

14. Ren, Zhi, Tao Cui, Jumei Zeng, Lulu Chen, Wenling Zhang, Xin Xu, Lei Cheng, et al. "Molecule Targeting Glucosyltransferase Inhibits Streptococcus Mutans Biofilm Formation and Virulence." *Antimicrobial Agents and Chemotherapy* 60, no. 1 (January 1, 2016): 126–35. https://doi.org/10.1128/AAC.00919-15.

15. Rosenblatt, A., T.C.M. Stamford, and R. Niederman. "Silver Diamine Fluoride: A Caries 'Silver-Fluoride Bullet.'" *Journal of Dental Research* 88, no. 2 (February 1, 2009): 116–25. https://doi.org/10.1177/0022034508329406.

16. Simón-Soro, Aurea, and Alex Mira. "Solving the Etiology of Dental Caries." *Trends in Microbiology* 23, no. 2 (February 2015): 76–82. https://doi.org/10.1016/j.tim.2014.10.010.

17. Strużycka, Izabela. "The Oral Microbiome in Dental Caries," n.d., 11.

18. Twetman, Svante, and Christina Stecksén-Blicks. "Probiotics and Oral Health Effects in Children." *International Journal of Paediatric Dentistry* 18, no. 1 (2008): 3–10. https://doi.org/10.1111/j.1365-263X.2007.00885.x.

19. Xiao, Cuicui, Shujun Ran, Zhengwei Huang, and Jingping Liang. "Bacterial Diversity and Community Structure of Supragingival Plaques in Adults with Dental Health or Caries Revealed by 16S Pyrosequencing." *Frontiers in Microbiology* 7 (July 22, 2016). https://doi.org/10.3389/fmicb.2016.01145.