

## Role of *Streptococcus anginosus* on the formation of dental caries

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### ABSTRACT

Generally, the etiology of dental caries is the cariogenic properties of bacteria, these are always associated with *Streptococcus mutans*. Glucosyltransferase fragment (Gtf) are also in other strains of *Streptococcus* such as *Streptococcus anginosus*, *Streptococcus milleri* which includes beta hemolysis. Genotypically B *Streptococcus anginosus* has genetic characteristics that are similar to *Streptococcus mutans*. The research objective was to determine the existence of Gtf B/C gene as a cause of caries in *Streptococcus anginosus*. The study was conducted in experimental laboratories with PCR technique by taking a sample of 20 children who had caries. The results showed there was amplification of *Streptococcus anginosus* with a level of homology 96% , 97% and 99% . The results of the Gtf genes amplification fragment B/C provided 600 pb ribbon. The conclusion was *Streptococcus anginosus* classified as cariogenic bacteria, because they had Gtf B/C genes.

**Key words:** *Streptococcus anginosus*, cariogenic, Gtf

### ABSTRAK

Umumnya penyebab terjadinya karies gigi selalu dihubungkan dengan sifat kariogenik *Streptococcus mutans*. Glucosyltransferase fragment (Gtf) terdapat juga pada *Streptococcus* strain lain seperti *Streptococcus anginosus* yang termasuk *Streptococcus milleri* beta hemolisis. Secara genotip, *Streptococcus anginosus* memiliki karakteristik genetic yang mirip dengan *Streptococcus mutans*. Tujuan penelitian adalah untuk melihat keberadaan gen Gtf B/C sebagai penyebab karies pada *Streptococcus anginosus*. Penelitian dilakukan secara eksperimental laboratories dengan teknik PCR dengan mengambil sampel dari 20 anak yang memiliki karies. Hasil penelitian menunjukkan bahwa terdapat amplifikasi *Streptococcus anginosus* dengan tingkat homologi 96%, 97% dan 99%. Hasil amplifikasi fragmen gen Gtf B/C memberikan pita 600 pb. Simpulan penelitian adalah *Streptococcus anginosus* termasuk bakteri kariogenik, karena mengandung gen Gtf B/C.

**Kata kunci:** *Streptococcus anginosus*, kariogenik, Gtf

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## INTRODUCTION

*Streptococcus mutans* is always generally referred to the specific bacteria as the dominant cause of dental caries, cariogenic properties of *Streptococcus mutans* is always associated with the presence of the enzyme Gtf B/C which converts sucrose into insoluble glucans.<sup>1-3</sup> it is more pathogenic due to the glucose polymers produced by both enzymes is a mediator of aggregation of *Streptococcus mutans* and other oral *Streptococci*.<sup>1,4,5</sup>

It is not impossible for other *Streptococcus* to play role in caries formation. Based on the sequence analysis of 16S rRNA gene, *Streptococcus viridans* are now divided into six genogroup: group pyogenic, mitis, bovis, salivarius, mutans, and anginosus. Anginosus group is also divided into three species: *S. anginosus*, *S. constellatus*, and *S. intermedius*.<sup>1,4,5</sup> Several studies have shown that these species are bacterial pathogen in humans with the ability to cause purulent infections, abscesses and dental caries.<sup>1,3,4,5</sup> Three species of the anginosus group are very difficult to identify because of the variety of biochemical and serological characteristics. Furthermore, the clinical spectrum associated to infection by this group varies. For this reason, some scientists prefer the genotype to phenotype characteristic to differentiate these species. DNA group data shows that *S. anginosus* can be divided into three species taxonomically with remaining heterogeneity on each species. 16S rRNA gene analysis is useful for identifying species of bacteria, especially for bacteria that is difficult to culture. Several studies used DNA sequences of 16S rRNA gene and hybridization with specific DNA probes to identified the *Streptococcus anginosus*.<sup>1,4,5</sup>

This study aimed to see the presence of Gtf B/C genes as a cause of caries in *Streptococcus anginosus*.

## METHODS

Bacteria used in this study were *Streptococcus anginosus* from 20 children's carious teeth. Purity of the bacteria was examined and it was sub cultured. Hemolysis was examined by growth observation on sheep blood agar plates, alpha and

gamma hemolytic was recorded as nonhemolytic.

In ordered to get the characteristics of the 16S rRNA, bacteria was grown in blood agar added with 5% sheep blood and the purity was then checked. Cells from the blood agar plate was then transferred into a micro centrifuge tube and washed twice. First, used a volume of 100 µl then used 900 µl volume buffer of 10mM Tris-150 mM NaCl. Supernatant was removed and the cells were inserted into 100 µl TE (10 mM Tris HCl, 1 mM EDTA ph 8.0) buffer and heated at 99° C for 10 minutes to lyse and release the entire DNA.<sup>1,3,4,6</sup>

For amplification 16S rRNA with PCR and PCR result purification, 16S rRNA gene was amplified with the standard conditions using universal primers (forward primer, 5'-GAG AGT TTG ATY MTG GCT CAG-3', and reverse primer, 5'-GAA GGA GGT GWT CCA RCC GCA-3').<sup>1,4,7</sup> PCR primer was not always found in bacterial species. However, universal primers were used to obtain wide filogenic type of the bacteria. PCR was placed in a thinwall tube with GeneAmp PCR system 9700 (ABI, Foster City, CA). 1 µl of lyse sample was added to the reaction mixture (final volume of 50 µl), contains of 20 pmol primer, 40 nmol deoxynucleoside triphosphates and 1 U platinum Taq polymerase (Invitrogen, San Diego, CA). In hot-start protocol, sample was heated at 95° C for 45 seconds, annealing at 60° C for 45 seconds and elongation at 72° C for 1.5 minutes with 15 second addition in each cycle. Thirty cycles were completed, followed by final elongation stage at 72° C for 15 minutes. PCR results were checked by electrophoresis in 1% agarose gel. DNA was stained with ethidium bromide and visualized under short-wavelength UV light.

Amplification of the gene encoding Gtf was completed using PCR method with forward and reverse primers that amplified encoding glucosyltransferase fragment (Gtf). Foward was 5'-AGATTT CCGT CCCTT ACTG 3', and Reverse was 5' ATCA TATTTGT CGCCAT CATA 3'.

Initial denaturation condition was at 94° C for 2 minutes, reaction cycle contains of denaturation at 94° C for 1 minute, annealing at 50° C for 1 minute and elongation at 72° C for 1 minute as many as 35 cycletime. During the final cycle, elongation was done at 72° C for 10 menit. PCR optimization was completed by setting the PCR



template concentration, the concentration of magnesium chloride, primer concentration, annealing temperature, and dNTP concentration. Optimization was performed based on the composition obtained according to the PCR, as many as 40 templates, 20 primer forward, 20 primer reverse, 9 µL magnesium chloride, 25 mM, 5 µL dapar Tag polimerase, 1 µL Tag polimerase enzyme and 1 µL dNTP. Lastly, sterile aquabidest was added until the volume reaches 50 µL.

PCR products validity was confirmed using 1% agarose gel electrophoresis (b/v) by comparing the DNA mark, positive control, and negative control. One percent agarose gel was created by dissolving 400 mg agarose and 40 mL buffer of TAE 1X (Tris-base; EDTA 0.5 M, pH 8.0; glacial acetic acid; and sodium hydroxide). Electrophoresis was performed at a voltage of 90 Volt for 45 minute. After electrophoresis completed, the PCR product was observed using UV transluminator.

Purification of PCR products was completed using GFX<sup>®</sup>. DNA strand was cut from the agarose gel and then inserted into the Eppendorf and the capture buffer was then added. Comparison between the weight of the gel with the capture buffer was 1:1 (1 mg gel: 1 µL capture buffer).

The sample was heated at a temperature of 50°C for 15 minutes (gel dissolves) then the sample was centrifuged for 1 minute at 13,000 rotation per minute (rpm). After the liquid was centrifuged in Eppendorf, GFX column was inserted into the collection tube while pipetted up and down as much as 4-5 times. Columns and collection tubes were centrifuged for 1 minute at 13,000 rpm.

Collection of fluid in the tube was removed and then 500 µL wash buffer was added and centrifuged for 1 minute, 13.000 rpm. GFX column was transferred into a sterile 1.5 mL sterile Eppendorf tube. As much as 25 µL of sterile aquabidest was inserted into the column and centrifuged for 1 minute at 13,000 rpm. This was done twice to obtain a fluid sample of 50 µL.

Determination of the nucleotide sequence was based on the Sanger dideoxy method. Mixture for the nucleotide sequence determination was purified PCR product with 10 ng/3 µL of forward primer, that has 10x Tag polimerase, Tag polimerase enzyme, DNTP, ddTP, and stop solution. The result of the nucleotide sequence determination was analyzed by comparing the results of the sequence

with the sequence of the Gtf genes nucleotide in the Gene Bank.

## RESULT

The result of 16S rRNA gene amplification was then sequenced and homogenized with each bacteria in Gen bank using DNA Star programme. Sequencing result showed that there were 4 *streptococcus anginosus* identified with high degree of homology.

Based on research results in the Table 1, only 4 samples were identified, namely *Streptococcus anginosus* on sample number: K 9, K 12, K 18, and

Table 1. Bacterial identification result using 16S rRNA approach

No	Sample	Sample category	Name of bacteria	Homology
1.	K 9	Tooth caries	<i>Streptococcus anginosus</i>	96 %
2.	K 12		<i>Streptococcus anginosus</i>	96 %
3.	K 18		<i>Streptococcus anginosus</i>	99 %
4.	K 19		<i>Streptococcus anginosus</i>	97 %

Note: K: sample on dental caries, %: shows degree of homology

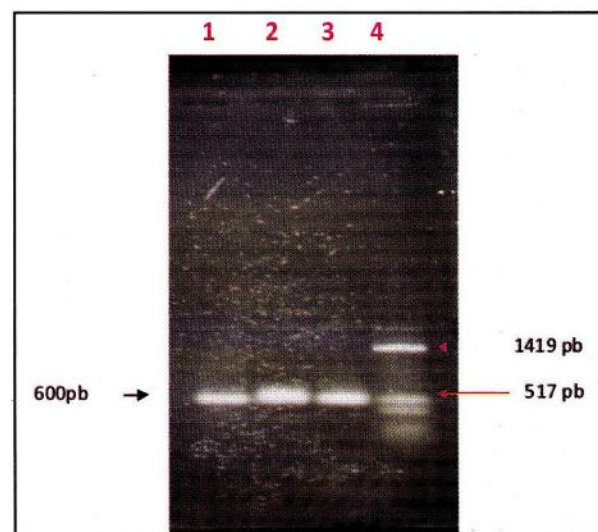


Figure 1. Gen fragment amplification GTF B/C with strands length of 600 pb: Line 1. PCR product isolate K9, *S. anginosus*; Line 2. PCR product isolate K12, *S. anginosus*; Line 3. PCR product isolate K18, *S. anginosus*; Line 4. PCR product isolate K19, *S. anginosus*.



Streptococcus anginosus strain ChDC YAS 16S ribosomal DNA gene, partial sequence Length=1517		
Score = 821 bits (414), Expect = 0.0 Identities = 480/499 (96%), Gaps = 4/499 (0%) Strand=Plus/Plus		
Query 1	TAGGGAATCTTCGGCAATGGGGGAAACCTGACCGAGCAACGCCGCTGAGTGAAGAAGG	60
Sbjct 265	TAGGGAATCTTCGGCAATGGGGGAAACCTGACCGAGCAACGCCGCTGAGTGAAGAAGG	424
Query 61	TTTTCGGATCGTAAAGCTCTGTTGTTAAGGAAGAACGAGTGTGAGANTGGAAAGTTCATA	120
Sbjct 425	TTTTCGGATCGTAAAGCTCTGTTGTTAAGGAAGAACGAGTGTGAGANTGGAAAGTTCATA	484
Query 121	CTGTGACGGTACTTAACCGAAGAGGACGGCTAACTACGTGCCAGCAGCCGCGGTAAATAC	180
Sbjct 485	CTGTGACGGTACTTAACCGAAGAGGACGGCTAACTACGTGCCAGCAGCCGCGGTAAATAC	544
Query 181	GTAGGTCCCGAGCGTTGTCCGATTTATTGGGCGTAAAGCGAGCGCAGCGGTTAGATAA	240
Sbjct 545	GTAGGTCCCGAGCGTTGTCCGATTTATTGGGCGTAAAGCGAGCGCAGCGGTTAGATAA	604
Query 241	GTCTGAAGTGAAGGCACTGGCTCAACCAATTGTACGCTTTGGAACTGTCTAACTTGACT	300
Sbjct 605	GTCTGAAGTGAAGGCACTGGCTCAACCAATTGTAGGCTTTGGAACTGTCTAACTTGACT	664
Query 301	GCAGAAGGGGAGAGTGGAAATTCATGTGTAGCGGTGAATGCGTAGATATATTGGAGGAA	360
Sbjct 665	GCAGAAGGGGAGAGTGGAAATTCATGTGTAGCGGTGAATGCGTAGATATA-TGGAGGAA	722
Query 361	CACCGGTGGCGAAGCGGCTCTCTGCTCTGTAAGTACGCT-NNGCTCAAGAGCGTTGGG	419
Sbjct 724	CACCGGTGGCGAAGCGGCTCTCTGCTCTGTAAGTACGCTGAGGCTCGAAGCGGTGGGG	783
Query 420	AGCGAACAGGATTAGATACCTGAGTACGAGCCGTAACGATTAAATTGCTAGGTGTTT	479
Sbjct 784	AGCGAACAGGATTAGATACCTGAGTACGAGCCGTAACGATTAAATTGCTAGGTGTTT	841
Query 480	AGGTCCCTTCGGGGATTA	498
Sbjct 842	AGGTCCCTTCGGGGACTTA	860

Figure 2. 96 % degree of homology

K 19 with a very high degree of homology.

Genes that play a role in the formation of dental caries was the Gtf gene B/C. The nature of gene B was expressed insoluble glucan while Gtf C gene expressing soluble and insoluble glucans. The length of the Gtf gene B/C was the total size of about 600 pb.

The results of the Gtf gene amplification fragment B/C by used specific primers gave a strands with sizes as expected at around 600 bp (Fig. 1). This suggested that the Gtf B/C gene had been amplified.

## DISCUSSION

A success in determining the sample as bacteria was shown by the amplification of bacterial gene encoding 16S rRNA using universal primer that recognize all type of bacteria. In determining the size of the 16S rRNA PCR product, pUC 19 DNA

marker that restricted by HinfI enzyme was used (pUC 19/HinfI). Restriction product was isolated from tooth caries. Research on *S. anginosus* was conducted to determine its role on caries formation and its cariogenic characteristic.

Bacteriological research on dental caries shows that *S. Anginosus* can be isolated from teeth. Some samples showed the growth of *S. anginosus* growth while some others show the mixed growth with other anaerob bacteria. Therefore, only 4 out of 20 samples were amplified.

This was presumably because many oral bacteria have been resistant to basitrasin. This was in accordance with the opinion expressed by Ruoff<sup>8</sup> who also said that oral bacteria have become resistant to basitrasin and it causes difficulty to isolate *S. anginosus*, thus, other bacterial colonies were obtained.

Some previous researchers found that the *Streptococcus* was very phenotypely heterogeneo-



us and have similar genetic characteristics that can be incorporated into a single species. They also acknowledge that the name of *S. anginosus* was the oldest name given to the members of "*Streptococcus milleri*", so the name of *S. anginosus* was set to represent this bacterial group.

According to Okayama<sup>9</sup>, *Anginosus* group was the most common organism found in abscesses. This group of organism is the first group found in oral infection.

*S. milleri* that was found and isolated from teeth has hemolytic characteristic and some species survive in pH of 5.0. Among other *Streptococcus*, the acid tolerant *Streptococcus* were *S. gordini*, *S. intermedius*, *S. mitis*, *S. oralis*, *S. salivarius*, and *S. sanguis*. Cariogenic character of *anginosus* group, according to Ruoff<sup>8</sup> was caused by its antigen protein, although it still has to be proven further.

Nevertheless, the important hallmark was that the acid tolerance is the characteristic of cariogenic bacteria. Based on this research, *S. Anginosus* is bacteria isolated from dental caries which is certainly a fairly acidic environment. Therefore, it is suspected that the bacteria is resistant to acid. This is similar to the characteristics of *S. mutans*, that is although its tolerance against dental plaque varies.<sup>8,9</sup> In conclusion, the presence of dental caries is not synonymous with the presence of *S. mutans*.

On *S. anginosus* cell membrane, glucans were found in high concentration. Glucan will be a source of energy for the bacteria through the process of glycolysis and produce acid.<sup>8,9</sup> This allegation causes difficulty in isolating the chromosome of *S. anginosus* that result in only 4 *S. Anginosus* isolated in this research.

Crystalized form of the colonies with uneven surfaces is presumably because these bacteria are able to bind to the sucrose fermentation product, glucan and fructan. According to this assumption, bacteria that is able to form crystalized colonies are those that have enzyme to break down sucrose into glucan, namely Gtf enzyme.<sup>8,9</sup>

Gtf B/C is a gene that expresses glucosyl-transferase enzyme. This enzyme is one cariogenic factor. This enzyme is an extracellular enzyme that is produced continuously. This enzyme has an ability to break down sucrose into glucans. Gtf B/

C gene which is owned by *S. anginosus* has the following characteristic; Gtf B encodes Gtf I enzyme, the insoluble enzyme, while Gtf C encodes Gtf SI which is soluble and insoluble. Gtf B/C gene is located in a sequence to form a tandem in which the Gtf C gene is located next to the Gtf B gene. According to Hamada & Kuramitsu<sup>10</sup>, if the bacteria have two genes, the bacteria are then said to have one of the cariogenic properties.

In molecular basis of Gtf B/C gene, it has a length that varies depending on the results of homology of each bacterial species, range from 400-900 pb (NCBI). The length of the Gtf B/C gene obtained from this research was between 450 pb, 500 pb, 600 pb, and 700 pb. It has been informed that the length of this strand was influenced by the degree of homology between the protein sequence of Gtf B and Gtf C gene so that the recombination of both gene arrangement may have different lengths.

## CONCLUSION

Bacteria that has Gtf B/C gene has one of the cariogenic properties that is able to synthesize insoluble glucans; as owned by the *S. anginosus*. *Streptococcus anginosus* is one of the cariogenic bacteria because it has Gtf B/C gene.

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