연쇄상구균 GS-5의 ag Ⅰ/Ⅱ와 gtfD 유전자 클로닝

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국문초록

치아 우식은 석회화 조직의 일부가 용해되고 파괴되는 감염성 세균 질환이다. 최근에 보고된 치아우식증 관련 미생물에 대한 연구는 대부분이 *Streptococcus mutans*와 같은 *Mutans streptococci*에 초점을 맞추고 있다. *Mutans streptococci*는 7가지의 서로 다른 종(*S. cricetus, S. downei, S. ferus, S. macacae, S. mutans, S. rattus, S. sobrinus*)과 8가지의 혈청형(serotype a-h)을 모두 포함하여 일컫는다. 산 생성으로 인한 치아의 법랑질 탈회뿐 아니라 치면에 접착하여 군집를 형성하는 것이 균의 독성에 있어 중요하다. 그러므로 우식은 치태와 밀접한 관련이 있다. 이런 초기 군집 형성은 antigen Ⅰ/Ⅱ (Ag Ⅰ/Ⅱ)와 glucosyltransferase(GTF)에 의해 이루어 진다. 그러므로, Ag Ⅰ/Ⅱ와 GTF는 *S. mutans* GS-5에 대한 백신 개발에 있어 적당한 목표가 된다. 본 실험은 *S. mutans* GS-5로부터 *ag Ⅰ/Ⅱ*와 *gtfD* 유전자를 복제하고 나열하였다. 핵산의 나열순서 분석결과 앞서 보고된 나열과 높은 일치도를 보였다. 복제된 Ag Ⅰ/Ⅱ와 앞서 보고된 *S. mutans* GS-5의 해당 부위의 280개의 핵산은 완벽하게 일치하였다. *gtfD*와 *S. mutans* UA159와 비교시, 105개의 아미노산 중 4부위에서 변화를 관찰하였다.

주요어 : 연쇄상 구균, Antigen Ⅰ/Ⅱ, Glucosyltransferase

Ⅰ. INTRODUCTION

Dental caries, also generally known as cavities, is a resultant of demineralization in tooth enamel by acids produced by bacteria. Among several hundreds bacteria in oral flora, only a small number of bacteria play key roles in tooth decay. So far, *Streptococcus mutans* is most well studied regarding its functions in the caries development[1-6]. In this study, I cloned antigen genes from *S. mutans* to prepare vaccine candidates for dental caries whose effective remedy is not yet available except tooth extraction.

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Streptococci are Gram-positive, facultative anaerobes and have no catalase activities. Based on surface antigens, Streptococci are classified into groups but *Streptococcus pneumoniae*, a major cause of human pneumonia, and viridans streptococci are two groups which have no group antigens. In the viridans streptococci, also called as the mutans streptococci, *Streptococcus sobrinus* and *S. mutans* are the major species[7,8]. The mutans streptococci have α-type hemolytic activities which partially hemolysis red blood cells with a green coloration. The mutans streptococci can grow at high temperature around 45℃. These are two critical indicators for identifying the mutans streptococci. The first strain in mutans streptococci, *S. mutans* Clarke, was isolated in 1924 and recently the whole genomic sequence was revealed for *S. mutans* UA159, a Bratthall serotype c strain of *S. mutans*[9,10].

S. mutans is a causative agent for dental caries. As

well as acid production yielding the demineralization of tooth enamel, adherence and colonization of *S. mutans* to the teeth are also important for its virulence[8,11]. Initial adherence occurs in a sucrose-independent way, while bacterial accumulation depends on sucrose. A surface fibrillar protein named as Antigen Ⅰ/Ⅱ or Ag Ⅰ/Ⅱ[12], also known as protein B[13], P1[14], Pac[15], and SpaP[16], plays a key role in the initial adherence, while a group of glucosyltransferases (GTF) are involved in the bacterial accumulation[17-19]. Once *S. mutans* forms a bacterial biofilm, the bacteria can be better protected from the host defense system, sequestered into a nutrient-rich niche, and beneficial from the community mode of growth[20]. A well-established biofilm can be better resistant to antibiotic agents or phagocytosis. That is why the prevention of bacterial colonization is of very importance for the prevention of dental caries. In this respect, Ag Ⅰ/Ⅱ and GTF will be good candidates for vaccine developments.

Ag Ⅰ/Ⅱ and GTF are involved in many key steps of caries development(Table 1) and many previous reports suggest that induction of antibodies against mutans streptococci in oral cavity effectively prevent dental caries[21-25]. Therefore, the determination of efficient epitopes in Ag Ⅰ/Ⅱ and GTF will be helpful for the vaccine development.

Ag Ⅰ/Ⅱ adhesion weighs around 185 kDa and is expressed as a transmembrane protein with the help of signal peptide at its amino terminus and transmembrane segment at its C-terminus as shown in Fig. 1[26,27]. Two domains in the central part of Ag Ⅰ/Ⅱ are involved in binding to salivary pellicle and these domains are rich in alanine and proline, respectively[28-32]. An epitope which can crossreact with human IgG exists adjacent to the proline-rich domain[30].

GTF proteins have three homologues encoded by *gtfB, gtfC and gtfD*. *gtfB* is in charge of insoluble glucan production, *gtfC* for soluble and insoluble glucan production, and *gtfD* encoding GTFd for soluble glucan production. GTF is expressed as extracellular or cell-associated protein. In addition to signal peptide, catalytic domain and glucan-binding domain exist in GTF(Fig. 1). Cell association and accumulation through glucan binding is critical for the cariogenic plaque formation and prevention of this process by salivary immunoglobulin will be one of ideal approaches to interfere caries development[33,34].

In oral cavity, secretory IgA(sIgA) antibodies act as safeguards against enormous challenges from oral bacteria and the principle role of sIgA is to reduce the chance of colonization of pathogens at mucosal surfaces[35]. Human salvia normally has about 0.25mg/ml of sIgA which is somewhat resistant to proteolytic degradation, allowing sIgA more effective at hostile mucosal environments[36-38].

Mucosal immunity can be induced at induction sites such as gut-associated lymphoid tissue (GALT) and nasal-associated lymphoid tissue (NALT) and specific IgA can be produced at effector sites such as lamina propria of gastrointestinal and respiratory tracts and various exocrine glands including salivary gland. Owing to the common mucosal immune system, mucosal immunity can be induced at the remote area other than local pathogen-abundant region[39-44]. Because of homing of specific B cell and antigenic activation at the local area,

Table 1. Roles of *S. mutans* proteins, Ag Ⅰ/Ⅱ and GTF, in dental caries development. The plus sign indicates the participation of Ag Ⅰ/Ⅱ or GTF in the step of pathogenesis.

Stages in caries development	AgI/II	GTF
Colonization by early colonizers		
Adhesion to early colonizer or salivary pellicle	+	+
Colonization		+
Evasion from phagocytosis	+	
Invasion of dental tubules	+	

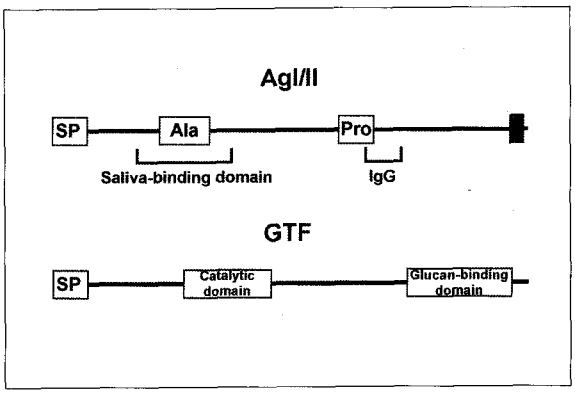

Fig. 1. Graphical presentation of two candidates for vaccine development against *S. mutans*. SP indicates signal peptides and the black box in the AgI/II represents anchoring domain in bacterial membrane.

mucosal immunity from the remote inductive sites can hold efficacy against the local infection. This property of mucosal immunity will be practical to develop vaccines against *S. mutans* by oral administration of bacterial antigens, such as Ag Ⅰ/Ⅱ and GTF, to induce antigen-specific B cell population at GALT and to generate sIgA against bacterial pathogens in oral cavity.

In this study, I cloned the genes of *ag Ⅰ/Ⅱ* and *gtfD* from *S. mutans* GS-5. The sequence from cloned *ag Ⅰ/Ⅱ* gene was compared with that of the sequence published from *S. mutans* GS-5, while the sequence of *gtfD* with that of the corresponding sequence from *S. mutans* UA159 in which the genomic DNA was completely sequenced recently.

Ⅱ. MATERIALS AND METHODS

1. Materials

All chemicals and plastic wares were purchased from Sigma (St. Louis, MO, U.S.A.) and Falcon Labware (Frankline Lakes, NJ, U.S.A.), respectively. Oligonucleotides were ordered from Genetech (Yusung, Korea) and T vector was supplied from Takara (Shiga, Japan). Restriction enzymes were obtained from KOSCHEM (Seongnam, Korea).

2. Bacterial culture

S. mutans GS-5 was grown in BHI medium for 16 h at 37℃ in a shaker and 200 ㎕ of overnight culture was transferred into 5ml of a fresh medium, leaving for further 24 hour incubation. *S. mutans* GS-5 had a longer lag phase than other bacteria such as *E. coli*.

3. Isolation of genomic DNA from *S. mutans* GS-5

The genomic DNA from *S. mutans* GS-5 was prepared as described previously[45]. Briefly, 5ml of *S. mutans* GS-5 was collected and resuspended in 4ml of TE buffer containing 1ml of 25% glucose. After 30min incubation at 37℃, 1mg of lysozyme was added into the bacterial suspension and further incubated at 37℃ for 1h. RNAs in the lysates were removed by adding 0.5 mg of RNase A and then proteins in the lysates were digested by 0.5mg of proteinase K. The cleared lysates was treated with SDS for 12h at 4℃ and then genomic DNA was precipitated by ethanol. Finally, the genomic DNA was resuspended in 1 ml of TE buffer.

4. Agarose gel electrophoresis

S. mutans chromosomal DNA, PCR products, and plasmids were separated in 1% agarose gel in Tris-acetate-EDTA buffer and visualized by staining with ethidium bromide. One kb DNA size marker was included in each gel

5. PCR amplification of bacterial genes

The genomic DNA was fragmented into smaller pieces by digestion with XhoI and SalI enzymes. The digested DNA was then used as templates for amplifying *ag Ⅰ/Ⅱ* and *gtfD* genes in 50㎕ of PCR reaction containing Tag polymerase, dNTP, and oligonucleotides. In addition to full-length genes, a portion of each gene was also amplified using primer as described in Table 2. The genomic DNA was denatured by 5min incubation at 95℃ and then target genes were amplified for 30 cycles of PCR re-

Table 2. List of oligonucleotides used to amplify *ag Ⅰ/Ⅱ* and *gtfD* genes. The upper case indicates the sequence from *ag Ⅰ/Ⅱ* and *gtfD*, while the small case shows the extra nucleotides to help gene cloning. The underlined sequences are the locations of restriction enzyme sites.

	Sequence
Ag Ⅰ/Ⅱ 5	acatGCATGCGGACAAAAGGTTTTGCCGATG
Ag Ⅰ/Ⅱ 3	tggGGTACCTCAAGCATTGTTTGTTACTCCCGT
Ag Ⅰ/Ⅱ M5	GTATACCAGAGCTAGCGAACCGGGATC
Ag Ⅰ/Ⅱ M3	GATCCCGGTTCGCTAGCTCTGGTATAC
gtfD 5	gatcGGATCCGAAACAGAACAGCAGACCTCAG
gtfD 3	tccCCCGGGTTAATAATATCTAGCGATACCCCAACG
gtfD M5	GATAAAAGTCATATGGATTGGGAT
gtfD M3	ATCCCAATCCATATGACTTTTATC

action(1min 95℃, 1min 55℃, 1min 72℃). The PCR product was then treated for 5min at 72℃.

6. Overlap PCR reaction

In order to obtain the full-length gene from two fragments amplified by PCR, PCR products from each reactions of 5' fragment and 3' fragment were mixed in a new PCR reaction tube and PCR was performed again.

7. Cloning of *ag I/II* and *gtfD* genes

About 2 μl of PCR products were ligated into T vector following manufacture's protocol and the ligation mixtures were transformed into competent Top 10cells. The resulting antibiotics resistant bacteria were selected from agar plates and screened by checking the plasmid from each bacterial colony. First, plasmids were compared in size with that of control vector in agarose gels to reveal the presence of any insert DNA. Second, digestions of plasmids with restriction enzymes were performed to further prove the presence of target genes. The plasmids containing expected insert size were then sequenced to confirm the presence of target gene. The DNA sequencing was performed by BMRC(Daejeon, Korea).

8. Sequence analysis of *ag I/II* and *gtfD* genes from *S. mutans* GS-5

The DNA sequence of cloned genes was analyzed by comparing with the previously known sequences. For *Ag I/II* sequence analysis, the known sequence from *S. mutans* GS-5 (GenBank # D78181) was used as standard, while the genomic sequence of *S. mutans* UA159 (GenBank # NC_004350) was compared to that of the cloned *gftD* gene. The program, Multalin[46] and ESPript[47], were used to generate sequence alignments for each gene.

9. Expression of *Ag I/II* and *gtfD* in bacterial expression system

In order to express cloned genes in *E. coil* system, gene fragments of *ag I/II* and *gtfD* were subcloned to pQE expression vector. BamHI and Sal I were used to transfer 5' fragment of *ag I/II* gene, Sph I and Sal I for 3' fragments of *ag I/II* gene and 5' fragment of *gtfD*, Sph I and Kpn I for 3' fragment of *gtfD*. Expression of each gene is in process.

Ⅲ. RESULTS

1. Isolation of genomic DNA from *S. mutans* GS-5

In order to clone *S. mutans* GS-5 antigen genes, the bacterial genomic DNA was isolated and the integrity of the purified DNA was confirmed in agarose gel(Fig. 2). The purified genomic DNA was trapped in the well due to its huge size about 2 Mbp long. A portion of the purified genome formed a fast-migrating band.

2. PCR amplification of *ag I/II* and *gtfD* genes

Because the genomic DNA of *S. mutans* is a circular form about 2 million base pairs long, the isolated genomic DNA was fragmented into smaller pieces by Xho I and Sal I to enhance the PCR reaction(Fig. 3). Xho I and Sal I were selected due the absence of those sites in the corresponding genes in *S. mutans* UA 159 revealed by the sequence analysis. The products of restriction enzyme digestion clearly showed that these enzyme sites were not abundant in the genomic DNA. Once chromosomal DNA was digested by each restriction enzyme, the resulting reaction mixtures were mixed to reduce the chance of unidentified enzyme sites in the antigen gene.

PCR was performed in three ways for each gene, yielding full-length, 5' fragment, and 3' fragment(Fig. 4). In both genes, smaller fragments were always amplified in a better quantity. This was may be caused by the PCR condition fitting better for about 2Kbp DNA amplification. The full-length gene of Ag I/II was also amplified as a single band, although the yield was not as good as that of smaller fragments. In addition to the expected band, at least two more bands were shown from the full-length amplification for *gtfD* gene. It was not clear why two smaller bands were generated in this reaction but there might be several possibilities. There could be some nonspecific sites which can allow primer binding or the full-length product was cleaved for unknown reasons.

Several other ways to generate the full-length *gtfD* gene in large quantity were applied but some of those were not successful (data not shown). One strategy was to perform several rounds of amplification in separated tubes containing one primer per tube and later assemble

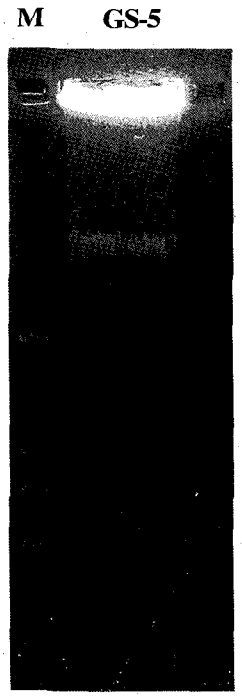

Fig. 2. *Isolation of genomic DNA from S. mutans GS-5. Purified* genomic DNA of *S. mutans* GS-5 was separated in agarose gel. M indicates size marker.

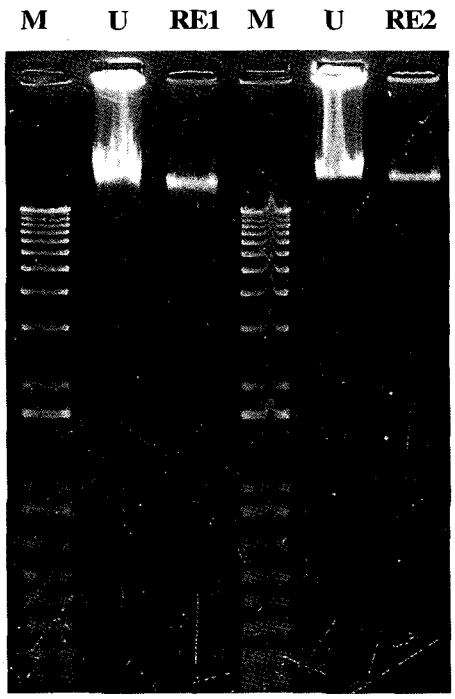

Fig. 3. *Restriction enzyme digestion of genomic DNA isolated from S. mutans* GS-5. The purified genomic DNA (U) was treated with XhoI (RE1) or SalI (RE2) and separated in an agarose gel. M indicates size marker.

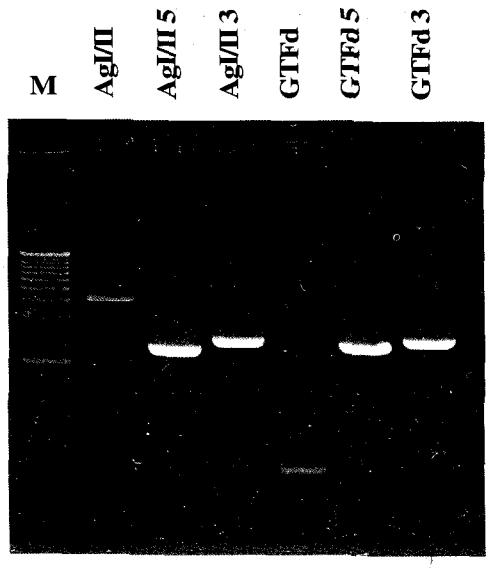

Fig. 4. PCR amplification of *S. mutans* GS-5 genes encoding AgI/II and GTFd. The genes for AgI/II and GTFd were amplified by PCR using the genomic DNA linearized by restriction enzyme digestion. In addition to full-length genes (*agI/II* and *gtfD*), truncated genes encoding 5' or 3' segments were also amplified.

the reaction mixtures to allow PCR amplification for the full-length product. This will be helpful to avoid any competition between primers to generate uneven amounts of early products which then interferes the following PCR amplification. The other measure is to perform overlap PCR reaction. Because smaller fragments were easily generated and one terminus of the PCR product from one reaction complements for that of the other reaction, these two PCR products can be combined to generate the full-length PCR product.

3. Cloning of *ag I/II* and *gtfD* genes

PCR products were ligated into T vector, yielding T vector cloned with *ag I/II* or *gtfD* gene. The cloned T vectors were transformed into an *E. coli* strain, Top 10. Plasmids were prepared from antibiotics-resistant colonies and separated in agarose gel(Fig. 5, 6). Two or more colonies contained T vector with expected inserts from partial fragment PCR products but none of colonies had full-length genes, meaning that antibiotic-resistant

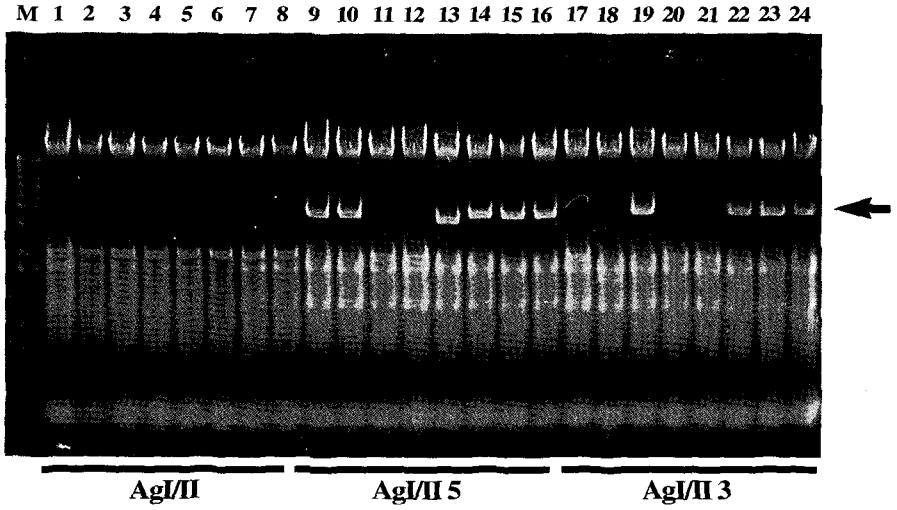

Fig. 5. Screening of plasmids cloned with PCR-amplified *agI/II* genes. The genes encoding full-length (AgI/II) or partial segments (AgI/II 5 or 3) were cloned into T vector and bacterial transformants were screened for the presence of insert DNA. Arrows indicated the positions of plasmids with insert DNA.

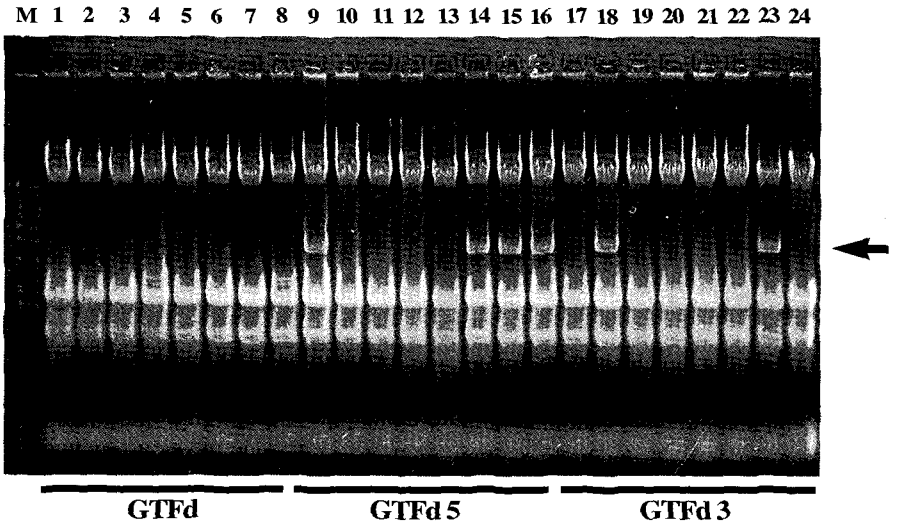

Fig. 6. Screening of plasmids cloned with PCR-amplified *gtfD* genes. The genes encoding full-length (GTFd) or partial segments (GTFd 5 or 3) were cloned into T vector and bacterial transformants were screened for the presence of insert DNA. Arrows indicated the positions of plasmids with insert DNA.

colonies from these samples resulted from self-ligated T vector without insert cloning. A couple of plasmids containing each of cloned genes were further confirmed by digestion with restriction enzymes which existed in the cloned genes, BamHI for *agI/II* and SphI for *gtfD*, respectively(Fig. 7). The linearized DNA revealed single or double bands depending on the direction of cloning. Finally, the sequences of cloned genes were confirmed by nucleotide sequencing.

Because there was no colonies positive for full-length gene cloning, the PCR products were investigated for their amounts and very little amount of full-length PCR

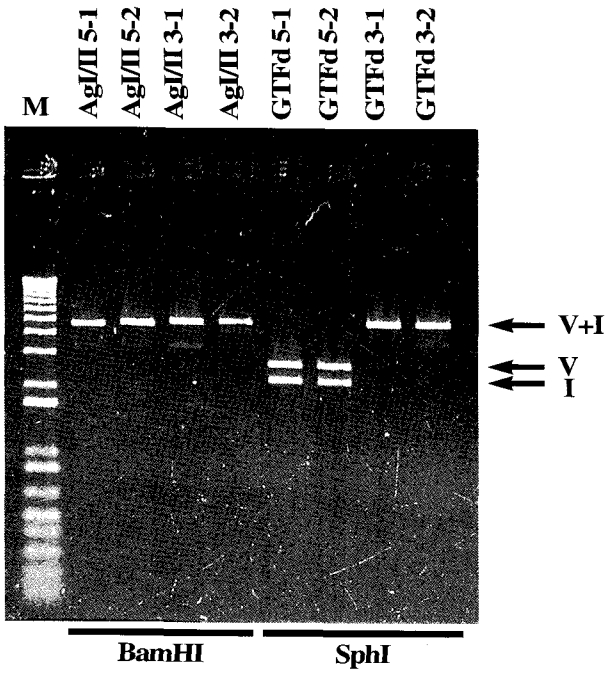

Fig. 7. Confirmation of the presence of insert DNA in the plasmids purified from transformed bacteria. Single or double fragments were generated according to the direction of an insert in each cloning vector after restriction enzyme digestion of plasmids. The locations of cloning vector (V), insert (I), and vector containing insert (V+I) were marked.

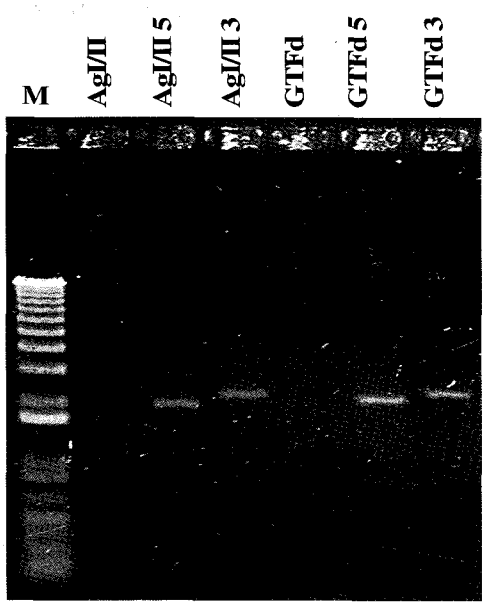

Fig. 8. Confirmation of the presence of insert DNA after extracting PCR-amplified DNA from agarose gel.

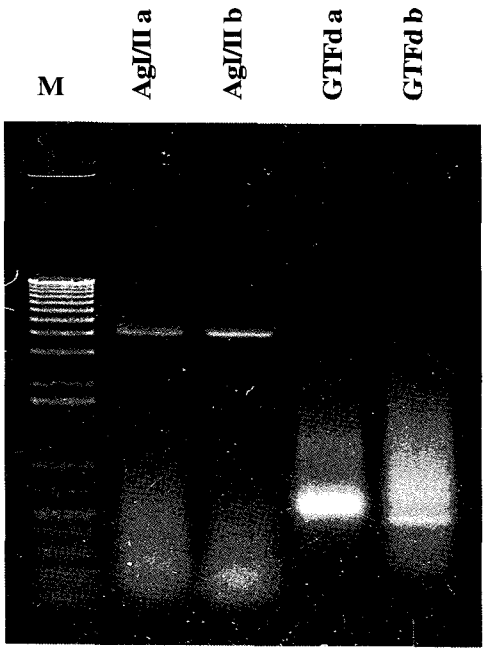

Fig. 9. PCR amplification of full-length *agI/II* and *gtfD* genes. PCR parameters were modified to obtain the full-length genes and the above is a representative picture from many trials.

products were shown from the samples which were previously extracted from agarose gel(Fig. 8). The full-length genes were amplified in several ways as described above. Those amplified genes were in the process of cloning(Fig. 9).

4. Sequence analysis of cloned genes

To prove the identity of the cloned genes, sequence analyses were accomplished using several programs such as Multalin[46] and ESPript[47]. For *agⅠ/Ⅱ* analysis, the corresponding sequence from *S. mutans* GS-5(GenBank # D78181) was compared with that of the cloned gene. The representative sequence alignment was shown in Fig. 10. In this 280bp sequence, there was no miss match at all. In a similar approach, there were seven miss matches out of 315 nucleotides, resulting four changes in amino acid sequences for *gtfD* sequence alignment when this sequence was compared with the corresponding sequence (GenBank # NC_004350) of *S. mutans* UA159 of which the nucleotide sequence was recently determined(Fig. 11, 12).

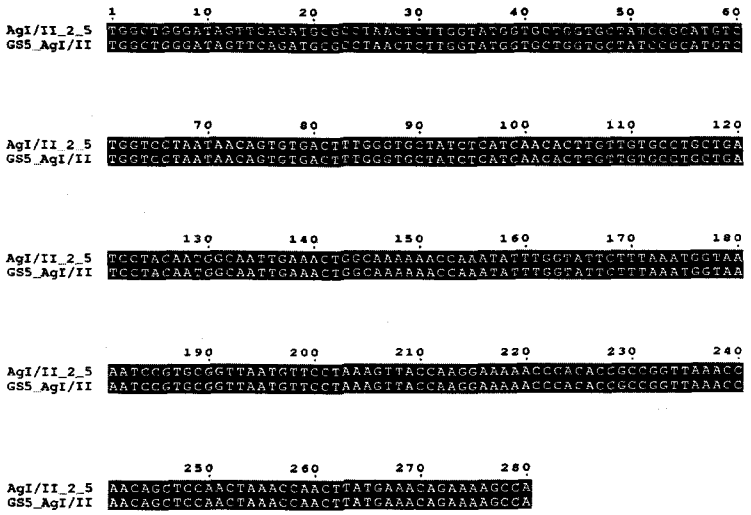

Fig. 10. Sequence alignment between cloned *agI/II* segment and the corresponding region in reported *agI/II* gene. The sequence of the cloned *agI/II* gene was compared to that of known *agI/II* gene (GenBank # D78181).

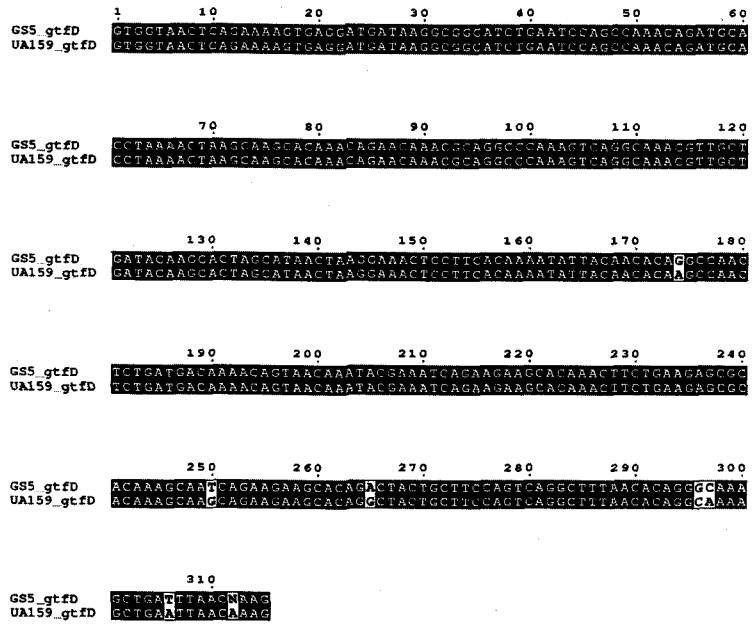

Fig. 11. Sequence alignment between cloned *gtfD* segment and the corresponding region in reported *gtfD* gene. The sequence of the cloned *gtfD* gene was compared to that of known *gtfD* gene (GenBank # NC_004350).

5. Expression of Ag I / II and GTFd in *E. coli* system

Once the genes for Ag I / II and GTFd were cloned and sequenced, these genes were transferred into an expression vector, pQE for their protein productions. Currently, the conditions to maximize the yield of target proteins were being tested. The purified protein will be used to make antibodies for the future experiment of vaccine development. Hopefully, segmented fragments will be also useful to determine an efficient epitope for the induction of protective immunity against dental caries.

Fig. 12. Comparison of peptide sequence deduced from cloned genes encoding GTFd. Amino acid sequence deduced from cloned *gtfD* of *S. mutans* GS-5 was compared with that of *S. mutans* UA159.

Ⅳ. DISCUSSION

In early competition between bacterial pathogens and host defense system, one of key steps for bacterial infection is the formation of a biofilm[20]. Once a bacterial biofilm was established, the bacterial growth in sessile mode has several advantages over the planktonic growth. In this mode of growth, bacteria will be better protected from the host defense system such as shear force generated by fluid flow, antibiotics, antibodies, phagocytosis, and changes in pH. Another benefit for the growth in a biofilm is that it offers a favorable environment for bacterial growths in the sense of nutrient-rich, relatively stable conditions.

Two genes have been well studied about adhesion and colonization of *S. mutans* in the development of dental plaque[8,11]. The bacterial fibrillar protein, Ag Ⅰ/Ⅱ, and glucosyltransferase, GTF, play key roles in the formation of a biofilm. Once the favorable niche was achieved, bacteria grow faster and generate lots of acids leading to demineralization of tooth enamel called as dental caries. In this view, prevention of biofilm process will be a reasonable target for vaccine development against dental caries. As an effort of this approach, genes for Ag Ⅰ/Ⅱ and GTFd were cloned in order to set the basis for antigen preparation in the future.

The genes for Ag Ⅰ/Ⅱ and GTFd were cloned as fragmented form rather than full-length. Fragmentation could be useful for vaccine development against dental caries, because saliva- and glucan-binding domains are in charge of bacterial adhesion and colonization and these domains are located at N-terminal or C-terminal half of each gene. By using smaller fragments, the specific antibodies against target epitope can be better induced by avoiding unnecessary competition through epitope dominance. Another advantage of using small fragment of Ag Ⅰ/Ⅱ is that the epitope adjacent to praline-rich region, mimicking human IgG can be discarded.

Because the overall sequence of *agⅠ/Ⅱ* gene was cloned in two fragments, it will offer a good opportunity to compare the controversial mismatch among research groups about the truncation of *agⅠ/Ⅱ* gene through nonsense mutation in the coding region[48,49]. For some reason, it is possible that the early report about this mutation may be caused by bacterial adaptation for solution culture which may enforce some laboratorial strain of *S. mutans* GS-5 to truncate its *agⅠ/Ⅱ* gene. This unintentionally-mutated strain will obtain the advantage of rapid growth in shaker culture but the strain in this study showed slow growth, implying that this strain might have the original activity of Ag Ⅰ/Ⅱ which is important for vaccine development.

To my knowledge, this study is the first report about the cloning of *S. mutans* GS-5 *gtfD*. This will set the basis for the basic study about the role of this protein in the pathogenesis of *S. mutans* GS-5. Comparing with the sequence of *gtfD* from *S. mutans* UA159, the corresponding nucleotide sequence of this strain showed some mismatches and the mismatches introduced changes in four residues out of 105 amino acids. One caused

changes from aspartic acid to glutamic acid allowing the same net charge of side chain. The other three included serine to alanine, threonine to alanine, and glycine to alanine. At this moment it is not clear what this difference can cause in terms of the protein function.

In conclusion, *ag Ⅰ/Ⅱ* and *gtfD* genes from *S. mutans* GS-5 were cloned and sequenced. Sequence analyses showed the nucleotides sequence of cloned genes had high homology to the sequences previously reported. Currently, the expression of cloned genes is being tested. In consideration of the importance of these proteins in the pathogenesis of *S. mutans* GS-5, vaccine development against these proteins will be promising and also vaccination through oral administration will be reasonable approach to stimulate mucosal immunity of the host.

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Abstract

IDENTIFICATION OF THE AG Ⅰ/Ⅱ AND GTFD GENES FROM *STREPTOCOCCUS MUTANS* GS-5

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Streptococci are Gram-positive, facultative anaerobes and have no catalase activities. Among mutans streptococci containing α-type hemolytic activity, *S. mutans* is a causative agent for dental caries. As well as acid production yielding the demineralization of tooth enamel, adherence and colonization of *S. mutans* to the teeth are also important for its virulence. These early colonization are accomplished by the bacterial fibrillar protein, Antigen Ⅰ/Ⅱ (Ag Ⅰ/Ⅱ), and glucosyltransferase (GTF). Therefore, Ag Ⅰ/Ⅱ and GTF are reasonable targets for the development of vaccine against *S. mutans* GS-5. The ag Ⅰ/Ⅱ and *gtfD* genes from *S. mutans* GS-5 were cloned and sequenced. Sequence analyses showed the nucleotides sequence of cloned genes had high homology to the sequences previously reported. The sequence alignment of 280 nucleotides between the cloned Ag Ⅰ/Ⅱ and the available sequence of the corresponding *S. mutans* GS-5 showed the perfect match. Comparing with the sequence of *gtfD* from *S. mutans* UA159, the corresponding nucleotide sequence of *S. mutans* GS-5 showed some mismatches and the mismatches introduced changes in four residues out of 105 amino acids, yielding four missense mutations.

Key words : *Streptococcus mutans*, Antigen Ⅰ/Ⅱ, Glucosyltransferase