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Biofilm Formation by *Streptococcus mutans* and Related Bacteria

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ABSTRACT

Caries is a disease of human dentition characterized by the loss of mineralized surfaces of the tooth; it is an infectious disease of the oral cavity in which biofilms play a causative role. Control of biofilms has traditionally relied on non-specific removal of plaque by mechanical means such as brushing, although it is difficult to remove biofilms by this method. Caries is also a widespread infection in children. *Streptococcus mutans* and *S. sobrinus* are important causative agents of caries. They produce a homologous exocellular polysaccharide called glucan, which strongly adheres to the enamel surface. This is a review of oral microbial biofilm formation by *S. mutans* and other related bacteria.

Keywords: Caries; *Streptococcus mutans*; *Streptococcus sobrinus*; Biofilm; Exocellular Polysaccharide; Glucan; Glucosyltransferase

1. Introduction

Caries has been characterized as an ecological collision in the mouth, involving infectious bacteria and readily available sugar in drinks and foods. *Streptococcus mutans* has been reported as the principal etiological agent of dental cavities and a normal inhabitant of dental plaque [1,2]. Oral microbial biofilms cause to form complex bacterial communities based on co-agglutination. Specific important pathogens in the oral biofilm include mutans streptococci, particularly *S. mutans* and *S. sobrinus*, which are highly associated with caries in humans. *S. mutans* readily metabolizes dietary sucrose to form insoluble polymers of glucose that aid in persistent colonization of solid surfaces. These bacteria can survive at low pH values that are toxic to most other bacterial species. Oral biofilms are exemplary and serve as a model system for bacterial adhesion [3]. In this review, we describe biofilm formation by *S. mutans* and related bacteria.

1.1. Dental Plaque and Decalcification in Caries

Dental plaque is caused by a complex and dynamic processes that involves the progressive destruction of tooth enamel, dentine and cementum by bacteria. Acid formation by cariogenic bacteria such as *S. mutans* drives the dissolution of calcium and phosphate in the hydroxyapatite crystal structure. Decalcification by acid from the bacteria is dependent on a variety of factors including the concentration of the carbon source, the amount and activity of the plaque microflora, the flow rate of saliva, and the nature of the tooth surface. The major species found in human dental plaque are *S. mutans* and *S. sobrinus*, *S. rattus*, *S. cricetus* and several mutans streptococci are cariogenic in animals [4,5]. The mutans streptococci are important etiological agents in dental caries.

1.2. Role of Biofilms in Caries

The etiology of caries disease is well established and bacterial colonization appears to be an important step for oral diseases, leading to biofilm formation [6-8]. Oral biofilms mostly consist of multiple bacterial strains. It has been recently shown that more than 700 bacterial strains are present in dental plaque [9]. Specific pathogens, including Gram-negative bacteria, can cause serious clinical diseases. The microflora in healthy individuals is dominated by the presence of Gram-positive bacte-

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ria, whereas patients with periodontitis have increased Gram-negative anaerobic rod-shaped bacteria [10]. There is communication between the multispecies in biofilms and the formation of biofilm can lead to antibiotic and oxygen resistance, resulting in failure to prevent or treat oral diseases.

Normally, the composition of human oral microflora is in a dynamic balance. When this balance is disturbed, the normal oral flora becomes disordered, leading to the development of dental plaque. High carbohydrate intake causes the accumulation of acidic metabolites within mature plaque, leading to a low pH environment. In addition, the number of acid-tolerant bacteria with cariogenic potential will increase significantly or bacterial virulence will be enhanced. In this circumstance, non-cariogenic plaque will change into cariogenic plaque producing a large number of pathogenic substances that can cause the development of dental caries. In particular, *S. sanguinis* and *S. oralis* are maintained under an ecological balance. *S. sanguinis* decreases after micro-ecological imbalance, which leads indirectly to the development of dental caries. The causative agents of acid production are not limited to *S. mutans*, but also include other bacteria in dental plaque, and it is actually the synergistic effect of oral bacteria in dental plaque that is the root cause of dental caries.

1.3. Metabolism by the Acidogenic Microflora and Acid Tolerance

Lactic acid is generated in the presence of high sugar concentrations, while low sugar concentrations lead to the production of acetic acid, formic acid and ethanol (**Figure 1**). Cariogenic bacteria such as *S. mutans* survive and grow in the mouths of caries-active patients due to the low resting pH in the plaque environment [11]. Survival in acidic environments depends on the ability of *S. mutans* and other acid-tolerant oral bacteria to maintain intracellular pH homeostasis using H^+/ATPase and acid end product efflux (**Figure 1**). Caries lesions can be near or below pH 4. Bowden and Hamilton [12] explained the acid tolerance response: 1) increased glycolytic activity provides proton-efflux; 2) the shift to a lower pH becomes optimum for glucose transport, the glycolytic pathway and proton impermeability; 3) decreased activity by the acid-sensitive, membrane-associated, sugar-specific enzyme IIs for the phosphotransferase sugar transport system is accompanied by an increase in the more acid tolerant, non-phosphotransferase transport activity; 4) increased specific activity of the H^+/ATPase responsible for proton efflux from cells in lower pH environments; 5) increased capacity to maintain pH at lower values; and 6) a shift to predominately homofermentative metabolism as indicated by increased lactate formation.

1.4. Glutamate Biosynthesis by *S. mutans*

S. mutans can be cultured anaerobically, but not aerobically, on minimal medium containing no amino acids with ammonia as the sole nitrogen source [13-15]. It is suggested that this organism possesses glutamate dehydrogenase (GDH), glutamine synthetase (GS) and glutamine oxoglutarate aminotransferase (glutamate synthase) (GOGAT) to assimilate ammonia [15,16]. The glutamate synthetic pathway that *S. mutans* uses under anaerobic conditions is illustrated in **Figure 2**. This

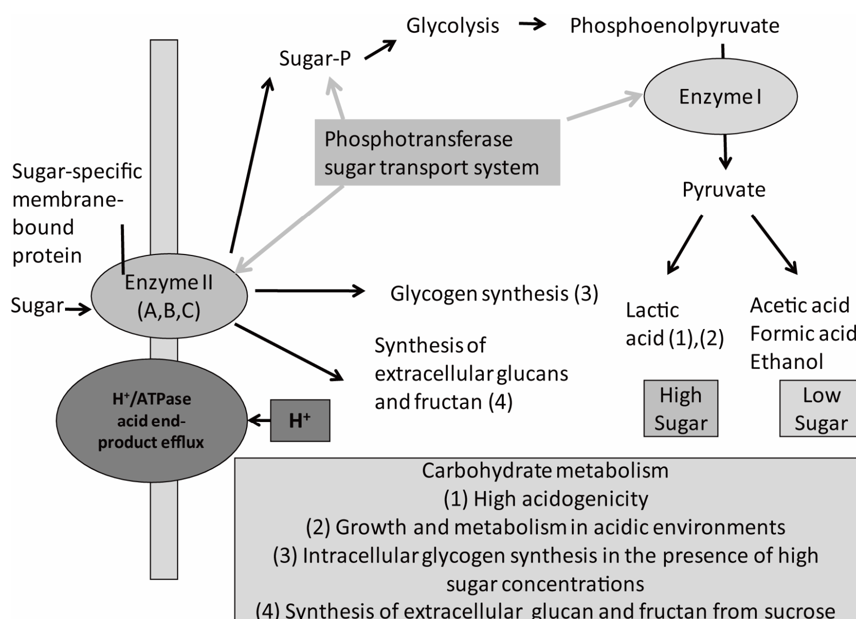

Figure 1. Sugar metabolism and acid formation in cariogenic bacteria.

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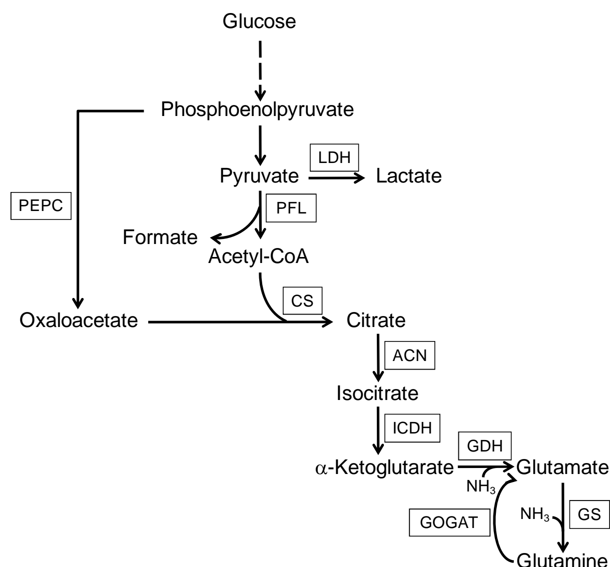

Figure 2. The glutamate synthesis pathway in *S. mutans*. Abbreviations: PEPC, phosphoenolpyruvate carboxylase; PFL, pyruvate formate-lyase; LDH, lactate dehydrogenase; CS, citrate synthase; ACN, aconitase; ICDH, isocitrate dehydrogenase; GDH, glutamate dehydrogenase; GS, glutamine synthetase; GOGAT, glutamine oxoglutarate aminotransferase (glutamate synthase).

organism can synthesize α-ketoglutarate, a substrate in the GDH reaction, using citrate synthase (CS), aconitase (ACN) and isocitrate dehydrogenase (ICDH) under anaerobic conditions [15-17]. However, under aerobic conditions, *S. mutans* is unable to synthesize α-ketoglutarate because PEPC, PFL and ACN enzymes lose their activity in the presence of oxygen [18].

Two major *S. mutans* virulence factors are acidogenicity (ability to produce acid via glycolysis) and aciduricity (ability to survive in a low pH environment) [19]. Aciduricity in *S. mutans* has been demonstrated in a number of studies [2]. Recently, it has been suggested that glutamate metabolism is associated with survival in low pH environments. Krastel *et al*. [20] reported a mutant deficient in the *glnQHMP* operon encoding a glutamine transporter which survived at pH 3.5 better than the parent strain. These results suggest that the glutamate transporter operon *glnQHMP* is involved in the acid-tolerance response in *S. mutans*.

1.5. Exocellular Polysaccharides (EPSs)

S. mutans is regarded as the main offending bacteria and producer of exocellular polysaccharides (EPSs) called glucans. EPSs are important in the first stage of caries development because they promote co-aggregation and colonization of cariogenic bacteria in biofilms.

Many *Streptococcus* strains can synthesize EPSs and they are generally produced as adhesins. *S. thermophilus* is the only "streptococcal" strain used in the food manufacturing process and is widely known as a yogurt starter. Bacterial EPSs are divided into homopolysaccharides and heteropolysaccharides according to whether they are composed of one or several kinds of sugar. *S. mutans* produces glucan, a type of homopolysaccharide, while heteropolysaccharides are synthesized by *S. thermophilus* strains.

1.6. Glucosyltransferase (GTF) and Glucan Formation in *S. mutans*

S. mutans synthesizes EPS (glucans) from the glucosyl residues of sucrose by secreting glucosyltransferases (GTFs). It is well known that *S. mutans* has at least three GTFs (GTF-B, C, and D) (**Table 1**). GTF-B and GTF-D mainly synthesize water-insoluble α-(1→3)- and water-soluble α-(1→6)-glucan. GTF-C associates with insoluble and soluble glucan synthesis, which is controlled by the genes *gtfB*, *gtfC*, and *gtfD* [21,22]. GTF-I, GTF-SI, and GTF-S are consistent in these genes. The correlation between the genetics and the properties of GTF-B, C, and D has been shown by various researchers [23-25]. In their reports, GTF-B, encoded by the *gtfB* gene on *S. mutans* GS-5, was a strongly hydrophilic protein consisting of 1475 amino acids with a molecular mass of 166 kDa. GTF-C was composed of 1375 amino acid residues (approx. 153 kDa) and was generally hydrophilic with three small hydrophobic domains. The *gtfC* gene (4218 bp) was located immediately downstream of the *gtfB* gene. A third enzyme, GTF-D, was found to have a molecular mass of 155 kDa and its kinetic parameters were identical to the GTF-S enzyme. These GTFs and glucansucrase (GS), produced by *Leuconostoc mesenteroides*, have high homology [26,27]. GTFs and GS have two functional domains: an N-terminal glucansucrase domain and a C-terminal glucan-binding domain (GBD). The N-terminal domain is noted as VR (variable region) as the properties of the signal peptide and core region vary according to changes in its composition and length [28]. The N-terminal domain catalyzes the transglycosylation reaction using sucrose as a substrate, transferring to low molecular mass acceptors [29,30]. On the other hand, the C-terminal domain of the GTFs binds to glucan polymers [29,31]. It seems that this domain plays a key role in the glucan structure. In *S. mutans* and *S. sobrinus*, the C-terminal domain has an acceptor site, called YG repeats, which binds both the 1,6-α-linked glucose residues of dextran and the 1,3-α-linked glucose residues. It has been also found YG repeats which were able to attach to either substrates [32,33]. Shar *et al*. showed that GBD of GTF I in *S. downei* was able to bind glucans with not only alternating α-1,3 and α-1,6 links, but also mainly α-1,3 or α-1,6 links [34] (**Table 1**). The detailed catalytic

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Table 1. Composition of GTFs and glucan patterns produced from *S. mutans* and other related bacteria.

Strain	Protein	Protein size (a.a.)	Gene	Ratio of glucan type	References
S. mutans GS-5	GTF B	1475	*gtfB*	*α*-(1→3) 87% *α*-(1→6) 13%	[23]
S. mutans GS-5	GTF C	1375	*gtfC*	*α*-(1→3) 85% *α*-(1→6) 15%	[24]
S. mutans GS-5	GTF D	1430	*gtfD*	*α*-(1→3) 30% *α*-(1→6) 70%	[25]
L. mesenteriodes NRRL B-1299		1290	*dsrA*	*α*-(1→3) 15% *α*-(1→6) 85%	[26]
L. mesenteriodes NRRL B-1299		1508	*dsrB*	*α*-(1→3) 5% *α*-(1→6) 95%	[27]
S. downei Mfe28	GTF I	1556	*gtfI*	*α*-(1→3) 88% *α*-(1→6) 12%	[34]
S. downei Mfe28	GTF S	1328	*gftS*	*α*-(1→3) 10% *α*-(1→6) 90%	[34]
S. sobrinus 6715	GTF-I	1592	*gtfIa*	*α*-(1→3)	[29,35]
S. sobrinus B13N	GTF-S_1		*gftU*	*α*-(1→6) *α*-(1→3,6)	[35,36]
S. sobrinus OMZ176	GTF-S_2	1542	*gftT*	*α*-(1→3) 16% *α*-(1→6) 73% *α*-(1→3,6) 5%	[35,37]
S. sobrinus	GTF-S_3		*gftS*		[35,36]

mechanisms and structures of GTFs and GS are not yet fully understood. Recently, it has been found that both GTF-B and GTF-C are necessary to synthesize glucans, and it is important to activate GTF-B on *S. mutans* for formation of microcolonies [21].

1.7. Glucan from *S. sobrinus*

Four types of GTFs occur in *S. sobrinus*, GTF-I, GTF-S_1, GTF-S_2, and GTF-S_3, which are encoded by *gtfI*, *gtfU*, *gtfT*, and *gtfS*, respectively [35,36] (**Table 1**). A high level of homology is recognized between the four enzymes and all members of GS proteins GH70. GTF-I (175 kDa) synthesizes water-insoluble glucan (*α*-1,3 glucan). GTF-S_1 (GTF-U) is activated by the addition of water-soluble glucan (*α*-1,6 glucan with *α*-1,3,6 branch linkages) and GTF-S_2 produces *α*-1,6 glucan with *α*-1,3 glucan. These two enzymes can hydrolyze sucrose and synthesize water-soluble glucan containing both *α*-1,3 and *α*-1,6 linkages. On the other hand, GTF-S_3 (155 kDa) hydrolyzes sucrose and synthesizes water-soluble *α*-1,6 glucan (oligo-isomaltosaccharides). Hanada *et al.* [37] showed that GTF-S_2 has three repeated sequences of 51 to 52 amino acids, a partial repeat of 18 amino acids and *gtfS* present in the region immediately upstream of the *gtfT* gene. They identified the *gtfU* gene and GTF-S_1 enzyme from the *S. sobrinus* strain B13N [36]. C-terminal fragments of the GBD of *S. sobrinus* GTF-I were prepared and shown that GBD bound tightly to a stretch of dextran chain through the combination of individually weak subsite/glucose interactions accompanied by the entropy change. Recently, glucan synthesis was correlated with its kinetic properties and the time course of saccharide production [38].

The sizes and structures of synthesized glucans vary according to the strain. Glucans are divided into four types based on their different main glucosidic linkages: *α*(1-6) glucosidic bonds (dextran); *α*(1-3) glucosidic bonds (mutan); *α*(1-4) glucosidic bonds (reuteran); and both *α*(1-6) and *α*(1-3) glucosidic bonds (alternan). Glucans vary due to branch linkage types, the degree of branching, the length of branch chains, and their spatial arrangement [22]. However, there is very little knowledge about the detailed structure of glucans to date.

1.8. Sugar Components and Structures of EPS in *S. thermophilus*

The sugar components of EPS synthesized from *S. thermophilus* are mainly galactose, glucose, and rhamnose. Furthermore, N-acetylgalactosamine, fucose, and ribose are also components of EPS (**Figure 3**) [39-49]. Galactose includes not only galactopyranose, but also galactofuranose. *S. thermophilus* synthesizes EPS via intracellular sugar nucleotide precursors (**Figure 4**). Metabolic flux via sugar nucleotide precursors of EPS in *S. ther-*

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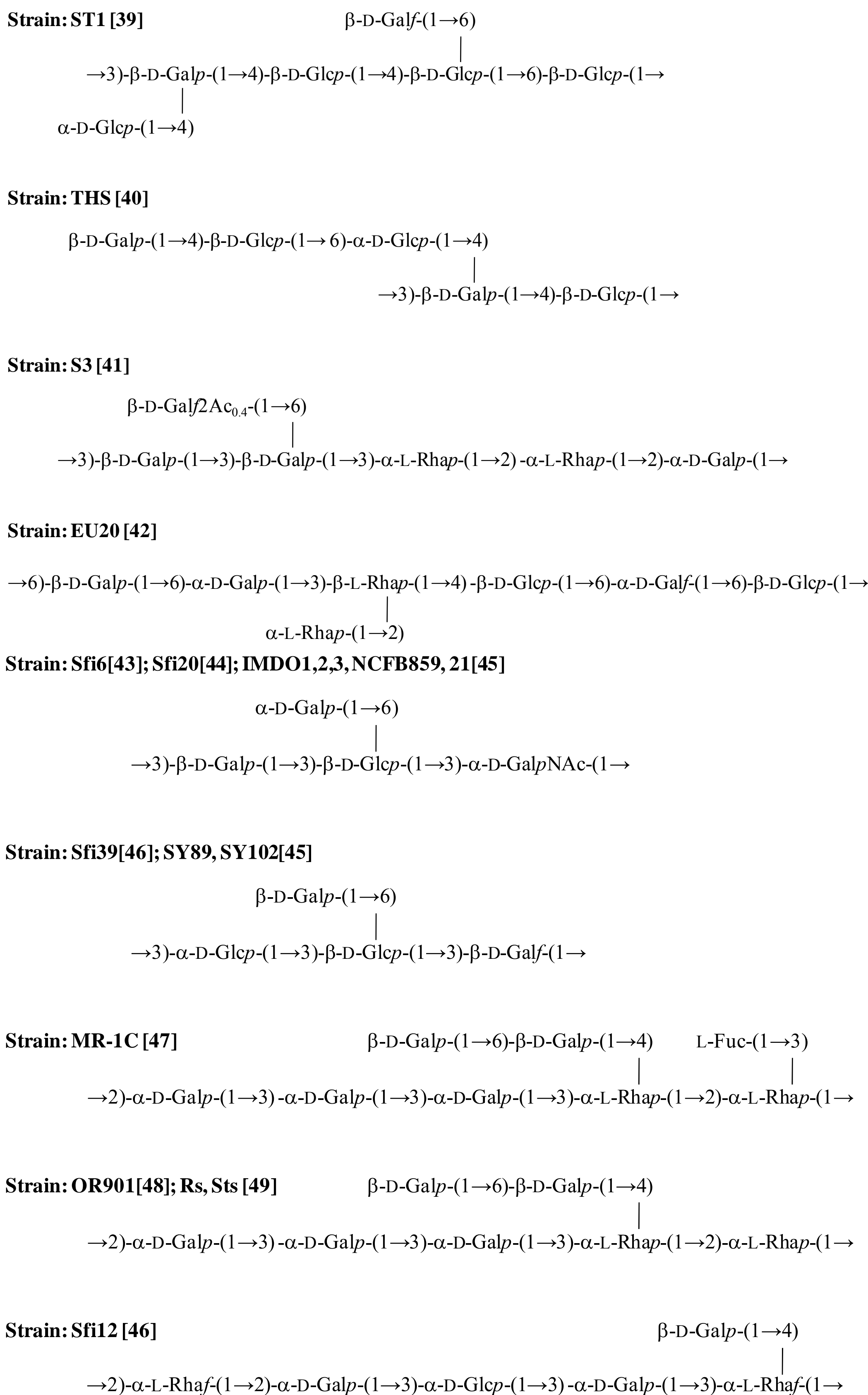

Figure 3. Structures of exocellular polysaccharide produced from *S. thermophilus*.

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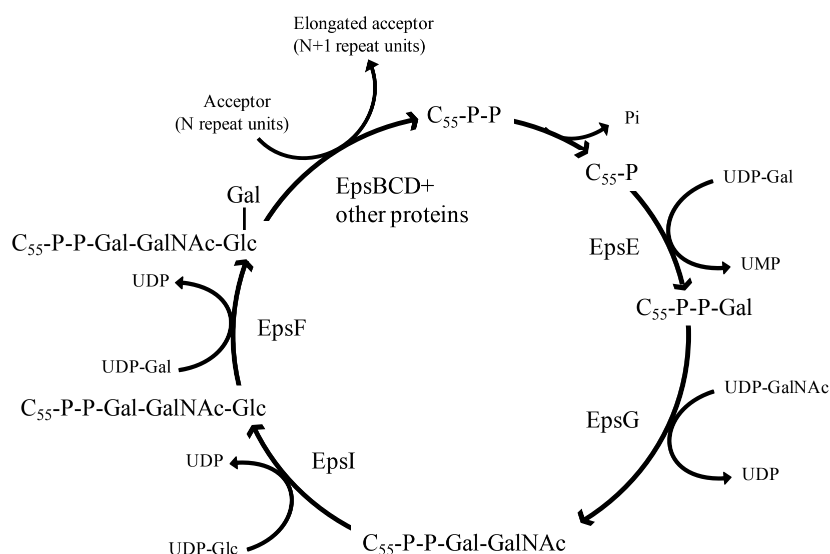

Figure 4. The model of EPS biosynthesis on *S. thermophilus*. The series of EPS metabolism was revealed by Stingele *et al.* [40,41].

mophilus has been found [43,50,51]. The mechanism of EPS production in this species is common in streptococcal systems. The EPS repeating unit is accomplished by multiple glycosyltransferases. These additional enzymes participate in *eps* gene regulation, membrane translocation and polymerization [50,52]. EPS gene cluster is found on chromosome. Stingele *et al.* reported the first genetic study of EPS production in lactic acid bacteria [43]. *S. thermophilus* Sfi6 has a gene cluster consisting of 13 ORFs (*epsA* to *epsM*; encoded within a 14.5 kb region). EPS expression is regulated by *epsA*, and *epsE*, *epsF*, *epsG*, *epsH* and *epsI*, which show homology with glycosyltransferases, catalyze the biosynthesis of the EPS repeating unit. The regions of EpsC to D and EpsJ to K play a role in the polymerization and export of the EPS. Furthermore, Germond *et al.* [53] identified a 20 kb gene cluster consisting of 15 ORFs with three IS-elements in *S. thermophilus* Sfi39. They demonstrated that *epsA* and *epsB* encode protein for regulating gene expression, on the other hand, *epsC* and *epsD* encode protein for chain length determination and export. Furthermore, *epsE*, *epsF*, *epsG* and *epsH* were found to contribute to the synthesis of EPS repeating units. The region encoding *orf*14.9 at the 3' end of the gene cluster is conserved in both strains. IS-elements and partial ORFs seem to provide genetic variability in *S. thermophilus* Sfi39.

2. Conclusion

Dental caries causes acid formation by cariogenic bacteria such as *S. mutans* and results from the interaction of *S. mutans* and other related bacteria by production of biofilm on tooth surfaces. Exocellular glucosyltransferases (GTFs) produced by these bacteria play a key role in biofilm formation. *S. mutans* has at least three GTFs, whereas *S. sobrinus* has four. Branch linkage types, the degree of branching, the length of branch chains vary according to the kind of GTFs and the strain. Engineering approaches of glucosyltransferase would provide valuable substances to prevent or treat oral diseases in the future.

REFERENCES

[1] S. Hamada and H. E. Slade, "Biology, Immunology, and Cariogenicity of *Streptococcus mutans*," *Microbiological Reviews*, Vol. 44, No. 2, 1980, pp. 331-384.

[2] J. A. Lemos and R. A. Burne, "A Model of Efficiency: Stress Tolerance by *Streptococcus mutans*," *Microbiology*, Vol. 154, Pt. 11, 2008, pp. 3247-3255. doi:10.1099/mic.0.2008/023770-0

[3] R. Bos, H. C. van der Mei and H. J. Busscher, "Physico-Chemistry of Initial Microbial Adhesive Interactions—Its Mechanisms and Methods for Study," *FEMS Microbiological Reviews*, Vol. 23, No. 2, 1999, pp.179-230. doi:10.1016/S0168-6445(99)00004-2

[4] F. Ota, M. Kiso, K. Okada, H. Kato, K. Hirota, K. Fukui, M. Yasuoka, M. Ono, K. Uegaki and Y. Morimoto, "*Streptococcus mutans* Serotype b Strain (*St. rattus*, Coykendall): First Isolation in Japan from Human Dental Plaque," *Microbiology and Immunology*, Vol. 29, No. 10, 1985, pp. 1005-1110.

[5] W. J. Loesche, "Dental Caries: A Treatable Infection," Charles C. Thomas Publisher, Springfield, 1982.

[6] H. J. Busscher and H. C. van der Mei, "Physico-Chemical Interactions in Initial Microbial Adhesion and Relevance for Biofilm Formation," *Advances in Dental Research*, Vol. 11, No. 1, 1997, pp. 24-32. doi:10.1177/08959374970110011301

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[7] W. J. Loesche, "Role of *Streptococcus mutans* in Human Dental Decay," *Microbiological Reviews*, Vol. 50, No. 4, 1986, pp. 353-380.

[8] D. H. Meyer and P. M. Fives-Taylor, "Oral Pathogens: from Dental Plaque to Cardiac Disease," *Current Opinion in Microbiology*, Vol. 1, No. 1, 1998, pp. 88-95. doi:10.1016/S1369-5274(98)80147-1

[9] J. A. Aas, B. J. Paster, L. N. Stokes, I. Olsen and F. E. Dewhirst, "Defining the Normal Bacterial Flora of the Oral Cavity," *Journal of Clinical Microbiology*, Vol. 43, No. 11, 2005, pp. 5721-5732. doi:10.1128/JCM.43.11.5721-5732.2005

[10] K. Hojo, S. Nagaoka, T. Ohshima and N. Maeda, "Bacterial Interactions in Dental Biofilm Development," *Journal of Dental Research*, Vol. 88, No. 11, 2009, pp. 982-990. doi:10.1177/0022034509346811

[11] J. van Houte, "Bacterial Specificity in the Etiology of Dental Caries," *International Dental Journal*, Vol. 30, No. 4, 1980, pp. 305-326.

[12] G. H. Bowden and I. R. Hamilton, "Survival of Oral Bacteria," *Critical Reviews in Oral Biology and Medicine*, Vol. 9, No. 1, 1998, pp. 54-85. doi:10.1177/10454411980090010401

[13] J. Carisson, "Nutritional Requirements of *Streptococcus mutans*," *Caries Research*, Vol. 4, No. 4, 1970, pp. 305-320. doi:10.1159/000259653

[14] B. Terleckj and D. Shockman, "Amino Acid Requirements of *Streptococcus mutans* and Other Oral Streptococci," *Infection and Immunity*, Vol. 11, No. 4, 1975, pp. 656-664.

[15] C. J. Griffith and J. Carlsson, "Mechanism of Ammonia Assimilation in Streptococci," *Journal of General Microbiology*, Vol. 82, No. 2, 1974, pp. 253-260. doi:10.1099/00221287-82-2-253

[16] E. J. St. Martin and C. L. Wittenberger, "Regulation and Function of Ammonia-Assimilating Enzymes in *Streptococcus mutans*," *Infection and Immunity*, Vol. 28, No. 1, 1980, pp. 220-224.

[17] D. G. Cvitkovitch, J. A. Gutierrez and A. S. Bleiweis, "Role of the Citrate Pathway in Glutamate Biosynthesis by *Streptococcus mutans*," *Journal of Bacteriology*, Vol. 179, No. 3, 1997, pp. 650-655.

[18] N. Takahashi, K. Abbe, S. Takahashi-Abbe and T. Yamada, "Oxygen Sensitivity of Sugar Metabolism and Interconversion of Pyruvate Formate-Lyase in Intact Cells of *Streptococcus mutans* and *Streptococcus sanguis*," *Infection and Immunity*, Vol. 55, No. 3, 1987, pp. 652-656.

[19] J. A. Lemos, J. Abranches and R. A. Burne, "Responses of Cariogenic Streptococci to Environmental Stresses," *Current Issues in Molecular Biology*, Vol. 7, No. 1, 2005, pp. 95-107.

[20] K. Krastel, D. B. Senadheera, R. Mair, J. S. Downey, S. D. Goodman and D. G. Cvvitkovitch, "Characterization of a Glutamate Transporter Operon, *glnQHMP*, in *Streptococcus mutans* and Its Role in Acid Tolerance," *Journal of Bacteriology*, Vol. 192, No. 4, 2010, pp. 984-993. doi:10.1128/JB.01169-09

[21] H. Koo, J. Xiao, M. I. Klein and J. G. Jeon, "Exopolysaccharides Produced by *Streptococcus mutans* Glucosyltransferases Modulate the Establishment of Microcolonies within Multispecies Biofilms," *Journal of Bacteriology*, Vol. 192, No. 12, 2010, pp. 3024-3032. doi:10.1128/JB.01649-09

[22] V. Monchois, R. M. Willemot and P. Monsan, "Glucansucrases: Mechanism of Action and Structure-Function Relationships," *FEMS Microbiology Reviews*, Vol. 23, 1999, pp. 131-151.

[23] T. Shiroza, S. Ueda and H. K. Kuramitsu, "Sequence Analysis of the gtfB Gene from *Streptococcus mutans*," *Journal of Bacteriology*, Vol. 169, No. 9, 1987, pp. 4263-4270.

[24] S. Ueda, T. Shiroza and H. K. Kuramitsu, "Sequence Analysis of the gtfC Gene from *Streptococcus mutans* GS-5," *Gene*, Vol. 69, No. 1, 1988, pp. 101-109. doi:10.1016/0378-1119(88)90382-4

[25] N. Hanada and H. K. Kuramitsu, "Isolation and Characterization of the *Streptococcus mutans* gtfD Gene, Coding for Primer-Dependent Soluble Glucan Synthesis," *Infection and Immunity*, Vol. 57, No. 7, 1989, pp. 2079-2085.

[26] V. Monchois, R. M. Willemot, M. Remaud-Simeon, C. Croux and P. Monsan, "Cloning and Sequencing of a Gene Coding for a Novel Dextransucrase from *Leuconostoc mesenteroides* NRRL B-1299 Synthesizing Only *α*(1-6) and *α*(1-3) Linkages," *Gene*, Vol. 182, No. 1-2, 1996, pp. 23-32. doi:10.1016/S0378-1119(96)00443-X

[27] V. Monchois, M. Remaud-Simeon, P. Monsan and R. M. Willemot, "Cloning and Sequencing of a Gene Coding for an Extracellular Dextransucrase (DSRB) from *Leuconostoc mesenteroides* NRRL B-1299 Synthesizing Only a *α*(1-6) Glucan," *FEMS Microbiology Letters*, Vol. 159, No. 2, 1998, pp. 307-315. doi:10.1111/j.1574-6968.1998.tb12876.x

[28] S. A. van Hijum, S. Kralj, L. K. Ozimek, L. Dijkhuizen and I. G. van Geel-Schutten, "Structure-Function Relationships of Glucansucrase and Fructansucrase Enzymes from Lactic Acid Bacteria," *Microbiology and Molecular Biology Reviews*, Vol. 70, No. 1, 2006, pp. 157-176. doi:10.1128/MMBR.70.1.157-176.2006

[29] N. Konishi, Y. Torii, T. Yamamoto, A. Miyagi, H. Ohta, K. Fukui, S. Hanamoto, H. Matsuno, H. Komatsu, T. Kodama and E. Katayama, "Structure and Enzymatic Properties of Genetically Truncated Forms of the Water-Insoluble Glucan-Synthesizing Glucosyltransferase from *Streptococcus sobrinus*," *Journal of Biochemistry*, Vol. 126, No. 2, 1999, pp. 287-295. doi:10.1093/oxfordjournals.jbchem.a022447

[30] G. Mooser, "Glycosidases and glycosyltransferases," In: D. Sigman, Ed., *The Enzymes*, 3rd Edition, Vol. XX, Academic Press, New York, 1992, pp. 187-233.

[31] H. Abo, T. Matsumura, T. Kodama, H. Ohta, K. Fukui, K. Kato and H. Kagawa, "Peptide Sequences for Sucrose Splitting and Glucan Binding within *Streptococcus sobrinus* Glucosyltransferase (Water-Insoluble Glucan Synthetase)," *Journal of Bacteriology*, Vol. 173, No. 3, 1991, pp. 989-996.

[32] P. M. Giffard and N. A. Jacques, "Definition of a Fundamental Repeating Unit in Streptococcal Glucosyltrans-

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ferase Glucan-Binding Regions and Related Sequences," *Journal of Dental Research*, Vol. 73, No. 6, 1994, pp. 1133-1141.

[33] K. B. Kingston, D. M. Allen and N. A. Jacques, "Role of the C-Terminal YG Repeats of the Primer-Dependent Streptococcal Glucosyltransferase, GtfJ, in Binding to Dextran and Mutan," *Microbiology*, Vol. 148, Pt. 2, 2002, pp. 549-558.

[34] D. S. Shah, G. Joucla, M. Remaud-Simeon and R. R. Russell, "Conserved Repeat Motifs and Glucan Binding by Glucansucrases of Oral Streptococci and *Leuconostoc mesenteroides*," *Journal of Bacteriology*, Vol. 186, No. 24, 2004, pp. 8301-8308. doi:10.1128/JB.186.24.8301-8308.2004

[35] A. Nanbu, M. Hayakawa, K. Takada, N. Shinozaki, Y. Abiko and K. Fukushima, "Production, Characterization, and Application of Monoclonal Antibodies Which Distinguish Four Glucosyltransferases from *Streptococcus sobrinus*," *FEMS Immunology and Medical Microbiology*, Vol. 27, No. 1, 2000, pp. 9-15. doi:10.1111/j.1574-695X.2000.tb01405.x

[36] N. Hanada, K. Fukushima, Y. Nomura, H. Senpuku, M. Hayakawa, H. Mukasa, T. Shiroza and Y. Abiko, "Cloning and Nucleotide Sequence Analysis of the *Streptococcus sobrinus* gtfU Gene that Produces a Highly Branched Water-Soluble Glucan," *Biochimica et Biophysica Acta*, Vol. 1570, 2001, pp. 75-79. doi:10.1016/S0304-4165(01)00240-9

[37] N. Hanada, Y. Isobe, Y. Aizawa, T. Katayama, S. Sato and M. Inoue, "Nucleotide Sequence Analysis of the gtfT Gene from *Streptococcus sobrinus* OMZ176," *Infection and Immunity*, Vol. 61, No. 5, 1993, pp. 2096-2103.

[38] H. Komatsu, Y. Abe, K. Eguchi, H. Matsuno, Y. Matsuoka, T. Sadakane, T. Inoue, K. Fukui and T. Kodama, "Kinetics of Dextran-Independent α-(1→3)-Glucan Synthesis by *Streptococcus sobrinus* Glucosyltransferase I," *The FEBS Journal*, Vol. 278, No. 3, 2011, pp. 531-540. doi:10.1111/j.1742-4658.2010.07973.x

[39] E. Säwén, E. Huttunen, X. Zhang, Z. Yang and G. Widmalm, "Structural Analysis of the Exopolysaccharide Produced by *Streptococcus thermophilus* ST1 Solely by NMR Spectroscopy," *Journal of Biomolecular NMR*, Vol. 47, No. 2, 2010, pp. 125-134. doi:10.1007/s10858-010-9413-0

[40] E. L. Nordmark, Z. Yang, E. Huttunen and G. Widmalm, "Structural Studies of an Exopolysaccharide Produced by *Streptococcus thermophilus* THS," *Biomacromolecules*, Vol. 6, No. 1, 2005, pp. 105-108. doi:10.1021/bm0496514

[41] E. J. Faber, M. J. van den Haak, J. P. Kamerling and J. F. Vliegenthart, "Structure of the Exopolysaccharide Produced by *Streptococcus thermophilus* S3," *Carbohydrate research*, Vol. 331, No. 2, 2001, pp.173-182. doi:10.1016/S0008-6215(01)00013-1

[42] V. M. Marshall, H. Dunn, M. Elvin, N. McLay, Y. Gu and A. P. Laws, "Structural Characterisation of the Exopolysaccharide Produced by *Streptococcus thermophilus* EU20," *Carbohydrate Research*, Vol. 331, No. 4, 2001, pp. 413-422. doi:10.1016/S0008-6215(01)00052-0

[43] F. Stingele, J. R. Neeser and B. Mollet, "Identification and Characterization of the eps (Exopolysaccharide) Gene Cluster from *Streptococcus thermophilus* Sfi6," *Journal of Bacteriology*, Vol. 178, No. 6, 1996, pp. 1680-1690.

[44] L. Navarini, A. Abatangelo, C. Bertocchi, E. Conti, M. Bosco and F. Picotti, "Isolation and Characterization of the Exopolysaccharide Produced by *Streptococcus thermophilus* SFi20," *International Journal of Biological Macromolecules*, Vol. 28, No. 3, 2001, pp. 219-226. doi:10.1016/S0141-8130(01)00118-0

[45] V. M. Marshall, A. P. Laws, Y. Gu, F. Levander, P. Rådström, L. De Vuyst, B. Degeest, F. Vaningelgem, H. Dunn and M. Elvin, "Exopolysaccharide-Producing Strains of Thermophilic Lactic Acid Bacteria Cluster into Groups According to Their EPS Structure," *Letters in Applied Microbiology*, Vol. 32, No. 6, 2001, pp. 433-437. doi:10.1046/j.1472-765X.2001.00937.x

[46] J. Lemoine, F. Chirat, J. M. Wieruszeski, G. Strecker, N. Favre and J. R. Neeser, "Structural Characterization of the Exocellular Polysaccharides Produced by *Streptococcus thermophilus* SFi39 and SFi12," *Applied and Environmental Microbiology*, Vol. 63, No. 9, 1997, pp. 3512-3518.

[47] D. Low, J. A. Ahlgren, D. Horne, D. J. McMahon, C. J. Oberg and J. R. Broadbent, "Role of *Streptococcus thermophilus* MR-1C Capsular Exopolysaccharide in Cheese Moisture Retention," *Applied and Environmental Microbiology*, Vol. 64, No. 6,1998, pp. 2147-2151.

[48] W. A. Bubb, T. Urashima, R. Fujiwara, T. Shinnai and H. Ariga, "Structural Characterisation of the Exocellular Polysaccharide Produced by *Streptococcus thermophilus* OR 901," *Carbohydrate research*, Vol. 301, No. 1-2, 1997, pp. 41-50. doi:10.1016/S0008-6215(97)00083-9

[49] E. J. Faber, P. Zoon, J. P. Kamerling and J. F. Vliegenthart, "The Exopolysaccharides Produced by *Streptococcus thermophilus* Rs and Sts Have the Same Repeating Unit but Differ in Viscosity of Their Milk Cultures," *Carbohydrate Research*, Vol. 310, No. 4, 1998, pp. 269-276. doi:10.1016/S0008-6215(98)00189-X

[50] J. R. Broadbent, D. J. McMahon, D. L. Welker, C. J. Oberg and S. Moineau, "Biochemistry, Genetics, and Applications of Exopolysaccharide Production in *Streptococcus thermophilus*: A Review," *Journal of Dairy Science*, Vol. 86, No. 2, 2003, pp. 407-423. doi:10.3168/jds.S0022-0302(03)73619-4

[51] F. Stingele, J. W. Newell and J. R. Neeser, "Unraveling the Function of Glycosyltransferases in *Streptococcus thermophilus* Sfi6," *Journal of Bacteriology*, Vol. 181, No. 20, 1999, pp. 6354-6360.

[52] A. D. Welman and I. S. Maddox, "Exopolysaccharides from Lactic Acid Bacteria: Perspectives and Challenges," *Trends in Biotechnology*, Vol. 21, No. 6, pp. 269-274. doi:10.1016/S0167-7799(03)00107-0

[53] J. E. Germond, M. Delley, N. D'Amico and S. J. Vincent, "Heterologous Expression and Characterization of the Exopolysaccharide from *Streptococcus thermophilus* Sfi39," *European Journal of Biochemistry/FEBS*, Vol. 268, No. 19, 2001, pp. 5149-5156.

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