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Dental Caries: Biochemical Aspects, Management and Treatment Using Probiotics and Prebiotics-An Updated Review.

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Abstract

Background: Dental caries, a common and concerning global public health issue, affects millions, including both primary and permanent dentition cases. The primary bacteria responsible for dental caries, *Streptococcus mutans*, creates biofilms that enable the bacteria to thrive and cause tooth decay. Current prevention strategies include dietary modifications, physical cleaning, and chemical treatments, but these are not always sufficient due to modern diets rich in sugars. Probiotic and postbiotic treatments, which aim to balance the oral microbiota and reduce the virulence of cariogenic bacteria, have become promising alternatives.

Aim: This review aims to explore the biochemical mechanisms involved in dental caries, the effectiveness of probiotics and postbiotics in preventing and managing dental caries by targeting the biofilm formation and virulence factors of *Streptococcus mutans* and other cariogenic bacteria.

Methods: A literature review was conducted to evaluate the mechanisms of action, application status, and challenges associated with the use of probiotics, prebiotics, synbiotics, and postbiotics in dental care. The review examines recent studies on the impact of these microbial preparations on dental health, focusing on their role in biofilm disruption and bacterial growth inhibition.

Results: Probiotics, postbiotics, and related microbial preparations have shown promise in reducing the growth of cariogenic bacteria such as *S. mutans* and preventing biofilm formation. These treatments promote a balanced oral microbiota, inhibiting the acid-producing and tooth-damaging activities of harmful bacteria. New advancements highlight the importance of targeting specific virulence factors of *S. mutans* for more effective treatments.

Conclusion: Microbial-based interventions, including probiotics and postbiotics, represent a viable strategy for preventing and managing dental caries. These treatments offer a less invasive, more targeted approach compared to traditional methods. However, further research is needed to standardize their use and ensure consistent, effective outcomes in dental caries prevention.

Keywords: Dental caries, *Streptococcus mutans*, biochemical, biofilm, probiotics, postbiotics, oral microbiota, prevention, treatment strategies.

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1. Introduction

Dental caries affects an alarming number of people globally and is a serious and urgent global public health concern. In particular, there are roughly 62.9 million cases pertaining to primary dentition and 64.6 million cases involving permanent dentition [1]. One of the main bacteria responsible for dental caries, Streptococcus mutans, produces collagen-binding proteins that allow it to efficiently infiltrate human umbilical vein endothelial cells [2], raising the possibility of infective endocarditis. Since biofilm is the primary mediator of dental caries, biofilm-targeting therapies have become an essential preventative tool. One successful preventive strategy has been to alter consumption of fermentable carbohydrates, especially sucrose [3]. It is quite difficult to completely eliminate cariogenic items in the current diet because of the prevalence of highly processed and sugary meals. Physical cleaning techniques (like brushing and the use of interdental instruments), chemical inhibition (like the use of povidone-iodine or chlorhexidine), and biological treatments (like probiotics) are further preventive strategies [4]. Modern approaches to dental caries prevention should concentrate on fostering a varied and balanced oral microbiota while inhibiting the proliferation of particular cariogenic bacteria by focusing on their virulence factors [5]. Microbial preparations, such as probiotics, prebiotics, synbiotics, and postbiotics, have attracted a lot of interest among these strategies because they are less intrusive and more targeted than physical and chemical therapies.

Probiotics were first introduced to the dental sector by Meurman and associates [6]. Since then, there has been a growing interest in microbial preparations as possible supplemental treatments for dental caries management and prevention. These treatments have shown significant effectiveness in preventing cariogenic bacteria from growing and forming biofilms. With a focus on the virulence factors of the cariogenic bacterium S. mutans, this article first gives a summary of the history and pathogenic mechanisms behind dental caries. The use

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of probiotics, prebiotics, synbiotics, and postbiotics in the prevention of dental caries is then summarized, along with new developments in the mechanisms of action, application status, and difficulties. The paper concludes by suggesting future paths for this field's advancement, with the goal of offering more standardized, scientifically supported, and successful recommendations for dental caries prevention from an academic perspective.

Dental Caries Background

With its diverse warm, humid microenvironments, the oral cavity serves as a complex ecosystem that fosters the proliferation of microorganisms [7]. According to a recent study, the oral microbiome is one of the most complex microbial habitats, second only to the colon [9], with 1,591 different species of bacteria, fungi, viruses, archaea, and protozoa [8]. Over the course of seven years, the core oral microbiota in healthy people stays largely constant [10], but an unbalanced microbiota can result in tooth caries and other oral illnesses [11]. Furthermore, people with severe dental caries have a substantially less diverse oral microbial community than people in good health [12]. A complicated interaction between the host, diet, and microbes leads to a change in the oral microbiota, which in turn causes dental caries [13].

Fermentable carbohydrates, which are frequently included in sweetened meals, are recognized as one of the most significant dietary contributors to dental caries among the many other factors [14]. The pH of biofilm is significantly lowered to as low as 4 or lower when sweetened foods are consumed because they quickly raise the concentration of carbohydrates in the oral cavity [15]. Studies have demonstrated a substantial association between the very acidic pH zones produced by biofilms and regions of rapid demineralization on enamel surfaces [16]. In the closed microenvironment of the biofilm, these frequent local pH drops upset the equilibrium between tooth mineralization and demineralization [17], resulting in tooth mineral loss as well as the formation of white spots, cavitation, pulp infections, and, in extreme situations, tooth loss. People with dental caries and those with healthy teeth have been found to have different oral microbiota. The genera Rothia, Neisseria, and Haemophilus, for example, were found to be among the first to colonize the oral cavity after birth [18] and are linked to dental health [19] in a study on children's oral microbiomes. On the other hand, children who have dental caries are more likely to have species such Prevotella spp., S. mutans, and Human herpesvirus 4 (EB virus) [19]. Lactobacillus was the most common genus in only 25% of carious lesions, while Actinomycetota (35.8%) and Bacillota (31.2%) were the most common phyla in deep dentin carious lesions [20]. It is becoming increasingly clear that dental caries is not caused by a single pathogen but rather results from an unbalanced microbiota in the biofilm, sometimes referred to as dental plaque [21].

Biochemical Mechanisms of Dental Caries:

Dental caries (DC) is a prevalent chronic condition impacting over 60% of children and a significant portion of adults in both industrialized and developing nations. It is a multifactorial disorder involving various elements such as cariogenic bacteria and genetic predisposition. Despite extensive research, the precise molecular mechanisms and key genes responsible for the initiation and progression of this condition remain insufficiently understood. The current study aimed to uncover potential biomarkers, master regulatory elements, protein kinases, transcription factors (TFs), signaling pathways, and biological processes (BPs) linked to the pathogenesis of DC. Gene expression data was gathered from pooled RNA samples of pulp tissue from 12 healthy individuals and 11 patients with DC, the latter group exhibiting deep dentinal lesions and pulp exposure. Analysis of the data identified 196 differentially expressed genes (DEGs), including 146 overexpressed and 50 underexpressed genes. Criteria such as a false discovery rate (FDR) below 0.001 and a |Log2 FC| greater than 1 were employed in this analysis. Subsequently, significant pathways and BPs associated with DC were identified using tools like the Reactome and DAVID online databases. A protein-protein interaction (PPI) network was constructed and analyzed based on these DEGs, shedding light on critical hub genes and their interactions. Among these hub genes, certain key players were identified, such as PTPRC, ITGB2, TYROBP, MMP-9, and others, which play roles in the development of dental caries.

Transcription factors regulating these hub genes were also highlighted. For example, BPTF, TROVE2, TCF12, SPI1, and CBFB were among the top-ranked TFs significantly associated with these genes. Additionally, protein kinases such as MAPK1, MAPK8, PRKCD, and MAPK3 were found to phosphorylate these TFs, further influencing the regulation of genes central to DC pathogenesis. The study also delved into the major histocompatibility complex (MHC), a set of genes crucial to the immune response in jawed vertebrates. Located on chromosome 6 (6p21.3) in humans, the MHC includes over 200 genes encoding glycoproteins known as human leukocyte antigens (HLAs). These HLAs are essential for immune defense, as they present peptide fragments to T cells. HLA alleles have been implicated in various conditions, including autoimmune diseases, inflammation, cancer, and even behavioral traits. However, the mechanisms underlying their diverse roles are not yet fully understood.

HLAs are categorized into two main classes: HLA class I and HLA class II. The former is found on all nucleated cells and presents peptides from degraded or abnormal proteins, such as those originating from viruses, to CD8+ T cells. This process is pivotal for activating the immune defense system. On the other hand, HLA class II proteins are primarily expressed on B cells and other antigen-presenting cells. They are involved in presenting extracellular antigens to CD4+ T cells, which play a central role in adaptive immunity. Through pathway and network analyses, this study provided insights into the molecular landscape of dental caries, emphasizing the involvement of specific signaling pathways, TFs, and protein kinases in its progression. For instance, pathways associated with immune response and inflammation, such as those mediated by the MAPK family of kinases, were highlighted. These findings not only enhance the understanding of DC but also pave the way for future research aimed at identifying therapeutic targets and developing novel interventions. In summary, the research identified significant molecular players and regulatory

networks underlying dental caries. By mapping out the relationships between DEGs, TFs, protein kinases, and immune pathways, the study contributes to the growing body of knowledge on the pathogenesis of DC. This information holds promise for advancing diagnostic tools and therapeutic strategies for managing this widespread condition (**Figure 1**).

Microorganisms Associated with Dental Caries

To varied degrees, cariogenic bacteria are a contributing factor to dental caries. Streptococcus sobrinus and S. mutans have been identified as the main causes of tooth caries for many years [22]. Significantly, *S. sobrinus* is more acidogenic and aciduric than S. mutans, although it is less able to adapt to the biofilm environment [23]. The oral microbiota's cariogenic bacteria are not independent entities; rather, they interact and have an impact on one another. In carious lesions, S. mutans contributes to the formation of a lactic acid-rich environment that encourages the growth of Veillonella species [12], which have been demonstrated to further S. mutans growth in biofilm investigations [24].

Furthermore, as common fungal representatives of cariogenic bacteria, Candida species have been identified as strong secondary cariogenic agents and have been isolated from 40–60% of adult and pediatric caries cases [25]. As an opportunistic caries yeast, Candida depends on the synthesis of proteinases and short-chain carboxylic acids, as well as its capacity to cling to abiotic surfaces and create biofilms [25]. In the oral cavity, a crucial interaction between bacteria and fungi takes place between Candida albicans and S. mutans. C. albicans stimulates S. mutans development, causing significant alterations in gene expression and improving the metabolism of carbohydrates [26]. Interestingly, 393 differently expressed genes in S. mutans are found in a dual-species biofilm as opposed to a mono-species biofilm made up entirely of S. mutans [26]. By attaching to Candida albicans and facilitating the conversion of sucrose into exopolysaccharides (EPS), the glucosyltransferases (Gtf) produced by S. mutans can provide binding sites for S. mutans [27].

According to one study, Veillonella parvula, Fusobacterium nucleatum, Prevotella denticola, and *Leptotrichia wadei* may be part of the core microbiota of early childhood caries (ECC) [28]. In addition to promoting S. mutans proliferation and acidogenicity by encouraging the production of biofilms with restricted acidogenic capability, this core microbiota also raises the cariogenic potential of enamel in vivo and promotes enamel demineralization in vitro [28]. Additionally, *Streptococcus gordonii, Leptotrichia buccalis, V. parvula, Actinomyces gerencseriae, Propionibacterium acidifaciens, Hallella multisaccharivorax, and Parascardovia denticolens* have all been strongly associated with dental caries in metagenomic investigations [29, 30]. S. mutans is one of the most well-researched species of the microbes linked to tooth caries. Unsurprisingly, the majority of preventive measures focus on S. mutans because they were once thought to be the main cause of dental caries [31].

Streptococcus mutans

Streptococcus mutans (S. mutans) has better characteristics than other first invaders of the oral cavity, mainly because of its ability to build a dense biofilm and its distinct virulence factors [32]. S. mutans is a prominent cariogenic bacteria that is controlled by the quorum-sensing system. S. mutans is the center core of the three-dimensional (3D) spherical biofilm that forms in dental caries of primary teeth, with other bacterial species encircling it in the outer layers [16]. Serious enamel demineralization is a result of the acidic pH that is fostered by this confined biofilm environment [16]. The oral microbiota's diversity declines with the progression of dental caries [33], creating a microbial imbalance that promotes the onset and spread of dental caries. It is commonly known that S. mutans and dental caries are related. Even though S. mutans naturally inhabits the human mouth [34], a rise in its number could be a symptom of dental caries [35]. Rats infected with human-derived S. mutans exhibit dental caries in experimental models [36], and S. mutans has been associated with recurrent early childhood caries (ECC) [37]. These results highlight how crucial S. mutans is to the development and spread of dental caries. Therefore, more research into S. mutans' pathogenic pathways (**Fig. 1**) is essential to creating efficient caries preventive and treatment plans. The virulence factors and quorum-sensing mechanisms of S. mutans will be discussed in the sections that follow.

Virulence Factors

The virulence of *S. mutans* can be categorized into four primary groups: extracellular polymeric substance (EPS) synthesis, adhesion, acid production, and acid resistance.

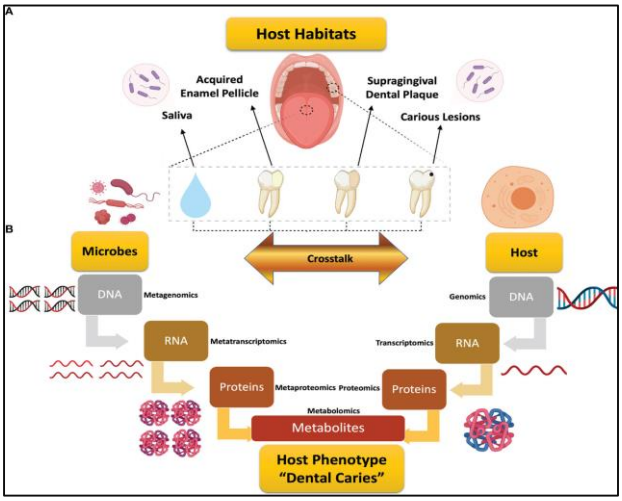

Figure 1: Biochemistry of Dental Caries.

EPS Synthesis

The production of EPS, a crucial component of biofilms, is the main factor responsible for S. mutans' pathogenicity [38]. Glucan is the main component of extracellular polysaccharide (EPS), which is made up of DNA, lipoteichoic acid, and extracellular proteins [39]. Glucosyltransferases (Gtf) produce glucosyltransferases produce glucosyltransferases produce glucosyltransferases [40], which gives microbes places to bond [38]. In order to enable microbial adhesion to inanimate surfaces, resist shear forces, elude host immune responses, tolerate antimicrobial agents, and preserve an acidic microenvironment that supports the growth of caries-associated biofilms, EPS helps the biofilm matrix to form highly structured chemical and physical barriers [42]. Mature biofilms are challenging to mechanically remove due to their increased viscoelasticity, which is a

result of EPS synthesis [42]. By controlling complement resistance and restricting the entry of effector molecules from the innate and adaptive immune systems, EPS also helps with immune evasion [43][44]. While sucrose, the precursor to glucan production diffuses readily with the help of EPS's negative charge, chlorhexidine, a common antibiotic in oral care, has limited penetration into deep biofilm layers because of its positive charge [45]. The glucan structure aids in the sequestration of protons on the negatively charged surface of S. mutans cells covered with EPS. This buildup is essential for establishing an acidic environment inside the biofilm, which promotes adaptability and acid retention [46]. In rodent models of dental caries, S. mutans's pathogenicity is greatly decreased when the expression of the gtf gene is disrupted [47]. In conclusion, S. mutans's cariogenic capacity depends on EPS, and preventing caries may be possible by focusing on EPS synthesis [48].

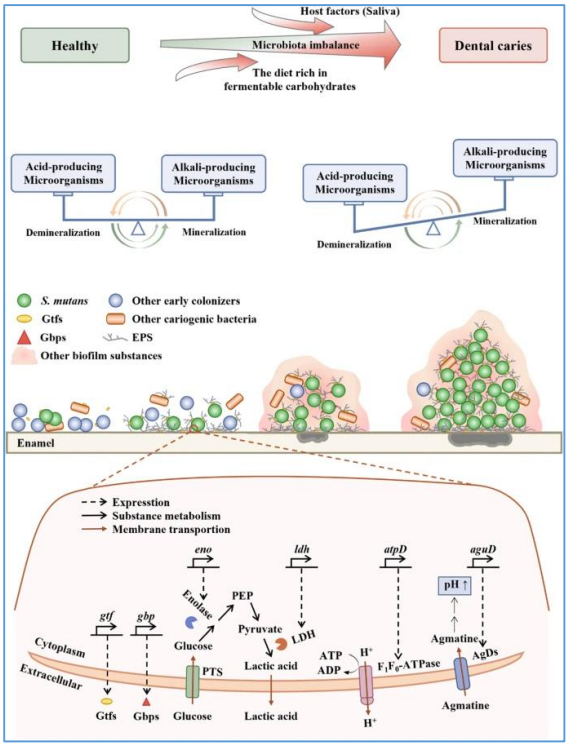

Figure 2: Cariogenic biofilm formation.

Adhesion

S. mutans adheres to teeth through both sucrose-independent and sucrose-dependent pathways [47]. The initial adhesion is facilitated by the sucrose-independent pathway, which is later reinforced by the sucrose-dependent pathway, stimulating glucan synthesis and biofilm formation [47]. Glucan-binding proteins (GBPs) mediate the binding of glucans produced by glucosyltransferases from sucrose, with GbpA playing a particularly crucial role in cariogenicity [49]. GbpA contributes to the integrity of the biofilm structure and is integral in linking glucan molecules, facilitating bacterial adhesion to tooth surfaces.

Acid Production

Upon glucose metabolism, *S. mutans* ferments dietary carbohydrates into organic acids, a critical process that contributes significantly to its pathogenicity and the progression of dental caries [50]. The enzyme enolase, encoded by the gene eno, is a central component of the phosphotransferase system responsible for glucose uptake [51]. Lactate dehydrogenase (LDH), encoded by the ldh gene, rapidly converts glucose into organic acids [52]. This acid production is a key factor in the cariogenic potential of *S. mutans*.

Acid Resistance

S. mutans utilizes a number of acid-resistant mechanisms to combat the stress brought on by acid generation. The atpD-encoded F1F0-ATPase proton pump produces ATP to sustain bacterial growth and survival and aids in the expulsion of intracellular protons to preserve pH homeostasis [53]. Reduced acid adaption and increased cytoplasmic acidity are the results of atpD expression inhibition in S. mutans [51]. Additionally, S. mutans exports acids out of the cell and generates alkali to neutralize them. The production of alkali to counteract acid stress is largely dependent on the agmatine deiminase system, specifically the agmatine-putrescine antiporter (AguD), which is encoded by the aguD gene [54]. As was previously mentioned in the section on EPS formation, the buildup of protons on the surface of S. mutans cells, which are encased in EPS, greatly enhances their resistance to acid [46].

Quorum-Sensing (QS) System

By releasing, detecting, and interacting with signaling molecules in response to cell density in the surrounding environment, the QS system in S. mutans controls virulence and biofilm formation [55]. Instead of operating as individual cells, S. mutans may communicate and work as a collective thanks to this arrangement. Bacteria can control gene expression in response to environmental cues thanks to the two-component signal transduction systems (TCSTS), which are essential to this process [56]. In S. mutans, key TCSTS including VicRKX and ComCDE are essential for controlling the formation of biofilms, acid resistance, and acid generation [57][58]. The cariogenic potential of S. mutans may be reduced by malfunctions in these regulatory systems.

Dental Caries Prevention Measures—Biological Interventions

Although dental plaque is a natural component of the oral environment from an evolutionary, biological, and nutritional standpoint, an imbalance within the oral microbiome, particularly in the pathological biofilm, can contribute to the development of dental caries [59]. Acid-producing cariogenic bacteria, especially *Streptococcus mutans*, play a significant role in damaging tooth enamel when fermentable carbohydrates are present [38].

In recent years, innovative biological interventions have emerged as potential strategies for preventing dental caries. One such approach involves utilizing microbial predators like *Bdellovibrio*, *Bacteriovorax*, and *Peredibacter* to target and eliminate anaerobic Gram-negative bacteria associated with periodontal diseases [60,61]. This contrasts with the fact that beneficial bacteria in the oral cavity are predominantly Gram-positive [62]. Additionally, biological interventions include the application of specific inhibitors targeting *S. mutans* proteins, vaccination strategies, and passive immunization using neutralizing bacteria [25,29]. While these novel methods show promise, the use of microbial preparations such as probiotics, prebiotics, synbiotics, and postbiotics remains

a more widely accepted and established approach for preventing dental caries.

Probiotics

Background:

The concept of probiotics dates back to 1908 when researchers first discovered their potential health benefits. Since then, the understanding of how probiotics impact host health has evolved significantly. In 2013, The International Scientific Association of Probiotics and Prebiotics (ISAPP) defined probiotics as "live microorganisms that, when administered in adequate amounts, confer a health benefit on the host" [64]. Today, probiotics are commonly used to support general health and well-being, with a well-established reputation for promoting gastrointestinal health. Increasing evidence also suggests that probiotics play an effective role in preventing and treating various oral diseases, including dental caries, oral mucositis, and halitosis [65].

Probiotics were first introduced to the dental field by Meurman and colleagues [6], who identified that *Lacticaseibacillus rhamnosus* GG ATCC 53103 could colonize the human mouth. Further studies have highlighted the potential of probiotics to prevent dental caries. For instance, one study demonstrated that Lactobacillus isolated from the oral cavities of subjects could inhibit the growth of *S. mutans* [66]. Among the most effective strains of *S. mutans* inhibitors are *Lacticaseibacillus paracasei* and *Lactiplantibacillus plantarum*, which are also frequently isolated from human subjects. The use of probiotics in oral health has been shown to restore microbial balance in the mouth, reducing *S. mutans* levels in both dental plaque and saliva [67]. Since each probiotic strain possesses unique characteristics, understanding these specifics is crucial for their application in the prevention and treatment of dental caries. For example, *L. rhamnosus* GG is a homofermentative Lactobacillus strain, which is not considered cariogenic as it cannot ferment sucrose or lactose [68]. On the other hand, *Limosilactobacillus reuteri*, an obligate heterofermentative species, produces broad-spectrum antimicrobial compounds such as reuterin and reutericyclin, which exhibit strong acid-base stability [69,70]. Apart from *Lactobacillus* spp., *Bifidobacteria* spp. also show promise as potential probiotics for caries prevention and treatment. For instance, yogurt containing *Bifidobacterium DN-173010* has been shown to significantly reduce *S. mutans* levels [71].

Mechanisms to Prevent Dental Caries

The mechanisms through which probiotics prevent dental caries are comparable to their actions within the gastrointestinal tract. These mechanisms primarily involve the synthesis of bioactive metabolites, inhibition of cariogenic microbial biofilm formation, competitive adhesion and colonization, coaggregation with pathogens, and modulation of the immune system.

Production of Active Metabolites

Bacteriocin

The antimicrobial qualities of bacteriocins, which are cationic antibacterial peptides produced by ribosomes, have been thoroughly investigated [72]. Bacteriocins fall into four groups, with groups I and II being the most pertinent to studies on probiotics [74]. A tiny, positively charged protein (2–5 kDa), nisin is a Type A bacteriocin belonging to Class I that creates membrane pores in target cells [72]. Class II bacteriocins work by increasing membrane permeability, which allows cell contents to escape [75]. Bacteriocins also have the ability to prevent the formation of biofilms, the creation of cell walls, and the degradation of bacterial DNA and RNA by DNase and RNase [72]. In biofilm simulations, studies have demonstrated that a protein-protein interaction between the bacteriocin generated by L. paracasei SD1 and the GtfB and LuxS proteins of S. mutans decreases microbial density and biofilm development [76]. Furthermore, lipid II and the bacteriocin Mersacidin produce a combination that prevents the production of cell walls [77]. Bacterial genetic material is broken down by the DNase and RNase activities of colicins, such as those from E2 to E9; colicin E2 has strong, long-lasting bactericidal effects [78]. Bacteriocins have been demonstrated to control the composition of the microbiota, influence the host's immunological response, and enable the long-term colonization of producer bacteria in particular niches [80]. Nisin and probiotics that produce it both lower the number of pathogens in biofilms and aid in reestablishing a balanced microbial diversity [81]. For bacteriocins to remain active during manufacturing and use, temperature is an essential component. The bacteriocin activity of Streptococcus oralis subsp. dentisani 7746 (AB-Dentisanium®), for instance, is at its peak between 30 and 45°C, with only a minor decrease at 60°C [82]. This emphasizes how crucial it is to regulate temperature properly when using bacteriocins for a variety of uses.

Enzymes

Probiotics release a diverse range of enzymes in addition to bacteriocins, which destroy biofilms and affect bacteriocin activity, hence contributing to their positive effects. For instance, the lipase produced by Lactobacillus acidophilus breaks down biofilms [83]. Similarly, the dextranase enzyme expressed by Streptococcus salivarius JH hydrolyzes S. mutans' extracellular polysaccharides (EPS), boosting the anti-S. mutans action of zoocin A, a muralytic bacteriocin [84]. Proteases that resemble challisin are produced by other probiotic bacteria, including Streptococcus sp. A12, and they prevent S. mutans from producing bacteriocin [85]. Additionally, S. salivarius M18 produces dextranase and urease, which lessen the production of plaque and balance out the saliva's acidic conditions [86].

Biosurfactants (BS)

Microorganisms produce biosurfactants, which are amphiphilic molecules made up of both hydrophilic and hydrophobic components. They are frequently made of proteins, lipids, and carbohydrates [87]. Techniques including nuclear magnetic resonance, Fourier transform infrared spectroscopy, and thin layer chromatography can be used to describe these substances [88]. Due to the intricacy of these molecules, it is noteworthy that 50% of the 40 biosurfactant studies that were examined did not analyze their structure [87]. Surfactin-type biosurfactants, which are generally produced by Lactobacillus species, are becoming more and more useful in the prevention of oral illnesses because of their strong anti-adhesion and anti-biofilm characteristics [87]. For instance, the adherence and biofilm development of Streptococcus oralis and S. mutans on titanium surfaces can be inhibited in a dose-dependent manner by biosurfactants generated by L. reuteri DSM 17938, L. acidophilus DDS-1, L. rhamnosus ATCC 53103, and L. paracasei B21060 [92].

Moreover, S. mutans's chain length is shortened, its ability to form biofilms on glass slides is hindered, and the expression of the gtfB and gtfC genes is downregulated by the protein-based biosurfactants that are produced by L. acidophilus DSM 20079 [93]. Cell lysis may result from L. rhamnosus-produced biosurfactants that disrupt the protein conformation or physical structure of the biofilm [94]. Biosurfactants have low cytotoxicity and good stability in addition to their antibacterial effectiveness. For example, rhamnolipids, which are typically thought to be non-toxic, show comparable low cytotoxicity to biosurfactants made from Lactobacillus species [87]. Furthermore, the stability of L. paracasei-derived biosurfactants, which maintain their surface activity over a pH range of 6–10 and can tolerate incubation at 60°C for up to 120 hours, shows how stable these biosurfactants are [95].

Organic Acids

Lactobacillus species in the human gastrointestinal system and other regions of the body create organic acids including lactic acid and butyric acid, which are well known for their health benefits. To some degree, these acids may have a bacteriostatic effect on oral pathogenic microbes. For instance, L. paracasei Lpc-37 generates organic acids that can prevent Streptococcus mutans from growing and forming biofilms [96][97]. However, there is worry about how the acids produced by probiotics interact with those produced by cariogenic bacteria, like S. mutans, given the clear correlation between regular exposure to high concentrations of acid and tooth demineralization [16]. From the larger viewpoint of the caries environment, where cariogenic microbes create a well-structured acidic barrier, this problem can be examined [42]. Instead of demineralizing the whole surface of the teeth, prolonged exposure to high amounts of acid causes selective demineralization. Probiotics may be able to break down this acidic barrier and lessen acid buildup if the organic acids they create hinder cariogenic bacteria and their biofilms. Because the mouth cavity maintains its own acid-base microbial balance [54], acids that contribute to this equilibrium rather than build up would probably be less dangerous.

Hydrogen Peroxide

Hydrogen peroxide is a byproduct of the antibacterial properties of several probiotic species, such as *Bifidobacterium bifidum*, *Lactobacillus johnsonii*, *Lactobacillus crispatus*, and *Lactobacillus jensenii* [98]. Pathogenic bacteria are killed by hydrogen peroxide because it targets their epithelium [98]. Additionally, it may be able to control the species composition in the oral cavity [99], with cariogenic species such as S. mutans being especially susceptible to its harmful effects [100]. dramatically, following catalase treatment, the antibacterial activity of L. paracasei cell-free supernatant (CFS) was dramatically reduced, underscoring the part hydrogen peroxide plays in its bacteriostatic properties [101]. It is crucial to remember that because hydrogen peroxide is prone to decomposition, it may lose its bacteriostatic qualities following specific procedures, like freeze-drying [102].

Inhibition of Cariogenic Microbial Biofilm

Biofilm development is frequently linked to dental caries. One important feature of probiotics is their capacity to prevent or eradicate the establishment of biofilms and harmful microbes in the oral cavity. It has been demonstrated that several probiotic strains, including Bifidobacterium longum subsp. longum, Limosilactobacillus fermentum TCUESC01, L. acidophilus 4A, and S. oralis 89a, have anti-biofilm action [103]. For instance, by down-regulating important genes like gtfB, gtfC, and gtfD in S. mutans, Lacticaseibacillus casei ATCC 393, L. reuteri ATCC 23272, L. plantarum ATCC 14917, and Ligilactobacillus salivarius ATCC 11741 can inhibit S. mutans biofilms [104]. Probiotics have also been shown to stop fungus from changing into harmful forms. A critical step in the pathophysiology of Candida albicans, the early stages of hyphal production, can be hampered by strains such as L. rhamnosus LR32, L. casei L324m, and L. acidophilus NCFM [105]. Lactobacillus helveticus CBS N116411, Lactobacillus plantarum SD5870, and S. salivarius DSM 14685 were the probiotics that significantly reduced the expression of genes involved in the yeast-to-hypha transition in Candida albicans. These genes included ALS3 (adhesin/invasin), SAP5 (secreted protease), EFG1 (hyphae-specific gene activator), and HWP1 (hyphal wall protein) [106]. Various models are employed according on the objectives of the research, and in vitro biofilm models are constantly improving to more accurately replicate human situations. For example, these studies have used experimental abutments that mimic the macro- and microstructures of dental implants [107]. Furthermore, it has been discovered that in the early phases of biofilm development, the combination of collagen peptides and L. rhamnosus considerably raises the medium's pH [108]. According to qPCR analysis, this combination down-regulated eno, ldh, and atpD, among other important genes linked to acid production and tolerance. Probiotics may also interfere with quorum sensing (QS) to produce antimicrobial effects. According to one study, exposure to Lactobacillus containing CFS caused the comD, vicR, and vicK genes to be down-regulated in both planktonic and biofilm forms of S. mutans. This could account for the decreased adherence and biofilm formation of S. mutans observed in scanning electron microscopy experiments.

Competitive Adhesion and Colonization

Probiotics' capacity to outcompete oral pathogens in terms of adherence and colonization is a crucial component that contributes to their positive health benefits [109]. For instance, out of the eight probiotic strains studied, L. reuteri LR6 showed the strongest adhesion capabilities, which is equivalent to a stronger ability to prevent pathogen adherence to Caco-2 cells [110]. Plaque scores and S. mutans levels decreased, indicating that S. salivarius M18's increased colonization efficacy produced higher anti-caries activity [111]. By decreasing extracellular polymeric substance (EPS) synthesis, cell surface hydrophobicity, and self-aggregation, Levilactobacillus brevis KCCM 202399 prevented S. mutans KCTC 5458 from adhering [112]. It's interesting to note that probiotics can lessen pathogen adherence without coming into direct touch with it. Probiotic-treated saliva, for instance, has been demonstrated to reduce S. mutans's adherence to hydroxyapatite surfaces, which act as a model for enamel [113]. Subsequent investigation revealed that this impact was associated with the lack of two proteins in the

salivary membrane: salivary peroxidase, a natural defense component found in human saliva, and salivary lectin gp340, the main receptor for S. mutans [114].

Coaggregation with Pathogens

Coaggregation, the ability of probiotics to form a barrier that impedes pathogen colonization, is another beneficial property of these microorganisms [115]. A study identified six out of 624 lactic acid bacteria that exhibited specific coaggregation with *S. mutans* in vitro [116]. The species identified as capable of this coaggregation were *L. paracasei* and *L. rhamnosus*. This coaggregation mechanism was found to be resilient to hyperthermia and protease treatment and was not reliant on lectins, nor was it influenced by saliva.

Regulation of the Immune System

Probiotics are known to activate and modify the host's immune system in addition to their direct effects on pathogenic bacteria and biofilms [117]. According to clinical studies, patients with severe early childhood caries (ECC) who consumed L. paracasei SD1 daily or triweekly had significantly higher salivary levels of human neutrophil peptides 1-3, which have broad bactericidal activity, and lower levels of S. mutans, which may slow the progression of caries [118]. Additionally, six months of consuming milk containing L. paracasei SD1 raised salivary immunoglobulin A levels, which were favorably connected with the L. paracasei load [119]. It has been demonstrated that some strains of Streptococcus thermophilus, such as ST1342, ST1275, and ST285, stimulate the release of pro-inflammatory cytokines by monocytes, including interleukin-1β, tumor necrosis factor-α, interleukin-6, and interferon-γ, and activate the innate immune response, which aids in the removal of pathogens [103]. By increasing the production of tumor necrosis factor-α, interleukin-6, and Chemokine (C-C motif) ligand 20 in human monocyte leukemia cells, commercial L. paracasei DG also exhibits immunostimulatory activity [120]. According to these results, probiotics may strengthen the host's defenses against harmful microbes, providing a possible means of preventing and curing infectious illnesses.

The delivery vehicle for probiotics plays a significant role in their colonization within the oral cavity, influencing their effectiveness. A variety of vehicles are available for probiotic administration, including dairy products, ice cream, cereal, pacifiers, chewing gum, curd, juice, and mouthwash [68]. Among these, dairy products are considered ideal carriers due to their inherent health benefits [121]. Within the category of dairy products, liquid forms such as milk and yogurt have been found to be particularly effective in reducing *Streptococcus mutans* (S. mutans) levels [122]. For individuals with dairy allergies, alternative carriers may be considered. Milk's buffering capacity aids in reducing acid production, while its colloidal properties offer protection for enamel [123]. Additionally, milk contains calcium and calcium lactate, which may contribute to caries prevention [124] and the reduction of pathogenic microbial colonization [125]. Furthermore, milk and cheese facilitate the dominance of casein phosphopeptides, which are integral to the process of biomineralization [126]. A systematic review and meta-analysis have demonstrated that dairy products containing probiotics have a significant impact in lowering S. mutans levels and increasing salivary pH [122].

The slow release of probiotics can be achieved by utilizing suitable embedding materials. For instance, formulations using L. paracasei 28.4-gellan have been shown to release the probiotic over a period of 24 hours [127]. In this formulation, L. paracasei effectively inhibited S. mutans both in its floating and biofilm states, significantly reduced the production of extracellular polysaccharides (EPS), and downregulated several key genes, including luxS, brpA, gbpB, and gtfB.

Despite the potential benefits, there remains some controversy regarding the use of probiotics in preventing dental caries, with particular focus on safety and possible cariogenic effects. Many probiotics are not sourced from the oral microbiota but rather from fecal samples, and some are derived from animals [82]. Therefore, it is essential to rigorously evaluate their safety before clinical application. Probiotics may pose a risk to individuals with compromised immunity or damaged barriers, such as those susceptible to bacteremia [128], particularly when administered at high concentrations. Regarding the potential cariogenic effects, a meta-analysis of 50 studies concerning dental caries and periodontal diseases concluded that there is insufficient evidence to support the use of probiotics in caries treatment [129]. In this meta-analysis, individuals with active dental caries exhibited higher levels of S. mutans, *Actinomyces* sp. strain B19SC, and *Lactobacillus* spp., as identified by PCR-based methods [130]. Lactic acid bacteria commonly identified in carious lesions in both adults and children include *Lactobacillus fermentum*, *Lactobacillus casei*/*paracasei*, *Lactobacillus salivarius*, *Lactobacillus rhamnosus*, *Lactobacillus plantarum*, and *Lactobacillus gasseri* [131]. Furthermore, *Bifidobacterium dentium*, a known late marker for dental caries progression, was absent in the oral cavities of caries-free individuals but detected in 30.8% of caries cases in a study of 56 participants [132].

It is unreasonable to infer a causal link between lactic acid bacteria and dental caries based on a strong correlation between these bacteria and caries scores [133]. Lactic acid bacteria exhibit a relatively low affinity for teeth, and their capacity to form biofilms in vitro is considerably weaker than that of S. mutans [131]. The attachment and proliferation of secondary invaders, including *Bifidobacterium* and other lactic acid bacteria, is facilitated by the initiation of caries by major caries-promoting organisms, such as S. mutans, which create an anaerobic, acidic environment rich in carbohydrates [132, 133]. The destruction of dentin requires more than just lactic acid; proteolytic activity is also necessary, as the primary component of dentin is the extracellular matrix, which is dominated by type I collagen [134]. Interestingly, lactic acid bacteria such as *Lactobacillus rhamnosus*, *Lactobacillus casei*/*paracasei*, *Lactobacillus salivarius*, *Lactobacillus vaginalis*, *Lactobacillus gasseri*, *Limosilactobacillus oris*, and *Lactobacillus fermentum* exhibit a greater tendency to bind to collagen rather than degrade it, based on genomic analysis [133]. In conclusion, the efficacy of probiotics in preventing dental caries remains a subject of ongoing debate. A promising avenue for further research is to explore the roles of different microbial populations in the transition from a healthy oral microbiota to a cariogenic one. Specifically, it is essential to consider factors such as dietary composition, the host immune environment, and the

physical and chemical characteristics of the oral cavity, particularly the teeth. Consequently, there has been growing interest in the potential of prebiotics, synbiotics, and postbiotics in caries prevention, offering potential advantages over probiotics in maintaining oral health.

Prebiotics

In 1995, prebiotics were defined as "non-digestible food ingredients that beneficially affect the host by selectively stimulating the growth and/or activity of one or a limited number of bacteria already resident in the colon" [135]. However, with advancements in scientific understanding, the International Scientific Association for Probiotics and Prebiotics (ISAPP) proposed a more refined definition in 2017, describing prebiotics as "a substrate that is selectively utilized by host microorganisms conferring a health benefit" [136]. Prebiotics offer a safe and effective alternative to probiotics because they are non-living and not subject to environmental factors that may affect the survival and efficacy of probiotic microorganisms. This section explores the various types of prebiotics, their mechanisms of action, efficacy, and other pertinent aspects.

Sugar

Certain sugars have been identified as exhibiting prebiotic properties. D-tagatose, a non-cariogenic sugar, is particularly noteworthy due to its lower calorie content and glycemic index compared to sucrose [137]. Notably, the saliva of individuals with optimal oral health is enriched with D-tagatose [138]. D-tagatose has been shown to inhibit the growth of *Streptococcus mutans* and *Streptococcus gordonii* by interfering with glycolysis and its downstream metabolic pathways, although it does not affect *Streptococcus oralis* [138]. Chewing gum containing D-tagatose has also been demonstrated to hinder the growth of *S. mutans* [139]. In addition to D-tagatose, other sugars such as xylose and arabinose are being considered as potential prebiotics. These sugars not only inhibit the growth of *S. mutans*, but also promote the growth of *Lactobacillus* species [140], suggesting their dual action in restoring the balance of the oral microbiome. While the promising prebiotic potential of these sugars warrants further investigation, additional research is necessary to evaluate their in vivo efficacy and any potential adverse effects.

Sugar Alcohol

Sugar alcohols, including xylitol, sorbitol, maltitol, and erythritol, exhibit prebiotic properties that are beneficial for oral health. Xylitol, a five-carbon polyol sweetener, is regarded as an oral-specific prebiotic according to the 2017 definition [142,143]. Xylitol offers multiple benefits, such as enhancing remineralization, lowering the pH of dental plaque, reducing *S. mutans* levels in saliva, diminishing the amount of insoluble dextran in the *S. mutans* biofilm, and lowering the incidence of dental caries [144,145,146]. However, xylitol loses its efficacy in the presence of fructose or sucrose [147]. Other sugar alcohols, including sorbitol, maltitol, and erythritol, have similarly demonstrated their ability to inhibit dental caries [148,149].

Oligosaccharides

Oligosaccharides are also gaining attention as potential prebiotics. Human milk oligosaccharides (HMOs), which are the third most abundant component in human milk, are commonly added to infant formula [150]. Galacto-oligosaccharides (GOS) and 2′-fucosyllactose, the predominant HMOs, have been shown to reduce the extracellular polysaccharide (EPS)-mediated adhesion of *S. mutans* DSM 20523 to glass surfaces, suggesting their potential as prebiotics for promoting oral health [150]. Furthermore, GOS, glucomannan hydrolysates, and mannose have demonstrated the ability to inhibit pathogen adhesion to epithelial cells by binding to the pathogen's lectins/pili [151]. However, while non-digestible and/or non-absorbable sugar alcohols and oligosaccharides confer health benefits, excessive intake can lead to significant gastrointestinal distress, such as diarrhea [152]. Further studies are required to determine the optimal dosage and duration of consumption to minimize these adverse effects.

Arginine

Arginine is a widely studied oral prebiotic known for its beneficial effects on oral health [38]. These effects include promoting alkaline substance production, mitigating tooth demineralization, and suppressing biofilm formation. Specifically, arginine has been shown to inhibit the growth of *Candida* [153] and reduce enamel demineralization [154]. Additionally, L-arginine enhances the alkali-producing capacity of arginine-solubilizing bacteria, such as *Streptococcus sanguinis* and *S. gordonii* [155], which elevates the pH of the biofilm environment, making it inhospitable to cariogenic microorganisms. Interestingly, L-arginine was found to reduce insoluble EPS levels by a factor of three, specifically targeting the *gtfB* gene [156]. A variety of studies have explored the mechanism underlying the prebiotic effects of arginine in oral health, particularly through the use of arginine-containing toothpaste. One such in vivo study revealed that the presence of arginine in toothpaste enhanced the arginolytic capacity of human saliva while reducing its sucrose metabolic activity [157]. Moreover, it led to a shift in the composition of the salivary microbiota towards a healthier ecological balance. Toothpaste formulations containing both fluoride and arginine were found to be more effective at preventing and reversing early caries lesions, with significantly increased remineralization compared to fluoride-only toothpaste [158]. Additionally, fluoride and arginine toothpaste was linked to an increase in gene expression related to the arginine deiminase pathway, as evidenced by metagenomic data [160]. The combined use of fluoride and arginine toothpaste also promoted healthier microbial communities and reduced the presence of caries-associated bacteria. Intriguingly, arginine may also stimulate the production of beneficial substances by probiotics. Exogenous arginine has been shown to upregulate the expression of the *S. gordonii spxB* gene, which encodes pyruvate oxidase (SpxB), leading to increased hydrogen peroxide production [156]. Furthermore, using magnesium ions (Mg^{2+}) as a cofactor for SpxB activity has been demonstrated to promote hydrogen peroxide production and enhance the abundance of SpxB in *S. sanguinis* and *S. gordonii* [161].

Urea and Nitrates

Urea and nitrates are being explored as potential oral prebiotics. Urea acts as a prebiotic due to its conversion into ammonia or ammonium and bicarbonate ions by urease-producing bacteria, such as *S. salivarius*, *Actinomyces naeslundii*, and *Haemophilus* species, which

helps neutralize acids in the oral cavity [163]. Nitrate-reducing bacteria in the oral cavity convert salivary nitrate into nitrite, which is subsequently reduced to nitric oxide [164]. These compounds have been shown to inhibit the growth of pathogenic bacteria [165,166], and nitrate has demonstrated the ability to reduce caries incidence and inhibit bacteria typically associated with dental caries, such as *S. mutans* NCTC 10499, *L. casei*, and *A. naeslundii*, as well as periodontal disease-related bacteria like *Fusobacterium nucleatum*, *Eikenella corrodens*, and *Porphyromonas gingivalis* [167]. Additionally, nitrate has been shown to promote oral health by supporting nitrate respiration in anaerobic microorganisms, increasing oral pH through several mechanisms, including competition for carbon sources with acid-producing fermentation processes and the generation of hydroxyl ions [168,169]. Beyond its effects on dental caries, dietary nitrate has been shown to reduce gum inflammation [170], lower systemic blood pressure [171,172], and improve vascular health [172], with potential benefits for patients with hypercholesterolemia [173, 174]. Given the broader health benefits of nitrate, further research is needed to better understand its role in preventing dental caries. Both probiotics and prebiotics offer distinct health benefits, and their combined use represents a promising strategy for co-administration in therapeutic contexts. The following section will delve into this combination approach.

Conclusion:

Dental caries continues to be a significant public health issue, with millions of cases globally affecting both primary and permanent dentition. The traditional approaches to managing dental caries, such as physical cleaning and chemical agents, are essential but often insufficient due to the prevalent sugary diets in modern societies. Probiotics, postbiotics, and other microbial preparations offer promising alternative solutions for caries prevention and management by targeting the root causes of the disease: biofilm formation and bacterial virulence. *Streptococcus mutans* plays a pivotal role in dental caries, largely due to its ability to form biofilms that foster an acidic environment conducive to tooth demineralization. The presence of virulence factors such as extracellular polymeric substance (EPS) synthesis, adhesion, and acid production enables *S. mutans* to thrive in the oral cavity, making it a central focus of research and treatment. Recent studies have highlighted the potential of probiotics and postbiotics in disrupting this biofilm formation, reducing bacterial load, and promoting a more balanced oral microbiota. The application of probiotics in dental care is increasingly being recognized for its effectiveness in maintaining a healthy oral ecosystem. Probiotic strains such as *Lactobacillus* and *Bifidobacterium* have been shown to inhibit the growth of cariogenic bacteria, enhance the natural defenses of the oral cavity, and reduce inflammation. Postbiotics, by providing beneficial metabolic byproducts from probiotic bacteria, have also demonstrated antibacterial properties that help in combating *S. mutans* and preventing caries development. Despite their promising potential, several challenges remain, such as the variability in probiotic efficacy, the need for further standardized clinical trials, and the long-term sustainability of these treatments. Moreover, while microbial-based therapies represent a less invasive option compared to traditional chemical treatments, there is a need for more research to refine their application in routine dental care. Moving forward, integrating probiotics and postbiotics into dental practice could offer a more sustainable and effective approach to dental caries prevention, provided ongoing studies address the current gaps in knowledge and application. In conclusion, probiotics and postbiotics hold considerable promise as complementary treatments for dental caries. These microbial strategies can work synergistically with other preventive measures, contributing to a comprehensive and more effective approach to managing oral health. The future of dental care may increasingly involve these biotherapeutic agents, creating a paradigm shift towards a more balanced and microbiome-friendly method of caries management.

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