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Can Acids Produced From Probiotics Demineralize the Tooth and Cause Progression of Caries: A Critical Review

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ABSTRACT

Background: Probiotics are known to provide various health benefits to the host in adequate concentrations. Lactobacilli, Propionibacterium, and Bifidobacterium are common "Lactic Acid-producing bacteria (LAB)" present in various probiotic formulations. These probiotic bacteria release large amount of organic acids, mainly acetic acid, lactic acid, and propionic acid, into their surrounding environment. Increased concentration of these inorganic and organic acids can reduce the saliva's critical pH to below 5.5, and can initiate demineralization of the tooth surface. Thus, the present review aims to critically appraise the existing evidence on this emerging clinical perspective and double edged nature of the probiotic bacteria, and explain as to how probiotic supplements can increase the risk of enamel demineralization and tooth decay.

Results*: Lactobacilli* and *Bifidobacterium* are associated with rampant caries in preschool children and progression of caries. All probiotics were found to be acidogenic in nature as they reduced the pH of the saliva, and eroded the enamel. Probiotics release extracellular polysaccharides that help in plaque formation and can cause leaching of essential elements like calcium and phosphorous from the enamel surface. The lactic acid-producing efficiency of probiotics is increased when combined with supragingival plaque.

Conclusions: Acids released from probiotic bacteria can reduce the critical pH of the oral environment and play an important role in initiating enamel demineralization and progression of caries.

Keywords: Probiotics; Lactobacilli; Tooth; Caries; Enamel; Demineralization; Oral health; Acid; Critical pH.

Probiyotiklerden Üretilen Asitler Dişi Demineralize Edebilir ve Çürüklerin İlerlemesine Neden Olabilir mi?: Kritik Bir İnceleme

Süreç

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ÖZ

Arka plan: Probiyotikler, yeterli konsantrasyonlarda konakçıya çeşitli sağlık yararları sağlayan canlı mikroorganizmalardır. Lactobacilli, Propionibacterium ve Bifidobacterium, çeşitli probiyotik formülasyonlarda bulunan yaygın "Laktik Asit üreten bakterilerdir (LAB)". Bu probiyotik bakteriler, çevrelerine büyük miktarda organik asit, özellikle laktik asit, asetik asit ve propiyonik asit salgılarlar. Bu inorganik ve organik asitlerin artan konsantrasyonu tükürüğün kritik pH'ını 5,5'in altına düşürebilir ve diş yüzeyinin demineralizasyonunu başlatabilir. Bu nedenle, bu gözden geçirme, ortaya çıkan bu klinik perspektif ve probiyotik bakterilerin çift kenarlı doğası hakkındaki mevcut kanıtları eleştirel olarak değerlendirmeyi ve probiyotik takviyelerin emaye demineralizasyonu ve diş çürümesi riskini nasıl artırabileceğini açıklamayı amaçlamaktadır.

Bulgular: Lactobacilli ve Bifidobacterium, okul öncesi çocuklarda yaygın çürükler ve çürüklerin ilerlemesi ile ilişkilidir. Tüm probiyotikler, tükürüğün pH'ını düşürdükleri, emayeyi demineralize ettikleri ve aşındırdıkları için asidojenikti. Probiyotikler, plak oluşumuna yardımcı olan ve kalsiyum ve fosfor gibi temel elementlerin emaye yüzeyinden sızmasına neden olabilecek hücre dışı polisakkaritleri serbest bırakır. Supraggival plak ile birleştirildiğinde probiyotiklerin laktik asit üreten verimliliği artar.

Sonuçlar: Probiyotik bakterilerden salınan asitler, ağız boşluğunun kritik pH'ını düşürebilir ve emaye demineralizasyonunun ve çürüklerin ilerlemesinin başlatılmasında önemli bir rol oynayabilir.

Anahtar Kelimeler: Probiyotikler; Lactobacilli; Diş; Çürük; Emaye; Demineralizasyon; Ağız sağlığı; Asit; Kritik pH.

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Introduction

Probiotics are known to provide various health benefits to the host upon intake in adequate concentrations.[1-2] The microorganisms present in probiotic formulations include Bifidobacterium longum, *Lactobacillus reuteri*, Lactobacillus acidophilus, *Lactobacillus rhamnosus*, *Streptococcus thermophiles*, *and Saccharomyces boulardil*.[3] Probiotics are commercially available in the forms of fermented milk, lozenges, tablets, powders, yogurt, chewing gums, liquid, syrups, and chips.[4-6] In the last decade, probiotic consumption has rapidly increased around the globe. According to the 2012 National Health Interview Survey (NHIS), probiotic consumption was found to be common with around 4 million U.S adults (1.6 percent) reportedly consuming probiotics in the preceding 30 days. With a rapid increase in the awareness and consumption of probiotics supplements, probiotics are among the most commonly consumed dietary supplement and superfood across the globe.[5]

Probiotic supplements are commonly prescribed to manage gastrointestinal, vaginal, urogenital, oropharyngeal, and atopic disorders.[5-7] In dentistry, probiotic bacteria are used as an adjunct to mechanical periodontal debridement, to treat oral and periodontal diseases. Probiotics can inhibit or replace the pathogens in the oral cavity and inhibit plaque formation. Probiotic bacteria also release various antimicrobial substances that can prevent attachment of primary coloniziers to the tooth surface, and reduce biofilm formation.[5-7] For example, probiotic can replace the primary colonizers such as *Streptococcus mutans*, *Streptococcus salivarius,* and *Actinomyces* viscosus and reduce the risk of caries and gingival inflammation.[7-20]

Although the role of probiotics in treating caries, halitosis, periodontal and peri-implant diseases is established, there is an emerging perspective that probiotic supplements could demineralize the tooth surface, and increase the risk of tooth decay.[21-31] This perspective is based on the acidogenic and aciduric nature of probiotic bacteria.

Probiotic microorganisms, particularly Bifidobacteria, *Lactobacilli*, and *Propionibacterium,* are classified as "Lactic Acid-producing bacteria" (LAB), as they release high amounts of organic acids in their surrounding environment.[28-33] Probiotics also produce extracellular polysaccharides (EPS) that aid in plaque formation, and in turn increase the overall acidity of the biofilm. Beighton *et al*. (2005) and Faraz *et al*. (2019) confirmed the strong acidogenic nature of probiotic bacteria and stated that 'lactic acid-producing bacteria,' may be a risk factor for demineralization of the enamel surface".[34-36] Since existing literature primarily focus on the ability of probiotics to replace the cariogenic pathogens, the demineralizing and erosive potential of probiotics are often ignored. Thus, the present review aims to explore this emerging clinical perspective and double edged nature of the probiotic bacteria. The paper is of paramount importance to the public, dentists, food scientists, and pharmaceutical companies, as it highlights the mechanisms, and existing evidence which confirm that probiotics may detrimental effect on the health of the enamel.

Methodology, Search Strategy, and Data Collection Process:

The following search strategy was used in following database: PubMed, Scopus, Web of Science database, Open Science and Dentistry for searching the relevant articles: (Probiotic* OR Probiotic supplements OR probiotic supplementation* OR Lactobacilli OR Bifidobacterium OR Propionibacterium) AND (Tooth OR Teeth OR Caries OR Decay OR Demineralization OR Enamel Loss OR Non-carious cervical lesion OR Erosion OR Roughness OR Strength OR Critical pH OR Lactic Acid). All articles up to July 2021 were included. The search strategy was modified accordingly in Scopus, Web of Science, ProQuest (Dentistry and Open access) to search for relevant articles. The cross-reference of all studies was searched to include any relevant data. Articles written in English language only were included. Two reviewers independently performed the initial screening for including the relevant articles.

All in-vitro, animal and in-vivo studies (randomized, quasi-randomized and non-randomized clinical trials, cross-sectional, prospective, retrospective, and observational studies) were included. The data from all the articles was compiled and presented as a narrative synthesis.

Results and Discussion

The search yielded 7055 articles in PubMed, 48 in Scopus, 1405 in Web of Science, 1027 in Open Sciences and dentistry, and 308 in ProQuest Health & Medical Complete (Proquest Medical Library). Of all the articles, only 40 were included for the review. Of the 40 articles, 13 articles directly explained the effects of probiotics and their role in decreasing the critical pH and progression of caries (Table 1a, b). The studies showed that probiotics can increase the acidity of the oral cavity and can induce erosive changes in the enamel.

Two main mechanisms that were found to be associated with increased demineralization of enamel surface and risk tooth decay were: *a) Increased acid production, b) Increased production of Exopolysaccharide (EPS)*

a) Increased Acid Production From Probiotic Microorganisms

Due to their capability to produce copious amounts of lactic acid, and other short-Chain fatty acids (SCFA) like acetic acid in their environment, Lactobacilli are considered as one of the most important cariogenic pathogens.[19,20] In 1998, Lankaputhra and Shah reported that lactic acid (780 – 3276 µg/ml), acetic acid (58-75 µg/ml), and pyruvic acid (6 – 22 µg/ml) are the most common organic acids produced by probiotic bacteria.[28]

Table 1a. Studies confirming the role of probiotics in reducing the critical pH, increasing the acidity of oral cavity.

A/Y	NP/PB	Aims and Objectives	Methodology	Results and Conclusion
A1	1	Lactobacillus salivarius was checked for its cariogenicity in rats	Six groups were infected with L. Salivarius with and without Streptococcus mutans.	L. salivarius proliferated and caused significant amount of dental caries within 5 days of infection. Higher caries score was observed in rats superinfected with both L. salivarius and S mutans and than those infected with either S. Mutans or L. salivarius alone. An inherent cariogenic activity was seen in relation to L salivarius strain after adherence to the tooth surface.
A2	2	Determine the profile of the bacterial species related to carious lesions in permanent and deciduous teeth, and variation with state of disease.	Plaque samples were collected from 39 healthy patients (control group). Plaque was collected from 51 subjects with high caries rate from healthy enamel with either whitespot lesions, or deep-dentinal lesions. The 16S rRNA was sequenced to the bacterial species.	197 bacteria with 22 new phylotypes were identified. In individuals having *S. mutans*, additional species of *Atopobium*, Lactobacillus, and *Propionibacterium* were noted in significantly amounts compared to *S. mutans*. These species played an crucial role in caries progression.
A3	3	To evaluate the presence of Bifidobacterium in root carious lesions.	Bifidobacterium was assessed in active carious lesions of the root, remineralizing lesions and healthy root surfaces.	*Bifidobacteria* was isolated from all soft active carious lesion on the root, five plaque samples collected from sound exposed root surfaces, and, 13 of 15 leathery lesions. The proportion of *Bifidobacteria* was significantly higher in infected dentin of carious lesions, and least in the plaque obtained from healthy root surfaces.
A4	2	The reduction in the pH level upon use of 14 different probiotics and dairy bacterial strains containing xylitol, lactose glucose, sorbitol, sucrose was assessed.	The pH levels were recorded for 30 mins at time intervals of 5, 10, 15, and 30 mins. Acid levels were measured after addition of different sugars (glucose, sucrose, lactose), and sugar alcohols (xylitol, sorbitol) to the fermentation medium.	The reduction in pH was dependent on sugar or sugar alcohol and the bacterial strain involved. The decrease in pH was fastest with glucose. After 5 min the pH was below 5 for all strains tested. None of the Bifidobacteria fermented sugar alcohols. All the strains were found to be acidogenic.
A5	1	To assess the mechanisms of probiotic bacteria and sum up the overall effects on the oral environment.	The role of salivary pellicle, attachment of bacteria, and the activation of peroxidase system was evaluated. The levels of Streptococcus gordonii, Streptococcus mutans, Aggregatibacter actinomycetemcomitans, and Helicobacter pylori were used noted	Probiotic bacteria affected the oral microbiota based on the specific nature of strain or species. Probiotics are acidogenic in nature. Probiotics may use either sugar alcohol or sugar for forming acid.
A6	4	To evaluate and compare the bacterial profiles for extensively carious teeth and healthy teeth in young permanent dentition.	Dental plaque was obtained from intact enamel in healthy subjects and from subjects with dental caries. The plaue was collected from the sound enamel; white spot lesions; carious/cavitated lesions; carious dentin. The compoistion of bacteria was noted from healthy and carious teeth using 16S rRNA genes.	*Lactobacillus* was the most prevalent species observed in increased caries. The Lactobacilli count increased markedly with progression of caries. The count of Propionibacterium FMA5 was not found to be high, but it was associated with the progression of caries. In contrast, S.mutans was not significantly associated with caries progression. The bacterial diversity reduced with progression of caries with the following species reduced in count: Capnocytophaga gingivalis, Campylobacter rectus, Corynebacterium matruchotii, Lachnospiraceae sp. C1, Eubacterium IR009, *Streptococcus gordonii; Streptococcus mitis; S.pneumoniae-S.infantis* group, and Streptococcus cristatus.
A7	1	To characterize two probiotic *Lactobacillus reuteri* strains, ATCC PTA 5289 and ATCC 55730, from a cariogenic perspective	Evaluation for adhesion and biofilm formation was done on saliva-coated hydroxyapatite. Arginine metabolism was estimated by measuring Ph values in the presence of glucose and arginine.	ATCC 55730 strains of Lactobacilli was found in low levels in both adhesion and biofilm formation compared to ATCC PTA 5289 strain. Both strains were found to be arginolytic and raised the pH in the presence of arginine.

Table 1b. Studies confirming the role of probiotics in reducing the critical pH, increasing the acidity of oral cavity.

A/Y	NP/PB	Aims and Objectives	Methodology	Results and Conclusion
A8	3	Bifidobacteria concentrations in caries-free and caries-active children were compared.	Salivary samples from 22 clinically caries free children and 38 caries-active children. Children were tested for levels of *Lactobacilli, Bifidobacteria, Streptococci mutans,* and yeasts	Bifidobacteria were isolated in 9% of the children without caries compared to 95% in children with active caries lesions. The nature of diet with level of sugar intake, frequency of consumption of sugar, and nature of oral hygiene practice were found to have a significant corelation to levels of Bifidobacteria in saliva.
A9	1	To assess the effect of lactobacilli present in the probiotic on lactic acid production in plaque.	*L. reuteri* and *L. plantarum* were added to the supragingival plaque which was obtained from 25 young healthy adults. The prodiction of lactic acid was evalauted after fermentation utilizing xylitol or fructose. 18 subjects were instructed to consume lozenges containing probiotic lactobacilli (*L. reuteri* DSM and ATCC PTA 5289), or placebo for two weeks. The concentration of lactic acid in supragingival plaque samples was compared at baseline and at 2 weeks. Salivary counts of Streptococci mutans and Lactobacilli were estimated .	Plaque with *L. reuteri* formed less lactic acid compared to *L. plantarum* and controls. Fructose induced more lactic acid concentrations compared to xylitol. No significant difference was noted for lactic acid production at 2 weeks compared to baseline in any of the groups. No significant changes were observed in relation to levels of S mutans. The levels of lactobacilli count increased significantly in the test group.
A10	5	Estimated the oral Lactobacilli levels among Japanese preschool children with differing prevalence of caries; and (2) to reveal the traits of these isolated *Lactobacillus* species.	Samples were collected from 74 preschool children with varied caries progression in Japan. The resistance to acidic environments and saliva-induced agglutination rate were measured.	High prelevance of dental caries was observed in preschool children with *Lactobacillus* (*L.*) *salivarius* or *Strepto coccus mutans* strains. The growth ability of L. Salivarius in acidic environment co-related positively with the scores of caries in the pre-school children with L. Salivarius strain.
A11	6	Compared the effectiveness of probiotic enriched yogurt extract and traditional yogurt extract in inhibiting dental enamel demineralization and promoting its remineralization.	60 caries-free human premolars divided into three groups comprising 20 teeth were checked for *in-vitro* demineralization. At a pH of 4.8, probiotic enriched yogurt extracts and traditional yogurt extracts was added and evaluated by confocal laser scanning microscopy and atomic absorption spectrophotometer.	Traditional yogurt has more inhibitory effect than probiotic enriched yogurt on dental enamel demineralization. The amount of calcium released into the solutions post emersion was more for probiotic groups (367.75 pg/ml) compared to the non-probiotic group (238.30 pg/ml). The difference was least for the traditional Non- probiotic group.
A12	1	To assess and compare the lactic acid-producing ability of L. Plantarum, and L. Acidophilus, in the presence or absence of dental plaque.	26 samples of Supragingival plaque with and without L.acidophilus and L.plantarum from three control groups and two test groups were collected. The concentration of the lactic acid was determined after acid production with sucrose.	The lactic acid production was greatest for the plaque group and least for L plantarum group. The lactic acid-producing ability of pure suspensions of L. .plantarum and L. acidophilus increased when mixed with the supragingival plaque.
A13	7	To evaluate the erosive activity on tooth enamel resulting from intake of six probiotic drinks.	48 extracted premolars, caries free, and not having hypocalcifications were used in the study to assess the erosive activity. Parameters like overall acidity, pH, weight loss of the tooth, and the rate at which calcium is released were noted when premolars were exposed daily for 5mins over a period of 1 week.	Probiotic drinks exhibit low erosive activity. Use of probiotics did not result in any calcium loss from tooth enamel although pH was seen to be lowered. All the tested samples showed continued weight loss of minerals for over 7 days' exposure.

A/Y: Author/year; *NP/PB:* Nature of Probiotics/ Probiotic bacteria

A1: Matsumoto *et al*., 2005; A2: Aas *et al*., 2008; A3: Mantzourani *et al*., 2008; A4: Haukioja *et al*., 2008; A5: Haukioja *et al*., 2009; A7: Jalasvuori *et al*., 2012; A8: Kaur *et al*.,2013; A9: Keller and Tweetman, 2012; A10: Shimada *et al*., 2015; A11: Singh and Doyle, 2016; A12: Faraz *et al*., 2019; A13: Zulkapli *et al*., 2020,

1: Lactobacilli; 2: Lactobacilli and Bifidobacteria; 3: *Bifidobacteria; 4: Lactobacillus , Propionibacterium; 5:* Lactobacillus; 6: Probiotic yogurt (Mother Dairy B-Active) containing Lactobacilli; 7: Different probiotic drinks;

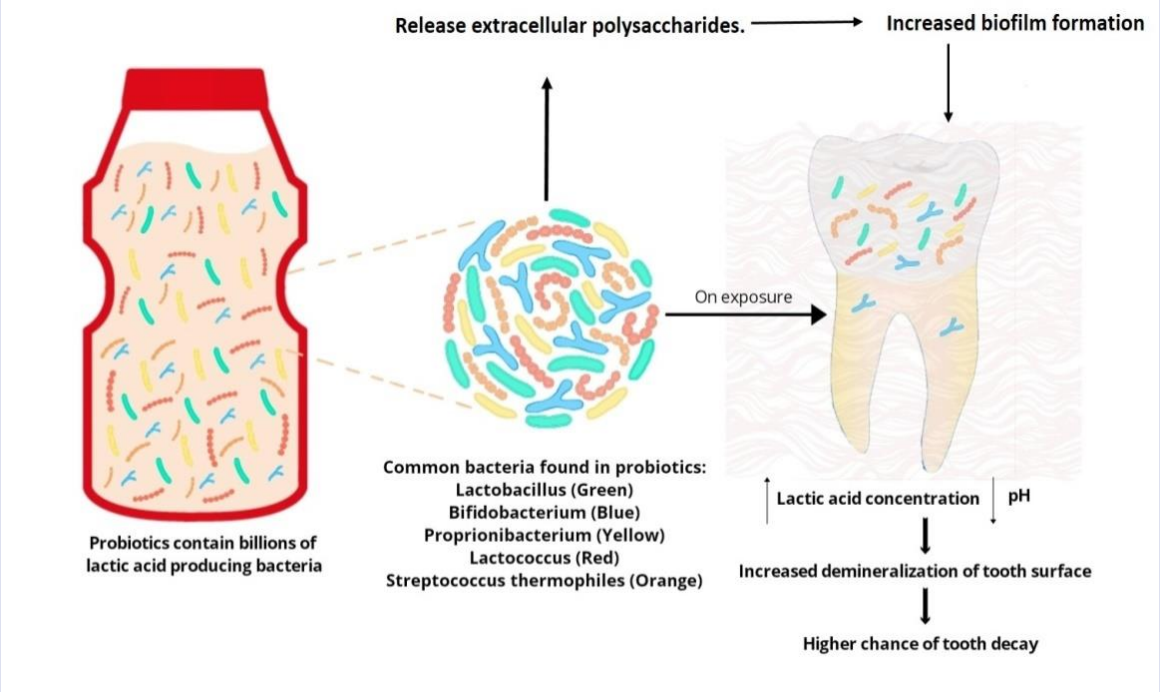

Figure 1. Schematic representation of the effect of probiotics on the Tooth surface: Probiotic supplements contain live microorganisms, particularly Lactobacilli, Propionibacterium, and Bifidobacteria. These microorganisms are classified as "Lactic Acid-producing bacteria" (LAB) as they release many organic acids using the glycolysis pathways in their surrounding environment. The increased acidic environment and alteration in microbiota induce structural and environmental changes that make the tooth susceptible to caries.

Increased acid production can lower the critical pH and increase the risk of enamel demineralization, microerosion, softening of the enamel and tooth decay.[24-36] The areas of demineralization are prone for caries formation as bacteria can easily colonize these demineralized areas and penetrate the dentin. This role of lactic acid in enamel demineralization can be explained by the classic 'chemoparasitic theory (acidogenic theory)', which states that 'the pathogenic microorganisms utilize the fermentable carbohydrates in the oral cavity to increase the production of formic acid, lactic acid, and succinic acid reducing the pH of the oral cavity.[4] The theory also stated that any 'extrinsic factors such as diet can influence the demineralization process in the oral cavity. Intake of dietary acid through food or drink, along with microbial attack from existing bacteria in the mouth, can induce structural and environmental changes in the enamel'.[4,22-32] Therefore, an increase in the overall acidity of the saliva and alteration in the microbiome should be considered the root cause for change in the critical pH value of the oral cavity. Marsh *et al*. (2003), in their ecological plaque hypothesis, stated that the demineralization of enamel could be attributed to any species, conditions, or habit, which can increase the acidic load in the oral environment, and thus should be considered as a risk factor for caries.[30-36] Moreover, since recent studies have shown that probiotics can colonize the oral cavity and integrate into the biofilm, the effect of probiotic on the tooth surface should be explored.

There is sufficient evidence linking the probiotic bacteria such as *Lactobacilli* and *Bifidobacterium* with demineralization of tooth and progression of caries.[37-53] High levels of *Lactobacilli* have been isolated from initial to deep carious lesions.[51] However, there is contradicting data pertaining to the salivary *Lactobacilli* levels after probiotic usage, with few studies reporting no effects[54], while others reporting a statistically significant increase in counts of salivary *Lactobacilli* after probiotic comsumption.[20,24-26] Matsumoto *et al*., 2005, evaluated the ability of *Lactobacillus salivarius* to cause caries when infected with and without *S. mutans* and observed that *Lactobacillus salivarius* can 'establish itself in the buccal cavity and induce dental caries as early as five days'. Additionally, the caries score of rats superinfected with *S. mutans* and *Lactobacillus salivarius* was considerably greater than those infected with either S.mutans or *Lactobacillus salivarius*. This was linked to the propensity of *Lactobacillus salivarius* to cling to the saliva-coated hydroxyapatite and its 'inherent cariogenic activity'.[24] Studies have also correlated the role of probiotic-containing Bifidobacteria or *Lactobacilli* to the *Streptococci mutans* count in saliva.[8-22] Studies have shown that the decline in the *Streptococci mutans* count in the saliva was not dependent on the demineralizing potential of the product or strain used.[15-23] In a recent systematic review and meta-analysis, Wang *et al*. (2021) concluded that "there was no statistically significant decrease in *Lactobacilli* and *Streptococcus mutans* counts in saliva in people consuming probiotics with

Bifidobacterium[57]. *Bifidobacterium* was not found to be effective in reducing *Lactobacillus* and Streptococcus mutans count in the saliva or dental plaque, nor in reducing the incidence of caries in deciduous teeth. Thus, further investigations should be carried out regarding the suitability of *Bifidobacterium* to prevent dental caries.

Numerous papers have documented a positive correlation between salivary *Bifidobacterium* species, particularly *B. dentium and B. longum,* active root carious lesions and restored tooth surfaces.[10,11,51-55] The ability of *Bifidobacterium* to persist and proliferate in the acidic environment of carious lesions could be attributed to their 'ability to preserve pH despite extracellular acidification, durability of cell membrane, and intracellular alkaline production. It also contributes to their ability to proliferate in carious lesions even in the presence of *Streptococci* and *Lactobacilli*.[54] Therefore, based on this evidence, the role of *Bifidobacterium* in inducing inflamed gums, carious lesions, endodontic infections, and infected human dentin should not be ignored.[52-55] Mantzourani *et al*. (2008) observed that high proportion of *Bifidobacteria* in the infected dentine from soft lesions (7.88 +/- 1.93) compared to those in leathery lesions ((1.61 +/- 0.91) and in plaque from sound exposed root surfaces (0.05 +/- 0.39).[53]

Studies have shown that the progression of demineralization and caries are higher when acid drinks are involved, along with the presence of cariogenic bacteria.[37,38] Therefore, even if *Lactobacilli* may not trigger the onset of caries, acid produced from probiotic strains can certainly induce demineralization and increase the risk of caries progression.[38] Dental caries has been observed in gnotobiotic rats inoculated with *Lactobacillus casei* alone, in the sulcular regions, without any plaque accumulation.[39-43] However, the acidity of the plaque increases in the presence of the probiotic bacteria.[28,32,35,36] Ahmed *et al*. (2019) evaluated "the lactic acid-producing ability of *Lactobacillus acidophilus* and *Lactobacillus plantarum,* with and without dental plaque." and concluded that the efficiency of acid production increased on combination with supragingival plaque.[35] Approximately 0.35 µg/dl amount of acid was found to be produced by Lactobacillus plantarum after fermentation of sucrose and glucose.[35] A reduction in pH was observed by Hedberg *et al.* on fermentation of sucrose and glucose at 5.2-6.8 pH by *Lactobacillus plantarum.*[42] The fall in the pH is also linked with the creation of antimicrobial substances. Few of these antimicrobial substances have also been associated with the buffering action, and innate defense mechanisms.

A small number of in-vivo studies that have established that 'continuous administration of probiotic drinks can cause superficial loss of calcium and phosphorous from enamel, and increase the risk of carious and erosive lesions formation'.[35-48] A recent study by Singh and Dole (2016) showed that traditional non-probiotic yogurt has more protective effects than probiotic enriched yogurt in preventing enamel demineralization.[50] Zulkapli *et al*. (2020) compared the erosive effects of different probiotic drinks and concluded that all probiotic drinks can lower the pH to below 5.[52] However, they exhibited low erosive activity as a consequence of the buffering action caused by the the high calcium content in the drink. Although no calcium loss was noted, steady loss in weight was measured over 7 days, except on repetitive exposure on days 3 and 5. It should be noted, however, that the extent of demineralization will be depend on the overall acidity of the drink, residence of the probiotic bacteria in oral cavity, and the nature of acids produced by the oral bacteria.[35]

Increased EPS Production

Apart from increased acid production, lactic acid bacteria (LAB) also extravasate large amounts of extracellular polysaccharides(EPS) that have been linked with increased acid production. EPS are 'long-chain polysaccharides consisting of repetitive units of sugars or sugar derivatives secreted into their surroundings in form of loose slime. The EPS commonly secreted by probiotic bacteria include mannose, galactose, glucose, N-acetyl galactosamine , N-acetylglucosamine, and rhamnose. The rate of biofilm formation is increased by EPS by providing the essential molecules needed for acid production. Studies have proven that cariogenic bacteria using the glycolysis pathway can convert the galactose and glucose to lactate, formate, and acetate and produce acids. The lactate is subsequently converted into lactate ions which react with enamel hydroxyapatite to initiate the release of calcium and phosphate ions. The accumulation of EPS even favors the proliferation and firm anchorage of other bacteria to the enamel surface that can increase the growth of the cariogenic biofilm. *Streptococcus mutans* and *Streptococcus sobrinus* can utilize the dextran present in the EPS for biofilm formation.[40] The fructans and glucans, commonly added in probiotic formulations, have shown to affect the adhesion of *Actinomyces viscosus, Streptococcus gordonii, Streptococcus mutans,* and *Streptococcus sobrinus,* to the hydroxyapatite, apart from other cariogenic bacteria, thereby contributing to dental plaque formation and increased risk of tooth decay.[40-45]

Conclusions and Future Perspectives

In conclusion, 3D modeling may affect the surgeons preoperative decision-making processes, especially regarding the operation duration, osteotomy boundaries/defect size and complication risk. In complex cases, this novel methodology can be utilized in order to provide a better surgical outcome both for the surgeons and the patients. The findings of this preliminary study need validation on larger sample groups, with addition of 3D printing process to the present test protocol.

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