

The *Streptococcus mutans* ability to survive in biofilms and during dental caries formation: scoping review

Anisauma UTAMANINGYAS¹, Hening Tjaturina PRAMESTI^{2*}, Felisha Febriane BALAFIF³

¹Bachelor of Dentistry, Faculty of Dentistry, Padjadjaran University, Indonesia

^{2,3}Department of Oral Biology, Faculty of Dentistry, Padjadjaran University, Indonesia

*Correspondence: hening.tjaturina@unpad.ac.id

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ABSTRACT. Caries are the deterioration of dental hard tissue caused by acidic byproducts of bacterial carbohydrate fermentation. The formation begins within the bacterial biofilm that covers the tooth's surface. *Streptococcus mutans* is the dominant bacteria in the biofilm, forming a multispecies biofilm on the tooth surface, growing, and surviving within it. *S. mutans* colony formation and acid formation can lead to tooth demineralization. The purpose of this scoping review is to determine the ability of *S. mutans* to survive in biofilms and during the formation of dental caries using articles from the chosen database. Articles published from 2016 until 2021 were searched for using the keywords: "*Streptococcus mutans* and caries or dental caries and survival ability or survivability and survival factor" in the PubMed, ScienceDirect, Cochrane, and Google scholar databases. Using PRISMA-Scr, existing articles were chosen based on inclusion and exclusion criteria. There were ten articles found that were suitable for review. The data presented in the article vary according to the study's location, purpose, method, and samples. The finding revealed that *S. mutans* survive in biofilms and caries formation due to their ability to activate enzymes, virulence factors of *S. mutans*, and the environmental conditions of the oral cavity. Aciduric; acidogenic; quorum sensing; ability to form GTFs, GBPs, ATPase, CSP, eDNA; and the ability to produce bacteriocin and autolysins all contribute to *Streptococcus mutans*' ability to survive in biofilms and during the formation of dental caries

KEYWORDS: biofilm, caries, survival ability, survival factor, *Streptococcus mutans*.

INTRODUCTION

In their early stages, dental biofilms are non-cariogenic. *Streptococcus mitis*, *S. gordonii*, and *Actinomyces sp.* are the first colonizers of dental biofilm. They adhere to the tooth surface with the help of salivary proteins and form a non-cariogenic three-dimensional biofilm with neutral pH. Commensal bacteria will produce H₂O₂ and antimicrobial proteins to prevent pathogenic bacteria like *S. mutans* from overgrowing. *S. mutans* overgrowth in the biofilm causes an imbalance of microbial biofilms, and the biofilm becomes cariogenic, making *S. mutans* the primary etiologic agent in human dental caries. The biofilm becomes cariogenic, so *S. mutans* is the primary etiologic agent in human dental caries.¹ The main natural habitat of *S. mutans* is the tooth surface of the biofilm.² These bacteria multiply and survive in the natural ecosystem of the dental biofilm, being the

dominant species in the multispecies biofilm along with other bacteria.

S. mutans dominance in dental biofilm development is characterized by the production of the enzyme fucosyltransferase (FTF), and three glucosyltransferases (GTFs); GTF-B, GTF-C, and GTF-D. *S. mutans* also encodes several cell surface-associated glucan binding proteins (GBPs) such as GBP-A, GBP-B, GBP-C, and GBP-D.³ GTFs and GBPs activity contributes to the structure of the intracellular and extracellular matrix that underpins construction and biofilm development. *S. mutans* uses the GBPs enzyme to support sucrose-dependent tooth surface attachment as a biofilm foundation. Surface-adsorbed GTF-B and GTF-C use dietary sucrose to synthesize insoluble and soluble glucans and provide an insoluble matrix for biofilm formation. GTF-D forms a soluble polysaccharide. Then acts as a primer for GTF-B synthesis,

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increasing exopolysaccharide synthesis. GTF-produced glucan molecules serve as avid binding sites on surfaces for *S. mutans* (and other microorganisms), mediating tight bacterial clustering and adherence to tooth enamel.^{4,5}

The presence of carbohydrates and sucrose in the oral cavity will cause *S. mutans* to ferment and produce lactic acid. An acidogenic factor is the ability to make this acid.⁶ Lactic acid in large quantities in the oral cavity can cause a pH drop above the critical limit (4.5-5) and can demineralize teeth. *S. mutans* is also an aciduric bacteria, allowing it to live at this critical pH. And it evens developing existing biofilm structures and colonizing with more aciduric bacteria, increasing the acidity level of the oral cavity.⁷ *S. mutans* ability to tolerate low pH or high acidity levels is a significant virulence factor in caries formation associated with dental biofilms.

The proton enzyme F-ATPase helps *S. mutans* survive in the acidic conditions of the dental biofilm. F-ATPase is a multimeric membrane-inserted enzyme that contains a functional subunit of the F₀ protein and the F₁ protein complex encoded by the *AtpF* and *AtpD* genes. The activity of F-ATPase keeps the biofilm's acidogenic and aciduric properties stable.⁸ Bacteriocin (mutacin) and autolysins are two other factors that contribute to caries formation. Bacteriocin, a peptide with antimicrobial activity against other bacterial chains, is produced by the *S. mutans* mutant. Bacteriocin contributes to *S. mutans* competitiveness for survival in multispecies oral biofilms, so *S. mutans* is considered an important pathogen of dental caries.⁹ Autolysis of *S. mutans* is required for biofilm maturation and biogenesis of a normal cell surface.¹⁰

MATERIALS AND METHODS

This study used scoping review method from January to May 2022. Figure 1 shows the research procedure was divided into three stages: preparation, implementation, and evaluation.¹⁶ The preparation stage begins with research questions centered on the PCC criteria. The study population was *S. mutans* that survive in biofilm and during caries formation. The concept was to examine *S. mutans* ability to survive in biofilm and during caries formation. The research context was a cross-sectional study, prospective cohort study, and randomized controlled trials articles used for this study.

The implementation phase used a prism scoping review procedure that began with identification. Articles were searched using the keywords: "*Streptococcus mutans* and caries or dental caries and survival ability or survivability and survival factor" in the PubMed, ScienceDirect,

The research findings show that *S. mutans* can survive in dental biofilms and is a dominant bacteria that cause dental caries. Valdez et al. found an increase in the prevalence of *S. mutans* with caries severity. *S. mutans* cell chains with a typical phenotype are found in higher concentrations in the biofilms of early caries children (ECC) and S-ECC (highly acid-tolerant) than in caries-free (CF) children. There was an association between the severity of children's caries and the number of *S. mutans*.¹¹ According to Esberg et al., 48% of children infected with *S. mutans* at the onset of caries had a twofold increase in def-s scores at 12 and 17 years of age. As well as an increase in caries compared to uninfected children aged five years.¹² Ghazal, T. S. et al. conducted a study that compared DMF-T values per person in the presence of 94% *S. mutans*.¹³

According to Basic Health Research, tooth decay/cavities/pain due to caries account for 45.3% of dental health problems in Indonesia.¹⁴ More than 90% of the world's population is affected by caries.¹⁵ *S. mutans* is the dominant bacteria that cause caries, as previously stated. So, one way to address the problem of rising caries prevalence in Indonesia and around the world is to prevent *S. mutans* overgrowth in the oral cavity, particularly in the dental biofilm. Prevention of *S. mutans* growth in dental biofilm will be most effective if we understand how *S. mutans* survive in that environment. The ability of *S. mutans* to survive in the dental biofilm and during the formation of dental caries is described in this scoping review study.

Cochrane, and Google Scholar databases. The research inclusion criteria, which include: articles published between 2016 and 2021 in both Indonesian and English; study subjects aged 3-21 years in healthy teeth, never had caries, and had no restorations; investigate *S. mutans* survival ability during the early stages of caries formation; and articles in the form of observational and randomized controlled trials. Exclusion criteria included paid full-text articles, previously restored teeth, malocclusion, use of orthodontic appliances, and systemic disease. The article was screened through several stages, including screening for article duplication using the Mendeley application. Screening for article titles and abstracts using the Rayyan website (inclusion/exclusion criteria) and screening for article eligibility by reading the entire article.

The evaluation stage consists of extracting the final screening articles one at a time and summarizing the results in a table based on an analytical framework that concludes a summary of the research to answer research questions. The data extraction investigated is depicted in Table 1. Each chosen article was described separately based on predetermined

criteria. The final findings included the existence of *S. mutans* during the caries formation process, from tooth attachment to the onset of caries formation. The effect of *S. mutans* and their virulence and what factors influence *S. mutans* ability to survive on biofilm formation and caries formation.

RESULTS

The results of the article identification stage yielded 1,379 articles with details in each database: 1,041 in PubMed, 40 in ScienceDirect, 134 in Cochrane, and 164 in Google Scholar. The database articles were then filtered for duplication, leaving 1,289 articles. The results of the duplication screening are then filtered based on the title and abstract's suitability with the previously determined inclusion and exclusion criteria. The second screening resulted in the removal of 19 articles. Ten articles were extracted to answer the research questions based on the eligibility criteria screening. Figure 2. depicts the article selection process using PRISMA's scoping review.

Table 1. contains the article extraction results, which include the title, author's name, year of publication, location, method, purpose, population or sample, and results of the selected research articles. Ten articles are obtained for extracting stage in this study. Five articles discussed the aciduric ability of *S. mutans*. Aciduric refers to *S. mutans*' ability to live in acidic or low-pH environments. It described that F-ATPase-mediated lipid membrane shift increases *S. mutans* virulence and viability in an acidic environment.¹⁷⁻²¹ Five articles discuss *S. mutans*' acidogenic ability, or ability to produce acid. The formation of low pH-dependent GTFs aids acid formation.^{19,22} The capability of *S. mutans* to produce acid from various fermentable sugars contributes to its virulence and tooth demineralization.^{17,21,22}

Four articles discuss *S. mutans*' ability to form GTF enzymes. GTFs can produce lactic acid and provide tight binding sites for *S. mutans* and

other microorganisms. GTFs catalyze the formation of water-soluble and water-insoluble glucans, which initiate biofilm attachment and colonization. Antibacterial compounds such as epigallocatechin-3-gallate (EGCG) and dextranase can be suppressed and increased in effective doses by GTFs protein.^{17,22} GBPs are glucan-binding proteins associated with acidity by *S. mutans* discussed in three articles. GBPs are attachments to tooth surfaces, facilitating sucrose-dependent bacterial colonization and biofilm aggregation.^{17,21,22} The formation of ATPase by *S. mutans* is discussed in five articles. The *atpF* gene encodes F-ATPase, which plays a vital role in acid tolerance in multispecies subunit complexes.¹⁷⁻²¹ F-ATPase is an enzyme that degrades ATP molecules into ADP and Pi ions, releasing energy while moving cell metabolites and exporting toxins to inhibit normal cell functions.

Other bacteria added to the biofilm did not reduce the dominance of *S. mutans* and autolysin in the presence of low pH, which caused cells to die and increased biofilm formation. In response to adverse physiological changes such as antibiotic exposure, *S. mutans* produces an autolysin that degrades its cell walls.^{17,22-25} *S. mutans* also produce bacteriocins, peptides with antimicrobial activity against other bacterial chains and help *S. mutans* survive in multispecies biofilms. Furthermore, *S. mutans* communicates with other bacteria via quorum sensing in the biofilm. The *S. mutans*' ability to communicate with other bacteria allows for the coordination of cell community in biofilm formation and expression of virulence gene.^{17,23}

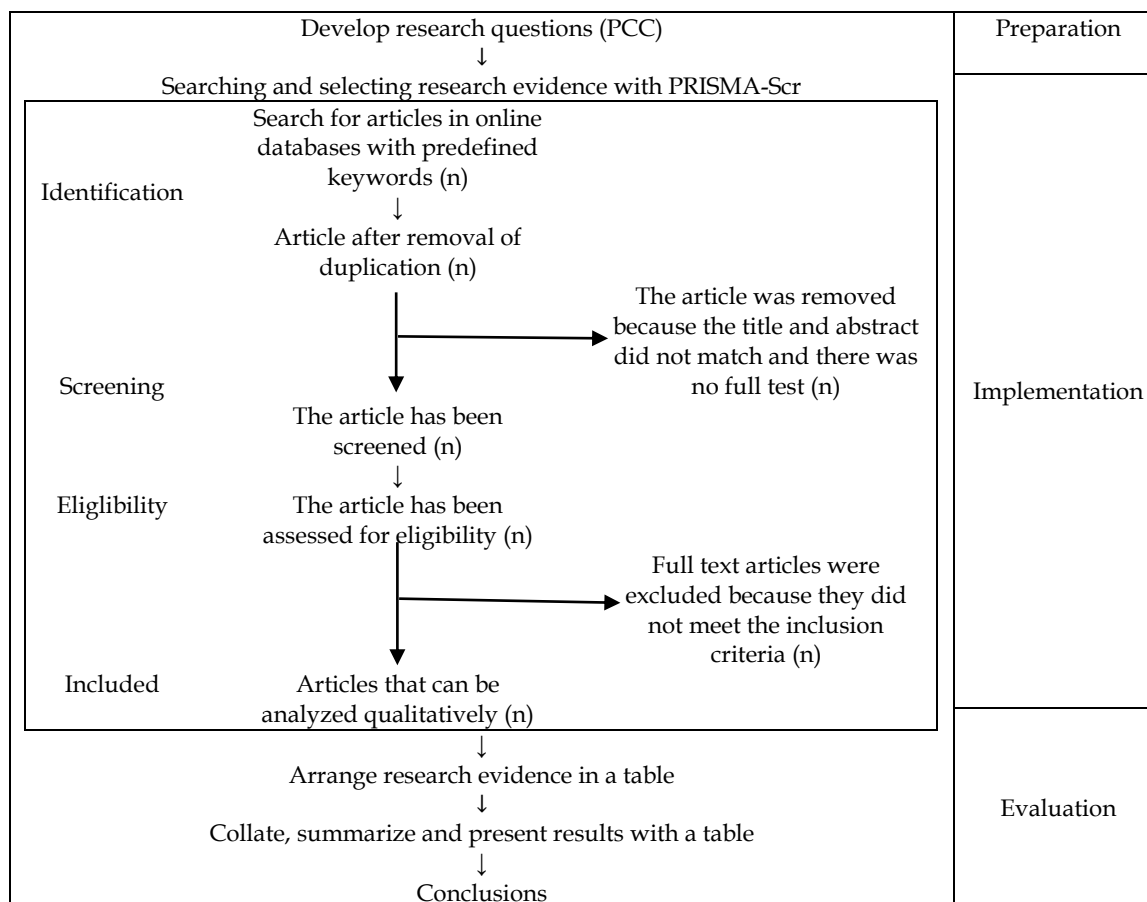


Figure 1. Stages of the research procedure

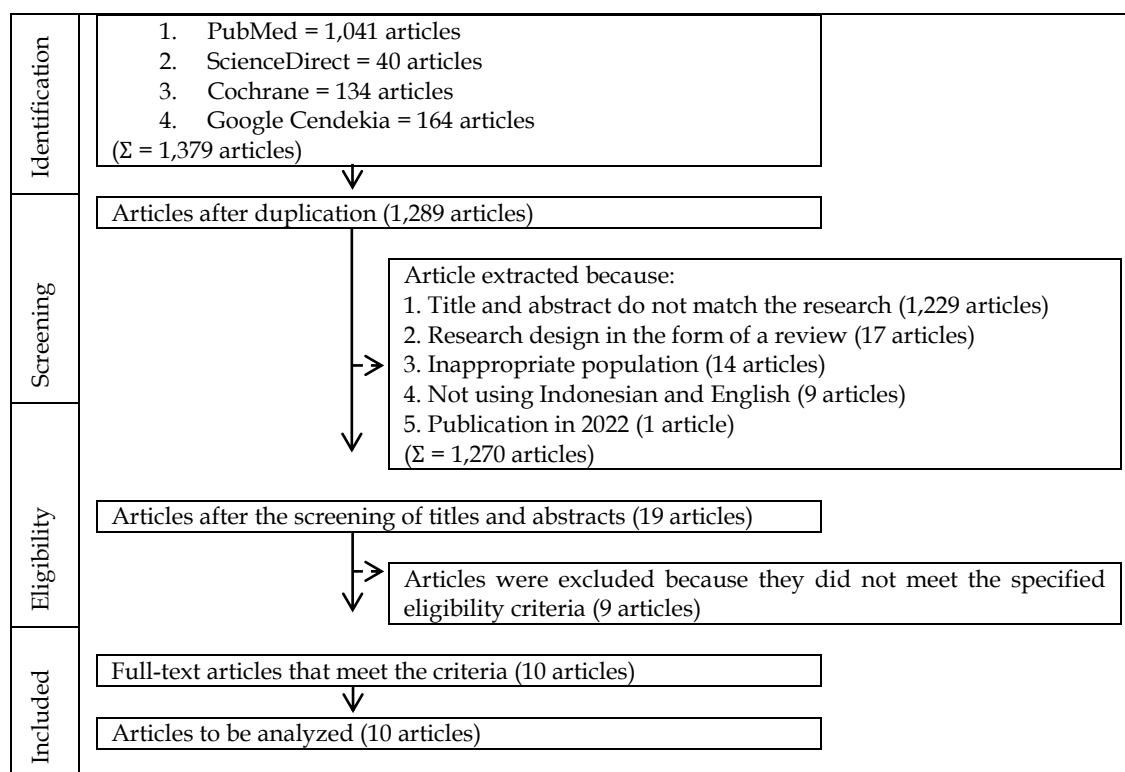


Figure 2. Flowchart of searching articles using PRISMA-Scr

Table 1. Extraction of data from 10 articles of final search

No	Author (Year)	Research Title	Research sites	Research methods	Research purposes	Population/Research Sample	Research result	
							Healthy teeth	Early dental caries
1	Rodrigu es et al. (2020)	Antimicrobial activity of <i>Lactobacillus fermentum</i> TcUESC01 against <i>Streptococcus mutans</i> UA159	Brazil	1. 16SrRNA gene sequencin g 2. Statistical test: kruskal-wallist, anova, bonferoni	To evaluate the effect of the fermentation product of <i>L. fermentum</i> on the viability and virulence factors of <i>S. mutans</i> UA159 in planktonic and biofilm models.	1. <i>S. mutans</i> UA 159 2. <i>L. fermentum</i> TcUESC01	The fermentation product of <i>L. fermentum</i> did not reduce the cell, acid production, cell membrane permeability, acid tolerance, and polysaccharide production of <i>S. mutans</i>	The tested biofilms inhibited <i>S. mutans</i> virulence factors by biosurfactant production, showing impaired adhesion and formation of <i>S. mutans</i> biofilms.
2	Valdez et al. (2016)	Comparative in vitro investigation of the cariogenic potential of bifidobacteria	Brazil	Statistical test: anova and tukey	Assessed in vitro cariogenic potential of several species of bifidobacterium compared with caries-causing bacteria.	1. <i>Bifidobacterium species</i> 2. <i>Lactobacillus species</i> 3. <i>Streptococcus species</i> 4. <i>Actinomyces species</i>	The percentage of living cells decreased at pH 2.8, except for <i>B. animalis</i> , <i>B. dentium</i> , <i>L. acidophilus</i> and <i>L. casei</i> . The ability to form biofilms was lower as a single species compared to multiple and multispecies species.	Multiple species and multispecies biofilms cause loss of surface hardness. The aciduric nature of bifidobacteria allows them to live in acidic carious lesions.
3	Villegas, Arango, dan Arias (2018)	Effects of a food enriched with probiotics on <i>Streptococcus mutans</i> and <i>Lactobacillus spp.</i> salivary counts in preschool children: a cluster randomized trial	Colombia	Statistical test: mann whitney U dan X ²	Evaluated milk supplementation with probiotic bacteria and standard milk, measuring levels of <i>S. mutans</i> and <i>Lactobacillus spp.</i>	Children aged 3-4 years, without systemic disease, intolerance to milk and antibiotics, and brushing teeth with fluoride paste min. 1 time/day.	Differences in CFU/mL of <i>S. mutans</i> , dental plaque, and pH were insignificant, while salivary buffering capacity was significant in the probiotic and control groups after 9 months.	Consumption of probiotics did not show any significance. There was no relationship between consumption of probiotics and the appearance of early caries during the intervention.
4	Wu et al. (2018)	Inhibitory effects of tea catechin epigallocatechin-3-gallate against biofilms formed from <i>Streptococcus mutans</i> and a probiotic lactobacillus strain	Taiwan	1.RNA extraction and qPCR 2. Statistical test: ANOVA and Tukey test	To determine the effect of catechin epigallocatechin-3-gallate (EGCG) on biofilm formation by <i>S. mutans</i> and probiotic <i>Lactobacillus casei</i> (LcY) on yakult.	1. <i>S. mutans</i> 2. <i>LcY</i>	EGCG increased the pH of the <i>Sm+LcY</i> and <i>LcY</i> biofilm culture media but reduced the <i>Sm</i> and <i>Sm+LcY</i> biofilm biomass. The presence of <i>LcY</i> and Gtfb increased the effective concentration of EGCG in inhibiting biofilm formation.	-
5	Chi et al (2016)	Milk Sweetened with Xylitol: A Proof-of-Principle Caries Prevention Randomized Clinical Trial	Peru	Statistical test:: chi-square, anova, cochran-mantel-hazel	Evaluating the efficiency of xylitol-sweetened milk as a caries prevention strategy.	153 kindergarten to 4th grade elementary school children from low-income families.	Xylitol milk does not prevent tooth decay over a short period in children at high caries risk.	Constant sugar intake over time makes many carious lesions contain colonies of <i>S. mutans</i>
6	Sekiya et al. (2019)	Proton-pumping F-ATPase plays an important role in <i>Streptococcus mutans</i> under acidic conditions	Japan	Enzyme test and Bacterial survival ability test	Reported the inhibitory effect of <i>E. coli</i> proton pumping F-type ATPase on <i>S. mutans</i> enzyme activity, growth and survival of <i>S. mutans</i> under acidic conditions.	F-ATPase from <i>E. coli</i> and <i>S. mutans</i> .	Enzyme activity, growth, and survival under acidic conditions suggest that <i>S. mutans</i> F-ATPase plays an important role in acid tolerance. Piceatannol, curcumin, and DMC significantly inhibited <i>S. mutans</i> F-ATPase under acidic	-

No	Author (Year)	Research Title	Research sites	Research methods	Research purposes	Population/Research Sample	Research result	
							Healthy teeth	Early dental caries
7	Miao, Wang, Zhang, Mode, dan Huangsh (2019)	Regulation of SmpB on acidogenic/aciduric ability and expression and activity of aciduric virulence factor in <i>Streptococcus mutans</i> from caries-sensitive children	China	Statistical test:: t test and anova	Exploring the role of SmpB on acidogenic and aciduric abilities and aciduric virulence factor expression in <i>S. mutans</i> from caries-sensitive children.	Saliva children aged 3-5 years.	Knockout SmpB inhibited the growth of <i>S. mutans</i> , SmpB deletion significantly reduced the acidogenic/aciduric ability of <i>S. mutans</i> .	SmpB regulates the acidogenic/aciduric ability and aciduric virulence factor of <i>S. mutans</i> ECC, which is sensitive at low pH, causing tooth demineralization
8	Kawarai et al. (2016)	<i>Streptococcus mutans</i> biofilm formation is dependent on extracellular DNA in primary low pH conditions	Japan	Statistical test:: t test	Observed the effect of low pH on primary culture conditions comparing results at pH 6 and 7.	1. <i>S. mutans</i> MT8148 2. <i>S. mutans</i> MT8148 gtfb	Increased biofilm formation with stimulation of CSP, TSB with glucose; production of eDNA without insoluble glucans; and decreased insoluble glucans at pH 6.	-
9	Bojanich dan Calderon (2017)	<i>Streptococcus mutans</i> membrane lipid composition: Virulence factors and structural parameters	Argentina	Statistical test:: kruskal-wallis.	Analyzed the location of the dental biofilm associated with a shift in the fatty acid membrane profile, and whether the shift could affect certain virulence factors of the <i>S. mutans</i> chain.	<i>S. mutans</i> was isolated from dental biofilms of children with a mean age of 6.2 years.	The control chain showed cell growth at pH 5 with the percentage of unsaturated fatty acids>saturated fatty acids.	The acidic environment promotes a shift in the membrane lipid profile. It is associated with higher aciduric properties and more developed virulence of <i>S. mutans</i> .
10	Cai, Meng, Liu, Cao, dan Wang (2021)	Suppressive effects of gecko cathelicidin on biofilm formation and cariogenic virulence factors of <i>Streptococcus mutans</i>	China	1. Real-time qPCR 2. Statistical test: t test	To determine the effectiveness of the gecko cathelicidin Gj-CATH2 on biofilm formation and cariogenic virulence factors of <i>S. mutans</i> , and to examine the mechanism of its function.	Gj-CATH2 peptide	Gj-CATH2 prevents <i>S. mutans</i> UA159 biofilm formation by downregulating the expression of biofilm genes, acidogenicity-associated genes, and all QS system-associated genes.	The effect of Gj-CATH2 was insufficient to prevent tooth demineralization caused by bacterial acid because it controlled F-ATPase as the main determinant of acid tolerance in <i>S. mutans</i> .

DISCUSSION

The oral cavity is covered by saliva for masticatory activity and to keep it warm and moist at 35-36° C.^{22,26} The oral cavity is at pH between 6.75 and 7.25, depending on the needs of microorganism growth. The salivary flow rate varies from person to person per day.²⁷ Physical and chemical changes in the composition of saliva, especially in buffering against dietary acids as well as metabolic acids and the ability to remineralize tooth enamel play a role in the development and progression of caries.²⁸ Consumption of a carbohydrate diet resulting in the acid of bacterial metabolism in the oral cavity causes a decrease in pH. The lower pH of the oral cavity causes the mineral crystals of the teeth to undergo local demineralization in the biofilm of the involved tooth surface.²⁹

The oral cavity is also a habitat for various bacteria with different compositions and numbers, including *Streptococcus mutans*.³⁰ *S. mutans* exist as a biofilm complex with other species in response to changes in the oral environment. *S. mutans* is a normal opportunistic flora. The imbalance of normal flora and host immunity will support the rapid growth or multiplication of *S. mutans*. This situation initiates the formation of pathogenic or cariogenic biofilms that have the potential to damage teeth and cause caries. Caries is a chronic disease that develops over time, is initially reversible, and can be stopped even when the dentin or enamel is destroyed to form a cavity as long as the cause of caries is removed.³⁰ Caries cannot develop without a cariogenic (pathogenic) biofilm and frequent exposure to carbohydrates, particularly free sugar.³¹ Caries in the oral cavity are frequently associated with bacterial metabolism in the biofilm, which causes the demineralization of teeth.¹⁷

The ability of *S. mutans* to produce GTFs enzymes is the first step in biofilm formation. GTFs secreted by *S. mutans* were incorporated into the pellicle and adsorbed on the bacterial surface, even without GTF production. GTF enzyme synthesis of both water-soluble and water-insoluble glucans of dietary sucrose. Changes in the amount of insoluble and soluble glucans between pH 6.0 and 7.0 are linked to a different reliance on biofilm formation and altered biofilm morphology.²³ Biofilm is attached to the tooth surface in two ways: dependent and independent of sucrose. The mechanism of GTFs in glucan synthesis initiates the sucrose-independent attachment to salivary components in the biofilm. While the sucrose-dependent extension is responsible for colonizing the tooth surface or bacterial immobilization on hard surfaces.^{24,32} Increasing GTF and GBP expression, EPS production, and *S.*

mutans adhesion sucrose-dependent, ultimately enhanced biofilm formation.²⁶ The maximum activity of GTF was seen between pH 5.5 and 6.5 and decreased at pH below 5.5.²³ The expression levels of GTF-B and GTF-D biofilm *Sm+LcY* were higher than the *Sm* biofilm when grown with 250 g/ml EGCG. This expression is an interaction between *S. mutans* and *LcY* which mediates the decrease in the inhibitory effect of EGCG on biofilm formation.³³

Streptococcus mutans and other bacteria that mediate selective bacterial aggregation and attachment to enamel use the glucan molecules as a strong adhesive of the surface binding site. GTFs and GBPs increase bacterial colonization by acting as acid producers in response to *S. mutans*' acidogenic ability. At the same time, the ability to tolerate acid in the biofilm maturation stage depends on membrane-bound F-ATPase. The *atpF* gene encodes an F-ATPase that regulates intracellular homeostasis by inducing proton pump activity and H⁺ transport from cells into extracellular media, which helps to maintain extracellular pH.

The maturation of the biofilm stimulates the formation of QS, which contains various enzymes such as bacteriocin (mutacin) and autolysin. *S. mutans* and other bacteria compete for adhesion sites and modify salivary pellicle protein composition. *S. mutans*' bacteriocin prevents the attachment of other bacteria so that other bacteria cannot bind to teeth, and *S. mutans* becomes the dominant species in the biofilm. Autolysin work in acidic conditions. These enzymes damage its *S. mutans* cell walls which lead to cell death. The autolysis mechanism decreases the number of cells in the biofilm community and increases antibiotic resistance. The ultimate goals is to maintain biofilm community balance. During biofilm formation, *S. mutans* also release extracellular DNA (eDNA) and become one of the components of the extracellular matrix. It maintains biofilm structural integrity, initiating adhesion to the dental surface, and facilitating horizontal gene transfer in QS.²³

This scoping review study shows that the experimental result of reviewed articles varies. The results depend on research locations, objectives, methods, samples, and populations. All articles did not discuss the exact number associated with the increase of *Streptococcus mutans* at the beginning of caries formation. All articles discussed virulence factors of *S. mutans* causing demineralization of the hard tissues of teeth. The research on bacterial culture can be biased between the bacterial culture environment and the oral cavity environment. Future studies can systematically assess the number of additions of *Streptococcus mutans* at the beginning of

caries formation compared to the number of caries-free teeth. Furthermore, further research was carried out on the FTF factor because there was no discussion

CONCLUSION

The ability of *Streptococcus mutans* to survive in the biofilm and during the formation of dental caries are: aciduric; acidogenic; quorum sensing; ability to form

of its virulence factors in biofilms and during caries formation.

GTFs, GBPs, ATPase, CSP, eDNA; and the ability to produce bacteriocin (mutacin) and autolysins.

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