2007년 2월

박사학위논문

Isolation and characterization of the mutans streptococci from Koreans

조 선 대 학 교 대 학 원

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A thesis submitted of the faculty of the Chosun University in partial fulfillment of the requirements for the Degree of Doctor of Philosophy in the Department of Dentistry

Gwangju, Korea

2007

Approved by

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한국인으로부터 mutans streptococci의 분리와 특성

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이 논문을 치의학 박사학위신청 논문으로 제출함

2006년 10월 일

조 선 대 학 교 대 학 원

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ABSTRACT

Isolation and characterization of the mutans streptococci from Koreans

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Mutans streptococci have been implicated as cariogenic bacteria in dental caries because they can produce high levels of dental caries-causing lactic acid and extracellular polysaccharide. The aim of this study was to isolate and characterize the mutans streptococci from the dental plaque obtained from Koreans using conventional methods including a biochemical test and molecular biological methods.

The dental plaque samples were collected from the anterior and molar teeth of both jaws in 155 subjects (aged 2 to 33.2 years, average age 13.7±4.7 years). The samples were diluted by 100-fold in 1 X PBS and plated on mitis-salivarius bacitracin (MSB) agar plates. The mutans streptococci were grown on MSB plates and screened by polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) targeting dextranase gene (*dex*). The biochemical tests were carried out to biotype the mutans streptococci. The mutans streptococci were identified at the species level using a 16S rDNA sequencing comparison method. Using the genomic DNAs of the clinical isolates, PCR was performed to evaluate the specificity of the *S. mutans*- or *S. sobrinus*-specific PCR primers based on 16S rDNA

and *dex*.

Ninety-eight strains of the mutans streptococci out of 358 colonies, which were derived from 141 subjects, were isolated. Of them, 80 strains and 18 strains were *S. mutans* and *S. sobrinus*, respectively. The biotyping data showed that 64, 1, 20, 10, and 3 strains were biotypes I, II, IV, V and variant, respectively. Of three strains of variant biotype, two strains were similar to biotype IV except that they tested positive to the arginine hydrolysis test. This is a new biotype and we classified the two strains as biotype VII.

In conclusion, *S. mutans* and biotype I are frequently detected in Korean dental plaque. The mutans streptococci strains isolated in this study might be useful for the study of the pathogenesis and the prevention of dental caries.

Ⅰ. INTRODUCTION

Dental caries is one of the most common infectious diseases in the human oral cavity. The enamel and dentin are demineralized by acids, such as lactic acid, which are produced as a by-product of the carbohydrate metabolism by cariogenic bacteria in dental plaque (Gjermo *et al.*, 1973). The major factors associated with the etiology of dental caries are the host, oral bacteria, diet, and time. The formation of dental caries is caused by the colonization and accumulation of oral bacteria. Adherence is the first step in the colonization process (Gibbons, 1984). In particular, among the oral bacteria, mutans streptococci have been implicated as cariogenic bacteria because they produce high levels of dental caries-causing lactic acid and extracellular polysaccharides (Loesche, 1986; Maiden *et al.*, 1992). In a study of the genus *Streptococcus* based on the sequence comparisons of 16S ribosomal RNA gene (16S rDNA), a total of six species groups were demonstrated, the anginosus, mitis, mutans, salivalius, bovis, and pyogenic group (Kawamura *et al.*, 1995). Mutans streptococci are divided into seven species: *Streptococcus mutans*, *S. sobrinus*, *S. downei*, *S. rattus*, *S. cricetus*, *S. ferus*, and *S. macacae* (Kawamura *et al.*, 1995). Of these, *S. mutans* and *S. sobrinus* are strongly associated with human dental caries because they are frequently isolated from human dental plaque, where they produce large amounts of acids and extracellular polysaccharides (Loesche *et al.*, 1986). It had been previously thought that *S. downei* was only isolated from monkeys. Interestingly, *S. downei* has also been isolated from human dental plaque (Yoo *et al.*, 2005). *S. downei* and *S. sobrinus* are closely related species with similar characteristics but they are distinct species phylogenetically (Whiley *et al.*, 1988; Bentley *et al.*, 1991).

Most mutans streptococci possess the glucosyltransferase (GTF), which synthesizes extracellular polysaccharides (glucan) from the dietary sucrose, and dextranase (DEX), which can break down glucans to isomaltosaccharides

3-4 glucose units long by cleaving the α(1-6) linkages within the dextran chain (Colby *et al.*, 1997). These enzymes facilitate the adhesion and accumulation of oral bacterial cells to the tooth surfaces. Therefore, these enzymes are believed to be one of the virulent factors in the pathogenesis of dental caries (Yamashita *et al.*, 1993). Therefore, it is essential to inhibit the activities of these enzymes in order to control dental caries.

Many chemical antiplaque agents and antibiotics including chlorhexidine, spiramycin and vancomycin have been used against *S. mutans* to treat plaque-mediated diseases including dental caries (Cragg *et al.*, 1997). However, antibiotics using chemical compounds have several side effects such as vomiting, diarrhea, and tooth staining (Wu-Yuan *et al.*, 1988). Recently, natural products have been investigated in an attempt to prevent oral diseases, particularly plaque-related diseases such as dental caries. The ethyl acetate soluble extract of *Sophora flavescens* Ait. and the methanol extract of *Polygoni radi*x (root) showed significantly inhibitory GTF activity and adhesion of *S. mutans* (Lee *et al.*, 2001; Lim *et al.*, 2003; Kim *et al.*, 2005). However, most studies on dental caries had been performed using the strains of mutans streptococci derived from Caucasians. It is unclear if natural products used in previous studies have a similar effect on the mutans streptococci isolated from the dental plaque in Koreans. Lim *et al.* (2003) reported that the leaf-extract from *Camellia sinensis* has an antimicrobial effect on mutans streptococci. There were differences in the different susceptibility between the type strains and the clinical isolates of mutans streptococci. In addition, the effectiveness of the leaf-extract from *Camellia sinensis* differed according to the clinical isolates. Therefore, in order to test the anti-cariogenic effect of natural extracts, it would be necessary to evaluate the antibacterial activity against the strains of the mutans streptococci isolated from the dental plaque obtained from Koreans.

Various methods have been used to both detect and identify the oral streptococcal species including biochemical tests, immunological tests, DNA

probes, polymerase chain reaction (PCR) and 16S rDNA sequencing comparison methods. Among them, the PCR method is currently being applied to the detection of putative pathogens and the identification of human cariogenic bacteria because it is more rapid, sensitive and simple. The target genes for the PCR are often related to virulence factors, which are sometimes species-specific and provide an additional detection marker to avoid ambiguous PCR results caused by the high similarity of the species-specific rRNA gene sequences. The dextranase gene (*dex*) encodes an enzyme that hydrolyzes glucan in a plaque matrix and is involved in the pathogenesis of dental caries (Colby *et al.*, 1997). Therefore, *dex* is one of the virulent factors in mutans streptococci. Various species-specific PCR primers targeting *dex* have been used to detect *S. mutans* (Igarashi *et al.*, 1996). 16S rDNA can be used effectively as a target gene in PCR because the bacterial 16S rDNA gene contains highly conserved and specific regions within bacterial species (Woese, 1987). Those regions can be useful in the development of species-specific PCR primers for the identification of bacteria. Species-specific primers based on the 16S rDNA sequences have also been used to detect *S. mutans* and *S. sobrinus* (Rupf *et al.*, 1999, 2001; Sato *et al.*, 2003; Kato *et al.*, 2004). However, *S. mutans* 16S rRNA gene-specific primers developed by Rupf *et al.* (1999) have also been detected in *S. sobrinus, S. downei,* and *S. rattus* (Ali *et al.*, 2006). According to Kim (2006), who used the strains of *Fusobacterium nucleatum* and *F. periodonticum* isolated from the subgingival plaques in Koreans, the *F. nucleatum*-specific PCR primers introduced by several groups did not have specificity for the species. Therefore, in order to assess the epidemiological studies for dental caries in Korea, it is essential to confirm the specificity of the PCR primers against the clinical strains of mutans streptococci isolated from Koreans.

S. mutans and *S. sobrinus* strains isolated from Korean dental plaque are needed to perform epidemiological studies on the dental caries and the screening of anti-cariogenic natural products. Therefore, the aim of this study

was to isolate and characterize the mutans streptococci obtained from the dental plaque in Koreans.

Ⅱ. MATERIALS AND METHODS

1. Bacterial strains and growth conditions

The type strains of the bacteria used in this study are as follows: *Streptococcus mutans* ATCC 25175^T, *S. sobrinus* ATCC 33478^T, *S. downei* ATCC 33748^T, *S. rattus* KCTC 3655^T, *S. cricetus* KCTC 3640^T, *S. anginosus* ATCC 33397^T, *S. thermophilus* KCTC 3658^T, *S. mitis* KCTC 3556^T, *Staphylococcus aureus* KCTC 1621^T, *Actinobacillus actinomycetemcomitans* ATCC 33384^T, and *Fusobacterium nucleatum* ATCC 25586^T. All the strains were obtained from the American Type Culture Collection (ATCC, USA) or the Korean Collection for Type Cultures (KCTC, Korea). The clinical strains of the mutans streptococci isolated from the dental plaque of Koreans were identified at the species level using a 16S rDNA cloning and sequencing method.

S. mutans, *S. sobrinus*, *S. downei*, *S. rattus*, and *S. cricetus* strains were cultured on a medium composed of mitis salivarius agar (Difco, USA) supplemented with 0.0001% potassium tellurite, 0.2 units (2.8 μg)/ml of bacitracin (Sigma, USA), and 20% (w/v) sucrose (CJ, Korea) (MSB agar). The bacitracin was freshly prepared immediately before use. The MSB agar plates were stored at 4℃ and were used within 7 days after preparation. *S. anginosus*, *S. thermophilus*, *S. mitis*, and *S. aureus* strains were cultured on Brain Heart Infusion (BHI) agar (Difco, USA). The streptococci and staphylococci strains were also cultured on a BHI broth (Difco, USA). All the above species were grown in a 37℃ incubator in air containing 10% CO_2.

A. actinomycetemcomitans was grown in a medium containing TSB (Difco, USA) supplemented with 0.6% yeast extract, 5% horse serum, 75 g/ml of bacitracin, and 5 μg/ml of vancomycin (Sigma, USA). *F. nucleatum* was grown in Schaedler broth (Difco, USA). *A. actinomycetemcomitans* and *F. nucleatum* strains were grown at 37℃ in an anaerobic chamber containing 10% H_2, 5% CO_2, and 80% N_2.

2. Dental plaque collection

The dental plaque samples were collected from the anterior and molar teeth of both jaws in 155 subjects (aged 2 to 33.2 years, average age 13.7±4.7 years). A sterile paper point was used to collect the dental plaque. The plaque samples were stored in a 1.5 ml Eppendorff tube containing 500 μl of 1X phosphate buffered saline (PBS) at −20℃ before analysis. The dental plaque samples were diluted 100−fold in 1X PBS and plated onto MSB agar using a sterilized cotton balls. The plates were incubated for 2 days at 37℃ in a CO_2 incubator.

3. Bacterial genomic DNA preparation

The bacterial genomic DNAs were prepared using a G−spin™ Genomic DNA Extraction kit (iNtRON, Korea) according to the manufacturer's instructions. The DNA concentrations were determined by measuring the OD at 260 and 280 nm using an UV spectrophotometer (Ultraspec 2000, Pharmacia Biotech., UK).

4. Polymerase chain reaction−restriction fragment length polymorphism (PCR−RFLP)

PCR-RFLP based on *dex* was performed to screen the mutans streptococci growing on the MSB agar plate, as described elsewhere (Igarashi *et al.*, 2001). The PCR reaction was carried out using an *AccuPower*® PCR PreMix (Bioneer, Korea) containing 5 nmole of each deoxynucleoside triphosphate, 0.8 μmole KCl, 0.2 μmole Tris−HCl (pH 9.0), 0.03 μmole $MgCl_2$, and 1 unit of *Taq* DNA polymerase. The bacterial genomic DNA and 20 pmoles of each primer were added to a PCR PreMix tube. The PCR was carried out in a final volume of 20 μl. The PCR reaction was run for 27 cycles on a Peltier thermal cycler (Model PTC−200 DNA engine™, MJ Research Inc., USA) under the following conditions: denaturation at 94℃ for 2 min, primer annealing at 55℃ for 1 min, and extension at 72℃ for 1 min. The final cycle

included an additional extension time of 10 min at 72℃. 2 μl of the reaction mixture was analyzed by 1.5% agarose gel electrophoresis in a Tris-acetate buffer (0.04 M Tris-acetate, 0.001 M EDTA, [pH 8.0]) at 100V for 30 min. The amplification products were stained with ethidium bromide and visualized using a UV transilluminator. The PCR products were digested with the restriction enzyme, *Hae*III (Bioneer, Korea). Table 1 lists the size and the number of the generated fragments.

Table 1. Size of the restriction fragments generated from PCR products of mutans streptococci

Species	Size (bp) of the PCR products digested with *Hae*III
S. mutans (534)[a]	412+122
S. sobrinus (525)	351+174
S. downei (525)	210+174+141
S. rattus (534)	325+209
S. cricetus (525)	210+141+109+65

[a]Indicate the size (bp) of PCR product.

5. 16S rDNA cloning and sequencing

16S rDNA cloning and sequencing were also carried out to identify the mutans streptococci screened by the PCR-RFLP at the species level. In order to amplify the 16S rDNA from the bacteria, the PCR was performed with the 27F and 1492R primers using an *AccuPower*® PCR PreMix (Bioneer, Korea). The PCR reaction was run for 30 cycles on a Peltier thermal cycler (Model PTC-200 DNA engine™, MJ Research Inc., USA). The PCR conditions were the same as those described previously (Lane *et al.*, 1985). Briefly, the reaction mixture (20 μl) was denatured at 94℃ for 2 min

followed by a series of amplications: denaturing at 94℃ for 1 min, annealing at 55℃ h for 30 sec and extension at 72℃ for 45 sec. The final cycle included an additional extension time of 10 min at 72℃. The PCR products were purified using an *Accu*Prep® PCR purification kit (Bioneer, Korea), and were ligated directly with the pGEM-T easy vector (Promega, USA). The nucleotide sequencing of the 16S rDNA was determined using the dideoxy chain termination method with a Big Dye Terminator Cycle Sequencing Kit (Applied Biosystems, USA) and an ABI PRISM 310 Genetic Analyzer (Applied Biosystems, USA). Table 2 lists the primers used in nucleotide sequencing. All the sequences were compared with similar sequences from the reference organisms provided by BLAST (a genome database of the National Center for Biotechnology Information). The 16S rDNA nucleotide sequences of the mutans streptococci were registered on the GenBank database (National Center for Biotechnology Information).

Table 2. The primers used for the 16S rDNA sequencing

Primer names	oligonucleotide sequences (5' → 3')
ChDC-GEM-F	TCC CCA GTC ACG ACG TTG TAA AA
Seq-F1	CCT ACG GGA GGC AGC AG
Seq-R2	GAC TAC CAG GGT ATC TAA TCC
Seq-F16	TAG ATA CCC YGG TAG TCC
ChDC-GEM-R	GTG TGG AAT TGT GAG CGG ATA AC

6. Biochemical tests

The biochemical tests were carried out to determine the biotypes of the mutans streptococci, as described previously (Shklair *et al.*, 1974, 1976). A phenol red broth base (BBL, USA) was used as the basal medium used for fermenting mannitol, sorbitol, raffinose, and melibiose. The carbohydrates

were sterilized by Millipore filtration (0.22 μm pore size) and added aseptically to the warm basal media. The final concentration of the carbohydrates was 1.0%. The media were dispensed into sterile screw cap tubes that had been inoculated with the organisms to be tested, and read after 48 hrs of either aerobic (for all tubes of carbohydrates) or anaerobic (for the mannitol tube) incubation. The level of ammonia production from L-arginine was determined using the medium described by Facklam (1977). After 48 hrs incubation, 0.1 ml of the Nessler's reagent was added directly to the medium, and the production of ammonia was indicated by the development of an orange-yellow color. The biochemical tests were repeated with the cultures of type strains to determine the reproducibility and reliability.

7. Species-specific PCR

PCR was carried out to compare the species-specificity of the PCR primers based on the dextranase gene reported previously with those based on the 16S rDNA gene developed in this study using the genomic DNAs of the mutans streptococci strains isolated from Koreans. The pairs of PCR primers based on the dextranase gene, SD1 and SD2 (Ono *et al.*, 1994), SOF14 and SOR1623 (Igarashi *et al.*, 2000), were used to detect the clinical isolates of *S. mutans* and *S. sobrinus,* respectively (Table 3). The PCR primer pairs targeting the 16S rDNA gene, ChDC-SmF2 and ChDC-SmR2, were used to detect *S. mutans* (Table 3). The PCR conditions were the same as those reported elsewhere (Choi *et al.*, 2006).

In this study, the oligonucleotide forward (ChDC-SsF2) and reverse primers (ChDC-SsR2) were designed based on the 16S rDNA genes of *S. sobrinus* using the PrimerSelect program (DNASTAR, USA) (Table 3).

Table 3. PCR primer sequences used in this study

Target gene	Primer names	Oligonucleotide sequences (5' → 3')	Size of amplicons
16 rDNA gene			
Universal	27F	AGA GTT TGA TCM TGG CTC AG	1465 bp
	1492R	TAC GGY TAC CTT GTT ACG ACT T	
S. mutans	ChDC-SmF2	TGG GAC GCA AGG GAA CAC A	356 bp
	ChDC-SmR2	GCG GCG TTG CTC GGT CAG A	
S. sobrinus	ChDC-SsF2	CAT TGG TAA CAC CGG ACT TGC	500 bp
	ChDC-SsR2	CGC CTG CGC TCC CTT TAC	
Dextranase gene			
	MSSD1467F	TGT CGG WGC YTA YAT GAA AG	530 bp
	MSSD2000R	AAT ARR TTG GTT TGC TCR TC	
S. mutans	SD1	TAT GCT GCT ATT GGA GGT TC	1272 bp
	SD2	AAG GTT GAG CAA TTG AAT CG	
S. sobrinus	SOF14	TGC TAT CTT TCC CTA GCA CTG	1610 bp
	SOR1623	GGT ATT CGG TTT GAC TGC	

The 16S rDNA sequences of *S. sobrinus*, which were stored in the GenBank database, were used as DNA templates. The specificity of the PCR primers was evaluated using the type strains of 11 oral bacteria species, *S. sobrinus* ATCC 33478^T, *S. mutans* ATCC 25175^T, *S. downei* KCTC 3634^T, *S. rattus* KCTC 3655^T, *S. cricetus* KCTC 3640^T, *S. anginosus* ATCC 33397^T, *S. thermophilus* KCTC 3658^T, *S. aureus* KCTC 1621^T, *S. mitis* KCTC 3556^T, *F. nucleatum* ATCC 25586^T, and *A. actinomycetemcomitans* ATCC 33384^T. The sensitivity test was determined by serial dilution of a genomic DNA mixture from *S. sobrinus* ATCC 33478^T. The sensitivities ranged from 4 fg to 4 ng using 10-fold dilutions. The PCR was performed using an *AccuPower*® PCR

PreMix (Bioneer, Korea). The PCR was carried out in a final volume of 20 μl. The PCR reaction was run for 30 cycles on a Peltier thermal cycler (Model PTC-200 DNA engine^{TM}, MJ Research Inc., USA) using the following conditions: denaturation at 95℃ for 1 min, primer annealing at 66℃ for 30 sec, and extension at 72℃ for 1 min. The final cycle included an additional extension time of 10 min at 72℃. A 2 μl aliquot of the reaction mixture was then analyzed by 1.5% agarose gel electrophoresis in a Tris-acetate buffer (0.04 M Tris-acetate, 0.001 M EDTA, [pH8.0]) at 100 V for 30 min. The amplification products were stained with ethidium bromide and visualized using a UV transilluminator.

Ⅲ. RESULTS

1. Isolation of the mutans streptococci

Three hundred and fifty-eight bacteria were recovered on MSB from 141 out of 155 persons (aged 2 to 33.2 years old). In order to isolate the mutans streptococci, 358 colonies were selected based on the colony morphology (data not shown).

The mutans streptococci were identified using PCR-RFLP targeting dextranase gene (*dex*). Of the 358 clinical isolates, 98 strains (from 80 persons) were mutans streptococci (Table 4). The PCR-RFLP profile resulting from the *Hae*III-digested 530 bp DNA fragments clearly differentiated each clinical isolate of mutans streptococci at the species level (Fig. 1). According to the PCR-RFLP data, there were 80 and 18 strains of *S. mutans* and *S. sobrinus*, respectively (Table 4). The size of the PCR products and their *Hae*III-fragments were all matched to those of the type strains (Fig. 1 and Table 4).

In the 98 clinical isolates of the mutans streptococci were identified at the species level by cloning and sequencing the 16S rDNA. Of the 98 isolates, 80 and 18 isolates were *S. mutans* and *S. sobrinus* (Table 4 and 5), respectively. 16S rDNA nucleotide sequences of the mutans streptococci were registered on the GenBank database (National Center for Biotechnology Information). Table 4 shows the GenBank accession numbers.

2. Biochemical characteristics of the mutans streptococci

The biotyping data of the 98 strains showed that there were 64, 1, 20, and 10 strains of biotype I, II, IV, and V, respectively. Two variant strains isolated from two persons, were similar to biotype IV except that they tested positive to the arginine hydrolysis test (Table 4 and 6). One strain, *S. mutans* YM217, could not ferment mannitol, sorbitol, raffinose, and melibiose and could not hydrolyze arginine (Table 4 and 6).

3. New PCR primers based on 16S rDNA sequence for the detection of *S. sobrinus*

The specificity and sensitivity of the new PCR primers for the detection of *S. sobrinus* were tested by performing PCR with the genomic DNAs of the type strains of 5 mutans streptococci species and *S. anginosus* ATCC 33397^T, *S. thermophilus* KCTC 3658^T, *S. aureus* KCTC 1621^T, *S. mitis* KCTC 3556^T, *F. nucleatum* ATCC 25586^T, and *A. actinomycetemcomitans* ATCC 33384^T. The specificity data of the *S. sobrinus*-specific primers (ChDC-SsF2 and ChDC-SsR2 primers) showed that the PCR product amplified only the *S. sobrinus* type strain (ATCC 33478^T) (Fig. 2). The detection limit with ChDC-SsF2 and the ChDC-SsR2 primers was 40 pg of the purified genomic DNA of *S. sobrinus* ATCC 33478^T (Fig. 3).

4. Validation of specificity of the species-specific PCR primers for the detection of *S. mutans* or *S. sobrinus* using clinical isolates from Koreans

The specificity of the *S. mutans*- or *S. sobrinus*-specific PCR primers reported previously and the new PCR primers for the *S. sobrinus* in this study was confirmed by PCR with the genomic DNA of the clinical isolates from Koreans.

The *S. mutans*-specific PCR primers (ChDC-SmF2 and ChDC-SmR2) and *S. sobrinus*-specific PCR primers (ChDC-SsF2 and ChDC-SsR2) detected *S. mutans* and *S. sobrinus* isolated from Koreans, respectively (Fig. 4 and Table 4). The PCR primers (SOF14 and SOF1623), which were based on *dex* for the detection of *S. sobrinus,* also detected the type strain and clinical isolates of *S. sobrinus* (Fig. 5 and Table 4). The PCR primers (SD1 and SD2), which were based on *dex* for the detection of *S. mutans* detected the two *S. sobrinus* strains (YS11 and YS201) as well as the *S. mutans* strains used in this study (Fig. 6).

Table 4. The determination of the species, biotype and PCR of the mutans streptococci isolated from the dental plaques in Koreans

Species[a] & Strains [Accession No.]	Biotype	PCR-RFLP	PCR		No.	Gender (year, month)
		dex (Igarashi *et al.*, 2001)	*dex* (Igarashi *et al.*, 1996,2000)	16S rDNA (Choi *et al.*, 2006)		
S. mutans YM1 [DQ677758]	I	M	M	M	1	Male (19, 10)
S. mutans YM3 [AY691526]	I	M	M	M	2	Male (26, 2)
S. mutans YM6 [DQ677777]	I	M	M	M	3	Male (28, 0)
S. mutans YM9 [AY691527]	I	M	M	M	4	Female (29, 4)
S. mutans YM12 [DQ677756]	I	M	M	M	5	Female (14, 4)
S. mutans YM14 [DQ677755]	I	M	M	M	9	Female (15, 0)
S. mutans YM15 [DQ677788]	I	M	M	M	9	Female (15, 0)
S. mutans YM16 [DQ677787]	Ⅶ	M	M	M	9	Female (15, 0)
S. mutans YM20 [DQ677786]	I	M	M	M	10	Female (21, 3)
S. mutans YM22 [DQ677785]	I	M	M	M	13	Male (20, 9)
S. mutans YM25 [DQ677784]	I	M	M	M	14	Male (27, 11)
S. mutans YM26 [DQ677782]	I	M	M	M	16	Male (18, 11)
S. mutans YM29 [DQ677783]	I	M	M	M	17	Male (27, 2)
S. mutans YM30 [DQ677780]	I	M	M	M	18	Female (18, 1)
S. mutans YM31 [DQ677781]	I	M	M	M	20	Male (15, 3)
S. mutans YM34 [DQ677779]	Ⅴ	M	M	M	20	Male (15, 3)
S. mutans YM37 [DQ677778]	Ⅳ	M	M	M	21	Female (15, 3)
S. mutans YM40 [DQ677774]	I	M	M	M	21	Female (15, 3)
S. mutans YM41 [DQ677773]	Ⅴ	M	M	M	22	Male (17, 1)
S. mutans YM42 [DQ677776]	Ⅶ	M	M	M	22	Male (17, 1)
S. mutans YM44 [DQ677775]	Ⅴ	M	M	M	25	Male (19, 4)
S. mutans YM45 [DQ677772]	I	M	M	M	27	Male (16, 0)
S. mutans YM47 [DQ677771]	I	M	M	M	28	Female (31, 5)
S. mutans YM49 [DQ677770]	I	M	M	M	30	Female (22, 9)
S. mutans YM51 [DQ677769]	I	M	M	M	31	Female (21, 7)
S. mutans YM53 [DQ677768]	Ⅱ	M	M	M	32	Female (22, 7)

M, *S. mutans*.

[a]Identified by the nucleotide sequencing of 16S rDNA gene.

(*Continued on next page*)

Table 4. *(Continued in previous page)*

Species[a] & Strains [Accession No.]	Biotype	PCR-RFLP	PCR		No.	Gender (year, month)
		dex (Igarashi *et al.*, 2001)	*dex* (Igarashi *et al.*, 1996,2000)	16S rDNA (Choi *et al.*, 2006)		
S. mutans YM54 [DQ677767]	I	M	M	M	32	Female (22, 7)
S. mutans YM55 [DQ677766]	V	M	M	M	32	Female (22, 7)
S. mutans YM57 [DQ677765]	I	M	M	M	33	Female (24, 3)
S. mutans YM60 [DQ677764]	V	M	M	M	33	Female (24, 3)
S. mutans YM61 [DQ677763]	I	M	M	M	36	Female (21, 3)
S. mutans YM62 [DQ677762]	V	M	M	M	36	Female (21, 3)
S. mutans YM63 [DQ677761]	I	M	M	M	37	Female (15, 9)
S. mutans YM64 [DQ677760]	Ⅳ	M	M	M	38	Male (14, 4)
S. mutans YM67 [DQ677759]	I	M	M	M	40	Female (20, 6)
S. mutans YM69 [DQ677757]	I	M	M	M	41	Female (22, 7)
S. mutans YM70 [DQ677754]	I	M	M	M	42	Female (26, 10)
S. mutans YM71 [AY691528]	V	M	M	M	43	Male (24, 1)
S. mutans YM72 [DQ677753]	I	M	M	M	44	Female (14, 3)
S. mutans YM75 [DQ677752]	I	M	M	M	45	Female (20, 11)
S. mutans YM77 [DQ677751]	I	M	M	M	48	Male (14, 1)
S. mutans YM79 [DQ677750]	I	M	M	M	49	Female (33, 2)
S. mutans YM81 [DQ677749]	I	M	M	M	50	Female (22, 7)
S. mutans YM82 [DQ677748]	I	M	M	M	52	Female (23, 2)
S. mutans YM85 [DQ677747]	I	M	M	M	53	Female (16, 4)
S. mutans YM87 [DQ677746]	I	M	M	M	55	Female (14, 6)
S. mutans YM89 [DQ677744]	I	M	M	M	56	Female (22, 5)
S. mutans YM90 [DQ677745]	I	M	M	M	57	Female (16, 0)
S. mutans YM95 [DQ677742]	I	M	M	M	61	Female (18, 11)
S. mutans YM96 [DQ677741]	I	M	M	M	62	Male (18, 5)
S. mutans YM97 [AY691532]	V	M	M	M	62	Male (18, 5)
S. mutans YM99 [DQ677740]	I	M	M	M	63	Male (16, 10)
S. mutans YM101 [DQ677739]	I	M	M	M	35	Female (21, 8)
S. mutans YM102 [DQ677738]	I	M	M	M	3	Male (28, 0)
S. mutans YM201 [DQ677737]	I	M	M	M	PD4	Male (8, 10)
S. mutans YM202 [DQ677736]	I	M	M	M	PD9	Male (11, 3)

M, *S. mutans*.

[a]Identified by the nucleotide sequencing of 16S rDNA gene.

(*Continued on next page*)

Table 4. *(Continued in previous page)*

Species[a] & Strains [Accession No.]	Biotype	PCR-RFLP	PCR		No.	Gender (year, month)
		dex (Igarashi *et al.*, 2001)	*dex* (Igarashi *et al.*, 1996,2000)	16S rDNA (Choi *et al.*, 2006)		
S. mutans YM203 [DQ677735]	I	M	M	M	PD24	Male (8, 4)
S. mutans YM204 [DQ677734]	I	M	M	M	PD32	Male (8, 4)
S. mutans YM205 [DQ677733]	I	M	M	M	PD59	Female (11, 3)
S. mutans YM206 [DQ677732]	I	M	M	M	PD65	Male (3, 8)
S. mutans YM207 [DQ677731]	V	M	M	M	PD69	Female (15, 1)
S. mutans YM209 [DQ677730]	I	M	M	M	PD1018	Female (2, 3)
S. mutans YM210 [DQ677729]	I	M	M	M	PD1019	Male (3, 2)
S. mutans YM211 [DQ677728]	I	M	M	M	PD1022	Female (5, 9)
S. mutans YM212 [DQ677727]	I	M	M	M	PD1026	Male (7, 4)
S. mutans YM213 [DQ677726]	I	M	M	M	PD1027	Male (5, 0)
S. mutans YM214 [DQ677725]	I	M	M	M	PD1035	Male (4, 6)
S. mutans YM215 [DQ677724]	I	M	M	M	PD1036	Male (2, 4)
S. mutans YM216 [DQ677723]	V	M	M	M	PD1045	Male (3, 8)
S. mutans YM217 [DQ677722]	-	M	M	M	PD1047	Female (3, 9)
S. mutans YM218 [DQ677721]	I	M	M	M	PD1049	Male (2, 4)
S. mutans YM219 [DQ677720]	I	M	M	M	PD1053	Female (3, 1)
S. mutans YM220 [DQ677719]	I	M	M	M	PD1056	Male (3, 2)
S. mutans YM222 [DQ677718]	I	M	M	M	PD1062	Male (3, 8)
S. mutans YM223 [DQ677717]	I	M	M	M	PD1063	Female (3, 5)
S. mutans YM225 [DQ677716]	I	M	M	M	PD1111	Female (3, 6)
S. mutans YM226 [DQ677715]	I	M	M	M	PD1115	Male (3, 5)
S. mutans YM228 [DQ677714]	I	M	M	M	PD1140	Female (4, 0)
S. mutans YM229 [DQ677713]	I	M	M	M	PD1145	Male (5, 5)
S. mutans YM230 [DQ677712]	I	M	M	M	PD1163	Male (3, 7)

M, *S. mutans*.

[a]Identified by the nucleotide sequencing of 16S rDNA gene.

(*Continued on next page*)

Table 4. *(Continued in previous page)*

Species[a] & Strains [Accession No.]	Biotype	PCR-RFLP	PCR		No.	Gender (year, month)
		dex (Igarashi *et al.*, 2001)	*dex* (Igarashi *et al.*, 1996,2000)	16S rDNA		
S. sobrinus YS1 [AY691533]	Ⅳ	S	S	S	1	Male (19, 10)
S. sobrinus YS4 [DQ677801]	Ⅳ	S	S	S	57	Female (16, 0)
S. sobrinus YS5 [DQ677805]	Ⅳ	S	S	S	58	Female (14, 8)
S. sobrinus YS6 [DQ677804]	Ⅳ	S	S	S	59	Female (14, 0)
S. sobrinus YS7 [DQ677803]	Ⅳ	S	S	S	59	Female (14, 0)
S. sobrinus YS11 [DQ677802]	Ⅳ	S	S	S	45	Female (20, 11)
S. sobrinus YS12 [DQ677800]	Ⅳ	S	S	S	40	Female (20, 6)
S. sobrinus YS13 [DQ677799]	Ⅳ	S	S	S	40	Female (20, 6)
S. sobrinus YS201 [DQ677798]	Ⅳ	S	S	S	PD14	Female (6, 1)
S. sobrinus YS202 [DQ677797]	Ⅳ	S	S	S	PD54	Male (14, 0)
S. sobrinus YS203 [DQ677796]	Ⅳ	S	S	S	PD57	Male (9, 0)
S. sobrinus YS205 [DQ677795]	Ⅳ	S	S	S	PD1010	Male (5, 6)
S. sobrinus YS206 [DQ677794]	Ⅳ	S	S	S	PD1017	Female (5, 2)
S. sobrinus YS207 [DQ677793]	Ⅳ	S	S	S	PD1021	Female (4, 1)
S. sobrinus YS209 [DQ677792]	Ⅳ	S	S	S	PD1051	Female (3, 7)
S. sobrinus YS210 [DQ677791]	Ⅳ	S	S	S	PD1135	Female (4, 6)
S. sobrinus YS211 [DQ677790]	Ⅳ	S	S	S	PD1164	Male (2, 6)
S. sobrinus YS212 [DQ677789]	Ⅳ	S	S	S	PD1109	Male (2, 1)

S, *S. sobrinus*.

[a]Identified by the nucleotide sequencing of 16S rDNA gene.

Table 5. The frequency of the mutans streptococci species isolated from the dental plaques in Koreans

	Mutans streptococci		Total
	S. mutans	*S. sobrinus*	
Percentage (Number of individuals)	86.3% (69)	20.0% (16)	80

Table 6. The frequency of the mutans streptococci biotypes isolated from the dental plaques in Koreans

Species	Percent frequency of biotypes (n)				
	I	II	IV	V	VII
S. mutans	65.3 (64)	1.02 (1)	2.04 (2)	10.2(10)	2.04 (2)
S. sobrinus	-	-	18.4 (18)	-	-
Total	65.3 (64)	1.02 (1)	20.4 (20)	10.2 (10)	2.04 (2)

Ⅳ. DISCUSSION

The mutans streptococci obtained from Koreans were isolated and characterized in order to provide basic data on the epidemiology and pathogenesis of the dental caries in Koreans.

The results showed that the most frequent mutans streptococci detected in Korean dental plaque (aged 2-33.2 years) was *S. mutans* (86.3%), followed by *S. sobrinus* (20.0%) (Table 4 and 5). According to the other epidemiological data in Korea, *S. mutans* and *S. sobrinus* were detected in 93.1% and 33.1% of the dental plaque samples from 137 Korean school children (10-11 years old) by biochemical tests (biotyping), respectively (Kim *et al.*, 1983). The detection frequencies of *S. mutans* and *S. sobrinus* were higher than those of this study. This might be due to the different ages of the subjects. In a recent study, the prevalence of *S. mutans* and *S. sobrinus* was 72.8% and 61.1% in 77 Japanese aged 3-5 years old, respectively, using a PCR method (Okada *et al.*, 2002). Wu *et al.* (2003) also reported the prevalence of *S. mutans* and *S. sobrinus* in 126 Chinese dental plaque samples (aged 25-55 years) to be 75.4% and 57.1%, respectively, using biochemical tests and PCR. In addition, *S. mutans* and *S. sobrinus* were detected in 65~95% and 0.4~36% of pre-school children aged 5-13 years old of the United States and Canada, respectively (Qureshi *et al.*, 1977). Although the ages of the subjects and detection methods used were different, the distribution and prevalence of *S. mutans* and *S. sobrinus* showed a similar tendency to those of the present study. However, 90% of the mutans streptococci isolated from 5- to 8-year-old schoolchildren in the U.K. was *S. mutans* with rare cases of *S. sobrinus* being detected (Beighton *et al.*, 1987). In Australians aged 10-25 years, *S. mutans* and *S. sobrinus* was detected in 30% and 35%, respectively (Rogers, 1973). Therefore, the distribution of *S. mutans* and *S. sobrinus* appears to be associated with differences in the geographical human populations.

In this study, the mutans streptococci were screened using the *dex*-targeted PCR-RFLP and biochemical tests, and the screened strains were identified at the species level using the 16S rDNA sequencing comparison method. Interestingly, the result of 16S rDNA sequencing comparison analysis was clearly consistent with that of PCR-RFLP analysis. Therefore, *dex*-targeted PCR-RFLP could be used to identify the mutans streptococci without the need for 16S rDNA sequencing comparison analysis. The clinical isolates that were not separated by PCR-RFLP were classified into non-mutans streptococcal organisms (non-MSO). According to previous studies, the majority of the non-MSO was *S. anginosus, S. sanguinis, Enterococcus faecalis,* and *Pantoea agglomerans* (Yoo *et al.*, 2005; Lee *et al.*, 2006).

The biotyping data showed biotype I (65.3%) to be the most common biotype in 98 strains of the mutans streptococci from Koreans (aged 2-33.2 years old), followed by IV (20.4%), V (10.2%), and II (1.02%) (Table 6), which is similar to the results of a previous study: biotype I (78.8%), IV (33.1%), V (19.5%), and II (1.7%) were isolated from 137 Korean school children (10-11 years old) (Kim *et al.*, 1983). However, detection frequencies of biotype isolated from the saliva of 114 preschool children in Taiwan were biotype I (72.9%), III (7.3%), IV (7.3%), V (7.3%), and II (5.2%) (Chen *et al.*, 1990). Several studies have reported biotype I to be most commonly detected in dental plaque of 49 San Diegan (17-22 years old), 25 Californian (17-22 years old), 55 Hawaiian school children (3-13 years old), and 217 Saudi Arabian Naval personnel (16-26 years old) (Bratthall *et al.*, 1972; Keene *et al.*, 1977).

Generally, biotype II is *S. rattus*, and biotype IV is *S. sobrinus* (Coykendall and Alan, 1989). Interestingly, in this study, 2 strains of *S. mutans* were biotype IV and 1 strain of *S. mutans* was biotype II (Table 4 and 6). In addition, in a previous study, biotype VII, which is similar to biotype IV except for a positive reaction to the arginine hydrolysis test, was isolated from two persons (Jeong, 2005). In this study, the two strains of biotype VII

were confirmed to be *S. mutans* using a 16S rDNA sequencing comparison method (Table 4). The reason for this is unclear but the oral environment of the hosts might affect the changes in the bacteria metabolic characteristics.

Of the mutans streptococci, *S. rattus* (biotype II) has the ability to hydrolyze arginine (Coykendall, 1974). In a recent study, the operon of the arginine deiminase system (ADS) was cloned from *S. rattus* FA-1 (Griswold *et al.*, 2004). The catabolism of arginine using the ADS produced ammonia (NH_3) and ATP (Curran *et al.*, 1995; Griswold *et al.*, 2004). The generation of ATP allows the use of arginine as the sole energy source. NH_3 produced within the cell combines with a proton to yield NH_4^+, and this reaction increases the pH value in plaque. PCR with the degenerative PCR primers based on the ADS operon of *S. rattus* FA-1 was performed to determine if the biotype II and VII strains isolated in this study have the ADS operon (data not shown). The PCR data did not detect any PCR amplicon (data not shown). In order to confirm the ability of the arginine hydrolysis in the biotype II and VII strains, the cloning and characterization of the ADS from the strains may be needed.

In this study, 10 of the *S. mutans* strains were biotype V (10.2%) (Table 4 and 6). It was reported that approximately 12% of *S. mutans* clinical isolates were melibiose negative (Beighton *et al.*, 1991). The PCR primers based on the GTF A gene (*gtf*A) were used to detect the melibiose-negative isolates (biotype V) (Colby *et al.*, 1995). The *gtf*A gene, which lies within the multiple sugar metabolism (msm) operon, disappears in the melibiose-negative isolates. The results suggest that PCR with the primers based on the genes of the enzymes corresponding to the biochemical tests may be useful for determining the biotype of mutans streptococci without biochemical tests.

The results of the specificity tests showed that the PCR primers based on the 16S rDNA gene were more specific than those based on the dextranase gene for the clinical isolates of *S. mutans* and *S. sobrinus* isolated from the dental plaque in Koreans (Table 4, Fig. 4, and 5). These results are

consistent with previous studies showing that the 16S rRNA gene-based nested PCR method is more sensitive in detecting *S. mutans* and *S. sobrinus* than that based on GTF and dextranase genes (Sato *et al.*, 2003; Matsuyama *et al.*, 2005). Therefore, these results suggest that the species-specific PCR primers based on the 16S rDNA gene can be used to both detect and identify mutans streptococci at the species level.

In conclusion, *S. mutans* and biotype I are frequently detected in Korean dental plaque. The mutans streptococci strains isolated in this study may be useful for examining the epidemiology and pathogenesis of dental caries.

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VI. FIGURE LEGENDS

Fig. 1. **Dextranase gene PCR-RFLP profiles of mutans streptococcal species from the dental plaque samples.** The PCR products amplified with primers MSSD1467F and MSSD2000R were digested with *Hae*III, and then electrophoresed in 3% agarose gel. Lanes; S, size maker (100 bp DNA ladder); 1, *S. mutans* ATCC 25175^{T}; 2, *S. mutans* YM210; 3, *S. mutans* YM211; 4, *S. mutans* YM215; 5, *S. sobrinus* ATCC 33478^{T}; 6, *S. sobrinus* YS1; 7, *S. sobrinus* YS206; 8, *S. sobrinus* YS210.

Fig. 2. **Specificity test of PCR with ChDC-SsF2 and ChDC-SsR2 primers and purified genomic DNA of type strains.** 4 ng of each bacterial genomic DNA was used as PCR templates. The PCR reactions were electrophoresed in 1.5% agarose gel. Lanes: S, size maker (100 bp DNA ladder); 1, negative control (distilled water); 2, *Streptococcus sobrinus* ATCC 33478^{T}; 3, *S. mutans* ATCC 25175^{T}; 4, *S. downei* KCTC 3634^{T}; 5, *S. rattus* KCTC 3655^{T}; 6, *S. cricetus* KCTC 3640^{T}; 7, *S. anginosus* ATCC 33397^{T}; 8, *S. thermophilus* KCTC 3658^{T}; 9, *S. aureus* KCTC 1621^{T}; 10, *S. mitis* KCTC 3556^{T}; 11, *Fusobacterium nucleatum* ATCC 25586^{T}; 12, *Actinobacillus actinomycetemcomitans* ATCC 33384^{T}.

Fig. 3. **The detection limits of PCR amplication with ChDC-SsF2, ChDC-SsR2 primers and purified genomic DNA of *S. sobrinus* ATCC 33478^{T}.** The PCR reactions were electrophoresed in 1.5% agarose gel. Lanes: S, size maker (100 bp DNA ladder); 1 through 7, purified genomic DNA serially diluted 10-fold from 4 ng to 4 fg. 1, 4 ng; 2, 400 pg; 3, 40 pg; 4, 4 pg; 5, 400 fg; 6, 40 fg; 7, 4 fg.

Fig. 4. **Detection of the 16S rDNA of mutans streptococcal species from the dental plaque samples.** The PCR products amplified with ChDC-SmF2, ChDC-SmR2 primer and ChDC-SsF2, ChDC-SsR2 primer. The PCR reactions were electrophoresed in 1.5% agarose gel. Lanes: S, size maker (100 bp DNA ladder); 1, negative control (distilled water); 2, *S. mutans* ATCC 25175^{T}; 3, *S. mutans* YM201; 4, *S. mutans* YM204; 5, *S. mutans* YM213; 6, *S. mutans* YM225; 7, *S. mutans* YM230; 8, *S. mutans* YM1; 9, *S. mutans* YM20; 10, *S. mutans* YM49; 11, *S. mutans* YM89; 12, *S. mutans* YM96; 13, distilled water; 14, *S. sobrinus* ATCC 33478^{T}; 15, *S. sobrinus* YS201; 16, *S. sobrinus* YS203; 17, *S. sobrinus* YS205; 18, *S. sobrinus* YS206; 19, *S. sobrinus* YS210; 20, *S. sobrinus* YS1; 21, *S. sobrinus* YS7; 22, *S. sobrinus* YS9; 23, *S. sobrinus* YS11; 24, *S. sobrinus* YS14.

Fig. 5. **Detection of the dextranase gene of mutans streptococcal species from the dental plaque samples.** The PCR products amplified with SD1-SD2 and SOF14-SOR1623 primers. The PCR reactions were electrophoresed in 1.5% agarose gel. Lanes: S, size maker (100 bp DNA ladder); 1, negative control (distilled water); 2, *S. mutans* ATCC 25175^{T}; 3, *S. mutans* YM201; 4, *S. mutans* YM204; 5, *S. mutans* YM213; 6, *S. mutans* YM225; 7, *S. mutans* YM230; 8, *S. mutans* YM1; 9, *S. mutans* YM20; 10, *S. mutans* YM49; 11, *S. mutans* YM89; 12, *S. mutans* YM96; 13, distilled water; 14, *S. sobrinus* ATCC 33478^{T}; 15, *S. sobrinus* YS201; 16, *S. sobrinus* YS203; 17, *S. sobrinus* YS205; 18, *S. sobrinus* YS206; 19, *S. sobrinus* YS210; 20, *S. sobrinus* YS1; 21, *S. sobrinus* YS7; 22, *S. sobrinus* YS9; 23, *S. sobrinus* YS11; 24, *S. sobrinus* YS14.

Fig. 6. **Detection of the dextranase gene of mutans streptococcal species from the dental plaque samples.** The PCR products amplified with SD1-SD2 primer. The PCR reactions were electrophoresed in 1.5% agarose gel. Lanes: S, size maker (100 bp DNA ladder); 1, negative control (distilled water); 2, *S. mutans* ATCC 25175^{T}; 3, *S. sobrinus* YS11; 4, *S. sobrinus* YS201.

VII. FIGURES

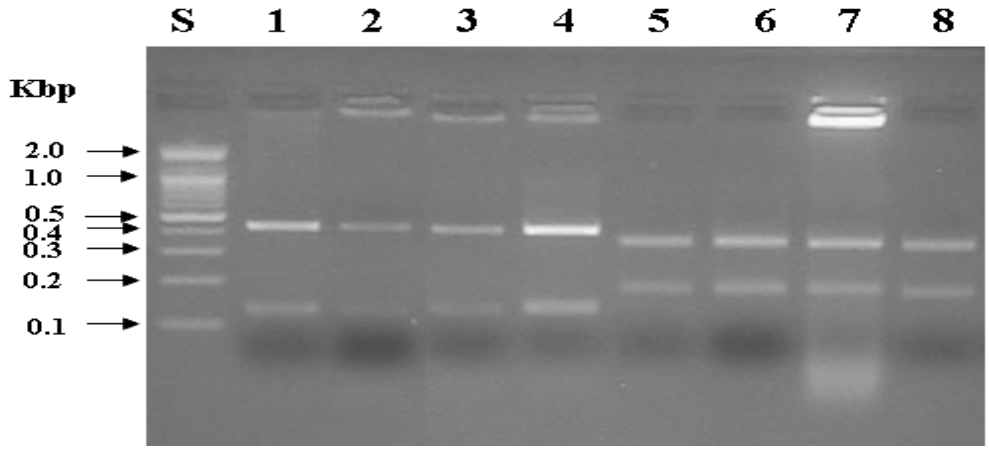

Fig. 1. Dextranase gene PCR-RFLP profiles of mutans streptococcal species from the dental plaque samples.

Fig. 2. Specificity test of PCR with ChDC-SsF2 and ChDC-SsR2 primers and purified genomic DNA of type strains.

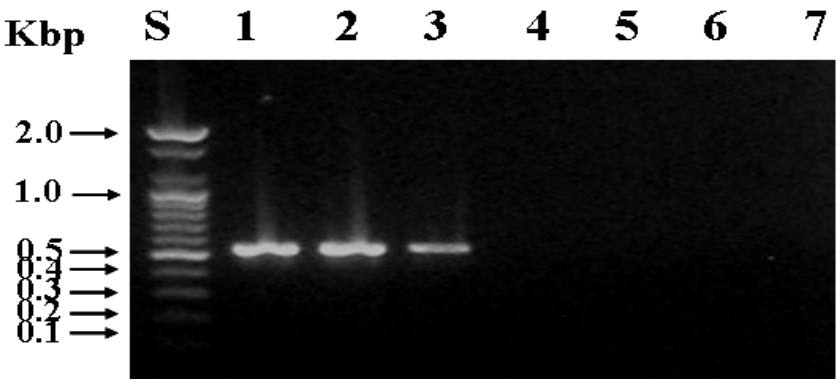

Fig. 3. The detection limits of PCR amplication with ChDC-SsF2, ChDC-SsR2 primers and purified genomic DNA of *S. sobrinus* ATCC 33478^{T}.

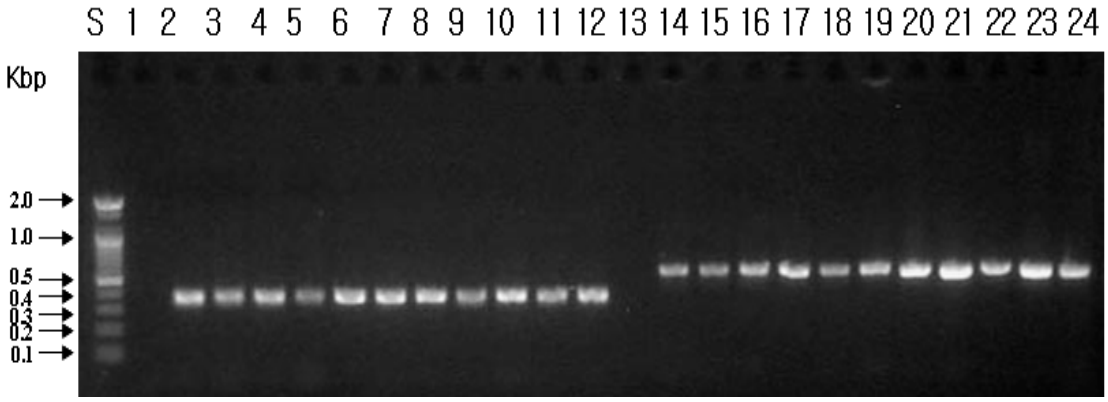

Fig. 4. Detection of the 16S rDNA of mutans streptococcal species from the dental plaque samples. *S. sobrinus* YS11; 24, *S. sobrinus* YS14.

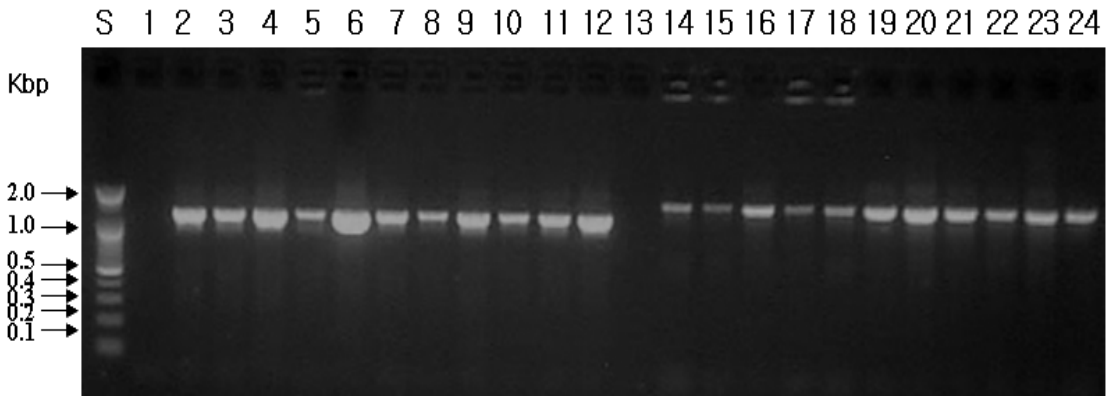

Fig. 5. Detection of the dextranase gene of mutans streptococcal species from the dental plaque samples.

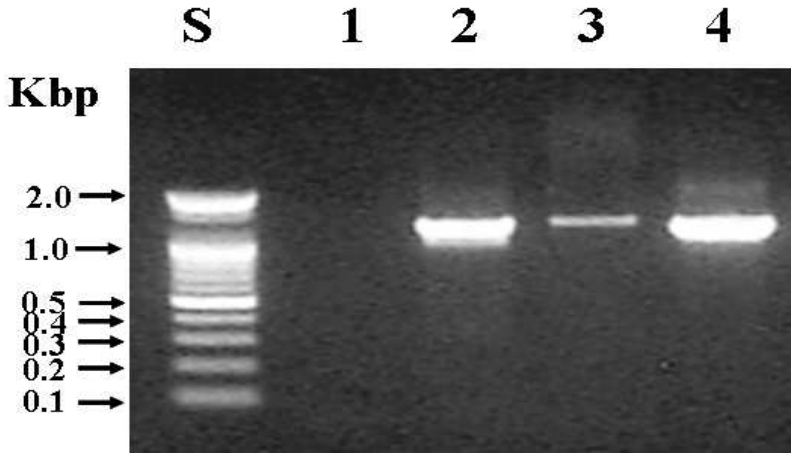

Fig. 6. Detection of the dextranase gene of mutans streptococcal species from the dental plaque samples.

ABSTRACT in KOREAN

한국인으로부터 mutans streptococci의 분리와 특성

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(지도교수: 국 중 기)

본 연구는 한국인을 대상으로 하는 치아우식증 역학 연구 및 병인론 연구에 기초적인 자료를 제공하기 위해 한국인의 치면세균막에서 mutans streptococci를 분리하고 생화학적인 특성을 알아보고자 시행하였다.

총 155명(2-33.2세, 평균 13.7±4.7세)을 대상으로 하여 상·하악 대구치 및 전치부의 협면에서 치면세균막을 채취하여 MSB 선택배지에 배양하였다. MSB 선택배지에서 성장한 균주들이 mutans streptococci인지 확인하기 위해 dextranase gene을 표적으로 한 PCR-RFLP를 시행하였다. mutans streptococci의 생물형을 결정하기 위해 생화학 검사를 실시하였으며, 종 수준에서의 동정을 위해 16S rDNA 염기서열비교법을 시행하였다. 16S rDNA와 dextranase 유전자를 바탕으로 한 *S. mutans*와 *S. sobrinus* 종-특이 PCR 프라이머의 특이성을 검증하기 위해 한국인의 치면세균막에서 분리한 *S. mutans*와 *S. sobrinus* 균주들을 이용하여 PCR을 실시하였다.

총 155명을 대상으로 하여 141명의 치면세균막으로 부터 358개의 세균 군락을 MSB 배지에서 분리하여 colony 형태에 따라 1차 구분한 후, mutans streptococci를 확인하기 위해 PCR-RFLP을 실시한 결과, 80명의 치면세균막에서 총 98 균주의 mutans streptococci를 얻었고, 16S rDNA 염기서열비교법으로 98% 이상의 상동성

을 갖는 80 균주의 *S. mutans*와 18 균주의 *S. sobrinus*를 얻었다. 생물형은 제 I형, II형, IV형 및 V형이 각각 64, 1, 20 및 10 균주가 분리되었다. 제 IV형과 당 발효능이 유사하고 arginine을 가수분해 할 수 있는 제 VII형이 2 균주 분리되었다. 종-특이 PCR 결과, dextranase gene보다는 16S rDNA 유전자를 표적으로 한 PCR 프라이머가 한국인에서 분리한 *S. mutans*와 *S. sobrinus* 균주에 대해 좀 더 종-특이성을 보였다.

이상의 결과로서 한국인의 치면세균막 내에 존재하는 mutans streptococci 중 *S. mutans* 종의 발현이 가장 높았으며 생물형은 제 I형이 가장 많음을 알 수 있었다. 본 연구의 결과는 한국인의 치아우식증을 예방하기 위한 치아우식증 역학연구와 병인론 연구에 기초적인 자료로 이용될 수 있을 것으로 사료된다.

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논문제목	한글 : 한국인으로부터 mutans streptococci의 분리와 특성 영문 : Isolation and characterization of the mutans streptococci from koreans				

본인이 저작한 위의 저작물에 대하여 다음과 같은 조건아래 조선대학교가 저작물을 이용할 수 있도록 허락하고 동의합니다.

- 다 음 -

1. 저작물의 DB구축 및 인터넷을 포함한 정보통신망에의 공개를 위한 저작물의 복제, 기억장치에의 저장, 전송 등을 허락함
2. 위의 목적을 위하여 필요한 범위 내에서의 편집·형식상의 변경을 허락함. 다만, 저작물의 내용변경은 금지함.
3. 배포·전송된 저작물의 영리적 목적을 위한 복제, 저장, 전송 등은 금지함.
4. 저작물에 대한 이용기간은 5년으로 하고, 기간종료 3개월 이내에 별도의 의사 표시가
   없을 경우에는 저작물의 이용기간을 계속 연장함.
5. 해당 저작물의 저작권을 타인에게 양도하거나 또는 출판을 허락을 하였을 경우에는 1개월 이내에 대학에 이를 통보함.
6. 조선대학교는 저작물의 이용허락 이후 해당 저작물로 인하여 발생하는 타인에 의한 권리 침해에 대하여 일체의 법적 책임을 지지 않음
7. 소속대학의 협정기관에 저작물의 제공 및 인터넷 등 정보통신망을 이용한 저작물의 전송·출력을 허락함.

동의여부 : 동의(v) 반대( )

2007년 2월 일

저작자: 유 소 영 (서명 또는 인)

조선대학교 총장 귀하