Japanese Dental Science Review 58 (2022) 328–335

Contents lists available at ScienceDirect

Japanese Dental Science Review

journal homepage: www.elsevier.com/locate/jdsr

The findings of glucosyltransferase enzymes derived from oral streptococci

Tomonori Hoshino [a,*], Taku Fujiwara [b]

[a] Division of Pediatric Dentistry, Department of Human Development and Foresting, Meikai University School of Dentistry, Sakado, Saitama, Japan
[b] Department of Pediatric Dentistry, Nagasaki University Graduate School of Biomedical Sciences, Nagasaki, Japan

ARTICLE INFO

Article history:
Received 28 August 2022
Received in revised form 18 October 2022
Accepted 21 October 2022

Keywords:
Glucosyltransferase
Streptococcus
Anti-caries vaccine
Phylogenetic analysis
Dental caries
Plaque biofilm (6 key words)

ABSTRACT

Glucosyltransferase enzymes (Gtfs) distribute among some streptococcal species in oral cavity and are known as key enzymes contributing to the development of oral biofilm such as dental plaque. In 18 streptococcal species, 45 glucosyltransferase genes (*gtf*) are detected from genome database. Gtfs catalyze the synthesis of the glucans, which are polymers of glucose, from sucrose and they are main component of oral biofilm. Especially, the Gtfs from *Streptococcus mutans* are recognized as one of dental caries pathogens since they contribute to the formation of dental plaque and the establishment of *S. mutans* in the tooth surface. Therefore, Gtfs has been studied particularly by many researchers in the dentistry field to develop the anti- caries vaccine. However, it is not still accomplished. In these days, the phylogenetic and crystal structure analyses of Gtfs were performed and the study of Gtfs will enter new situation from the technique in the past old viewpoint. The findings from those analyses will affect the development of the anti-caries vaccine very much after this. In this review, we summarize the findings of oral streptococcal Gtfs and consider the perspectives of the dental caries prevention which targeted Gtf.

Contents

1. Introduction

The genus *Streptococcus* comprises many species of Gram-positive bacteria living in human or animal oral cavity and/or alimentary tact and sometimes associated with pathogens that have a severe influence on human health [1,2]. According to the phylogenomic analyses, the genus *Streptococcus* divided into eight monophyletic groups, Mitis

Abbreviations: Gtf, glucosyltransferase; gtf, glucosyltransferase gene; WSG, water-soluble glucan; WIG, water-insoluble glucan

* Correspondence to: Division of Pediatric Dentistry, Department of Human Development and Foresting, Meikai University School of Dentistry, 1-1 Keyakidai, Sakado, Saitama 350-0283, Japan.
E-mail address: thoshino1@dent.meikai.ac.jp (T. Hoshino).

https://doi.org/10.1016/j.jdsr.2022.10.003

group, Sanguinis group, Anginosus group, Pyogenic group, Bovis group, Mutans group, Downei group, and Salivarius group [3,4].

Among these groups, some streptococcal species produce glucosyltransferases (Gtfs), which catalyze the synthesis of the glucans, polysaccharide, from sucrose as a substrate [5–7]. The produced glucans play an important role in developing oral biofilm [8]. Therefore, acquisition of the ability to produce polysaccharide from sucrose, i.e., the *gtf* gene encoding Gtf, is the key evolutionary event enabling dental biofilm formation by streptococci [9–11]. Especially, the glucan biofilms produced by the Gtfs from *Streptococcus mutans* are recognized as main components of cariogenic plaque and those enzymes are known as one of dental caries pathogens since those enzymes play an important role in the formation of dental plaque and the establishment of *S. mutans* on the tooth surface [12–14]. Thus, many researchers in the dentistry fields have studied streptococcal Gtfs to prevent dental caries [15–19].

On the other hand, immunization of *S. mutans* whole cells was attempted as the primary etiologic agent in rhesus monkeys to develop a vaccine against dental caries and were successfully prevent dental caries [20,21]. However, it was reported that the antibodies induced by *S. mutans* whole cells showed cross-reactivity with the human heart muscle [22,23]. Thus, GtfB and PAc (protein antigen c) from *S. mutans* were adopted on another occasion [24,25] since the components derived from *S. mutans* were used as the targets of anti-caries vaccine to prevent induction of the antibodies that cross-reacted with human tissue. PAc, a protein antigen on the cell surface of *S. mutans* would be more important factor as the antigen against anti-caries vaccine. However, we will be focused on GtfB since this review summarizes the Gtfs derived from oral streptococci. Moreover, GtfB was truncated to the catalytic region (CAT) and the glucan-binding domain (GBD), and these were employed as antigenic components [26,27]. These components were combined with the cholera toxin B subunit [28] or both [29] to further enhance immunogenicity. In spite of those successful reports, the anti-caries vaccine which targets to GtfB as antigen has not yet been put to practical use. One reason might be that CAT and GBD were employed as vaccine targets. These were selected due to their association with enzyme function [30,31]. However, there are no studies that the immunogenicity of CAT and GBD is verified by immunological, biochemical, or bioinformatic methods. Mora et al., (2018) suggested that effective screening for candidate vaccine antigens should include *in silico* analysis followed by in vitro and in vivo evaluations of antigenicity to reduce time and cost [32]. Those methods should be adopted also in the development of anti- caries vaccine against GtfB.

During this decade, *in silico* approaches have been clarified the necessity of reevaluation in the immunogenicity region of GtfB [33] and the phylogenetic relationships among streptococcal Gtfs and/or glycosyl hydrolase family 70 enzymes from lactic acid bacteria [9–11]. Crystal structure analysis of Gtf180-ΔN from *Lactobacillus reuteri* 180 [34] was performed and then Crystal structure of Gtf-SI (GtfC) was analyzed [35]. Thus, the 3D-structure and domains of streptococcal Gtfs has been estimated [36]. Those findings would be available for the development of anti-caries vaccine or other caries prevention measure.

In this review, we summarize the findings of oral streptococcal Gtfs, the distribution in the genus *Streptococcus*, phylogenetic relationships, and the immunogenicity based on 3D-structure analysis. Then, we make a survey of the development possibility of the anti-caries reagent and/or vaccine which targeted Gtf from *S. mutans*.

2. Gtfs among streptococcal species

2.1. Distribution of Gtfs in the genus Streptococcus

As early studies of Gtfs, it was reported that three different Gtfs, GtfB, GtfC, and GtfD, were identified from *S. mutans* GS-5 [37–39]. From *Streptococcus sobrinus* and *Streptococcus downei,* four different Gtfs, GtfI, GtfS, GtfT, and GtfU were reported [16,40–44]. GtfG, GtfR, and GtfP, which synthesize water soluble glucan, were identified from *Streptococcus gordonii*, *Streptococcus oralis*, and *Streptococcus sanguinis*, respectively [8,45–47]. In *Streptococcus salivarius*, four different Gtfs, GtfJ, GtfK, GtfL, and GtfM were identified [5,48,49]. Among those streptococcal species that produce Gtfs, the Mutans and Downei groups are known as caries-promoting species [50] and Gtfs from these groups contain water insoluble glucan (WIG) synthesizing Gtfs, GtfB, GtfC, and GtfI (Table1). These WIG-synthesizing Gtfs are recognized as a pathogen of initial caries since those play an important role in the colonization of Mutans and Downei group streptococci on tooth surface [51]. In this point, the role of the Gtfs may be limited in the initial formation of dental caries. However, it would be important that the biochemical, molecular biological, and immunological characters of WIG-synthesizing Gtfs were studied since the obstruction of dental plaque formation in the early stage was most effective in the prevention of dental caries. Other than those WIG-synthesizing Gtfs, *S. salivarius* GtfJ and GtfL are also responsible for synthesizing WIG. However, they are not actually closely related to the WIG synthesizing Gtfs of Mutans and Downei groups [11].

Previously, by the sequence of 16 S rRNA, the genus *Streptococcus* was divided into 6 groups, Mitis group, Anginosus group, Pyogenic group, Bovis group, Mutans group, and Salivarius group [52]. By phylogenomic analysis, Sanguinis and Downei group was separated from Mitis and Mutans group, respectively, and the genus *Streptococcus* was divided into eight monophyletic groups [3,4]. Among these eight groups, Gtfs were detected in 7 groups except Anginosus group. Xu et al. (2018) surveyed 37 streptococcal species from the genome sequences on the NCBI (National Center for Biotechnology) Microbial Genomes resource site (http://www.ncbi.nlm.nih.gov/genome/microbes/) and 18streptococcal species that possess *gtf* genes and their 45 Gtfs were picked up [11] (Table 1). The Gtfs derived from human alimentary tract and oral cavity of horse, ruminants, rodents, and monkeys were additionally joined in the list of those Gtfs. Thus, it was suggested that Gtf enzymes were distributed among the genus *Streptococcus*.

2.2. Purification of WIG synthesizing Gtfs from S. mutans

To the induction of antibody and the crystal structure analysis, the purification of target protein is indispensable. Purification of oral streptococcal Gtfs was performed by many researchers [8,53–57]. It was also reported that WIG synthesizing Gtfs, GtfB or GtfC from *S. mutans* was successfully purified [17,37,58], it was practically difficult. This is one of reasons that the development of the anti-caries vaccine against GtfB has been delayed. Although we succeeded in the purification of GtfB and GtfC [33], the purified product was small amount and low concentration. Only about 0.5 mg of purified product was recovered from the whole cell collected from 5 L of *S. mutans* culture. In this study, it was revealed that GtfB from *S. mutans* was easy to aggregate by concentrating. It was thought that the character was disadvantageous to crystal structure analysis since it needs the purity and density of tested protein.

2.3. Three Gtfs from S. mutans and other streptococcal Gtfs in glucan biofilm formation

Ooshima et al. (2001) reported that three Gtfs, GtfB, GtfC, and GtfD, contributed cooperatively to adherence of *S. mutans cells* to smooth surface by reconstitution experiment of sucrose-dependent adherence using the resting cells and its three recombinant Gtfs (rGtf) and that the highest level of sucrose-dependent adherence was found at the ratio of 20 rGtfB:1 rGtfC:4 rGtfD in both the resting cells of Gtf-deficient mutants and insoluble glucan synthesized by

Table 1
18 streptococcal species that produce Gtfs and 45 streptococcal Gtfs.

Streptococcal species	Gene name / protein name	Isolation source	Coupling scheme of glucan
Mitis group			
S. oralis	*gtfR / GtfR*	human oral cavity	α-1,6
Sanguinis group			
S. gordonii	*gtfG / GtfG*	human oral cavity	α-1,6
S. cristatus	*gtf / Gtf*	human oral cavity	α-1,6
S. sanguinis	*gtfP /GtfP*	human oral cavity	α-1,6
Mutans group			
S. mutans	*gtfB / GtfB*	human oral cavity	α-1,3
	gtfC / GtfC		α-1,3 / α-1,6
	gtfD / GtfD		α-1,6
S. ratti	*gtf1 / Gtf1*	caries lesion in rat	unknown
	gtf2 / Gtf2		unknown
S. devriesei	*gtf1 / Gtf1*	teeth of horses	unknown
	gtf2 / Gtf2		unknown
S. orisasini	*gtf1 / Gtf1*	donkey oral cavities	unknown
	gtf2 / Gtf2		unknown
S. troglodytte	*gtfB / GtfD*	oral cavity of a chimpanzee (Pan troglodytes)	unknown
	gtfC / GtfD		unknown
	gtfD / GtfD		unknown
S. macacae	*gtf1 / Gtf1*	dental plaque of monkeys (Macaca fascicularis)	unknown
	gtf2 / Gtf2		unknown
	gtf3 / Gtf3		unknown
Downei group			
S. sobrinus	*gtf1 · gtfI / GtfI*	human oral cavity	α-1,3
	gtf2 / Gtf2		unknown
	gtf3 · gtfS / GtfS		α-1,6
	gtf4 · gtfU / GtfU		α-1,3,6
	gtf5 · gtfT / GtfT		α-1,6
S. downei	*gtf1 · gtfI / GtfI*	dental plaque of monkeys (Macaca fascicularis)	α-1,3
	gtf2 / Gtf2		α-1,6
	gtf3 · gtfS / GtfS		α-1,6
	gtf4 · gtfU / GtfU		α-1,6
	gtf5 · gtfT / GtfT		α-1,6
S. criceti	*gtf1 · gtfI / GtfI*	caries lesion in hamster	α-1,3
	gtf2 / Gtf2		unknown
	gtf3 · gtfS / GtfS		α-1,6
	gtf4 · gtfU / GtfU		α-1,6
	gtf5 · gtfT / GtfT		α-1,6
Salivarius group			
S. salivarius	*gtf1 · gtfL/GtfL*	human oral cavity	α-1,3 / α-1,6
	gtf2 · gtfM/GtfM		α-1,6
	gtf3 · gtf/Gtf3		unknown
	gtf4 · gtfK/GtfK		α-1,6
	gtf5 · gtfJ/GtfJ		α-1,3
	gtf6/Gtf6		unknown
Pyogenic group			
S. urinalis	*gtf / Gtf*	human urine	unknown
Bovis group			
S. gallolyticus	*gtf1 / Gtf1*	alimentary tract of cattle, sheep, and other ruminants	α-1,6
	gtf2 /Gtf2		α-1,6
S. equinus	*gtf/Gtf*	alimentary canal of a horse	unknown
S. infantarius	*gtfA / GtfA*	feces from healthy infant	unknown

*This table was made based on the eight monophyletic groups by Richards et al.{Richards, 2014 #111}. *Among the streptococci which were isolated from human oral cavity, Anginosus group streptococci do not possess *gtf* gene.

rGtfs [59]. And then, Fujiwara et al., (2002) indicated that the mRNA expression ratios of *gtfB*:*gtfC*:*gtfD* in the early-, middle-, and late-exponential phases were 16:1:10, 15:1:12, and 69:1:25, respectively, by real time RT-PCR analyses of mRNA expression of three *gtfs* from *S. mutans* [60]. For these reasons, it was suggested that the presence of all three Gtfs at the optimum ratio was necessary for sucrose-dependent adherence of *S. mutans*, and that GtfC and GtfD might play significant roles in the synthesis of adhesive and insoluble glucan from sucrose.

On the other hand, the effects of GtfR from *S. oralis,* which is a member of the human oral microbiota and an early-colonizing microorganism in human oral cavity, on the sucrose-dependent adhesion of *S. mutans* resting cells was verified experimentally [8]. As the results, it was revealed that the addition of a small amount of GtfR (1 mU/ml) resulted in firm adhesion of the *S. mutans* resting cells and that the adhesion was as strong as that of the *S. mutans* growing cells in sucrose-containing medium, although the cells incubated without GtfR adhered to smooth surface only loosely. Thus, it was suggested that other streptococcal Gtfs containing GtfR would play a significant role as a substitute for GtfD in glucan biofilm formation.

3. Evolution of Gtfs in the genus Streptococcus

3.1. Phylogenetic analysis of Gtfs

At first, in order to test for evidence of recombination between the four *gtf* alleles, *gtfJ*, *gtfK*, *gtfL*, and *gtfM* of *S. salivarius*, the phylogenetic tree was constructed among the 12 oral streptococcal Gtfs

containing *S. mutans, S. sobrinus*, and *S. salivarius* [5,49]. Then, Streptococcal Gtfs and their homologs in the genus *Leuconostoc* and *Lactobacillus*, which are belonging to the glycoside hydrolase family 70 (GH70), were analyzed and the phylogenetic tree was constructed [9]. As the results, it was suggested that all Streptococcal Gtfs share a common ancestor and that the ancestral Streptococcal Gtf gene was likely acquired from a lactic acid bacterium via horizontal gene transfer. Thereafter, more Gtf genes from various Streptococcal species became available, and a phylogeny covering 39 Gtf sequences from 16 Streptococcal species was carried out [10]. As the result, it was revealed that Streptococcal Gtfs had diverged into two major clades, with one comprising only Gtfs synthesizing WIG and the other including mainly Gtfs synthesizing WSG. Moreover, Xu et al., (2018) surveyed a total of 872 Streptococci genomes covering 37 species to detect Gtf genes and 45 sequences from 18 species to establish the updated Gtf gene phylogenetic tree (Fig. 1.) [11]. This report supported that Streptococcal Gtfs indeed share a common ancestry among all 45 sequences formed a monophyletic group and that the phylogeny includes two major clades, one WIG clade and the other WSG clade. In addition, the tree indicated that Clade I included *gtf* genes from nine caries-promoting species from the Mutans and Downei groups and that Clade II contained the *gtf* genes from all 18 species, which are mainly responsible for synthesizing WSG.

3.2. Alteration of glucan coupling by evolution of Gtf

It was reported that streptococcal Gtf came to produce soluble glucans as the result of the displacement of amino acids in the catalytic domain and/or a decrease in the number of repeating units in the glucan-binding domain [42,61]. Thus, Hoshino et al. suggested that streptococcal Gtf evolved to synthesize WSGs and that the ability to synthesize WIGs would only be acquired if the ancestral character was strongly retained and highly specialized [9]. The result phylogeny of Xu et al. (2018) also indicated that WIG Clade (I) existed in the upper stream of WSG Clade (II) (Fig. 1.). Thus, streptococcal Gtfs would become mainly responsible for synthesizing WSGs by evolution.

3.3. Gene transfer of gtf genes among streptococcal species

Homologues can be acquired through gene duplication (paralogs) or horizontal gene transfer (orthologs) [62,63]. In our previous study, it was assumed that evidence exists to support both methods of acquisition also in the GH70 genes. As evidence of horizontal gene transfer, it was shown that transposase encoding gene upstream or downstream of the *gtf* gene in both *Lactobacillus reuteri* and *Lactobacillus sakei* and that a sequence highly homologous to streptococcal transposase genes was also found downstream of *S. oralis gtfR* [9]. It was reported that intra-and interspecies genomic variation in streptococcal species was associated with transposase and that the bacterial population was a flexible gene pool [64]. Thus, it was suggested that the streptococcal *gtf* was acquired through horizontal gene transfer via transposons, while periodic extinguishing of transposable elements might explain their absence around the *gtf* in some species of bacteria [65]. Later, Wu et al. estimated it as follows. If one assumes that the ancestral Gtf gene originated in the common ancestor of the monophyletic clade secondarily constructed with the Salivarius group, the Downei group, the Mutans group, the Bovis group, the Pyogenic group in the phylogenomic tree of the genus *Streptococcus*, then the GtfR from *S. oralis*, GtfP from *S. sanguinis*, GtfG from *S. gordonii*, and Gtf from *S. cristatus* would more likely have been acquired secondarily from a Salivarius group species [11].

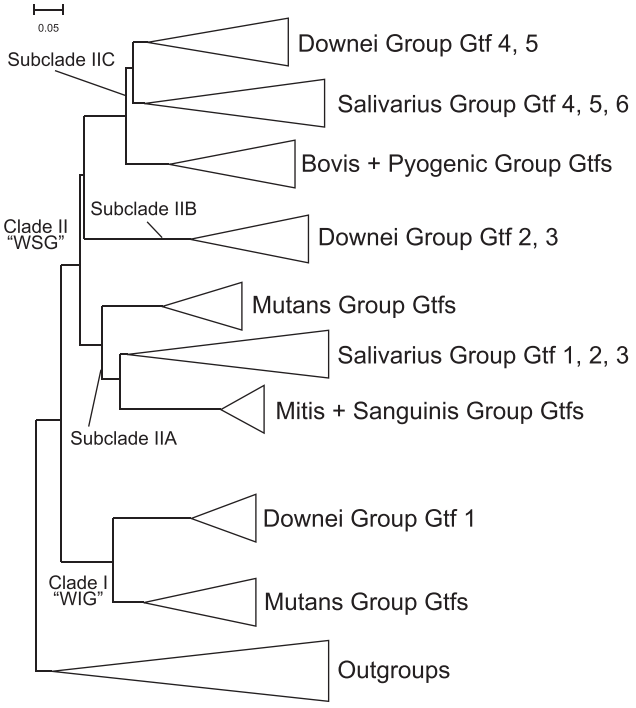

Fig. 1. Outline of phylogenetic analysis among 45 streptococcal *gtf* genes. The outlined phylogenetic tree was built with the Maximum-Likelihood method using the aligned homologous sequences (~3.9 kb) of *gtf* genes. WIG or WSG refers to in which Gtf are mainly responsible for synthesizing water- insoluble glucan or water-soluble glucan. The phylogenetic tree is constructed of WIG clade (I) and three WSG subclades (IIA, IIB, and IIC).
(adapted from Xu et al.2018 [11]).

4. Antigenic prediction of GtfB from S. mutans by in-silico and crystal structure analysis

4.1. Antigenic propensity of catalytic domain (CAT), glucan binding domain (GBD), and N-terminal region of GtfB by in-silico analyses

GtfB is roughly divided into 4 regions as follows: a ca. 40-amino-acid conserved signal sequence; a ca. 360-amino-acid variable region with unknown function that is species specific and not conserved among the other Gtfs; a ca. 500-amino-acid catalytic region that contains conserved amino acids that are necessary for sucrose hydrolysis [54,66,67]; a series of 6 direct repeats that function in glucan binding [16,42,54] (Fig. 2.). Since antigenicity prediction of these 4 regions from GtfB by using Kolaskar's method [68] estimated that primary catalytic site (DSIRVDAVDNVDADLLQIAGDYLKAAK) and glucan-binding site (repeat of NGQHLYFRANGVQVKG) were included in the group of highly antigenic candidates [33], two functional domains, CAT and GBD were employed as potential vaccine target regions in previous studies to develop a component vaccine against GtfB [69], owing to their association with enzyme function. CAT has the high degree of amino acid sequence conservation among streptococci (Fig. 3.). Since this high homogeny would induce cross-reactive antibodies among Gtfs of *S. mutans* and those of *S. sobrinus*, it was thought that the cross-reactivity was more effective in the prevention of dental caries [26]. Moreover, to make up for antigenicity, the diepitopic antigen consisting of both the CAT and GBD [70], and the chimera of CAT and the B subunit of cholera toxin [28] were applied. In spite of these successful reports, the anti-caries vaccine has not been put to practical use yet. For this reason, we

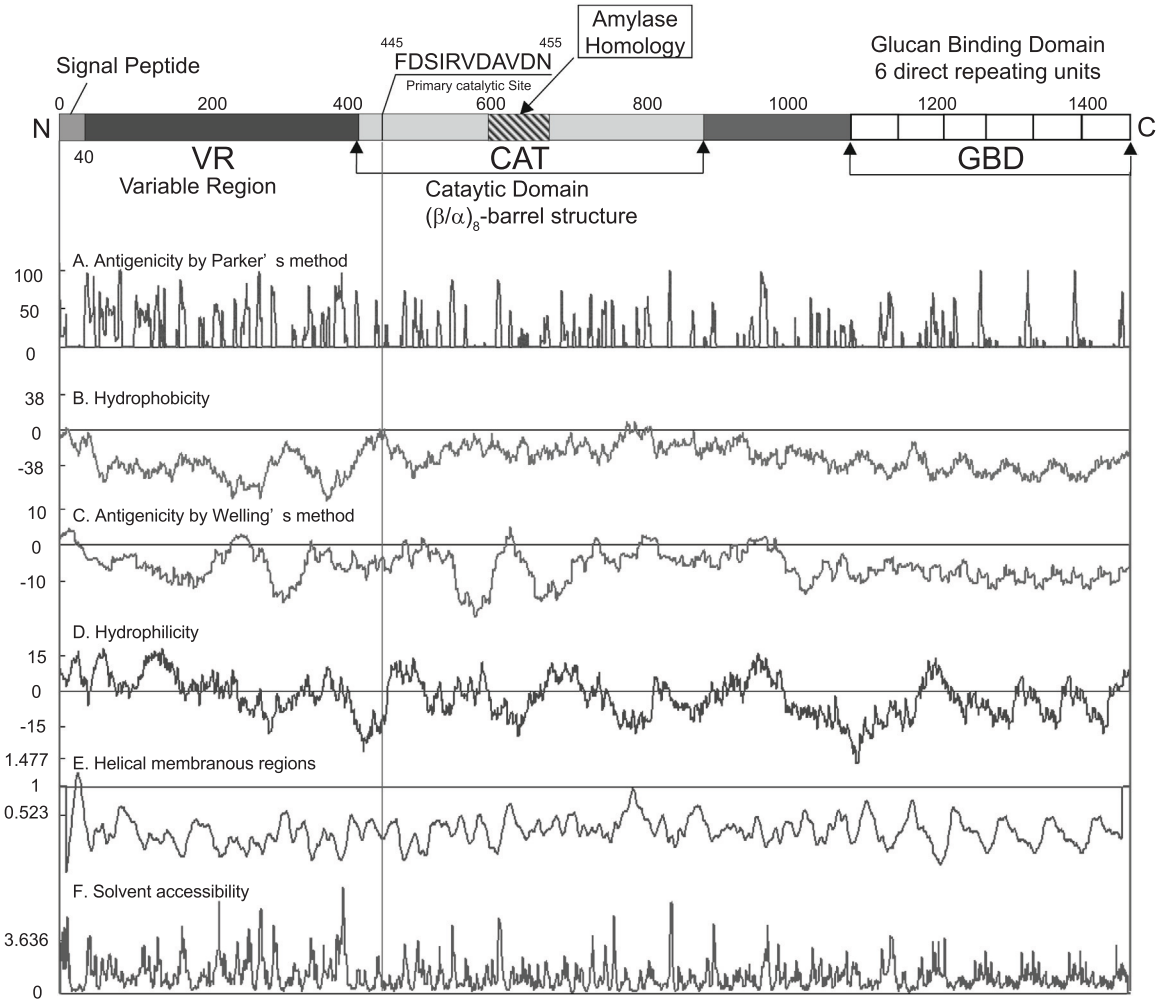

Fig. 2. GtfB antigenicity prediction using ANTHEPROT software. The deduced GtfB amino acid sequence was evaluated for antigenicity using Parker's method (A), hydrophobicity (B), Welling's method (C), hydrophilicity (D), helical membranous regions (E), and solvent accessibility (F) (adapted from Hoshino et al., 2011 [33]).

reevaluated the antigenic region of GtfB by the other *in-silico* predictions identified by the Parker's [71] and Welling's [72] methods by using the ANTHEPROT software [73] in our previous study [33] (Fig. 2.). As the results, it was suggested that the antigenicity of the primary catalytic site and the glucan biding site would be predicted to be low that the N-terminal variable region of GtfB was immunodominant, because of many antigenic peaks compared with those of CAT and GBD. On the other hand, because of high homogeny in the catalytic site as shown in Fig. 3., the antibodies induced by this site would cross-react not only between Gtfs of *S. mutans* and *S. sobrinus* but also among Gtfs of other oral streptococci. If the antibodies induced by the catalytic site have a sufficient effect, the establishment of other commensal streptococci in oral cavity will be inhibited. Since the healthy oral microbiota would be destroyed by this hypothesis, it was thought that the species-specific component should be adopted as a vaccine target.

4.2. 3D-structure analysis of Gtf-SI from S. mutans and antigenicity of GtfB

In the recent review of structure–function relationship studies about GH70 family enzymes [36], it was shown that the 3D-structure of GTF180-ΔN from *Lactobacillus reuteri* 180 confirmed the hypothesis of the circular permutation [34] and revealed an organization in five distinct domains: the domains A, B and C, structurally close to those of the glycoside hydrolase 13 family enzymes [74,75], and the domains IV and V, unique to GH70 family enzymes (Fig. 4. A). Each domain is built of a non-contiguous chain except for domain C, which forms the base of the U-shape fold [34] (Fig. 4. B). Ito et al., (2011) crystallized recombinant Gtf-SI (GtfC) from *S. mutans* and clarified 3D-sturucture of that [35] (Fig. 4. C). GtfB would also take the 3D-structure like GtfC because of high homology between them.

Streptococcal Gtfs are members of the α-amylase superfamily and contain a circularly permuted $(\alpha/\beta)_8$-barrel motif [76,77]. It was thought that the primary catalytic site existed in the $(\alpha/\beta)_8$-barrel structure (Fig. 4. C) would be difficult to be recognized as an antigen for the immune system although low-molecular-weight sucrose was able to pass through this structure as an enzyme substrate.

Previously, function of the N-terminal region in Gtfs was thought to be unknown as described above. However, it was clarified that this N-terminal region contained domains IV and V by the U-shape fold formation (Fig. 4. B). Domains IV and V are unique to GH70 Gtfs [78]. Domain IV was positioned next to domain B in the Gtf-SI (GtfC) structure. Domain V is thought to be composed of sequences upstream of domain IV and/or downstream of domain IV, which

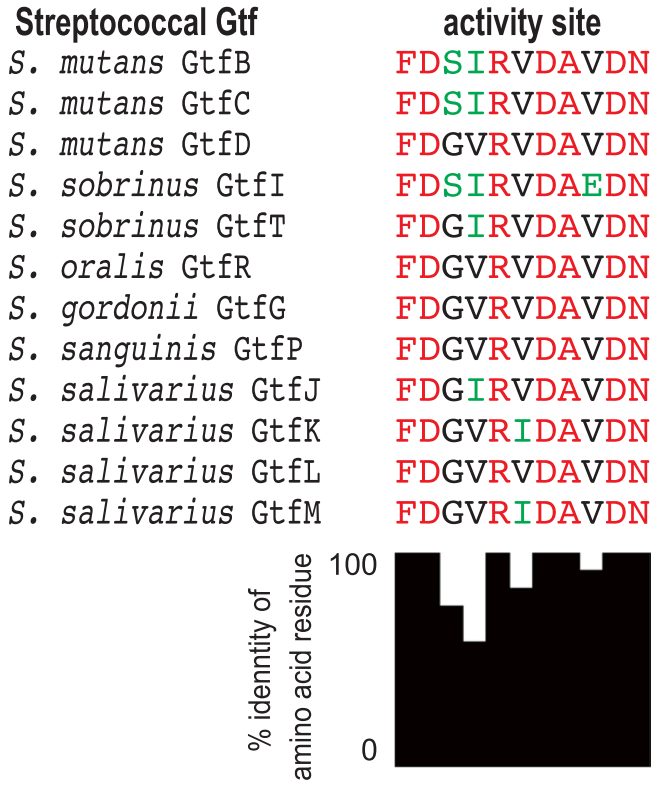

Fig. 3. Homologous region in catalytic site of Gtfs among the streptococci in human oral cavity. The rubric amino acid residues agree on all the selected Gtfs. Black and green characters indicate the amino acid residues of majority and minority, respectively.

implies that it is located next to domain IV. Although domains IV and V are not essential for the enzymatic activity of Gtfs [79], domain IV may serve as a hinge, swinging glucan-binding domain V toward and away from the catalytic domains to facilitate glucan extension [35]. For this reason, it would suppress the enzyme activity to assume then-terminal region of GtfB with a target of vaccine. In our previous study, it was shown that antibody against the N-terminal region of GtfB suppress glucan production [33].

5. Future perspectives and conclusions

The dental plaque is the oral biofilm which is the pathogen associated with periodontal disease e. g. dental caries and periodontitis, and systematic disease e. g. infective endocarditis and aspiration pneumonia [1,2]. Since oral streptococcal Gtfs play an important role by producing glucan that is main constituents of this oral biofilm, the biochemical, molecular biological, phylogenetic, and immunological studies of Gtfs lead to discover biofilm-formation inhibitor as a result. Especially, 3D-sturucture analysis of Gtf would be available for the discovery of the molecular inhibitor that could reduce glucan-producing activity as well as the development of anti-caries vaccine.

By the way, an outbreak of COVID-19 in these days is extremely a serious problem. However, the RNA and DNA vaccine were recognized by this outbreak [80–83]. These techniques would be effective in the development of the anti-caries vaccine and were adopted in the development of anti-caries vaccine [84,85]. Especially, in the case that the large-scale purification of antigen protein such as GtfB from *S. mutans* may be difficult, the method of vaccine application would be updated. Moreover, antigenic prediction of GtfB from *S. mutans* by *in-silico* and crystal structure analysis would give the new idea of anti-caries vaccine development different from

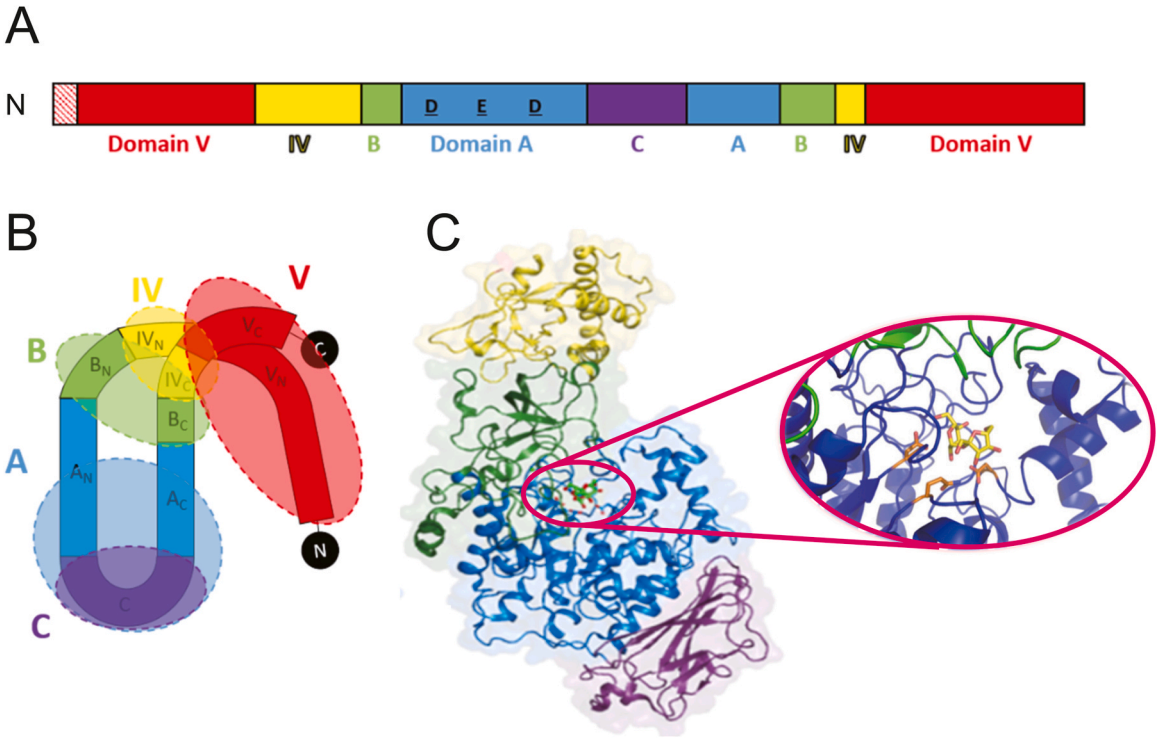

Fig. 4. Schematic representation of the U shape fold formed by the various domains of GTF-SI from *S. mutans* and the active site which sucrose substrate is bound in. A. Five distinct domains of GH70 family enzyme. B. The U-shape fold formation. The colors in the 3D structure indicate red: domain V; yellow: domain IV; green: domain B; blue: domain A; and purple: domain C, respectively. C. 3D-structure of GTF-SI from *S. mutans*. The magenta circle indicates the catalytic site.
(adapted from Vujičić-Žagar et al., 2010 [34], Ito et al.,2011 [35], and Molina et al., 2021 [36])

the measures that have been performed so far. Especially, in the future developmental study of anti-caries vaccine, the placement of the three-dimensional amino acid residues which seem to be recognized as the actual immunogen in vivo would come to be selected although the past vaccine development was performed by employing the two-dimensional amino acid sequence as antigen based on function.

Conflicts of interest

We declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported on this paper.

Acknowledgements

This work was supported by the Japan Society for Promotion of Science (JSPS) KAKENHI Grant Numbers, 11470451, 13470449, 15390639, 16592050, 16659574, 17390554, 18592243, 21659477, 23659968, and 24659912 and the JSPS International program for Young Researcher Oversea Visits.

References

[1] Coykendall AL. Classification and identification of the viridans streptococci. Clin Microbiol Rev 1989;2:315–28.
[2] Kohler W. The present state of species within the genera *Streptococcus* and *Enterococcus*. Int J Med Microbiol 2007;297:133–50.
[3] Shao ZQ, Zhang YM, Pan XZ, Wang B, Chen JQ. Insight into the evolution of the histidine triad protein (HTP) family in *Streptococcus*. PLoS One 2013;8:e60116.
[4] Richards VP, Palmer SR, Pavinski Bitar PD, Qin X, Weinstock GM, Highlander SK, et al. Phylogenomics and the dynamic genome evolution of the genus *Streptococcus*. Genome Biol Evol 2014;6:741–53.
[5] Simpson CL, Cheetham NW, Giffard PM, Jacques NA. Four glucosyltransferases, GtfJ, GtfK, GtfL and GtfM, from *Streptococcus salivarius* ATCC 25975. Microbiology 1995;141(6):1451–60.
[6] Simpson CL, Giffard PM, Jacques NA. *Streptococcus salivarius* ATCC 25975 possesses at least two genes coding for primer-independent glucosyltransferases. Infect Immun 1995;63:609–21.
[7] Fujiwara T, Terao Y, Hoshino T, Kawabata S, Ooshima T, Sobue S, et al. Molecular analyses of glucosyltransferase genes among strains of *Streptococcus mutans*. FEMS Microbiol Lett 1998;161:331–6.
[8] Fujiwara T, Hoshino T, Ooshima T, Sobue S, Hamada S. Purification, characterization, and molecular analysis of the gene encoding glucosyltransferase from *Streptococcus oralis*. Infect Immun 2000;68:2475–83.
[9] Hoshino T, Fujiwara T, Kawabata S. Evolution of cariogenic character in *Streptococcus mutans*: horizontal transmission of glycosyl hydrolase family 70 genes. Sci Rep 2012;2:518.
[10] Argimon S, Alekseyenko AV, DeSalle R, Caufield PW. Phylogenetic analysis of glucosyltransferases and implications for the coevolution of mutans streptococci with their mammalian hosts. PLoS One 2013;8:e56305.
[11] Xu RR, Yang WD, Niu KX, Wang B, Wang WM. An update on the evolution of glucosyltransferase (Gtf) genes in *Streptococcus*. Front Microbiol 2018;9:2979.
[12] Caufield PW. Dental caries: an infectious and transmissible disease. Compend Contin Educ Dent 2005;26:10–6.
[13] W.D. Miller. The microorganisms of the human mouth. Basel Switzerland: S Karger 1973.
[14] Touger-Decker R, van Loveren C. Sugars and dental caries. Am J Clin Nutr 2003;78:881S–892SS.
[15] Hamada S, Torii M. Effect of sucrose in culture media on the location of glucosyltransferase of *Streptococcus mutans* and cell adherence to glass surfaces. Infect Immun 1978;20:592–9.
[16] Ferretti JJ, Gilpin ML, Russell RR. Nucleotide sequence of a glucosyltransferase gene from *Streptococcus sobrinus* MFe28. J Bacteriol 1987;169:4271–8.
[17] Hamada S, Horikoshi T, Minami T, Okahashi N, Koga T. Purification and characterization of cell-associated glucosyltransferase synthesizing water-insoluble glucan from serotype c *Streptococcus mutans*. J Gen Microbiol 1989;135:335–44.
[18] Kuramitsu HK. Virulence factors of mutans streptococci: role of molecular genetics. Crit Rev Oral Biol Med 1993;4:159–76.
[19] Fujiwara T, Tamesada M, Bian Z, Kawabata S, Kimura S, Hamada S. Deletion and reintroduction of glucosyltransferase genes of *Streptococcus mutans* and role of their gene products in sucrose dependent cellular adherence. Micro Pathog 1996;20:225–33.
[20] Lehner T, Challacombe SJ, Caldwell J. Immunological and bacteriological basis for vaccination against dental caries in rhesus monkeys. Nature 1975;254:517–20.
[21] Lehner T, Challacombe SJ, Caldwell J. Immunologic basis for vaccination against dental caries in rhesus monkeys. J Dent Res 1976;55. C166-80.
[22] Van de Rijn I, Bleiweis AS, Zabriskie JB. Antigens in *Streptococcus mutans* cross reactive with human heart muscle. J Dent Res 1976;55. C59-64.
[23] Ferretti JJ, Shea C, Humphrey MW. Cross-reactivity of *Streptococcus mutans* antigens and human heart tissue. Infect Immun 1980;30:69–73.
[24] Taubman MA, Smith DJ. Effects of local immunization with glucosyltransferase fractions from *Streptococcus mutans* on dental caries in rats and hamsters. J Immunol 1977;118:710–20.
[25] Smith DJ, Taubman MA, Ebersole JL. Local and systemic antibody response to oral administration of glucosyltransferase antigen complex. Infect Immun 1980;28:441–50.
[26] Smith DJ, Shoushtari B, Heschel RL, King WF, Taubman MA. Immunogenicity and protective immunity induced by synthetic peptides associated with a catalytic subdomain of mutans group streptococcal glucosyltransferase. Infect Immun 1997;65:4424–30.
[27] Jespersgaard C, Hajishengallis G, Huang Y, Russell MW, Smith DJ, Michalek SM. Protective immunity against *Streptococcus mutans* infection in mice after intranasal immunization with the glucan-binding region of *S. mutans* glucosyltransferase. Infect Immun 1999;67:6543–9.
[28] Laloi P, Munro CL, Jones KR, Macrina FL. Immunologic characteristics of a Streptococcus mutans glucosyltransferase B sucrose-binding site peptide-cholera toxin B-subunit chimeric protein. Infect Immun 1996;64:28–36.
[29] Niu Y, Sun J, Fan M, Xu QA, Guo J, Jia R, et al. Construction of a new fusion anticaries DNA vaccine. J Dent Res 2009;88:455–60.
[30] Smith DJ, Taubman MA, Holmberg CF, Eastcott J, King WF, Ali-Salaam P. Antigenicity and immunogenicity of a synthetic peptide derived from a glucan-binding domain of mutans streptococcal glucosyltransferase. Infect Immun 1993;61:2899–905.
[31] Smith DJ, Taubman MA, King WF, Eida S, Powell JR, Eastcott J. Immunological characteristics of a synthetic peptide associated with a catalytic domain of mutans streptococcal glucosyltransferase. Infect Immun 1994;62:5470–6.
[32] Mora M, Telford JL. Genome-based approaches to vaccine development. J Mol Med 2010;88:143–7.
[33] Hoshino T, Kondo Y, Saito K, Terao Y, Okahashi N, Kawabata S, et al. Novel epitopic region of glucosyltransferase B from *Streptococcus mutans*. Clin Vaccin Immunol 2011;18:1552–61.
[34] Vujičić-Žagar A, Pijning T, Kralj S, Lopez CA, Eeuwema W, Dijkhuizen L, et al. Crystal structure of a 117 kDa glucansucrase fragment provides insight into evolution and product specificity of GH70 enzymes. Proc Natl Acad Sci USA 2010;107:21406–11.
[35] Ito K, Ito S, Shimamura T, Weyand S, Kawarasaki Y, Misaka T, et al. Crystal structure of glucansucrase from the dental caries pathogen Streptococcus mutans. J Mol Biol 2011.
[36] Molina M, Cioci G, Moulis C, Severac E, Remaud-Simeon M. Bacterial alpha-glucan and branching sucrases from GH70 family: discovery, structure-function relationship studies and engineering. Microorganisms 2021:9.
[37] Shiroza T, Ueda S, Kuramitsu HK. Sequence analysis of the *gtfB* gene from *Streptococcus mutans*. J Bacteriol 1987;169:4263–70.
[38] Ueda S, Shiroza T, Kuramitsu HK. Sequence analysis of the *gtfC* gene from *Streptococcus mutans* GS-5. Gene 1988;69:101–9.
[39] Honda O, Kato C, Kuramitsu HK. Nucleotide sequence of the *Streptococcus mutans gtfD* gene encoding the glucosyltransferase-S enzyme. J Gen Microbiol 1990;136:2099–105.
[40] Yamashita Y, Tsukioka Y, Nakano Y, Shibata Y, Koga T. Molecular and genetic analysis of multiple changes in the levels of production of virulence factors in a subcultured variant of *Streptococcus mutans*. FEMS Microbiol Lett 1996;144:81–7.
[41] Gilmore KS, Russell RR, Ferretti JJ. Analysis of the *Streptococcus downei gtfS* gene, which specifies a glucosyltransferase that synthesizes soluble glucans. Infect Immun 1990;58:2452–8.
[42] Abo H, Matsumura T, Kodama T, Ohta H, Fukui K, Kato K, et al. Peptide sequences for sucrose splitting and glucan binding within *Streptococcus sobrinus* glucosyltransferase (water-insoluble glucan synthetase). J Bacteriol 1991;173:989–96.
[43] Hanada N, Isobe Y, Aizawa Y, Katayama T, Sato S, Inoue M. Nucleotide sequence analysis of the *gtfT* gene from *Streptococcus sobrinus* OMZ176. Infect Immun 1993;61:2096–103.
[44] Colby SM, McLaughlin RE, Ferretti JJ, Russell RRB. Effect of inactivation of *gtf* genes on adherence of *Streptococcus downei*. Oral Microbiol Immunol 1999;14:27–32.
[45] Vickerman MM, Sulavik MC, Nowak JD, Gardner NM, Jones GW, Clewell DB. Nucleotide sequence analysis of the *Streptococcus gordonii* glucosyltransferase gene, *gtfG*. DNA Seq 1997;7:83–95.
[46] Vacca Smith AM, Ng-Evans L, Wunder D, Bowen WH. Studies concerning the glucosyltransferase of *Streptococcus sanguis*. Caries Res 2000;34:295–302.
[47] Xu P, Alves JM, Kitten T, Brown A, Chen Z, Ozaki LS, et al. Genome of the opportunistic pathogen *Streptococcus* sanguinis. J Bacteriol 2007;189:3166–75.
[48] Giffard PM, Simpson CL, Milward CP, Jacques NA. Molecular characterization of a cluster of at least two glucosyltransferase genes in *Streptococcus salivarius* ATCC 25975. J Gen Microbiol 1991;137:2577–93.
[49] Giffard PM, Allen DM, Milward CP, Simpson CL, Jacques NA. Sequence of the *gtfK* gene of *Streptococcus salivarius* ATCC 25975 and evolution of the gtf genes of oral streptococci. J Gen Microbiol 1993;139:1511–22.
[50] Kilian M, Chapple IL, Hannig M, Marsh PD, Meuric V, Pedersen AM, et al. The oral microbiome - an update for oral healthcare professionals. Br Dent J 2016;221:657–66.
[51] Park M, Sutherland JB, Rafii F. Effects of nano-hydroxyapatite on the formation of biofilms by *Streptococcus mutans* in two different media. Arch Oral Biol 2019;107:104484.

[52] Kawamura Y, Hou XG, Sultana F, Miura H, Ezaki T. Determination of 16S rRNA sequences of *Streptococcus mitis* and *Streptococcus gordonii* and phylogenetic relationships among members of the genus *Streptococcus*. Int J Syst Bacteriol 1995;45:406–8.
[53] Yamashita Y, Hanada N, Takehara T. Purification of a fourth glucosyltransferase from *Streptococcus sobrinus*. J Bacteriol 1989;171:6265–70.
[54] Mooser G, Wong C. Isolation of a glucan-binding domain of glucosyltransferase (1,6-alpha-glucan synthase) from *Streptococcus sobrinus*. Infect Immun 1988;56:880–4.
[55] Mayer RM. Dextransucrase: a glucosyltransferase from *Streptococcus sanguis*. Methods Enzym 1987;138:649–61.
[56] Kumada H, Umemoto T, Onisi M, Tsumori H, Shimamura A, Mukasa H. Purification and characterization of extracellular glucosyltransferase from *Streptococcus mutans* serotype *b* (subspecies rattus). J Gen Microbiol 1987;133:1435–41.
[57] Shimamura A, Tsumori H, Mukasa H. Purification and properties of *Streptococcus mutans* extracellular glucosyltransferase. Biochim Biophys Acta 1982;702:72–80.
[58] Kuramitsu HK, Wondrack L. Insoluble glucan synthesis by *Streptococcus mutans* serotype *c* strains. Infect Immun 1983;42:763–70.
[59] Ooshima T, Matsumura M, Hoshino T, Kawabata S, Sobue S, Fujiwara T. Contributions of three glycosyltransferases to sucrose-dependent adherence of *Streptococcus mutans*. J Dent Res 2001;80:1672–7.
[60] Fujiwara T, Hoshino T, Ooshima T, Hamada S. Differential and quantitative analyses of mRNA expression of glucosyltransferases from *Streptococcus mutans* MT8148. J Dent Res 2002;81(109–13).
[61] Monchois V, Vignon M, Escalier PC, Svensson B, Russell RR. Involvement of Gln937 of *Streptococcus downei* GTF-I glucansucrase in transition-state stabilization. Eur J Biochem 2000;267:4127–36.
[62] Ochman H, Lawrence JG, Groisman EA. Lateral gene transfer and the nature of bacterial innovation. Nature 2000;405:299–304.
[63] Jain R, Rivera MC, Moore JE, Lake JA. Horizontal gene transfer accelerates genome innovation and evolution. Mol Biol Evol 2003;20:1598–602.
[64] Hakenbeck R, Balmelle N, Weber B, Gardès C, Keck W, de Saizieu A. Mosaic genes and mosaic chromosomes: intra- and interspecies genomic variation of *Streptococcus pneumoniae*. Infect Immun 2001;69:2477–86.
[65] Wagner A. Periodic extinctions of transposable elements in bacterial lineages: evidence from intragenomic variation in multiple genomes. Mol Biol Evol 2006;23:723–33.
[66] Funane K, Shiraiwa M, Hashimoto K, Ichishima E, Kobayashi M. An active-site peptide containing the second essential carboxyl group of dextransucrase from *Leuconostoc mesenteroides* by chemical modifications. Biochemistry 1993;32:13696–702.
[67] MacGregor EA, Janecek S, Svensson B. Relationship of sequence and structure to specificity in the alpha-amylase family of enzymes. Biochim Biophys Acta 2001;1546:1–20.
[68] Kolaskar AS, Tongaonkar PC. A semi-empirical method for prediction of antigenic determinants on protein antigens. FEBS Lett 1990;276:172–4.
[69] Smith DJ, King WF, Rivero J, Taubman MA. Immunological and protective effects of diepitopic subunit dental caries vaccines. Infect Immun 2005;73:2797–804.
[70] Taubman MA, Holmberg CJ, Smith DJ. Diepitopic construct of functionally and epitopically complementary peptides enhances immunogenicity, reactivity with glucosyltransferase, and protection from dental caries. Infect Immun 2001;69:4210–6.
[71] Parker JM, Guo D, Hodges RS. New hydrophilicity scale derived from high-performance liquid chromatography peptide retention data: correlation of predicted surface residues with antigenicity and X-ray-derived accessible sites. Biochemistry 1986;25:5425–32.
[72] Welling GW, Weijer WJ, van der Zee R, Welling-Wester S. Prediction of sequential antigenic regions in proteins. FEBS Lett 1985;188:215–8.
[73] Deleage G, Combet C, Blanchet C, Geourjon C. ANTHEPROT: an integrated protein sequence analysis software with client/server capabilities. Comput Biol Med 2001;31:259–67.
[74] Moulis C, André I, Remaud-Simeon M. GH13 amylosucrases and GH70 branching sucrases, atypical enzymes in their respective families. Cell Mol Life Sci 2016;73:2661–79.
[75] Meng X, Gangoiti J, Bai Y, Pijning T, Van Leeuwen SS, Dijkhuizen L. Structure-function relationships of family GH70 glucansucrase and 4,6-α-glucanotransferase enzymes, and their evolutionary relationships with family GH13 enzymes. Cell Mol Life Sci 2016;73:2681–706.
[76] Devulapalle KS, Goodman SD, Gao Q, Hemsley A, Mooser G. Knowledge-based model of a glucosyltransferase from the oral bacterial group of mutans streptococci. Protein Sci 1997;6:2489–93.
[77] MacGregor EA, Jespersen HM, Svensson B. A circularly permuted alpha-amylase-type alpha/beta-barrel structure in glucan-synthesizing glucosyltransferases. FEBS Lett 1996;378:263–6.
[78] Monchois V, Willemot RM, Monsan P. Glucansucrases: mechanism of action and structure-function relationships. FEMS Microbiol Rev 1999;23:131–51.
[79] Kralj S, van Geel-Schutten GH, Dondorff MM, Kirsanovs S, van der Maarel MJ, Dijkhuizen L. Glucan synthesis in the genus *Lactobacillus*: isolation and characterization of glucansucrase genes, enzymes and glucan products from six different strains. Microbiology 2004;150:3681–90.
[80] Polack FP, Thomas SJ, Kitchin N, Absalon J, Gurtman A, Lockhart S, et al. Safety and efficacy of the BNT162b2 mRNA Covid-19 vaccine. New Engl J Med 2020;383:2603–15.
[81] Safar HA, Mustafa AS, McHugh TD. COVID-19 vaccine development: what lessons can we learn from TB? Ann Clin Microbiol Antimicrob 2020;19:56.
[82] Pitkänen HH, Jouppila A, Helin T, Dulipati V, Kotimaa J, Meri S, et al. COVID-19 adenovirus vaccine triggers antibodies against PF4 complexes to activate complement and platelets. Thromb Res 2021;208:129–37.
[83] Wack S, Patton T, Ferris LK. COVID-19 vaccine safety and efficacy in patients with immune-mediated inflammatory disease: Review of available evidence. J Am Acad Dermatol 2021;85:1274–84.
[84] Li H, Lu Y, Xiang J, Jiang H, Zhong Y, Lu Y. Enhancement of immunogenic response and protection in model rats by CSTM nanoparticles anticaries DNA vaccine. Nanomedicine 2016;11:1407–16.
[85] Jia R, Yan L, Guo J. Enhancing the immunogenicity of a DNA vaccine against Streptococcus mutans by attenuating the inhibition of endogenous miR-9. Vaccine 2020;38:1424–30.