EGAS MONIZ SCHOOL of HEALTH & SCIENCE | INSTITUTO UNIVERSITÁRIO EGAS MONIZ

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MESTRADO INTEGRADO EM MEDICINA DENTÁRIA

THE USE OF PREBIOTICS IN DENTISTRY: A LITERATURE REVIEW

Trabalho submetido por
Rémi Breau
para a obtenção do grau de Mestre em Medicina Dentária

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Professor Doutor Nuno Taveira

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Resumo

As patologias dentárias têm sido tratadas ao longo dos séculos através de atos mecânicos mais ou menos invasivos e, mais recentemente, desde o final do século XIX, com medicamentos como analgésicos, anti-inflamatórios ou antibióticos.

No entanto, nos últimos vinte anos, novos tipos de suplementos, como prébióticos, probióticos ou simbióticos, têm surgido para ajudar na cura de algumas dessas patologias. Esses produtos, destinados ao microbioma oral, visam selecionar certas bactérias benéficas e inibir outras patogénicas.

De facto, o microbioma oral é um ambiente composto por múltiplos sítios, cada um com uma proporção fisiológica específica de micro-organismos, e quando essas proporções são alteradas, podem surgir patologias.

O objetivo desta revisão narrativa foi apresentar o microbioma oral, diferentes prébióticos e estudos que demonstram a sua eficácia em certas patologias, nomeadamente cáries dentárias.

A conclusão é que os estudos realizados são muito promissores; os prébióticos orais poderiam desempenhar no futuro um verdadeiro complemento, ou até mesmo uma alternativa, aos tratamentos atuais, como os antibióticos, que, com a antibiorresistência, se tornam uma preocupação de saúde importante.

Palavras-chaves: prébióticos orais; microbioma; prébióticos dentisteria

Abstract

Dental pathologies have been treated for centuries through more or less invasive mechanical procedures and, more recently, since the late 19th century, with medications such as painkillers, anti-inflammatories, or antibiotics.

However, over the past twenty years, new types of supplements, such as prebiotics, probiotics, or synbiotics, have emerged to aid in the healing of certain dental conditions. These products, designed for the oral microbiome, aim to select beneficial bacteria and inhibit pathogenic ones.

Indeed, the oral microbiome is an environment composed of multiple sites, each with a specific physiological proportion of microorganisms, and when these proportions are altered, pathologies can arise.

The objective of this narrative review was to present the oral microbiome, various prebiotics, and studies demonstrating their effectiveness in certain pathologies, particularly dental caries.

The conclusion is that the studies conducted are very promising; oral prebiotics could play a significant role in the future as a complement or even an alternative to current treatments, such as antibiotics, which, with antibiotic resistance, are becoming a major health concern.

Key words: oral prebiotics; microbiome; prebiotics dentistry

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ABREVIATURAS

2'-FL - 2′-fucosyllactose

AD - arginine deiminase

ADS - arginine deiminase-system

AgDS - agmatine deiminase system

AUC - Area under the growth curve

BHI - brain heart infusion

cOTC - catabolic ornithine transcarbamylase

CA - active caries

CF - caries-free

CK - carbamate kinase

CLSM - confocal laser scanning microscope

Ctrl - control

CDM - chemically defined medium

DNRA - dissimilatory nitrate reduction to ammonium

ECC - early childhood caries

EHOMD - Expanded Human Oral Microbiome Database

EPS - extracellular polysaccharides

FOS - fructo-oligosaccharides

GBPs - Glucan Binding Proteins

GOS - galacto-oligosaccharides

GI - gingival index

Gtf - glucosyltransferase

HA - hydroxyapatite

HMOs - human milk oligosaccharide

HMP - Human Microbiome Project

ISAPP - International Scientific Association of Probiotics and Prebiotics

IOH - intra-oral halitosis

EOH - extra-oral halitosis

LDH - lactate dehydrogenase

NaF - sodium fluoride

N2O - nitrous oxide

NO - nitric oxide

NH4 - ammonium

OD - optical density

PEP - phosphoenolpyruvate

PTS - fructose-specific phosphotransferase system

RCTs - randomised controlled trials

RTCA - Real Time Cell Analyzer

SAOS-2 - osteosarcoma cells

TSB - Tryptic Soy Broth

VSCs - volatile sulphur compounds

Xyl - xylitol

HaCaT - human keratinocytes

1. Introduction

The gastrointestinal microbiome has garnered significant attention in recent years due to its profound impact on overall health and well-being. However, the oral microbiome, the second most important microbial ecosystem in the human body, remains relatively understudied despite its critical role in oral health and systemic diseases (Yu et al., 2024). The oral microbiome is a dynamic ecosystem comprising a diverse array of commensal bacteria that coexist in a delicate balance. This microbial community is not only essential for maintaining oral health but also seems to play a role in the development and progression of various systemic diseases, including diabetes, Alzheimer's disease, and cardiovascular disorders (Baker et al., 2024). The interplay between the oral microbiome and these systemic conditions underscores the importance of understanding and modulating this ecosystem to promote overall health.

Prebiotics, defined as non-digestible food ingredients that beneficially affect the host by selectively stimulating the growth and activity of beneficial microorganisms, have emerged as a promising strategy for modulating the oral microbiome(Verspecht et al., 2021). While the influence of prebiotics on gut health has been extensively studied, their role in oral health remains relatively unexplored. Preliminary research suggests that prebiotics have the potential to restore a healthy oral microbiome by promoting the growth of beneficial bacteria and inhibiting the proliferation of pathogenic species(Verspecht et al., 2021). This modulation of the oral microbiome could offer a novel approach to preventing and treating oral diseases, potentially serving as a complement to traditional oral care.

The purpose of this study is to present an overview of various prebiotics, their mechanisms of action, and to review the existing data suggesting that their use may offer a viable complement to conventional oral care practices. By examining the current state of research on prebiotics and their impact on the oral microbiome, this work aims to shed light on the potential benefits of incorporating prebiotics into oral health strategies.

2. The oral microbiome

2.1. Composition, dynamics and role in oral health and diseases

Since Antonie van Leeuwenhoek first observed and discovered oral bacteria (from his own dental plaque) with his first microscope in 1680 (X.-S. He & Shi, 2009), microbiology has continued to evolve, culminating in the recent discovery and understanding of human body microbiome through DNA sequencing. Indeed, at the beginning of the 21st century, the Human Microbiome Project (HMP) made it possible to identify many microorganisms living in the human body and enabling it to perform numerous bodily functions, such as digestion, immunity and protection against pathogens(Proctor et al., 2019). However, research on the human oral microbiome has not made as much progress as research on the gut microbiome, which is the largest in human body.

The oral cavity is a highly heterogeneous ecological system due to its various dental and mucosal surfaces. These habitats are colonised by diverse microbial communities (including bacteria, archaea, fungi, algae, and small protists) collectively known as the Oral Microbiome (Bacali et al., 2022). These microorganisms must engage and co-evolve with their neighbours and hosts and adapt to diverse and rapidly changing conditions. Despite this, the microbial composition is relatively stable and exhibits community functions such as resistance to colonisation (X. He et al., 2014). These characteristics require a complex level of interspecies communication.

Using next generation sequencing techniques, the scientist's community of eHOMD (Expanded Human Oral Microbiome Database) sequenced 774 bacterial species in the healthy oral microbiome (Human Oral Microbiome Database), which includes the human mouth and aerodigestive tract (pharynx, nasal passages, sinuses and oesophagus), and identified seven dominant phyla, which represent about 80-95% of oral microbiome: *Actinobacteria, Bacteroidetes, Firmicutes, Fusobacteria, Proteobacteria, Saccharibacteria* and *Spirochaetes*.

Regarding non-bacterial microorganisms, a study by Sharma et al. identified 85 fungal species in 20 healthy adults, with the dominant genera being *Candida, Aureobasidium, Saccharomycetales, Cladosporium, Aspergillus, Fusarium, and Cryptococcus* (Sharma et al., 2018).

The oral cavity, being composed of various anatomic structures (for example the hard surface of the tooth enamel, the keratinized surfaces of the palate, gingiva and tongue papillae and the soft surfaces such as the buccal mucosa), contains many distinct microenvironments and, consequently, distinct bacterial communities (Baker et al., 2024) as shown in Figure 1 below.

Saliva is primarily composed of bacteria originating from oral tissues. Indeed, after the shedding of epithelial cells, they are released into the saliva. However, the microbial profile of saliva is not similar to that of dental tissues, as the latter are hard tissues to which different bacteria adhere compared to soft tissues. The microbial composition of saliva can be useful in diagnosing certain diseases, such as oral cancer or dental caries (Megha Baghel et al., 2025).

The back of the tongue is also a significant bacterial niche, composed of multiple layers of bacteria, similar to dental plaque. These bacteria are closely linked to the occurrence of halitosis, or bad breath. The crevices and fissures of the tongue provide an ideal environment for anaerobic bacteria belonging to the phyla *Fusobacteria, Bacteroidetes, and Spirochaetes* (Lee & Hong, 2023).

The tooth surface is itself divided into two distinct niches, the supra-gingival and the sub-gingival (Baker et al., 2024). A systematic review (del Pilar Angarita-Díaz et al., 2024) identified bacterial species present in healthy subgingival dental plaque, including *Actinomyces viscosus, Actinomyces naeslundii, Haemophilus parainfluenzae, Rothia dentocariosa, Streptococcus sanguinis, Streptococcus mitis, Streptococcus oralis, Streptococcus gordonii, Streptococcus intermedius*, and *Prevotella nigrescens*. Regarding the supragingival microbiome, a review (Anderson et al., 2023) revealed that the genera *Actinomyces, Streptococcus, Veillonella, Corynebacterium*, and *Neisseria* were the most abundant in the supragingival biofilm of healthy individuals.

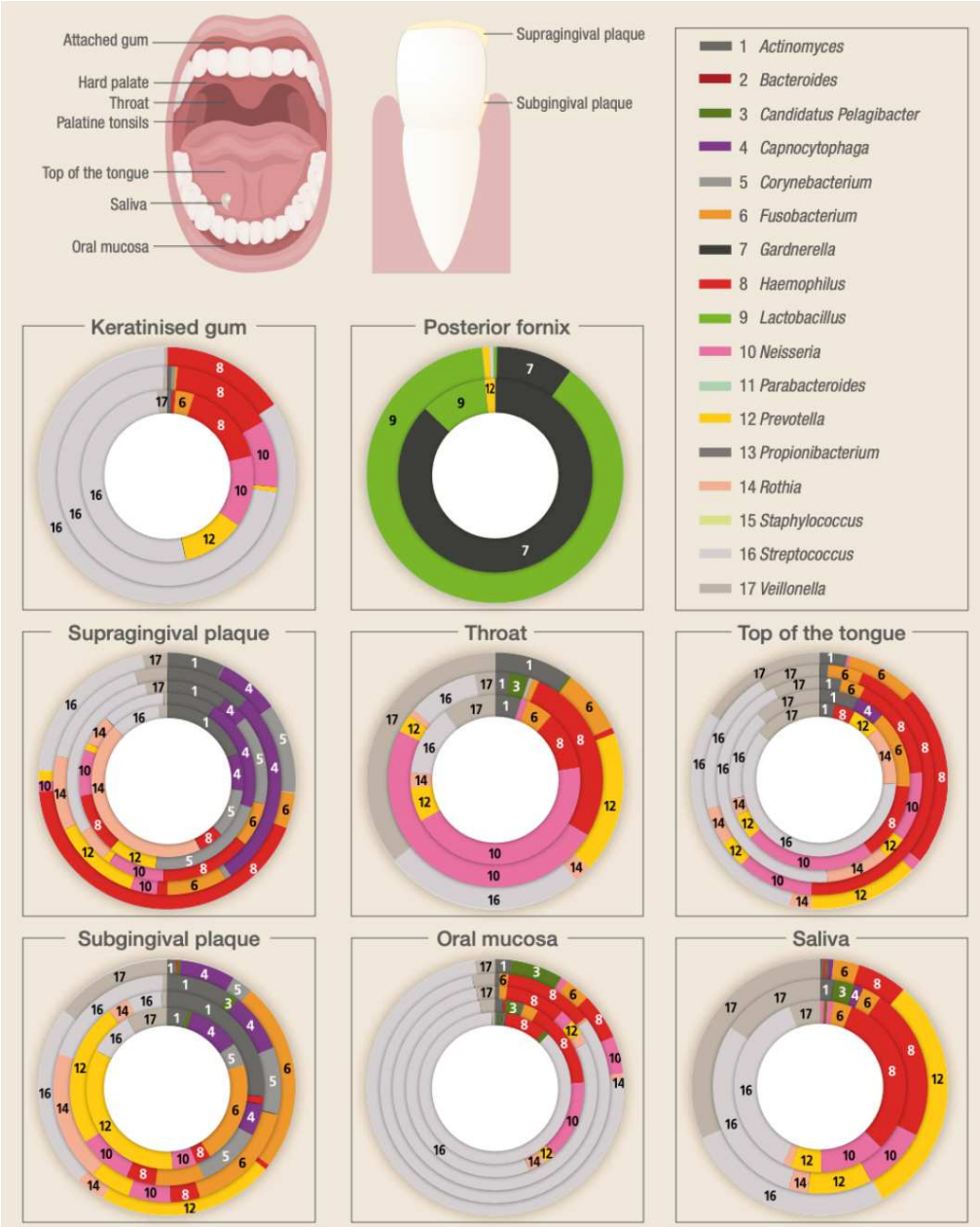


Figure 1: Comparison of bacterial genera from different sites of the oral microbiome in 2 to 5 healthy adult individuals. Microbial diversity is represented by doughnut charts, with the number of rings corresponding to the number of individuals analysed for each site. The sequencing technique used in this study is shotgun metagenomic sequencing, based on the total DNA of the samples.

This illustration and these graphs show that the predominant genera are *Streptococcus*, *Prevotella*, *Rothia*, *Neisseria* and *Haemophilus*, in oral health.

This illustration is inspired by the study conducted by Xi et al. (Xie et al., 2014).

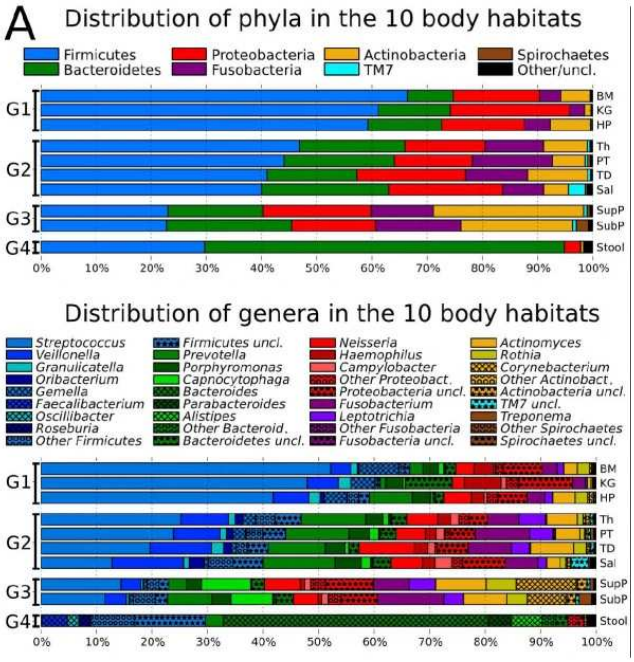


Figure 2: These charts show the abundance of phyla and genera for each site. The *Firmicutes* and *Streptococcus* are clearly abundant compared to others. Shotgun metagenomic sequencing was performed on 200 healthy adults, analysing ten sites of the digestive tract, including seven sites in the oral cavity. (Segata et al., 2012)

Group 1 (G1): Buccal mucosa, keratinised gingiva, hard palate (dominated by *Firmicutes*)

Group 2 (G2): Saliva, tongue, tonsils, throat (higher diversity, with significant proportions of *Bacteroidetes*, *Fusobacteria*, and *Actinobacteria*)

Group 3 (G3): Supragingival and subgingival plaque (higher proportion of Actinobacteria)

Group 4 (G4): Stool.

Illustration from (Segata et al., 2012).

The oral microbiome is an adaptive environment that can change under various conditions. Diet is a prime example, as it not only provides resources to the microbiome's bacteria but also acts as a selective pressure on them (Baker & Edlund, 2019). The introduction of agriculture 10,000 years ago, marking the transition from the Mesolithic to the Neolithic era, was a major turning point in human history, radically transforming dietary habits and, consequently, the composition of the oral microbiome (Andréa Quagliariello, 2023). This shift from a hunter-gatherer diet to an agricultural one introduced food such as refined vegetable oils, dairy products, processed grains, and farm-raised meats, thereby altering the microbial diversity present in the oral cavity (Megha Baghel et al., 2025).

Modern diets, often rich in sugars and refined carbohydrates, promote the proliferation of specific bacteria associated with dental caries and periodontal diseases. For example, the consumption of sugary and carbonated beverages is linked to an increase in populations of *Fusobacterium, Bacteroidetes, Veillonella*, and *Gammaproteobacteria* (Chumponsuk et al., 2021). These bacteria thrive in sugar-rich environments, which can lead to the acidification of dental plaque and promote the development of cavities.

Age is also a determining factor in the composition and diversity of the oral microbiome. In infants, the mode of delivery and type of feeding (breast milk or formula) influence the initial colonization of microorganisms in the oral cavity. By three months, infants delivered vaginally exhibit greater taxonomic diversity than those delivered by cesarean section (Oba et al., 2020). Breastfed children show higher proportions of *Streptococcus*, while those fed formula have higher proportions of *Actinomyces* and *Prevotella* (Oba et al., 2020). As children grow and begin consuming solid foods, the composition of the oral microbiome continues to evolve, reflecting changes in dietary habits and environment. In adults, dietary habits and lifestyle factors, such as smoking and alcohol consumption, can further alter the oral microbiome, influencing both oral and systemic health (Chumponsuk et al., 2021).

Smoking also has a strong impact on the oral microbiome, altering its composition and natural balance (Jia et al., 2021). Substances present in tobacco smoke, such as nicotine, can disrupt interactions between oral microorganisms and human tissues, promoting the growth of pathogenic bacteria. Cigarette smoke creates an anaerobic environment in the oral cavity, which favors the proliferation of anaerobic bacteria. Additionally, it increases levels of free iron and inhibits oral peroxidase activity, further facilitating the growth of these bacteria (Rajasekaran et al., 2024). These changes can disrupt microbial balance, increasing the risk of infections and oral diseases. Therefore, quitting smoking can help restore a healthier and more balanced oral microbiome.

Finally, alcohol consumption is also a factor in oral microbiome dysbiosis. The enrichment of *Prevotella* and *Moryella* and the depletion of *Lautropia*, *Haemophilus*, and *Porphyromonas* are significantly observed in individuals who consume alcohol (Liao et al., 2022). Furthermore, certain bacteria, such as *Rothia mucilagenosa*, can convert

ethanol into acetaldehyde, a compound known to be carcinogenic to humans (Amer et al., 2020).

In summary, the oral microbiota is influenced by various factors such as age, diet, smoking, and alcohol consumption, which can disrupt the balance of the oral microbiome and lead to dysbiosis. This can result in the proliferation of disease-associated bacteria, increased inflammation, and a higher risk of oral disorders such as tooth decay, gum disease, and other oral infections.

2.1.1. Periodontal disease

Numerous studies have highlighted the relationship between periodontal diseases, particularly periodontitis, and dysbiosis of the oral microbiome. The colonisation of the periodontal pocket, formed because of the loss of attachment between teeth and gums, by bacteria known as periodontopathogens, leads to the development of periodontitis (Doucette et al., 2024) (Figure 3). Notably implicated in the development of periodontitis are strict anaerobes and Gram-negative capnophilic bacteria, including *Porphyromonas gingivalis, Treponema denticola, Tannerella forsythia (the red complex), Actinobacillus actinomycetemcomitans, Prevotella intermedia,* and *Campylobacter rectus (Di Stefano et al., 2022).* The conventional treatment of periodontitis involves the mechanical removal of subgingival plaque: scaling and root planing (SRP) is considered the standard method of control (Suvan et al., 2020) and can be enhanced by the addition of antibiotics. However, several disadvantages are associated with antibiotic use: risk of resistance, increased host vulnerability, alterations in the commensal flora, and the potential for further dysbiosis (Ardila et al., 2023).

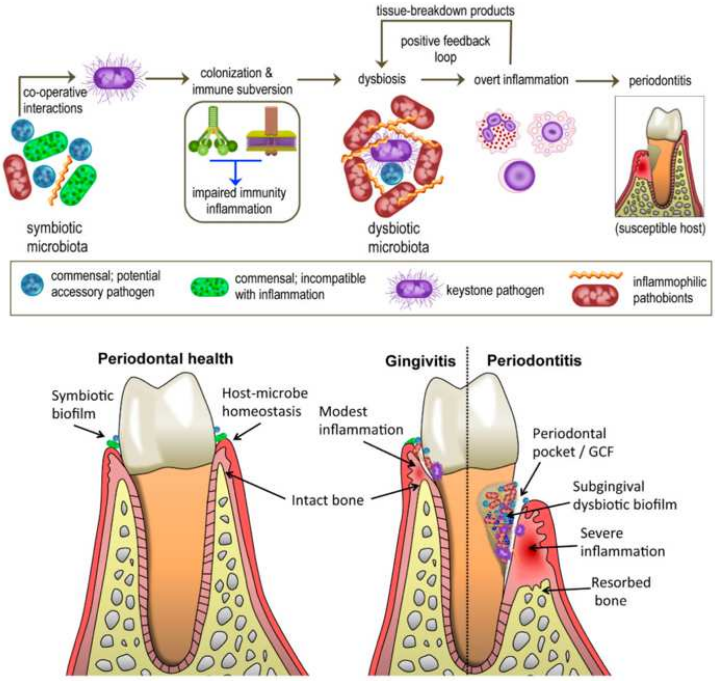


Figure 3: Periodontitis occurs in susceptible hosts due to a polymicrobial community where different microbes work synergistically to trigger destructive inflammation. Keystone pathogens, aided by accessory pathogens, disrupt the host's immune response, leading to a dysbiotic microbiota. This overactivation of inflammation results in tissue destruction, including alveolar bone resorption. Inflammation and dysbiosis create a feedback loop, as tissue breakdown products fuel the dysbiotic bacteria. The progression includes: healthy gums (shallow gingival crevice (≤2 mm)); gingivitis (inflammation without bone loss and gingival crevice ≤3 mm); periodontitis (periodontal pockets ≥4 mm with inflammatory bone loss). Deep pockets (up to 10-12 mm) form as collagenolytic enzymes degrade tissue. These pockets harbour up to 10^{10} bacteria, sustained by nutrients like collagen peptides and haem-containing compounds from the gingival crevicular fluid (Hajishengallis, 2015).
Image reproduced from (Hajishengallis, 2015) with permission from Springer Nature.

As previously explained, periodontitis is a disease largely caused by Gram-negative anaerobic bacteria. The most well-known and studied are undoubtedly the three that make up the "red complex": *Porphyromonas gingivalis, Treponema denticola* and *Tannerella forsythia*.

Porphyromonas gingivalis is a Gram-negative anaerobic bacterium strongly associated with severe forms of periodontitis. It produces various virulence factors, including gingipains (proteases capable of degrading host proteins) thus contributing to inflammation and periodontal tissue destruction. Moreover, *P. gingivalis* can evade the immune system and modulate the inflammatory response, thereby promoting disease progression (Gasmi Benahmed et al., 2022).

Treponema denticola is a strictly anaerobic spirochete bacterium characterised by high motility, allowing it to penetrate deep into periodontal tissues. It produces various enzymes, including proteases and haemolysins, which facilitate tissue invasion and destruction of the tooth-supporting structures. Its close interaction with *P. gingivalis* enhances the virulence of the subgingival biofilm, thereby exacerbating periodontitis (Pisani et al., 2023).

Tannerella forsythia, another Gram-negative anaerobic bacterium, is frequently found in advanced forms of periodontitis. It secretes enzymes capable of degrading connective tissues, thereby promoting periodontal destruction. Additionally, its glycosylated surface provides resistance to host immune defences, allowing it to persist in the periodontal environment (Schäffer & Andrukhov, 2024).

These three bacteria act synergistically within the subgingival biofilm, mutually enhancing their pathogenicity and playing a key role in the progression of periodontal disease (Mohanty et al., 2019).

2.1.2. Dental caries

Dental caries is the most common oral disease and one of the most prevalent chronic diseases, affecting approximately 2 billion adults and 520 million children in 2019, according to the Global Burden of Disease Study (Hernández et al., 2022). The primary cause of tooth demineralisation is acid production by bacteria in the supragingival dental plaque (Utamaningyas et al., 2023). Initially, a physiological dental biofilm forms, primarily composed of commensal bacteria such as *S. mitis*, *S. gordonii*, and *Actinomyces sp*., which adhere to the tooth surface with the help of salivary proteins and produce hydrogen peroxide (H_2O_2) and bacteriocins that inhibit the growth of cariogenic bacteria (UTAMANİNGYAS et al., 2023)(Radaic & Kapila, 2021). Subsequently, a small quantity of cariogenic bacteria can grow if homeostasis is disrupted, such as reduced salivary flow, diabetes increasing blood glucose levels, or a diet rich in carbohydrates without proper hygiene (Radaic & Kapila, 2021); this environment becomes conducive to the development of a cariogenic biofilm, composed mainly of aciduric bacteria like Streptococci, Lactobacilli, and Bifidobacteria (Hernández et al., 2022).

The key bacterium in caries formation is *S. mutans*, a Gram-positive bacteria capable of metabolising certain carbohydrates and sugars into pyruvate and subsequently into lactic acid, an acidic molecule that lowers the pH of the oral cavity, leading to tooth

demineralisation (Amargianitakis et al., 2021). Another virulence factor of this bacteria is its ability to thrive in acidic environments, unlike commensal bacteria, which are inhibited (UTAMANİNGYAS et al., 2023), due to the F_1F_0 ATPase, a proton pump that expels intracellular protons to maintain stable intracellular pH. Additionally, the agmatine deiminase system helps produce alkaline substances that neutralise acids and contribute to bacterial survival in acidic conditions (Luo et al., 2024).

Another strength of *S. mutans* is its synthesis of extracellular polysaccharides (EPS), formed from glucans synthesised by glucosyltransferase enzymes (Gtf), creating an adherent matrix that protects biofilm bacteria from antimicrobial agents, host immune responses, and shear forces from fluids like saliva (Luo et al., 2024). Once these EPS are in place, other cariogenic bacteria, primarily Gram-positive, adhere to this matrix and contribute to the biofilm's extension (Hernández et al., 2022). Finally, its fourth and last virulence factor is its ability to adhere to dental structures, made possible by the production of glucans as well (Luo et al., 2024). The mechanism of caries formation and the virulence factors of *S. mutans* are shown in Figure 4.

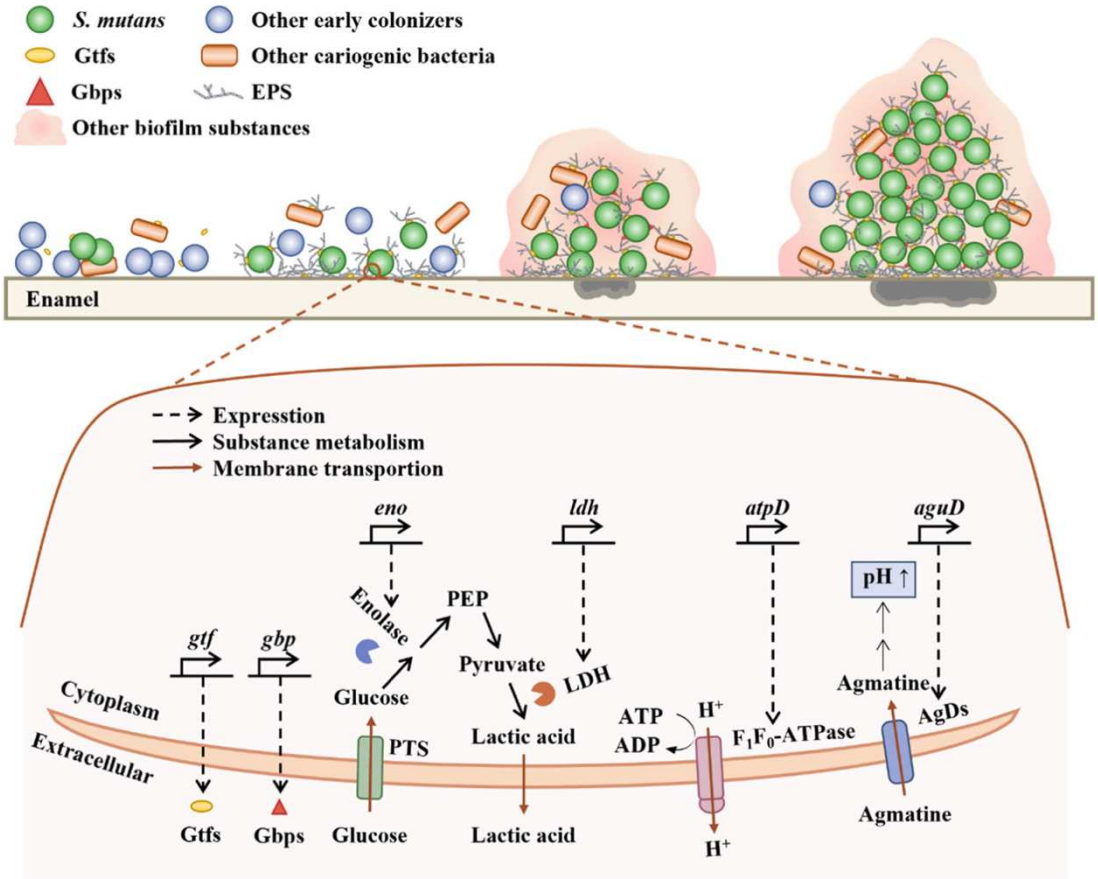


Figure 4: Mechanism of dental caries formation and virulence factors of *S. mutans*.

The first step in the formation of a cavity is the adhesion of *S. mutans*, facilitated by the formation of glucans by Glucan Binding Proteins (GBPs) encoded by the *gbp* genes. Subsequently, the formation of EPS by

glucosyltransferases (Gtf) creates a three-dimensional matrix that allows other bacteria to adhere and begin forming a biofilm. Finally, the ability of *S. mutans* to produce lactic acid from glucose enables the demineralisation of dental enamel. This metabolisation is facilitated by the presence of certain enzymes such as enolase, encoded by the *eno* gene, which converts glucose into phosphoenolpyruvate (PEP), and lactate dehydrogenase (LDH), encoded by *ldh*, which converts pyruvate into lactic acid. *S. mutans* manages to survive in an acidic environment thanks to an effective system, notably the *atpD* gene, which encodes for F1F0 ATPase that allows the expulsion of protons from the cell's cytoplasm, thereby increasing the pH and also generating energy for the bacterium. Additionally, the *aguD* gene encodes for the agmatine deiminase system (AgDS), which imports agmatine molecules that are subsequently converted into ammonium, further increasing the pH (Wang et al., 2019) (Luo et al., 2024).
Illustration from (Luo et al., 2024).

Interestingly, while *S. mutans* has long been considered the primary cause of dental caries, recent studies show a high prevalence of *S. mutans* in biofilms containing the fungal pathogen *Candida albicans*, indicating that interactions between these species may play a role in the development of cavities (Lu et al., 2023).

Candida albicans is one of the most abundant fungal species in the oral cavity (Lu et al., 2023b). Its ability to transition from a yeast form to a filamentous form is a key factor in its virulence, with this transformation occurring under specific conditions such as changes in temperature, pH, CO_2 concentration, serum presence, and malnutrition (Lopes & Lionakis, 2022). This species exhibits strong adhesion to tooth enamel and other bacteria within the biofilm through adhesins like *als1* and *als3* (Li et al., 2023). Additionally, *C. albicans* can produce acid from sugars like glucose or fructose even in highly acidic environments (down to pH 4), making it a highly cariogenic microorganism (Lu et al., 2023b).
C. albicans is strongly associated with early childhood caries (ECC), where it is frequently detected in large quantities in dental plaque and carious lesions, with its presence positively correlated with the severity of the lesions (Li et al., 2023). A close interaction is observed with *S. mutans*, a major cariogenic bacterium: *S. mutans* secretes the enzyme GtfB, which binds to the surface of *C. albicans*, leading to the synthesis of glucans on the fungal cells. This facilitates mutual adhesion and the establishment of a cohesive and highly virulent biofilm (Ellepola et al., 2019). Furthermore, the metabolic cross-feeding between the two microorganisms (e.g., *S. mutans* providing simple sugars, *C. albicans* enhancing glucide transport and catabolism in *S. mutans*) strengthens their

growth and virulence (Li et al., 2023). This interkingdom biofilm results in increased biomass, enhanced extracellular matrix production, and elevated resistance to environmental stresses, exacerbating the severity of carious lesions and complicating treatment (Lu et al., 2023).

Scanning electron microscope analysis of mixed-species biofilms grown on human teeth and hydroxyapatite confirmed the strong coadhesion between *C. albicans* and *S. mutans*, with *S. mutans* showing a strong affinity for the hyphae of *C. albicans* (Figure 5).

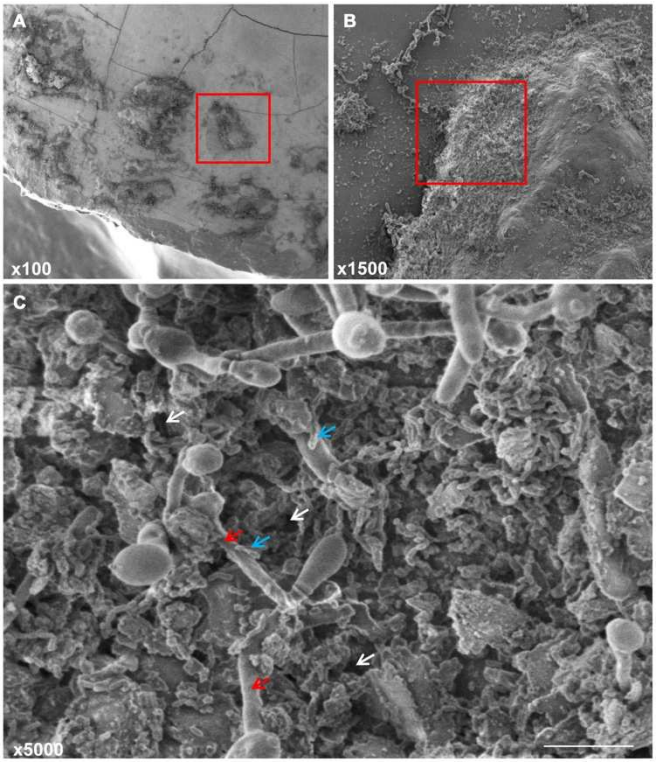


Figure 5: Photo showing the coadhesion between *C. Albicans* hyphae (red arrows) and *S. Mutans* cells (blue arrows) in a dental mature biofilm, viewed under an electron microscope. The white arrows indicate channels where a polymeric substance matrix flows to distribute nutrients and signalling molecules.
Image from (Metwalli et al., 2013).

2.1.3. Halitosis

Halitosis, commonly known as bad breath, is a widespread issue affecting between 22% and 60% of the global population (Anbari et al., 2019; Kumbargere Nagraj et al., 2019). It is primarily categorised into two types: extra-oral halitosis (EOH) and intra-oral halitosis (IOH). IOH is mainly caused by volatile compounds produced by anaerobic bacteria in the oral cavity, particularly volatile sulphur compounds (VSCs) such as hydrogen sulphide, dimethyl sulphide, dimethyl disulphide, and methyl mercaptan (Hampelska et al., 2020). The bacteria most frequently associated with halitosis include

Actinomyces spp., Bacteroides spp., Fusobacterium spp., and Porphyromonas spp., among others (Hampelska et al., 2020). Halitosis can be influenced by various factors such as periodontal diseases, dry mouth, smoking, alcohol consumption, dietary habits, diabetes, and obesity. It can also be exacerbated by stress, which increases hydrogen sulphide production by subgingival anaerobic bacteria (Wu et al., 2020). Treatments for halitosis often involve improved oral hygiene and targeted approaches against the responsible bacteria.

In recent years, to counteract these pathogenic bacteria and prevent dysbiosis, studies have focused on the mechanism and role that oral prebiotics could play, either as an alternative or a complement to conventional dental treatments.

3. Overview of prebiotics

3.1. Prebiotics, probiotics and synbiotics

It was in 1994 that the term "prebiotic" was mentioned for the first time, when Glenn R. Gibson and Marcel B. Roberfroid published a critical review on the modulation of the gut microbiota and the role these components could play. Prebiotics were defined as “non-digestible (by the host) food ingredients that have a beneficial effect through their selective metabolism in the intestinal tract”(Gibson & Roberfroid, 1995) . This review, like most research on prebiotics, focused on the gut microbiota, but in recent years, an increasing number of studies and research have focused on the effects of oral prebiotics to regulate the oral microbiota. More recently, Bindels et al. in 2015 defined prebiotic as "a non-digestible compound that, through its metabolisation by microorganisms in the intestine, modulates the composition and/or activity of the gut microbiota, thus conferring a beneficial physiological effect on the host". In 2017, the International Scientific Association of Probiotics and Prebiotics (ISAPP) consensus panel proposed the following definition of a prebiotic: a substrate that is selectively utilised by the host microorganisms, conferring a health benefit (Gibson et al., 2017a). When applied to the oral microbiome, a prebiotic should promote the growth of host’s bacteria associated with oral health and limit dysbiosis leading to conditions such as dental caries by increasing the pH, like urea or arginine, to eliminate cariogenic bacteria, (Natalia Molinaro García, 2024). They are mainly composed of carbohydrates, such as fructo-oligosaccharides (FOS) and galacto-

oligosaccharides (GOS), although non-carbohydrate substances, such as polyphenols and polyunsaturated fatty acids converted into conjugated fatty acids, have also been reported. These prebiotics can provide benefits for oral health by promoting the selective growth of probiotic bacteria beneficial to the oral microbiome (Santacroce et al., 2023).

Probiotics, discovered in 1908 by Élie Metchnikoff, (Mercenier et al., 2003). have been defined by ISAPP as "live microorganisms that, when administered in adequate amounts, confer a health benefit on the host." They are therefore not metabolites like prebiotics, but rather live bacteria directly ingested by the host to restore eubiosis. They are most used for intestinal dysbiosis, but since Meurman's discovery showing that *Lacticaseibacillus rhamnosus* GG ATCC 53103 could colonise the oral cavity, the use of probiotics in dentistry has been continuously evolving. Research has shown that probiotics have a remarkable ability to prevent dental caries, particularly *Lactobacillus* species (notably *Lacticaseibacillus paracasei* and *Lactiplantibacillus plantarum*) and *Bifidobacterium* DN-173010 (found in certain yoghurts), which effectively inhibit *S. mutans* (Luo et al., 2024). They are also beneficial in other oral diseases such as chronic periodontitis, halitosis, or oral mucositis (Saïz et al., 2021). Probiotics exert their effects through various mechanisms, as illustrated in Figure 6, which explains their efficacy.

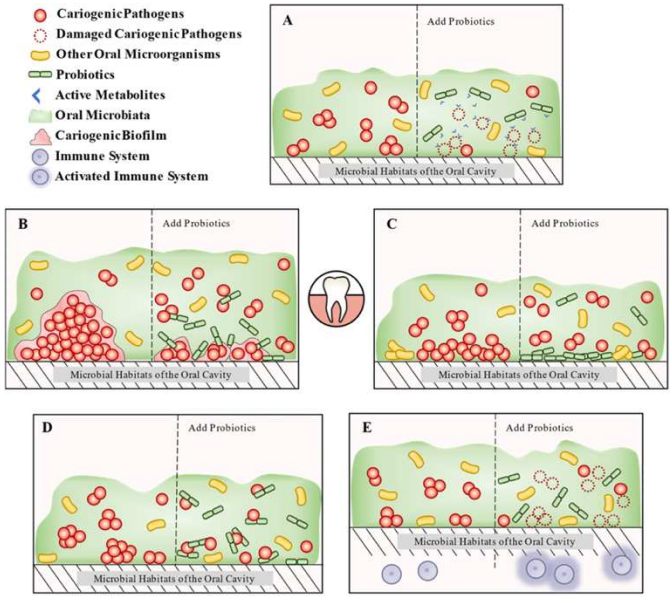


Figure 6: Mechanism of probiotics to prevent dental caries. It is roughly divided into five parts. (Luo et al., 2024)

A: Probiotics produce bioactive substances (like bacteriocins, enzymes, biosurfactants, organic acids, or hydrogen peroxide) that have antimicrobial properties and help suppress cariogenic bacteria.
B: They can disrupt or eliminate biofilms formed by cariogenic microbes in the mouth.
C: By adhering to surfaces in the oral cavity, probiotics prevent harmful bacteria from attaching and colonising.
D: They bind with pathogens, reducing their ability to settle and grow in the mouth.
E: Probiotics influence the host's immune system, boosting its response against cariogenic bacteria.
Illustration from (Luo et al., 2024).

Probiotics can be supplemented with prebiotics to enhance their effectiveness, a combination known as synbiotics. In 2020, the ISAPP defined them as "a mixture comprising live microorganisms and substrate(s) selectively utilised by host microorganisms that confers a health benefit on the host."
Although synbiotics are less well known than probiotics, as they were introduced and studied later, researchers have demonstrated their effectiveness. For example, Nunpan et al. showed that a synbiotic composed of *L. acidophilus* combined with GOS and FOS can significantly inhibit *S. mutans* (Nunpan et al., 2019).

3.2. Criteria for a substance to be considered prebiotic

According to the definition of prebiotics established by ISAPP, a prebiotic is a substrate that is selectively utilised by the host microorganisms, conferring a health benefit. It is not a complete diet but a specific ingredient that must be sufficiently studied and described to enable robust data comparisons and reproducible manufacturing. One of the fundamental criteria of a prebiotic is its selective utilisation by the host's microbiota, which can be demonstrated by a single microbial change, a modification of multiple taxa, or specific functional readouts. To be recognised as such, a prebiotic must also provide a demonstrated health benefit and must be validated through well-controlled studies, typically randomised controlled trials (RCTs) conducted on the target population (Gibson et al., 2017).
A mechanism of action explaining how microbiota modulation by prebiotics can lead to a health benefit must be proposed, based on the observed pattern of selective utilisation. However, it is not always necessary to establish a direct causal link between this selective utilisation and the observed benefit, although research in this direction is encouraged, particularly through causal mediation analysis strategies. In vitro studies and animal experiments on non-target hosts can be useful for exploring underlying mechanisms and

guiding clinical trials in the target host, but they alone are insufficient to prove the beneficial effects of a prebiotic on human health (Hutkins et al., 2025).

Another crucial aspect concerns the safety of prebiotic use, which must be rigorously assessed by monitoring potential adverse effects in studies conducted on the target host. Safety requirements vary according to regulatory categories and target populations. Furthermore, multiple confirmatory studies are needed to ensure the reproducibility of health effects and selective utilisation by the microbiota, thereby strengthening the reliability of the results obtained. Finally, a prebiotic must be administered in a sufficient quantity to guarantee its effectiveness while avoiding potential side effects such as gastrointestinal disturbances, toxicity, or choking risks. Precise recommendations on the dose or serving size must be provided to ensure optimal health benefits (Hutkins et al., 2025).

4. Examples of common prebiotics, their sources and mechanisms of actions

4.1. D-tagatose

Sugar is known to be the main component leading to dental caries, as it is metabolised by cariogenic bacteria into acid. However, some sugars do not have this effect and are even classified as prebiotics. D-tagatose, for instance, is a non-cariogenic six carbon ($C_6H_{12}O_6$, Fig. 7) sugar that provides fewer calories and has a lower glycemic index than fructose (Ortiz et al., 2024). It is an epimer of D-fructose and a "rare" sugar because is present in small quantities in nature (Smith et al., 2022). However, it can be obtained from the gum of plants such as *Sterculia setigera* or the lichen *Roccella (Roy et al., 2018)*. Additionally, it is found in fruits like apples and oranges, and it can also be industrially produced through enzymatic hydrolysis of lactose (Ortiz et al., 2024). A study by Mayumi et al. (Mayumi et al., 2021) revealed that the saliva of individuals with no dental biofilm and good oral hygiene contained D-tagatose. This study also demonstrated that D-tagatose inhibits pathogenic bacteria such as *S. mutans*, while

having little effect on the growth of commensal bacteria like *S. oralis* (Mayumi et al., 2021). Certain chewing gums containing D-tagatose may be effective in inhibiting dental biofilm (by preventing bacterial adhesion to dental surfaces and modifying the expression of certain genes related to biofilm formation) and reducing the risk of dental caries (notably by being less metabolised into acid compared to other sugars, which increases pH and inhibits cariogenic bacteria) (Nagamine et al., 2020).

Finally, Di Tinco et al. (Di Tinco et al., 2021) assessed the use of D-tagatose as a cleaning powder for the decontamination of titanium surfaces, like implant. D-tagatose reduced Pseudomonas aeruginosa biofilms, although its effectiveness was lower than that of glycine. The study concluded that D-tagatose could be an effective cleaning agent without altering the titanium surface or compromising cell viability.

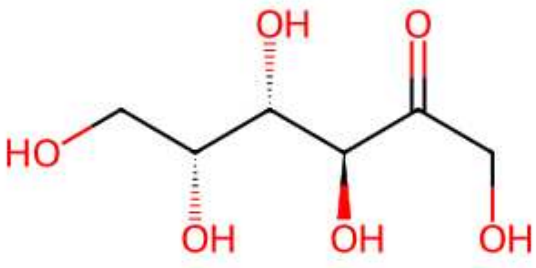


Figure 7: Chemical structure of D-tagatose

D-tagatose inhibits the growth of biofilms and pathogenic bacteria through several mechanisms, including antimicrobial selectivity via catabolism. Indeed, D-tagatose is not readily metabolised by pathogenic bacteria such as *Streptococcus mutans*. These bacteria lack the metabolic pathways required to effectively break down D-tagatose, limiting their ability to utilise it as an energy source (Hasibul et al., 2017).

The study by Mayumi et al. (Mayumi et al., 2021) showed that D-tagatose interferes with the glycolysis of pathogen bacteria like *S. mutans*, a process essential to produce energy and organic acids in bacteria. It reduces the levels of certain pyruvate-derived amino acids, such as branched-chain amino acids and alanine, in *S. mutans* and *S. gordonii*, but not in *S. oralis*. This indicates that D-tagatose disrupts glycolysis and downstream metabolic pathways, which are crucial for bacterial growth and survival. D-tagatose is taken up by *S. mutans* and *S. gordonii* via the fructose-specific phosphotransferase system (PTS), which converts it into D-tagatose-6-phosphate. The latter can accumulate and induce the expression of fructose-specific PTS genes, causing phosphorylated sugar stress that is harmful to the bacteria. Furthermore, D-tagatose reduces the viability of *S. mutans*

and *S. gordonii* in a dose-dependent manner, with a limited effect on *S. oralis*, and inhibits biofilm formation by disrupting the production of extracellular polysaccharides (EPS) and other biofilm matrix components.

The bacterial composition of the biofilm is also altered, making it heterogeneous, less cohesive, and more granular (as EPS production is disrupted). These characteristics facilitate removal through brushing with a toothbrush (Roy et al., 2018).

Mayumi et al. (Mayumi et al., 2021) explored in 2021 the effects of D-tagatose on three species of oral streptococci: *Streptococcus mutans, Streptococcus gordonii, and Streptococcus oralis*. The in-vitro studies conducted in this research highlighted several crucial aspects of the interactions between D-tagatose and these bacteria. Firstly, the researchers analysed and compared the planktonic growth of the bacteria (Fig. 8), using optical density at 600 nm, in the presence of D-tagatose and glucose in a static aerobic environment at 37°C in brain heart infusion (BHI) broth in a chemically defined medium (CDM). They observed that D-tagatose extended the growth lag phase of *S. gordonii* and *S. mutans* at concentrations of 0.5% or higher, while *S. oralis* was only affected at higher concentrations (5% and 10%). Additionally, D-tagatose was not used as an energy source by these bacteria, but it inhibited their growth in the presence of glucose, suggesting a specific inhibitory effect (Mayumi et al., 2021).

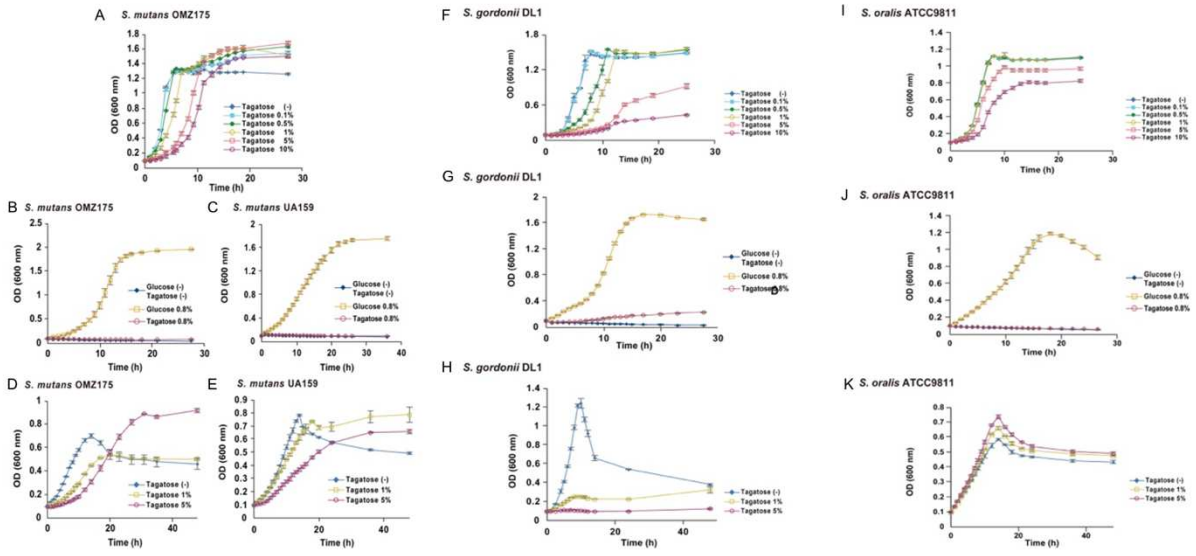


Figure 8: Growth of bacteria *S. mutans, S. gordonii*, and *S. oralis* under different conditions.

(A, F, I) Growth of the three bacteria in BHI medium containing various concentrations of D-tagatose; growth is inhibited in a dose-dependent manner by D-tagatose, with an extended lag phase and reduced maximum growth at higher concentrations (5% and 10%) for *S. mutans* and *S. gordonii*. In contrast, the

growth of *S. oralis* is less affected by D-tagatose, with notable inhibition only at higher concentrations (5% and 10%).

(B, C, G, J) Growth of two strains of *S. mutans, S. gordonii*, and *S. oralis* in CDM medium without sugar, with 0.8% D-glucose, or with 0.8% D-tagatose; bacteria do not grow in the presence of D-tagatose alone (0.8%) but grow in the presence of glucose (0.8%).

(D, E, H, K) Growth of two strains of *S. mutans*, *S. gordonii*, and *S. oralis* in CDM medium containing 0.2% G-glucose with 0%, 1%, or 5% D-tagatose; D-tagatose inhibits growth in the presence of glucose (0.2%) in a dose-dependent manner for *S. mutans* and *S. gordonii*. For *S. oralis*, growth is less affected by D-tagatose in the presence of glucose, with limited inhibition even at higher concentrations.

Image from (Mayumi et al., 2021)

Biofilm formation assays were measured using confocal imaging on CDM media containing glucose, sucrose, or D-tagatose as the sole carbon source. The results showed that *S. gordonii* and *S. mutans* formed negligible biofilms in the presence of D-tagatose, whereas *S. oralis* formed significant biofilms, suggesting a potential utilisation of D-tagatose by this strain (Figure 9) (Mayumi et al., 2021).

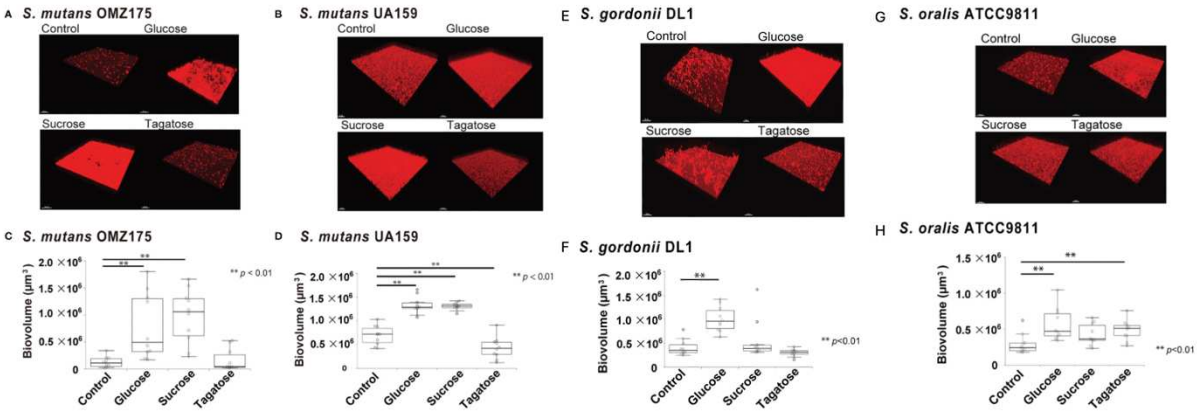


Figure 9: Effects of various sugars (glucose, sucrose, and D-tagatose) on biofilm formation.

(A, B, E, G) Representative confocal laser scanning microscope (CLSM) images showing the typical architecture of biofilms after reconstruction with Imaris software. *S. mutans, S. gordonii*, and *S. oralis* cells were stained with hexidium iodide (red) and incubated with saccharides or without any addition. The results show that biofilm formation is inhibited for *S. mutans* and *S. gordonii* in the presence of D-tagatose, but with glucose, the biofilm develops strongly. In the presence of sucrose, *S. mutans* develops a very dense biofilm, but not *S. gordonii*, where cells are fragile and detached. In contrast, biofilm formation is similar for *S. oralis* in the presence of the different sugars.

(C, D, F, H) Biovolume analysis of *S. mutans, S. gordonii*, and *S. oralis*. Ten fields per sample were randomly recorded with CLSM, and the biovolume of the bacteria was quantified using IMARIS software.

Image from (Mayumi et al., 2021).

These in-vitro results, among others, suggest that D-tagatose could be used as a prebiotic to modulate the composition and metabolism of oral biofilms, selectively inhibiting pathogens while promoting beneficial commensals.

A clinic study by Nagamine et al. in 2020 (Nagamine et al., 2020)aimed to evaluate the effect of D-Tagatose on oral bacteria, particularly *S. mutans*. It included an in vitro phase and a randomised, double-blind clinical trial. In the in vitro study, saliva from 10 healthy volunteers was cultured on specific media: BHI (for total bacteria) and MSB (for *S. mutans)*. These media contained either D-Tagatose, xylitol, or a combination of the two, and cultures were grown under both aerobic and anaerobic conditions. The results showed that D-Tagatose led to a complete and significant reduction in oral bacteria, even surpassing the effect of xylitol, known for its anti-cariogenic properties. Concurrently, the clinical trial involved 19 volunteers divided into three groups, who chewed gum containing D-Tagatose, xylitol, or both for 4 weeks. Each week, saliva samples were analysed to measure changes in the bacterial population. The results revealed that neither D-Tagatose alone nor xylitol alone significantly reduced *S. mutans*, but their combination led to a marked decrease in this bacterium in the participants' saliva. Additionally, no notable side effects were observed, particularly in terms of digestive issues or weight changes. The study suggests that D-Tagatose and xylitol could be promising agents for the prevention of caries and other oral diseases due to their impact on bacterial growth and biofilm formation.

4.2. Xylitol and 2'-fucosyllactose

Xylitol is one of the sugar alcohols, along with sorbitol, maltitol, erythritol and others. It is a five-carbon polyol ($C_5H_{12}O_5$, Figure 10), commonly used as a sweetener in sugar-free confectionery, produced artificially from vegetable materials rich in xylene, such as beech wood and birch (Ortiz-Sáez et al., 2024). It is also naturally found in certain fruits and vegetables (plums, strawberries, cauliflower, and pumpkin. It has a sweetness equivalent to that of sucrose and a similar sweetness-time intensity (Luo et al., 2024). It is considered a prebiotic for oral health as it enhances remineralisation, increases salivation, thereby inhibiting *S. mutans*, and reducing the incidence of dental caries.

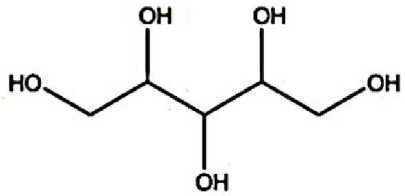


Figure 10: Chemical structure of xylitol

Indeed, xylitol is transported into the cell and is converted into xylitol-5-phosphate by the fructose-phosphoenolpyruvate phosphotransferase system of *S. mutans*. This leads to the formation of intracellular vacuoles and the degradation of the cell membrane. The xylitol-5-phosphate is then dephosphorylated and expelled from the cell. Since xylitol cannot be metabolised by *S. mutans* to produce energy, the cycle of transport, phosphorylation, and dephosphorylation results in a net energy expenditure without any gain. This leads to the depletion of the bacterium's energy resources and, consequently, to cell death (Jayadevan et al., 2019).

This molecule is considered prebiotic as it promotes the growth of non-pathogenic bacteria, in this case, non-cariogenic bacteria such as *S. sanguinis* or *S. mitis* (Bahador et al., 2012). The functioning of xylitol metabolism is not well studied, and further research would be necessary to understand it. However, other studies (Xiang et al., 2021) focused on the intestinal microbiome have shown that certain bacteria, such as *Bacteroides* and *Lachnospiraceae*, possess a system for metabolising xylitol. Xylitol is converted into D-xylulose by xylitol dehydrogenase, then into D-xylulose-5-phosphate by xylokinase, before being converted into D-ribulose-5-phosphate by xylulose phosphate isomerase. This ribulose can then enter the pentose phosphate pathway and, subsequently, produce energy. Non-cariogenic bacteria may therefore possess this mechanism, which is not present in cariogenic bacteria.

Other sugars are considered prebiotics, such as 2′-fucosyllactose (2′-FL), which is a human milk oligosaccharide (HMO), meaning a complex carbohydrate naturally present in human milk (Salli et al., 2020). It is a trisaccharide composed of fucose, galactose, and glucose. 2′-FL is one of the most abundant HMOs in human milk, with an average concentration of approximately 2.7 g/L (Bode, 2012).

To demonstrate the prebiotic properties of xylitol, particularly its ability to inhibit the formation of *S. mutans* biofilm, Loimaranta et al. in 2020 conducted several in-vitro tests

using an innovative approach with the xCELLigence Real Time Cell Analyzer (RTCA) instrument. This system measures the electrical impedance generated by bacteria adhering to the surface of a plate equipped with electrodes, allowing continuous monitoring of biofilm formation without labelling or disturbing the system. The biofilms were cultivated in a medium containing 1% sucrose to promote the production of extracellular polysaccharides (EPS), key elements of the biofilm matrix, and different concentrations of xylitol (1%, 2%, 5%) were tested and added after two hours of initial adhesion. The results showed a dose-dependent inhibition of biofilm formation by xylitol on the first strain NCTC 10449, with a more pronounced effect during the early hours (between 3h and 10h) and inhibition starting from 5% xylitol for the clinically isolated (CI) strain 2366 (Figure 11) (Loimaranta et al., 2020).

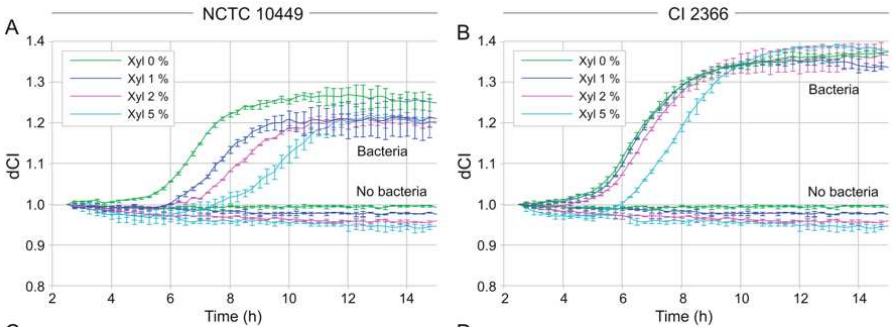


Figure 11: Real-time biofilm formation of (a) *S. mutans NCTC 10449* and (b) *S. mutans CI 2366* in BHI-sucrose medium or medium supplemented with 1, 2% or 5% xylitol.
Image from (Loimaranta et al., 2020).

Xylitol disrupts the formation of *S. mutans* biofilm by reducing bacterial adhesion and the production of polysaccharides necessary for the biofilm matrix. However, this disruption does not result in mere passive inhibition. In fact, the bacterium responds to this stress by activating an adaptive response. Gene expression analysis (Figure 12) shows that after 7 hours, there is no notable difference in the expression of genes responsible for the production of glucan-binding proteins (gbpB) or glucosyltransferases (gtfB, gtfC, gtfD), enzymes essential to produce the biofilm matrix, between media containing xylitol and those without it. However, after 10 hours, biofilms exposed to xylitol exhibit a strong increase in the expression of these genes (Loimaranta et al., 2020). This phenomenon indicates that *S. mutans*, after experiencing the inhibitory effect of xylitol in the early hours, attempts to compensate for this disruption by overactivating the mechanisms that

normally allow it to build a stable and functional biofilm. This type of adaptive response is typical of bacteria facing hostile conditions, as they alter their genetic expression to try to adhere, strengthen their matrix, and ensure their survival (Decker et al., 2014). However, despite this attempt at compensation, the quality and cohesion of the formed matrix remain impaired, indicating that the overall effect of xylitol goes beyond merely inhibiting bacterial growth and profoundly affects *S. mutans*' ability to structure a robust biofilm.

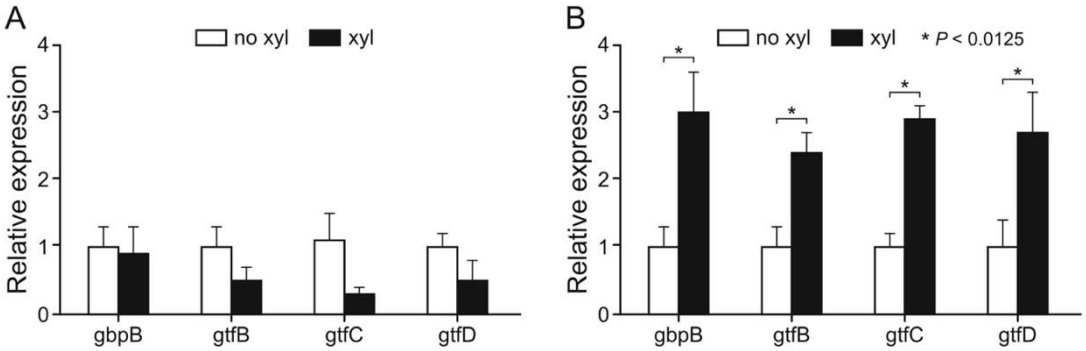


Figure 12: Expression of genes involved in glucose mediated adhesion and extra cellular polysaccharide formation in *S. mutans* 2366 biofilms at two different time points. A: 7 h, B: 10 h
Image from (Loimaranta et al., 2020).

Another recent study (2024) by Breban-Schwarzkopf et al. evaluated the cytotoxic effects of sodium fluoride (NaF) and xylitol (Xyl), both individually and in combination, on human keratinocytes (HaCaT) and osteosarcoma cells (SAOS-2). NaF, widely used in dental products to prevent caries, exhibits dose-dependent toxicity on healthy HaCaT cells (figure 13A), reducing their viability and triggering apoptosis through the activation of caspases 3, 7, and 9, nuclear condensation, and actin reorganisation. It also increases the expression of pro-apoptotic genes Bad and Bax, while decreasing that of the anti-apoptotic Bcl-2. In contrast, on tumour cells SAOS-2 (figure 13B), NaF paradoxically stimulates proliferation at high doses, with a moderate apoptotic effect. Xylitol acts in a biphasic manner: at low doses, it stimulates the viability and proliferation of HaCaT cells (figure 13A), whereas at high doses, it induces marked cytotoxicity, with morphological alterations and activation of apoptosis, particularly in tumour cells (figure 13B). When combining NaF and Xyl, the study observes partial protection by Xyl against NaF toxicity in healthy cells, while maintaining inhibition of tumour proliferation. This study suggests that the NaF-Xyl combination could offer an interesting compromise between the protection of healthy cells, caries prevention, and potential anti-tumour activity (Breban-Schwarzkopf et al., 2024).

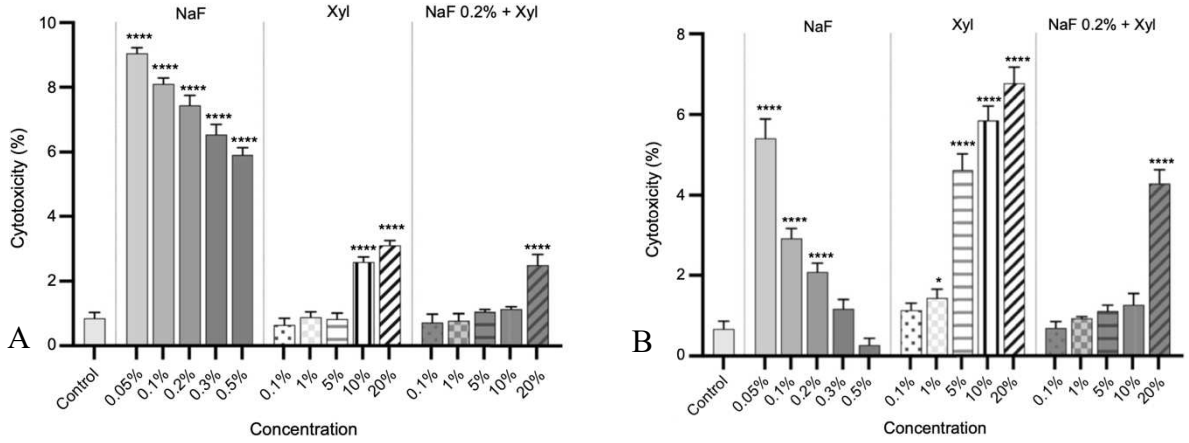


Figure 13: Evaluation of the cytotoxicity of NaF, Xyl, and their combination on (A) healthy HaCaT cells and (B) tumour SAOS-2 cells.

Image from (Breban-Schwarzkopf et al., 2024).

An in-vitro study by Salli et al. (Salli et al., 2020) specifically examined the impact of 2′-FL and xylitol on *Streptococcus mutans* and showed that it is not a fermentable carbon source for *S. mutans* and inhibits its adhesion to hydroxyapatite.

Growth tests (Figure 14) were measured in Tryptic Soy Broth (TSB) medium at 37°C with optical density monitoring at 600 nm every 30 minutes for 24 hours. The results show that 2'-FL cannot be used as a carbon source by any of the tested strains, unlike glucose, lactose, and GOS, which support their growth. Xylitol, on the other hand, significantly inhibits the growth of *S. mutans*. Regarding adhesion (Figure 15), it was measured after gentle agitation for 60 minutes of strains labelled with 35S-methionine on hydroxyapatite powder coated with parotid saliva. 2'-FL reduces the adhesion of the clinical strain CI 2366 to saliva-coated hydroxyapatite, potentially interfering with the formation of the extracellular matrix. Xylitol, however, has no notable effect on adhesion under these conditions. Overall, 2'-FL appears to be of interest in limiting the development and adhesion of certain *S. mutans* strains, while xylitol primarily acts by inhibiting their growth.

These properties could help reduce the risk of dental caries in infants, particularly when 2′-FL is included in infant formulas (Salli et al., 2020).

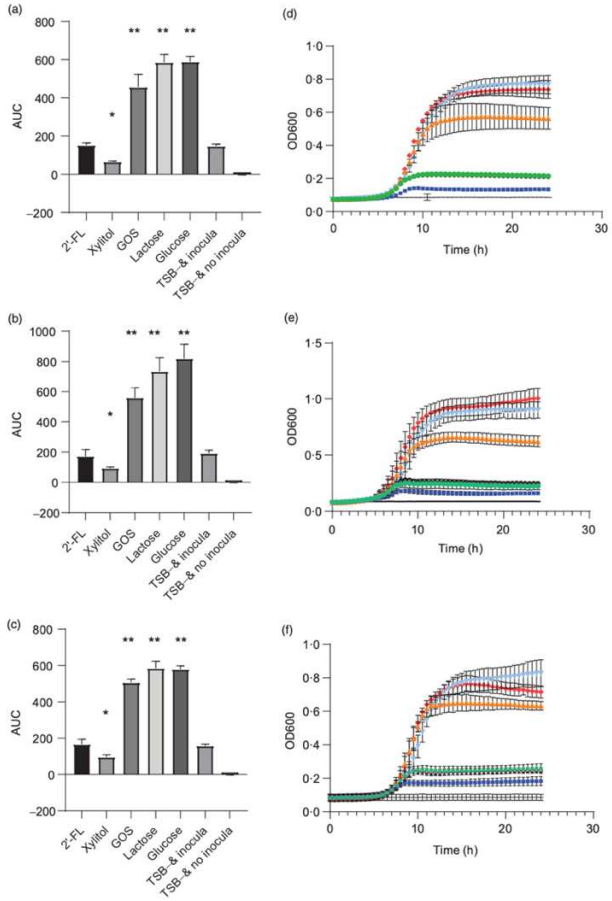


Figure 14: Graphs show the area under the growth curve (AUC) (a–c) and optical density (OD) growth curves (e and f) for *Streptococcus mutans* strains DSM 20523 (a, d), CI 2366 (b, e), and Ingbritt (c, f). The bacteria were cultured in modified Tryptic Soy Broth (TSB−) lacking glucose and supplemented with 1% of different carbohydrates: 2′-fucosyllactose (2-FL), xylitol, galacto-oligosaccharides (GOS), lactose, or glucose. Controls included TSB− with and without inoculum.
Images from (Salli et al., 2020).

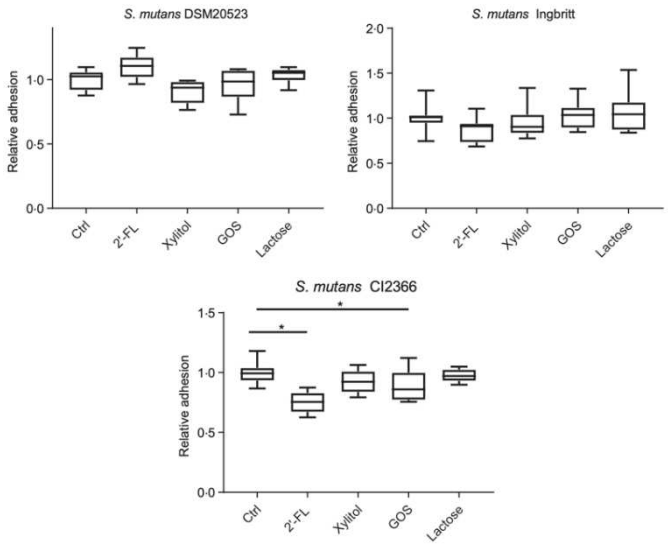


Figure 15: Relative adhesion to parotid saliva-coated hydroxyapatite (HA) for three *Streptococcus mutans* strains (DSM 20523, Ingbritt and CI 2366) with 1 % (2 -FL), xylitol, galacto-oligosaccharides (GOS), lactose and phosphate buffer as a control (Ctrl). Bacterial adhesion to HA was determined by scintillation count. Images from (Salli et al., 2020).

4.3. Arginine

Another molecule widely studied for its prebiotic properties is arginine, more specifically L-arginine. It is an essential amino acid, with the formula $C_6H_{14}N_4O_2$ (Figure 16), found in dietary proteins and added to toothpastes, but also produced by the human body through protein turnover (Nascimento, 2018).

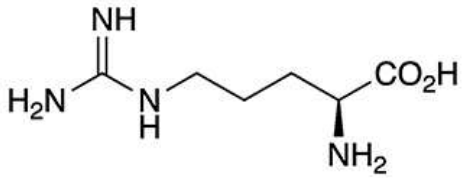


Figure 16: Chemical structure of L-arginine

L-arginine is beneficial for oral health as it inhibits the growth of *Candida* spp. and raises the pH of the oral cavity by alkali production, thereby reducing the demineralisation of tooth enamel and, consequently, cavities. Indeed, L-arginine enhances the ability of certain bacteria (*Streptococcus sanguinis, Streptococcus parasanguinis, and Streptococcus gordonii*) (Bijle et al., 2020) to produce alkaline substances like ammonia. Arginolytic bacteria first metabolise arginine into citrulline and ammonia via arginine deiminase (AD); the citrulline produced is then converted by catabolic ornithine transcarbamylase (cOTC) into ornithine and carbamyl phosphate in the presence of inorganic phosphate. Finally, carbamate kinase (CK) cleaves carbamyl phosphate into

ammonia and CO_2, while transferring a phosphate to ADP to produce ATP(Nüse et al., 2023). This net reaction, illustrated in Figure 17, thus produces two molecules of ammonia, which are basic, cross the cell membrane, and increase the pH of the biofilm, reducing the risk of dental caries (Goyal et al., 2023).

Ammonia neutralizes acids and restores the pH of the biofilm, creating a less favorable environment for cariogenic bacteria such as *S. mutans* while promoting the growth of arginolytic commensal bacteria such as *S. Sanguinis, gordonii*) (El Harram & Sqalli, 2024).

To benefit from the prebiotic properties of arginine, this molecule is added to certain toothpastes, coupled with fluoride (Kuriki et al., 2024).

Fluoride has been used for many years to combat dental caries. Indeed, with its properties, it prevents the formation of this pathology through three mechanisms: it inhibits the demineralisation of enamel, promotes its remineralisation by rapidly adsorbing onto partially demineralised enamel crystals and attracting supersaturated calcium and phosphate ions, favouring remineralisation preferentially in the form of fluorapatite (which is stronger) (Cate & Buzalaf, 2019), and it inhibits cariogenic bacteria such as *S. mutans* by interfering with their enzymatic activity and causing cell lysis (Veneri et al., 2024).

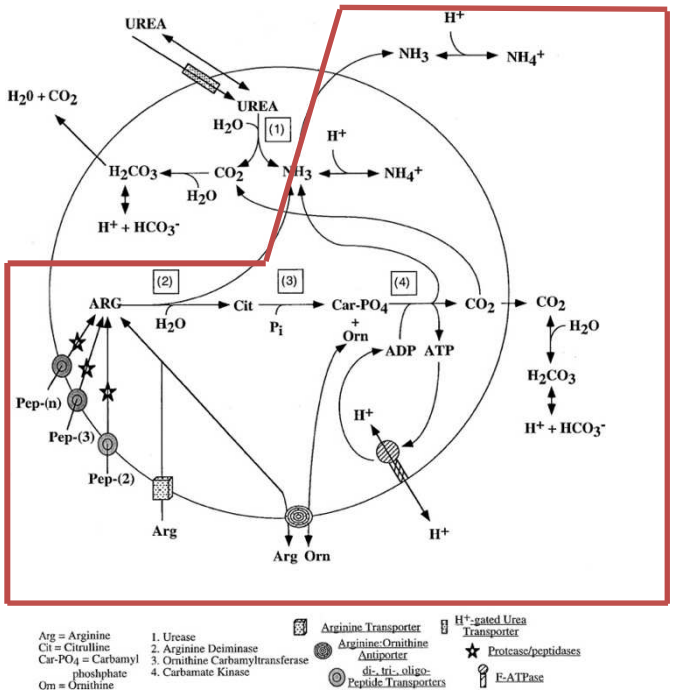


Figure 17: Diagram representing the metabolism of arginine by an arginolytic bacteria such as *S. sanguinis* and *S. gordonii*

Image reproduced from (Burne & Marquis, 2000) with permission from Oxford University Press.

The study by Tada et al. in 2016 (Tada et al., 2016) explores the potential of L-arginine acid (pH 3.5) to clean oral biofilms (dental plaque), a complex structure composed of bacteria and salivary proteins. This study focuses on a different property than inhibiting biofilm formation: the ability of L-arginine acid to destabilise and remove already formed biofilms. The researchers cultivated oral biofilms using human saliva mixed with a nutrient medium (BHI) and then washed them with a saline solution, a citrate solution (pH 3.5), or an L-arginine acid solution (0.5 M, pH 3.5). The cleaning efficacy was measured by crystal violet staining (figure 18a) and scanning electron microscopy (figure 18b). The results showed that L-arginine acid removes oral biofilms much more effectively than saline solution or simple citrate. Unlike citrate, which primarily targets streptococci, L-arginine acts more broadly, detaching a wide variety of bacteria from the biofilm surface. This capability of L-arginine acid could be explained by its effect on protein-protein interactions, reducing biofilm cohesion without having significant bactericidal activity. This property is particularly interesting for oral health, as it could effectively reduce dental plaque without excessively disrupting the natural balance of the oral microbiota. However, the use of acidic solutions in the mouth could pose a risk to dental enamel, an aspect that will need to be evaluated in future clinical studies. In conclusion, this study proposes L-arginine acid as a potential supplement to conventional mouthwashes, capable of destabilising mature oral biofilms and facilitating their removal during daily hygiene care, while respecting the beneficial microbial diversity of the oral cavity (Tada et al., 2016).

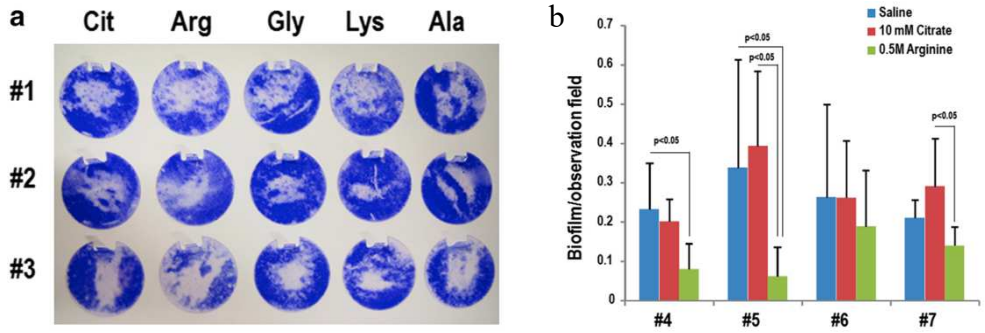


Figure 18: (a) Effect of L-arginine washing on salivary biofilm formed on plastic discs, measured by crystal violet staining; (b) Quantification of biofilm after washing with saline solution, citrate, and arginine on saliva samples from different individuals (4 to 7), measured by scanning electron microscopy and Photoshop CS6 colour detection software.

Images from (Tada et al., 2016).

Another study by Zheng et al. (Zheng et al., 2017) examined the impact of arginine on the oral microbiota and its potential in preventing dental caries. The study involved 21 caries-free individuals (CF) and 21 individuals with active caries (CA), who used a toothpaste containing 8% arginine for two weeks. Saliva and dental plaque samples were collected before and after treatment for analysis. The results showed that arginine treatment altered the microbial composition by reducing the cariogenic *S. mutans* and increasing the beneficial *S. sanguinis* in plaque and saliva samples from CA individuals. The *S. mutans/S. sanguinis* ratio was significantly reduced after treatment, indicating a healthier microbial balance. Additionally, the activity of arginine deiminase-system (ADS) and urease enzymes increased, while lactate dehydrogenase (LDH) activity decreased (Figure 19), suggesting increased alkali production and reduced acidogenicity. The combination of arginine and fluoride showed a synergistic effect, further reducing biofilm-induced enamel demineralisation compared to arginine or fluoride alone. These results suggest that arginine could be a promising agent for the ecological management of caries by modulating the oral microbiota, and its combined use with fluoride could offer an effective strategy for caries prevention by leveraging both ecological and remineralising effects.

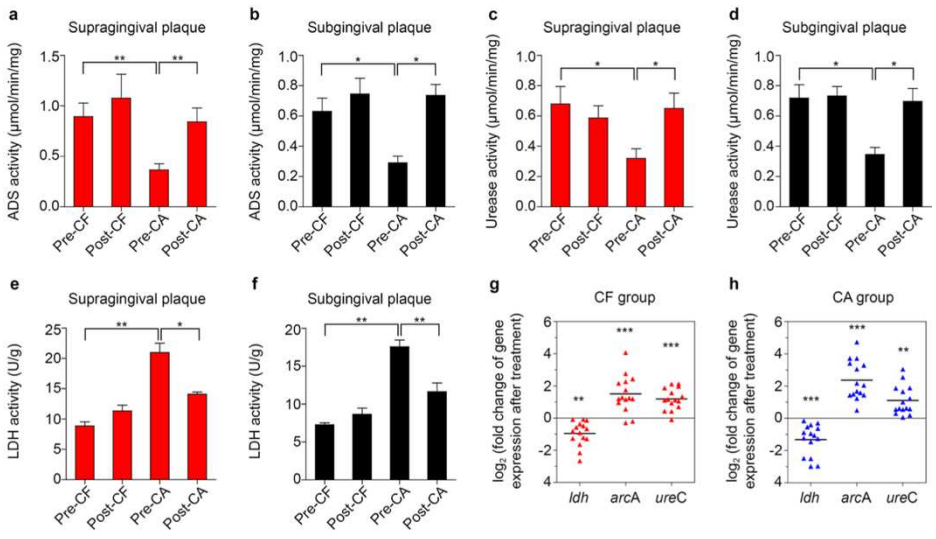


Figure 19: Graph comparing the enzymatic activities of ADS (a,b), urease (c,d), and LDH (e,f) in bacterial plaque samples from healthy adults (CF) and individuals with active caries (CA), before and after arginine treatment (Pre/post).

(g,h) Comparison of the expression levels of the genes ldh, arcA (arginine deiminase in ADS), and ureC (α-subunit of urease) in supragingival plaque samples after arginine treatment for the CF and CA groups; Consistent with the observed changes in the enzymatic activities of supragingival plaque samples, arcA and ureC transcripts within the supragingival microbiome were upregulated, while ldh was suppressed.

Image from (Zheng et al., 2017)

The clinic study by Souza et al. in 2013 in Brazil compared the efficacy of a toothpaste containing 1.5% arginine and 1450 ppm fluoride with that of a toothpaste containing only 1450 ppm fluoride in managing primary root caries in adults. The primary objective was to determine if the addition of arginine to fluoride could enhance the arrest and reversal of root caries lesions. 284 eligible patients were divided into two groups: an experimental group using the arginine toothpaste and a control group using the fluoride-only toothpaste. Participants were instructed to brush their teeth at least twice a day with the assigned toothpaste, and lesions were examined at the start of the study, at 3 months, and at 6 months. The results showed that after 6 months of use, 70.5% of root caries lesions improved in the group using the arginine toothpaste, compared to 58.1% in the control group, with a statistically significant difference ($p = 0.038$). Arginine, by neutralising plaque acids and stabilising the plaque biofilm, and the insoluble calcium compound, acting as a reservoir of calcium ions, contributed to improved remineralisation of the lesions. In conclusion, the study proved that the use of a toothpaste containing 1.5% arginine, an insoluble calcium compound, and 1450 ppm fluoride significantly improves the remineralisation of root caries lesions compared to a toothpaste containing only fluoride, suggesting that arginine can complement and enhance the beneficial effects of fluoride in caries prevention (Souza et al., 2013).

Another study by Nascimento et al. (Nascimento et al., 2019), published in the Journal of Dental Research in 2019, examines how the use of toothpastes containing arginine (1.5%) or fluoride (1,100 ppm F/NaF) influences the metabolic profile of supragingival dental plaque in adults with varying caries status. The study, conducted over 12 weeks with 83 participants, revealed that arginine significantly increases the activity of arginine deiminase (ADS), a metabolic pathway that produces ammonia, thereby helping to maintain a higher pH in the plaque and neutralising the acids produced by bacteria. In contrast, fluoride reduces lactate production from endogenous sources, thus decreasing plaque acidity. Metabolomic analyses showed that arginine affects the concentrations of 16 metabolites, including phenethylamine, agmatine, and glucosamine-6-phosphate, while fluoride influences 9, such as phenethylamine and N-methyl-glutamate. These results suggest that arginine and fluoride act through distinct but potentially complementary mechanisms to prevent caries: arginine promotes pH homeostasis, while fluoride enhances the resistance of dental minerals and reduces acid production. The study

underscores the importance of understanding the biochemical effects of dental treatments to develop more effective and targeted strategies for caries prevention.

4.4. Nitrate

Nitrate (NO_3^-) is an ion present in fruits, leafy green vegetables (such as lettuce, cabbage, etc.), and primarily beetroot, but it is also added to some processed meats for preservation (Shannon et al., 2021). It is considered a prebiotic because it provides numerous benefits to the oral microbiome; indeed, through its antimicrobial capabilities and alkaline power, it helps combat dental caries, periodontal diseases, and halitosis (Moran et al., 2024). After ingestion, nitrate is concentrated in saliva by the salivary glands, reaching high concentrations (5-8 mM) (Rosier et al., 2020), where it is reduced to nitrite (NO_2^-) by bacteria such as *Neisseria*, *Haemophilus*, *Aggregatibacter*, *Veillonella*, *Prevotella* and *Rothia* (Morou-Bermúdez et al., 2022). There are then two possible mechanisms for the metabolisation of nitrite: denitrification and dissimilatory nitrate reduction to ammonium (DNRA) (Morou-Bermúdez et al., 2022). Denitrification, carried out by bacteria such as *Neisseria, Haemophilus,* and *Aggregatibacter*, consists of four steps: nitrite is first converted by nitrite reductase into nitric oxide (NO), a molecule known for its antimicrobial and vasodilatory properties (Lima et al., 2024); it is then metabolised into nitrous oxide (N_2O) by nitric oxide reductase; finally, a molecule of dinitrogen (N_2) is produced. The second metabolic pathway is DNRA, carried out by bacteria such as *Veillonella, Prevotella,* and *Rothia*, where nitrite is converted into ammonium (NH_4^+) by ammonia-producing nitrite reductase. Since ammonium is a base, its presence increases the pH of the oral cavity and reduces the risk of dental caries (Rosier et al., 2020). Furthermore, the acidic breakdown of nitrite, which begins at a pH of around 5, a level frequently encountered in cariogenic biofilms following sugar consumption, could function as a negative feedback mechanism by producing nitric oxide, an antimicrobial agent (Bowles et al., 2024). Furthermore, some bacteria in the DNRA pathway, such as *Veillonella* and *Rothia*, can utilise lactate, an acidic molecule produced by *S. mutans* after sugar consumption, as an electron donor, which also makes the environment less acidic (Bowles et al., 2024). Nitrate is also beneficial for patients with halitosis, similar to what was hypothesized for lactate, nitrate-reducing bacteria could use hydrogen sulfide (one of the main VSCs involved in halitosis) as an electron donor (Rosier et al., 2020). It is also worth noting the benefits of nitrate for systemic health, such as its vasodilatory

action, which is beneficial for blood pressure, as well as its antimicrobial activities in the stomach. All these mechanisms are represented in Figure 20.

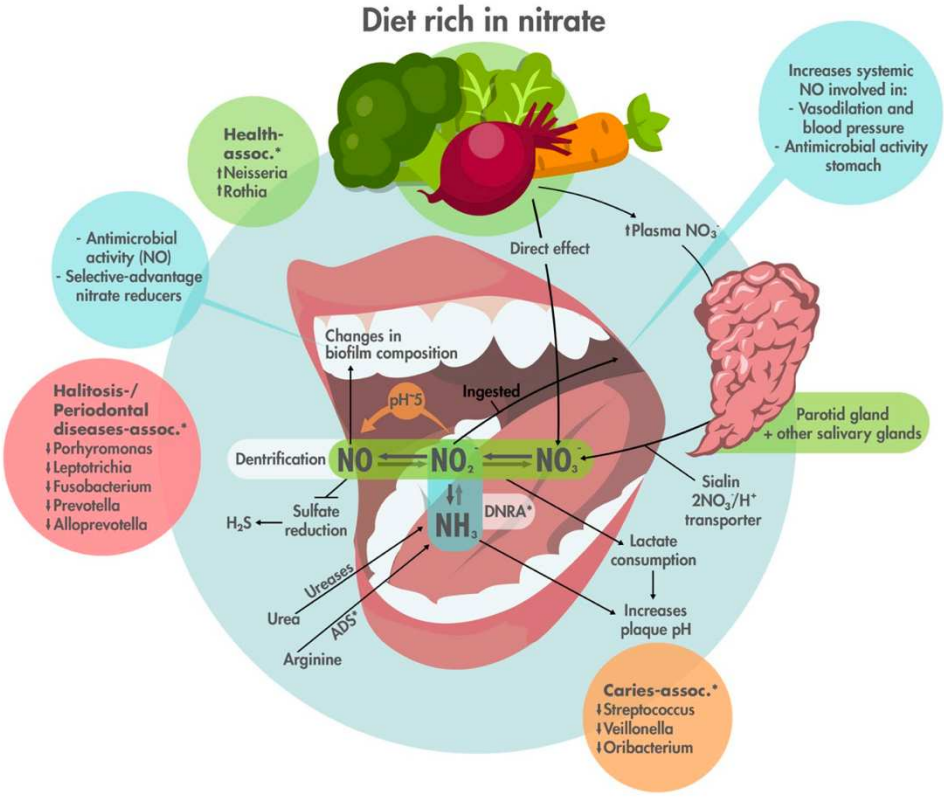


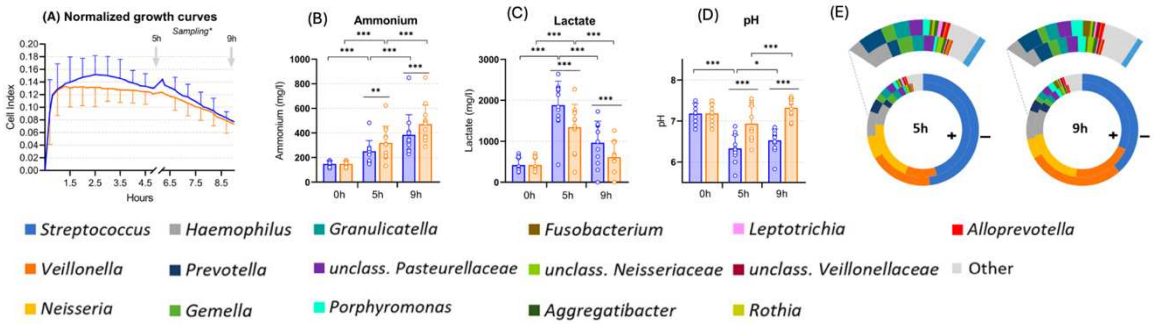
Figure 20: Illustration representing the sources of nitrate, its metabolism in the oral cavity, and its beneficial effects (reduction of bacteria responsible for dental caries, periodontal diseases, and halitosis, as well as the growth of beneficial bacteria for the microbiome). NO_3^-: nitrate; NO_2^-: nitrite; NO: nitric oxide; NH_3: ammonia; DNRA: dissimilatory nitrate reduction to ammonium; H_2S: hydrogen sulfide. Image from *(Rosier et al., 2020).*

The study by Rosier et al. in 2020 (Rosier et al., 2020) explores the potential role of nitrate as a prebiotic for the oral microbiome by evaluating its impact on biofilm growth, bacterial composition, and metabolic balance. Saliva from 12 healthy donors was collected and used to grow biofilms in vitro, with and without the addition of 6.5 mM nitrate, a physiological concentration after a vegetable-rich meal. Biofilm growth was monitored in real-time by impedance measurement, while samples were analysed at 5 h and 9 h to measure residual nitrate, nitrite, ammonium, lactate, and pH. Nitrate did not significantly affect biofilm growth (figure 21A), but it led to an increase in pH due to a decrease in lactate production and an increase in ammonium (figure 21BCD), indicating a more alkalising metabolism that could protect against dental caries. Concurrently, microbiota analysis by 16S rRNA gene sequencing revealed a significant increase in the

genera Neisseria and Rothia, known for their nitrate-reducing capacity, while bacteria associated with caries (Streptococcus or Veillonella) and periodontal diseases (Porphyromonas, Fusobacterium, or Prevotella) were significantly reduced (figure 21E). These changes were observed as early as 5 h, demonstrating a rapid effect of nitrate on the structure of the oral microbiota. Additionally, supplementary tests showed that nitrate inhibited the acidification of saliva due to glucose fermentation, suggesting a protective role against conditions favouring enamel demineralisation and the progression of periodontal diseases (Rosier et al., 2020). These results indicate that nitrate could play a key role in modulating the oral microbiota by promoting a more alkaline environment and stimulating the growth of beneficial bacteria, acting as a natural prebiotic that could be integrated into the diet or as a supplement to improve oral health by reducing the risk of caries, gingivitis, and periodontitis.

Figure 21: Measurements and comparisons with (orange) and without (blue) nitrate supplementation of (A) biofilm mass formed, (B) ammonium concentration, (C) lactate concentration, (D) pH, and (E) bacterial composition after 16S rRNA sequencing of in vitro saliva samples.

Figures from (Rosier et al., 2020).



Two clinic studies conducted by Jockel-Schneider et al. (2016 and 2021) explored the effect of a nitrate-rich diet on gingival inflammation and the oral microbiome.

The first study in 2016 was a randomised, placebo-controlled, double-blind clinical trial aimed at evaluating the impact of daily consumption of lettuce juice containing 200 mg of nitrate on gingival inflammation in patients undergoing periodontal monitoring. 44 patients were recruited and divided into two groups: a test group consuming nitrate-rich juice and a placebo group receiving an identical juice without nitrates. After 14 days, the test group showed a significant reduction in the gingival index (GI) compared to the placebo group, indicating a decrease in gingival inflammation. Additionally, salivary nitrate levels were significantly higher in the test group, with no significant change in plaque control (Jockel-Schneider et al., 2016).

The second study, published in 2021, continued this investigation by analysing the impact of the nitrate-rich diet on the composition of the subgingival microbiome. 37 patients with gingival inflammation were included, following the same dietary intervention protocol as in the previous study. Microbial samples were collected before and after the intervention and analysed by DNA sequencing targeting the V3-V4 region of the 16S rDNA gene. The results revealed a significant increase in Rothia and Neisseria bacteria, known for their role in nitrate reduction. Additionally, alpha diversity (reflecting the total number of bacterial species) decreased, while beta diversity (microbiome composition) was significantly altered. In contrast, no notable changes were observed in the placebo group (Jockel-Schneider et al., 2021).

In conclusion, the first study demonstrated that regular nitrate consumption reduces gingival inflammation, while the second study revealed that this effect could be attributed to changes in the oral microbiota, promoting the establishment of beneficial bacteria and potentially reducing pathogenic species. These studies suggest that incorporating a nitrate-rich diet could serve as an effective complementary approach to conventional periodontal care for managing gingival inflammation and modulating the oral microbiome.

All the prebiotics, along with their mechanisms and benefits, are summarised in Figure 22.

Prebiotic	Sources	Mechanism	Benefits
D-tagatose	- Fruits (apple, orange) - Gum of plants such as *Sterculia setigera* or the lichen *Roccella* - Enzymatic hydrolysis of lactose (industrially)	- Increases the expression of the gene coding for PTS, which converts fructose into fructose-1-phosphate and accumulates in *S. mutans* or *S. gordonii* but not *S. oralis*, leading to cellular stress and lysis	- Fewer calories and has a lower glycemic index than fructose - Inhibits cariogenic bacteria without affecting non-pathogenic bacteria
Xylitol	- Certain fruits and vegetables (plums, strawberries, cauliflower, and pumpkin) - Produced artificially from vegetable materials rich in xylene, such as beech wood and birch	- The transport, phosphorylation, and dephosphorylation cycles of xylitol to xylitol-5-phosphate do not produce energy for cariogenic bacteria like *S. mutans*, leading to their cell death	- Sweetness equivalent to sucrose - Enhances remineralisation - Increase salivation - Reducing the incidence of dental caries
2'-fucosyllactose	- Human milk	- Reduces the adhesion of *S. mutans* by interfering with the production of extracellular matrix.	- Reduces cavities in children by decreasing the adhesion of *S. mutans*
Arginine	- Essential amino acid found in dietary protein and added to toothpastes	- Converted into citrulline, then into carbamyl-phosphate before being metabolized into ammonium, a basic molecule that increases the pH of the environment	- Promotes pH homeostasis - Its combination with fluoride in toothpaste allows for effective reduction of cavities
Nitrate	- Fruits, leafy green vegetables (such as lettuce, cabbage), and primarily beetroot - added to some processed meats for preservation	- Metabolized into nitric oxide (antimicrobial and vasodilator) through the denitrification pathway or into ammonium (basic) through the DNRA pathway. - nitrate-reducing bacteria could use hydrogen sulfide as an electron donor	- Promotes the increase of oral pH - Antimicrobial action through nitric oxide - Reduces hydrogen sulfide levels in patients with halitosis

Figure 22: Summary table of the different prebiotics, their mechanisms of action, and their benefits for oral health.

5. Challenges and limitations

Research on prebiotics in dental medicine faces several challenges, including the complexity of the oral microbiome, with its multitude of species, and the variability in study methodologies. The oral environment is a dynamic ecosystem where bacteria interact with each other and the host in complex ways, making it difficult to isolate the specific effects of prebiotics. Additionally, interindividual variations in the composition of the oral microbiota complicate the establishment of standardised protocols for drawing generalisable conclusions. This is further compounded by the diversity of prebiotic types studied, modes of administration (toothpastes, mouthwashes, or oral supplements), and criteria for evaluating clinical outcomes, making the comparison of studies difficult. Consequently, current evidence remains limited and sometimes contradictory, highlighting the need for more robust studies with well-controlled, long-term clinical trials. The study of synbiotics, which combine prebiotics and probiotics, appears to be a promising avenue. By providing nutritional support to specific probiotic bacteria administered simultaneously, synbiotics could promote more effective and durable colonisation of the beneficial oral microbiome, thereby improving outcomes compared to the isolated use of prebiotics or probiotics. This synergistic approach could be particularly valuable in the prevention and management of oral diseases such as periodontitis or dental caries, where microbiota balance plays a key role. However, although preliminary results are encouraging, studies on synbiotics in dental medicine remain limited and require further investigation to better understand their efficacy and precise mechanisms of action.

6. Future directions

Currently, the teaching of oral microbiology primarily focuses on pathogenic agents and conventional treatments, while the role of prebiotics in oral microbiota balance remains largely unexplored in university curricula. A more in-depth integration of this topic into academic programmes would not only train future practitioners in innovative preventive approaches but also stimulate new research on the impact of prebiotics in oral health. To address these gaps, several research avenues need to be developed, including the establishment of rigorous clinical trials evaluating the efficacy of prebiotics on specific parameters such as the reduction of cariogenic bacteria, improvement of gingival health,

or stabilisation of oral pH. Furthermore, the development of prebiotic-based supplements and products, such as toothpastes, mouthwashes, or dietary supplements enriched with fermentable fibres, could open new perspectives in preventive and therapeutic dentistry. Additionally, promoting oral health through nutrition represents a promising field of application. Dentists could play a key role in raising awareness among their patients about the impact of their diet on the balance of the oral microbiota and encouraging them to consume foods rich in natural prebiotics, such as vegetables, fruits, and whole grains. Such a nutritional approach, combined with conventional hygiene practices, could enhance the prevention of periodontal and carious diseases, while aligning with a holistic vision of oral health. By integrating prebiotics into dentists' training and clinical recommendations, it would be possible to fully exploit their potential to improve oral health and prevent various dental pathologies in a natural and sustainable manner.

7. Conclusion

The increasing interest in prebiotics within dentistry is opening new doors for the prevention and management of oral diseases. Unlike conventional treatments, which often rely on antibiotics or antiseptics to indiscriminately eliminate harmful bacteria, prebiotics work by promoting a balanced oral microbiome. This strategy, influenced by research on the gut microbiome, seeks to foster the growth of beneficial bacteria while curbing the proliferation of microbes linked to cavities, periodontal disease, and other oral conditions.

The studies reviewed in this work indicate that various prebiotics, such as D-tagatose, xylitol, arginine, and nitrates, exhibit distinct yet complementary mechanisms of action. These compounds play a crucial role in inhibiting the formation of cariogenic biofilms, shifting the oral microbiome toward a healthier composition, and maintaining an optimal pH balance. By doing so, they help reduce the risk of enamel demineralization and gum inflammation.

Despite these promising findings, several obstacles must be overcome before prebiotics can be fully integrated into dental care strategies. One significant challenge lies in the individual variability of the oral microbiome, which complicates the process of applying laboratory findings to broader human populations. Additionally, most current studies

remain at the preclinical stage or involve short-term clinical trials, making it difficult to assess the long-term impact of prebiotic use on oral health. To bridge this gap, further research employing rigorous methodologies, such as large-scale randomized clinical trials and long-term observational studies, will be necessary to determine the true effectiveness of these compounds in everyday use.

Another critical factor is the development of suitable formulations that enable prebiotics to be seamlessly incorporated into clinical practice and daily oral hygiene routines. Integrating these substances into products like toothpaste, mouthwash, or chewing gum presents a promising avenue, yet additional studies are required to establish optimal dosages, compound stability, and potential synergies with other therapeutic agents, such as fluoride. Furthermore, the exploration of synbiotics enhance the efficacy of these interventions by supporting the colonization and persistence of beneficial bacteria within the oral ecosystem.

Lastly, raising awareness of prebiotics and their role in oral health is crucial for encouraging their adoption by both healthcare professionals and the patients. At present, dental education primarily focuses on pathogenic bacteria and conventional treatments, while the concept of microbiome modulation through prebiotics remains underexplored. Integrating these principles into university curricula, alongside public awareness campaigns, could help maximize the potential of prebiotics as part of a sustainable and preventive approach to oral healthcare.

In conclusion, while prebiotics alone cannot replace conventional dental treatments, they serve as a valuable complementary strategy for fostering a balanced oral microbiome and reducing the prevalence of oral diseases. Their incorporation into dental care could represent a significant shift in modern dentistry, offering less invasive solutions that respect the natural microbial ecosystem of the oral cavity. Future research will be essential to validate their clinical efficacy and refine their integration into daily oral care, paving the way for an innovative and sustainable approach to preventing dental and periodontal diseases.

8. References

Amargianitakis, M., Antoniadou, M., Rahiotis, C., & Varzakas, T. (2021). Probiotics, prebiotics, synbiotics and dental caries. New perspectives, suggestions, and patient coaching approach for a cavity-free mouth. In *Applied Sciences (Switzerland)* (Vol. 11, Issue 12). MDPI AG. https://doi.org/10.3390/app11125472

Amer, A., Whelan, A., Al-Hebshi, N. N., Healy, C. M., & Moran, G. P. (2020). Acetaldehyde production by *Rothia mucilaginosa* isolates from patients with oral leukoplakia. *Journal of Oral Microbiology*, *12*(1), 1743066. https://doi.org/10.1080/20002297.2020.1743066

Anbari, F., Ashouri Moghaddam, A., Sabeti, E., & Khodabakhshi, A. (2019). Halitosis: *Helicobacter pylori* or oral factors. *Helicobacter*, *24*(1). https://doi.org/10.1111/hel.12556

Anderson, A. C., von Ohle, C., Frese, C., Boutin, S., Bridson, C., Schoilew, K., Peikert, S. A., Hellwig, E., Pelz, K., Wittmer, A., Wolff, D., & Al-Ahmad, A. (2023). The oral microbiota is a reservoir for antimicrobial resistance: resistome and phenotypic resistance characteristics of oral biofilm in health, caries, and periodontitis. *Annals of Clinical Microbiology and Antimicrobials*, *22*(1), 37. https://doi.org/10.1186/s12941-023-00585-z

Andréa Quagliariello. (2023, September 4). *Ancient skeletal remains and the invention of agriculture: effects on the oral microbiome*. https://www.nature.com/articles/d43978-022-00165-3?utm_source=chatgpt.com

Ardila, C., Bedoya-García, J., & Arrubla-Escobar, D. (2023). Antibiotic resistance in periodontitis patients: A systematic scoping review of randomized clinical trials. *Oral Diseases*, *29*(7), 2501–2511. https://doi.org/10.1111/odi.14288

Bacali, C., Vulturar, R., Buduru, S., Cozma, A., Fodor, A., Chiș, A., Lucaciu, O., Damian, L., & Moldovan, M. L. (2022). Oral Microbiome: Getting to Know and Befriend Neighbors, a Biological Approach. In *Biomedicines* (Vol. 10, Issue 3). MDPI. https://doi.org/10.3390/biomedicines10030671

Bahador, A., Lesan, S., & Kashi, N. (2012). Effect of xylitol on cariogenic and beneficial oral streptococci: a randomized, double-blind crossover trial. *Iranian Journal of Microbiology*, *4*(2), 75–81.

Baker, J. L., & Edlund, A. (2019). Exploiting the Oral Microbiome to Prevent Tooth Decay: Has Evolution Already Provided the Best Tools? *Frontiers in Microbiology*, *9*. https://doi.org/10.3389/fmicb.2018.03323

Baker, J. L., Mark Welch, J. L., Kauffman, K. M., McLean, J. S., & He, X. (2024). The oral microbiome: diversity, biogeography and human health. In *Nature Reviews Microbiology* (Vol. 22, Issue 2, pp. 89–104). Nature Research. https://doi.org/10.1038/s41579-023-00963-6

Bijle, M. N., Ekambaram, M., & Yiu, C. K. Y. (2020). A Scoping Review on Arginine in Caries Prevention. In *Journal of Evidence-Based Dental Practice* (Vol. 20, Issue 3). Mosby Inc. https://doi.org/10.1016/j.jebdp.2020.101470

Bode, L. (2012). Human milk oligosaccharides: Every baby needs a sugar mama. *Glycobiology*, *22*(9), 1147–1162. https://doi.org/10.1093/glycob/cws074

Bowles, E. F., Burleigh, M., Mira, A., Van Breda, S. G. J., Weitzberg, E., & Rosier, B. T. (2024). Nitrate: “the source makes the poison.” In *Critical Reviews in Food Science and Nutrition*. Taylor and Francis Ltd. https://doi.org/10.1080/10408398.2024.2395488

Breban-Schwarzkopf, D., Chioibas, R., Macasoi, I., Bolintineanu, S., Marcovici, I., Draghici, G., Dinu, S., Buzatu, R., Dehelean, C., & Szuhanek, C. (2024). Comprehensive in vitro and in ovo assessment of cytotoxicity: Unraveling the impact of sodium fluoride, xylitol, and their synergistic associations in dental products. *Biomolecules and Biomedicine*, *24*(4), 923–938. https://doi.org/10.17305/bb.2024.10181

Burne, R. A., & Marquis, R. E. (n.d.). *Alkali production by oral bacteria and protection against dental caries*. www.fems-microbiology.org

Chumponsuk, T., Gruneck, L., Gentekaki, E., Jitprasertwong, P., Kullawong, N., Nakayama, J., & Popluechai, S. (2021). The salivary microbiota of Thai adults with metabolic disorders and association with diet. *Archives of Oral Biology*, *122*, 105036. https://doi.org/10.1016/j.archoralbio.2020.105036

Decker, E.-M., Klein, C., Schwindt, D., & von Ohle, C. (2014). Metabolic activity of Streptococcus mutans biofilms and gene expression during exposure to xylitol and sucrose. *International Journal of Oral Science*, *6*(4), 195–204. https://doi.org/10.1038/ijos.2014.38

del Pilar Angarita-Díaz, M., Fong, C., & Medina, D. (2024). Bacteria of healthy periodontal tissues as candidates of probiotics: a systematic review. *European Journal of Medical Research*, *29*(1), 328. https://doi.org/10.1186/s40001-024-01908-2

Di Stefano, M., Polizzi, A., Santonocito, S., Romano, A., Lombardi, T., & Isola, G. (2022). Impact of Oral Microbiome in Periodontal Health and Periodontitis: A Critical Review on Prevention and Treatment. *International Journal of Molecular Sciences*, *23*(9), 5142. https://doi.org/10.3390/ijms23095142

Di Tinco, R., Bertani, G., Pisciotta, A., Bertoni, L., Bertacchini, J., Colombari, B., Conserva, E., Blasi, E., Consolo, U., & Carnevale, G. (2021). Evaluation of Antimicrobial Effect of Air-Polishing Treatments and Their Influence on Human Dental Pulp Stem Cells Seeded on Titanium Disks. *International Journal of Molecular Sciences*, *22*(2), 865. https://doi.org/10.3390/ijms22020865

Doucette, H., Ward, R., Wheeldon, C., & Whelan, A. (2024). The impact of prebiotics and probiotics on the oral microbiome of individuals with periodontal disease: a scoping review. In *Can J Dent Hyg* (Vol. 58, Issue 3).

El Harram, S., & Sqalli, T. (2024). Could the Arginine-Fluoride Association Have a Real Impact on Caries Prevention? *Cureus*. https://doi.org/10.7759/cureus.72153

Ellepola, K., Truong, T., Liu, Y., Lin, Q., Lim, T. K., Lee, Y. M., Cao, T., Koo, H., & Seneviratne, C. J. (2019). Multi-omics analyses reveal synergistic carbohydrate metabolism in streptococcus mutans-candida albicans mixed-species biofilms. *Infection and Immunity*, *87*(10). https://doi.org/10.1128/IAI.00339-19

Gasmi Benahmed, A., Kumar Mujawdiya, P., Noor, S., & Gasmi, A. (2022). Porphyromonas Gingivalis in the Development of Periodontitis: Impact on Dysbiosis and Inflammation. *Archives of Razi Institute*, *77*(5), 1539–1551. https://doi.org/10.22092/ARI.2021.356596.1875

Gibson, G. R., Hutkins, R., Sanders, M. E., Prescott, S. L., Reimer, R. A., Salminen, S. J., Scott, K., Stanton, C., Swanson, K. S., Cani, P. D., Verbeke, K., & Reid, G. (2017a). Expert consensus document: The International Scientific Association for Probiotics and Prebiotics (ISAPP) consensus statement on the definition and scope of prebiotics. *Nature Reviews Gastroenterology & Hepatology*, *14*(8), 491–502. https://doi.org/10.1038/nrgastro.2017.75

Gibson, G. R., Hutkins, R., Sanders, M. E., Prescott, S. L., Reimer, R. A., Salminen, S. J., Scott, K., Stanton, C., Swanson, K. S., Cani, P. D., Verbeke, K., & Reid, G.

(2017b). Expert consensus document: The International Scientific Association for Probiotics and Prebiotics (ISAPP) consensus statement on the definition and scope of prebiotics. *Nature Reviews. Gastroenterology & Hepatology*, *14*(8), 491–502. https://doi.org/10.1038/nrgastro.2017.75

Gibson, G. R., & Roberfroid, M. B. (1995). Dietary Modulation of the Human Colonic Microbiota: Introducing the Concept of Prebiotics. *The Journal of Nutrition*, *125*(6), 1401–1412. https://doi.org/10.1093/jn/125.6.1401

Goyal, V., Damle, S., Puranik, M. P., Nuvvula, S., Kakanur, M., Marwah, N., Asokan, S., Suprabha, B. S., Sreenivasan, P., Wadgave, U., Shyam, S., & Thakur, D. (2023). Arginine: A New Paradigm in Preventive Oral Care. *International Journal of Clinical Pediatric Dentistry*, *16*(5), 698–706. https://doi.org/10.5005/jp-journals-10005-2693

Hajishengallis, G. (2015). Periodontitis: from microbial immune subversion to systemic inflammation. *Nature Reviews Immunology*, *15*(1), 30–44. https://doi.org/10.1038/nri3785

Hampelska, K., Jaworska, M. M., Babalska, Z. Ł., & Karpiński, T. M. (2020). The role of oral microbiota in intra-oral halitosis. In *Journal of Clinical Medicine* (Vol. 9, Issue 8, pp. 1–17). MDPI. https://doi.org/10.3390/jcm9082484

Hasibul, K., Nakayama-Imaohji, H., Hashimoto, M., Yamasaki, H., Ogawa, T., Waki, J., Tada, A., Yoneda, S., Tokuda, M., Miyake, M., & Kuwahara, T. (2017). D-Tagatose inhibits the growth and biofilm formation of Streptococcusï¿½mutans. *Molecular Medicine Reports*. https://doi.org/10.3892/mmr.2017.8017

He, X., McLean, J. S., Guo, L., Lux, R., & Shi, W. (2014). The social structure of microbial community involved in colonization resistance. *ISME Journal*, *8*(3), 564–574. https://doi.org/10.1038/ismej.2013.172

He, X.-S., & Shi, W.-Y. (2009). Oral Microbiology: Past, Present and Future. In *International Journal of Oral Science* (Vol. 1, Issue 2).

Hernández, P., Sánchez, M. C., Llama-Palacios, A., Ciudad, M. J., & Collado, L. (2022). *Strategies to Combat Caries by Maintaining the Integrity of Biofilm and Homeostasis during the Rapid Phase of Supragingival Plaque Formation*. https://doi.org/10.3390/antibiotics

Hutkins, R., Walter, J., Gibson, G. R., Bedu-Ferrari, C., Scott, K., Tancredi, D. J., Wijeyesekera, A., & Sanders, M. E. (2025). Classifying compounds as prebiotics — scientific perspectives and recommendations. *Nature Reviews Gastroenterology & Hepatology*, *22*(1), 54–70. https://doi.org/10.1038/s41575-024-00981-6

Jacques Amar, & Vincent Blasco-Baque. (2017). *microbiotes la revue des Periodontitis and cardiometabolic diseases*.

Jayadevan, D., Chakravarthy, D., Bal, D., & Dimple, D. (2019). Dental Caries and Sugar Substitutes: A Review. *IOSR Journal of Dental and Medical Sciences (IOSR-JDMS) e-ISSN*, *18*, 13–23. https://doi.org/10.9790/0853-1805081323

Jia, Y.-J., Liao, Y., He, Y.-Q., Zheng, M.-Q., Tong, X.-T., Xue, W.-Q., Zhang, J.-B., Yuan, L.-L., Zhang, W.-L., & Jia, W.-H. (2021). Association Between Oral Microbiota and Cigarette Smoking in the Chinese Population. *Frontiers in Cellular and Infection Microbiology*, *11*. https://doi.org/10.3389/fcimb.2021.658203

Jockel-Schneider, Y., Goßner, S. K., Petersen, N., Stölzel, P., Hägele, F., Schweiggert, R. M., Haubitz, I., Eigenthaler, M., Carle, R., & Schlagenhauf, U. (2016). Stimulation of the nitrate-nitrite-NO-metabolism by repeated lettuce juice consumption decreases gingival inflammation in periodontal recall patients: A randomized, double-blinded, placebo-controlled clinical trial. *Journal of Clinical Periodontology*, *43*(7), 603–608. https://doi.org/10.1111/jcpe.12542

Jockel-Schneider, Y., Schlagenhauf, U., Stölzel, P., Goßner, S., Carle, R., Ehmke, B., Prior, K., & Hagenfeld, D. (2021). Nitrate-rich diet alters the composition of the oral microbiota in periodontal recall patients. *Journal of Periodontology*, *92*(11), 1536–1545. https://doi.org/10.1002/JPER.20-0778

Kumbargere Nagraj, S., Eachempati, P., Uma, E., Singh, V. P., Ismail, N. M., & Varghese, E. (2019). Interventions for managing halitosis. *Cochrane Database of Systematic Reviews*, *2019*(12). https://doi.org/10.1002/14651858.CD012213.pub2

Kuriki, N., Asahi, Y., Okamoto, M., Noiri, Y., Ebisu, S., Machi, H., Suzuki, M., & Hayashi, M. (2024). Synergistic effects of arginine and fluoride on human dental biofilm control. *Journal of Dentistry*, *149*, 105307. https://doi.org/10.1016/j.jdent.2024.105307

Lee, Y.-H., & Hong, J.-Y. (2023). Oral microbiome as a co-mediator of halitosis and periodontitis: a narrative review. *Frontiers in Oral Health*, *4*. https://doi.org/10.3389/froh.2023.1229145

Li, Y., Huang, S., Du, J., Wu, M., & Huang, X. (2023). Current and prospective therapeutic strategies: tackling Candida albicans and Streptococcus mutans cross-kingdom biofilm. In *Frontiers in Cellular and Infection Microbiology* (Vol. 13). Frontiers Media S.A. https://doi.org/10.3389/fcimb.2023.1106231

Liao, Y., Tong, X.-T., Jia, Y.-J., Liu, Q.-Y., Wu, Y.-X., Xue, W.-Q., He, Y.-Q., Wang, T.-M., Zheng, X.-H., Zheng, M.-Q., & Jia, W.-H. (2022). The Effects of Alcohol Drinking on Oral Microbiota in the Chinese Population. *International Journal of Environmental Research and Public Health*, *19*(9), 5729. https://doi.org/10.3390/ijerph19095729

Lima, L., Gaspar, S., Rocha, B. S., Alves, R., & Almeida, M. G. (2024). Current clinical framework on nitric oxide role in periodontal disease and blood pressure. *Clinical Oral Investigations*, *28*(10), 521. https://doi.org/10.1007/s00784-024-05913-x

Loimaranta, V., Mazurel, D., Deng, D., & Söderling, E. (2020). Xylitol and erythritol inhibit real-time biofilm formation of Streptococcus mutans. *BMC Microbiology*, *20*(1). https://doi.org/10.1186/s12866-020-01867-8

Lopes, J. P., & Lionakis, M. S. (2022). Pathogenesis and virulence of Candida albicans. In *Virulence* (Vol. 13, Issue 1, pp. 89–121). Taylor and Francis Ltd. https://doi.org/10.1080/21505594.2021.2019950

Lu, Y., Lin, Y., Li, M., & He, J. (2023a). Roles of Streptococcus mutans-Candida albicans interaction in early childhood caries: a literature review. In *Frontiers in Cellular and Infection Microbiology* (Vol. 13). Frontiers Media S.A. https://doi.org/10.3389/fcimb.2023.1151532

Lu, Y., Lin, Y., Li, M., & He, J. (2023b). Roles of Streptococcus mutans-Candida albicans interaction in early childhood caries: a literature review. In *Frontiers in Cellular and Infection Microbiology* (Vol. 13). Frontiers Media S.A. https://doi.org/10.3389/fcimb.2023.1151532

Luo, S. C., Wei, S. M., Luo, X. T., Yang, Q. Q., Wong, K. H., Cheung, P. C. K., & Zhang, B. B. (2024). How probiotics, prebiotics, synbiotics, and postbiotics prevent dental caries: an oral microbiota perspective. In *npj Biofilms and Microbiomes* (Vol. 10, Issue 1). Nature Research. https://doi.org/10.1038/s41522-024-00488-7

Mayumi, S., Kuboniwa, M., Sakanaka, A., Hashino, E., Ishikawa, A., Ijima, Y., & Amano, A. (2021a). Potential of Prebiotic D-Tagatose for Prevention of Oral Disease. *Frontiers in Cellular and Infection Microbiology*, *11*. https://doi.org/10.3389/fcimb.2021.767944

Mayumi, S., Kuboniwa, M., Sakanaka, A., Hashino, E., Ishikawa, A., Ijima, Y., & Amano, A. (2021b). Potential of Prebiotic D-Tagatose for Prevention of Oral Disease. *Frontiers in Cellular and Infection Microbiology*, *11*. https://doi.org/10.3389/fcimb.2021.767944

Megha Baghel, Sneha Tripathi, Maneesh Kumar Maurya, & Shivani Mahra. (2025). *Oral Microbiome*. https://doi.org/10.1201/9781003478638

Mercenier, A., Pavan, S., & Pot, B. (2003). Probiotics as Biotherapeutic Agents: Present Knowledge and Future Prospects. *Current Pharmaceutical Design*, *9*(2), 175–191. https://doi.org/10.2174/1381612033392224

Metwalli, K. H., Khan, S. A., Krom, B. P., & Jabra-Rizk, M. A. (2013). Streptococcus mutans, Candida albicans, and the Human Mouth: A Sticky Situation. *PLoS Pathogens*, *9*(10). https://doi.org/10.1371/journal.ppat.1003616

Mohanty, R., Asopa, S., Joseph, Md., Singh, B., Rajguru, J., Saidath, K., & Sharma, U. (2019). Red complex: Polymicrobial conglomerate in oral flora: A review. *Journal of Family Medicine and Primary Care*, *8*(11), 3480. https://doi.org/10.4103/jfmpc.jfmpc_759_19

Moran, S. P., Rosier, B. T., Henriquez, F. L., & Burleigh, M. C. (2024). The effects of nitrate on the oral microbiome: a systematic review investigating prebiotic potential. In *Journal of Oral Microbiology* (Vol. 16, Issue 1). Taylor and Francis Ltd. https://doi.org/10.1080/20002297.2024.2322228

Morou-Bermúdez, E., Torres-Colón, J. E., Bermúdez, N. S., Patel, R. P., & Joshipura, K. J. (2022). Pathways Linking Oral Bacteria, Nitric Oxide Metabolism, and Health. In *Journal of Dental Research* (Vol. 101, Issue 6, pp. 623–631). SAGE Publications Inc. https://doi.org/10.1177/00220345211064571

Nagamine, Y., Hasibul, K., Ogawa, T., Tada, A., Kamitori, K., Hossain, A., Yamaguchi, F., Tokuda, M., Kuwahara, T., & Miyake, M. (2020a). D-Tagatose Effectively Reduces the Number of Streptococcus mutans and Oral Bacteria in Healthy Adult Subjects: A Chewing Gum Pilot Study and Randomized Clinical Trial. *Acta Medica Okayama*, *74*(4), 307–317. https://doi.org/10.18926/AMO/60369

Nagamine, Y., Hasibul, K., Ogawa, T., Tada, A., Kamitori, K., Hossain, A., Yamaguchi, F., Tokuda, M., Kuwahara, T., & Miyake, M. (2020b). D-Tagatose Effectively Reduces the Number of Streptococcus mutans and Oral Bacteria in Healthy Adult Subjects: A Chewing Gum Pilot Study and Randomized Clinical Trial. *Acta Medica Okayama*, *74*(4), 307–317. https://doi.org/10.18926/AMO/60369

Nascimento, M. M. (2018). Potential Uses of Arginine in Dentistry. In *Advances in dental research* (Vol. 29, Issue 1, pp. 98–103). https://doi.org/10.1177/0022034517735294

Nascimento, M. M., Alvarez, A. J., Huang, X., Browngardt, C., Jenkins, R., Sinhoreti, M. C., Ribeiro, A. P. D., Dilbone, D. A., Richards, V. P., Garrett, T. J., & Burne, R. A. (2019). Metabolic Profile of Supragingival Plaque Exposed to Arginine and Fluoride. *Journal of Dental Research*, *98*(11), 1245–1252. https://doi.org/10.1177/0022034519869906

Natalia Molinero García. (2024). Oral probiotics and prebiotics. *Anales de Microbiota Probioticos & Prebioticos*.

Nunpan, S., Suwannachart, C., & Wayakanon, K. (2019). Effect of Prebiotics-Enhanced Probiotics on the Growth of *Streptococcus mutans*. *International Journal of Microbiology*, *2019*, 1–7. https://doi.org/10.1155/2019/4623807

Nüse, B., Holland, T., Rauh, M., Gerlach, R. G., & Mattner, J. (2023). L-arginine metabolism as pivotal interface of mutual host–microbe interactions in the gut. *Gut Microbes*, *15*(1). https://doi.org/10.1080/19490976.2023.2222961

Oba, P. M., Holscher, H. D., Mathai, R. A., Kim, J., & Swanson, K. S. (2020). Diet Influences the Oral Microbiota of Infants during the First Six Months of Life. *Nutrients*, *12*(11), 3400. https://doi.org/10.3390/nu12113400

Ortiz, A. de C., Fideles, S. O. M., Reis, C. H. B., Pagani, B. T., Bueno, L. M. M., Moscatel, M. B. M., Buchaim, R. L., & Buchaim, D. V. (2024). D-Tagatose: A Rare Sugar with Functional Properties and Antimicrobial Potential against Oral Species. In *Nutrients* (Vol. 16, Issue 12). https://doi.org/10.3390/nu16121943

Ortiz-Sáez, B., Aguilella-Traver, M., Hernández-Pando, C., Martínez-Salmerón, E. M., Muñoz-Barrio, J. E., & Gómez-Moreno, G. (2024). Is xylitol effective in the prevention of dental caries? A systematic review. In *Journal of Clinical and Experimental Dentistry* (Vol. 16, Issue 10, pp. e1307–e1315). Medicina Oral S.L. https://doi.org/10.4317/jced.62008

Pisani, F., Pisani, V., Arcangeli, F., Harding, A., & Singhrao, S. K. (2023). Treponema denticola Has the Potential to Cause Neurodegeneration in the Midbrain via the Periodontal Route of Infection-Narrative Review. *International Journal of Environmental Research and Public Health*, *20*(11). https://doi.org/10.3390/ijerph20116049

Proctor, L. M., Creasy, H. H., Fettweis, J. M., Lloyd-Price, J., Mahurkar, A., Zhou, W., Buck, G. A., Snyder, M. P., Strauss, J. F., Weinstock, G. M., White, O., & Huttenhower, C. (2019). The Integrative Human Microbiome Project. *Nature*, *569*(7758), 641–648. https://doi.org/10.1038/s41586-019-1238-8

Radaic, A., & Kapila, Y. L. (2021). The oralome and its dysbiosis: New insights into oral microbiome-host interactions. In *Computational and Structural Biotechnology Journal* (Vol. 19, pp. 1335–1360). Elsevier B.V. https://doi.org/10.1016/j.csbj.2021.02.010

Rajasekaran, J. J., Krishnamurthy, H. K., Bosco, J., Jayaraman, V., Krishna, K., Wang, T., & Bei, K. (2024). Oral Microbiome: A Review of Its Impact on Oral and Systemic Health. *Microorganisms*, *12*(9), 1797. https://doi.org/10.3390/microorganisms12091797

Rosier, B. T., Buetas, E., Moya-Gonzalvez, E. M., Artacho, A., & Mira, A. (2020). Nitrate as a potential prebiotic for the oral microbiome. *Scientific Reports*, *10*(1). https://doi.org/10.1038/s41598-020-69931-x

Roy, S., Chikkerur, J., Roy, S. C., Dhali, A., Kolte, A. P., Sridhar, M., & Samanta, A. K. (2018). Tagatose as a Potential Nutraceutical: Production, Properties, Biological Roles, and Applications. *Journal of Food Science*, *83*(11), 2699–2709. https://doi.org/10.1111/1750-3841.14358

Saïz, P., Taveira, N., & Alves, R. (2021). Probiotics in oral health and disease: A systematic review. In *Applied Sciences (Switzerland)* (Vol. 11, Issue 17). MDPI. https://doi.org/10.3390/app11178070

Salli, K., Söderling, E., Hirvonen, J., Gürsoy, U. K., & Ouwehand, A. C. (2020). Influence of 2′-fucosyllactose and galacto-oligosaccharides on the growth and adhesion of *Streptococcus mutans*. *British Journal of Nutrition*, *124*(8), 824–831. https://doi.org/10.1017/S0007114520001956

Santacroce, L., Passarelli, P. C., Azzolino, D., Bottalico, L., Charitos, I. A., Cazzolla, A. P., Colella, M., Topi, S., Godoy, F. G., & D'Addona, A. (2023). Oral microbiota in human health and disease: A perspective. In *Experimental Biology and Medicine*

(Vol. 248, Issue 15, pp. 1288–1301). SAGE Publications Inc. https://doi.org/10.1177/15353702231187645

Schäffer, C., & Andrukhov, O. (2024). The intriguing strategies of Tannerella forsythia's host interaction. *Frontiers in Oral Health*, *5*, 1434217. https://doi.org/10.3389/froh.2024.1434217

Segata, N., Haake, S. K., Mannon, P., Lemon, K. P., Waldron, L., Gevers, D., Huttenhower, C., & Izard, J. (2012). Composition of the adult digestive tract bacterial microbiome based on seven mouth surfaces, tonsils, throat and stool samples. *Genome Biology*, *13*(6). https://doi.org/10.1186/gb-2012-13-6-r42

Shannon, O. M., Easton, C., Shepherd, A. I., Siervo, M., Bailey, S. J., & Clifford, T. (2021). Dietary nitrate and population health: a narrative review of the translational potential of existing laboratory studies. *BMC Sports Science, Medicine and Rehabilitation*, *13*(1), 65. https://doi.org/10.1186/s13102-021-00292-2

Sharma, N., Bhatia, S., Sodhi, A. S., & Batra, N. (2018). Oral microbiome and health. In *AIMS Microbiology* (Vol. 4, Issue 1, pp. 42–66). AIMS Press. https://doi.org/10.3934/MICROBIOL.2018.1.42

Smith, A., Avery, A., Ford, R., Yang, Q., Goux, A., Mukherjee, I., Neville, D. C. A., & Jethwa, P. (2022). Rare sugars: metabolic impacts and mechanisms of action: a scoping review. *British Journal of Nutrition*, *128*(3), 389–406. https://doi.org/10.1017/S0007114521003524

Souza, M. L. R., Cury, J. A., Tenuta, L. M. A., Zhang, Y. P., Mateo, L. R., Cummins, D., & Ellwood, R. P. (2013). Comparing the efficacy of a dentifrice containing 1.5% arginine and 1450 ppm fluoride to a dentifrice containing 1450 ppm fluoride alone in the management of primary root caries. *Journal of Dentistry*, *41*(SUPPL. 2). https://doi.org/10.1016/j.jdent.2010.04.006

Suvan, J., Leira, Y., Moreno Sancho, F. M., Graziani, F., Derks, J., & Tomasi, C. (2020). Subgingival instrumentation for treatment of periodontitis. A systematic review. *Journal of Clinical Periodontology*, *47*(S22), 155–175. https://doi.org/10.1111/jcpe.13245

Tada, A., Nakayama-Imaohji, H., Yamasaki, H., Hasibul, K., Yoneda, S., Uchida, K., Nariya, H., Suzuki, M., Miyake, M., & Kuwahara, T. (2016). Cleansing effect of acidic L-arginine on human oral biofilm. *BMC Oral Health*, *16*(1). https://doi.org/10.1186/s12903-016-0194-z

ten Cate, J. M., & Buzalaf, M. A. R. (2019). Fluoride Mode of Action: Once There Was an Observant Dentist . . . *Journal of Dental Research*, *98*(7), 725–730. https://doi.org/10.1177/0022034519831604

UTAMANİNGYAS, A., PRAMESTİ, H. T., & BALAFİF, F. F. (2023). The Streptococcus mutans ability to survive in biofilms and during dental caries formation: scoping review. *Journal of Syiah Kuala Dentistry Society*, *7*(2), 150–158. https://doi.org/10.24815/jds.v7i2.30295

Veneri, F., Vinceti, S. R., & Filippini, T. (2024). Fluoride and caries prevention: a scoping review of public health policies. *Annali Di Igiene Medicina Preventiva e Di Comunita*, *36*(3), 270–280. https://doi.org/10.7416/ai.2024.2593

Verspecht, T., Van Holm, W., Boon, N., Bernaerts, K., Daep, C. A., Masters, J. G., Zayed, N., Quirynen, M., & Teughels, W. (2021). Potential prebiotic substrates modulate composition, metabolism, virulence and inflammatory potential of an in vitro multi-species oral biofilm. *Journal of Oral Microbiology*, *13*(1). https://doi.org/10.1080/20002297.2021.1910462

Wang, S., Wang, Y., Wang, Y., Duan, Z., Ling, Z., Wu, W., Tong, S., Wang, H., & Deng, S. (2019). Theaflavin-3,3′-Digallate Suppresses Biofilm Formation, Acid

Production, and Acid Tolerance in Streptococcus mutans by Targeting Virulence Factors. *Frontiers in Microbiology*, *10*. https://doi.org/10.3389/fmicb.2019.01705

Wu, J., Cannon, R., Ji, P., Farella, M., & Mei, L. (2020). Halitosis: prevalence, risk factors, sources, measurement and treatment – a review of the literature. *Australian Dental Journal*, *65*(1), 4–11. https://doi.org/10.1111/adj.12725

Xiang, S., Ye, K., Li, M., Ying, J., Wang, H., Han, J., Shi, L., Xiao, J., Shen, Y., Feng, X., Bao, X., Zheng, Y., Ge, Y., Zhang, Y., Liu, C., Chen, J., Chen, Y., Tian, S., & Zhu, X. (2021). Xylitol enhances synthesis of propionate in the colon via cross-feeding of gut microbiota. *Microbiome*, *9*(1), 62. https://doi.org/10.1186/s40168-021-01029-6

Xie, G., Lo, C. C., Scholz, M., & Chain, P. S. G. (2014). Recruiting human microbiome shotgun data to site-specific reference genomes. *PLoS ONE*, *9*(1). https://doi.org/10.1371/journal.pone.0084963

Yu, X., Devine, D. A., & Vernon, J. J. (2024). Manipulating the diseased oral microbiome: the power of probiotics and prebiotics. In *Journal of Oral Microbiology* (Vol. 16, Issue 1). Taylor and Francis Ltd. https://doi.org/10.1080/20002297.2024.2307416

Zheng, X., He, J., Wang, L., Zhou, S., Peng, X., Huang, S., Zheng, L., Cheng, L., Hao, Y., Li, J., Xu, J., Xu, X., & Zhou, X. (2017). Ecological Effect of Arginine on Oral Microbiota. *Scientific Reports*, *7*(1). https://doi.org/10.1038/s41598-017-07042-w