First isolation of *Streptococcus downei* from human dental plaques

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Abstract

In this study, we isolated four bacterial strains grown on mitis-salivarius sucrose bacitracin agar. The strains had similar biochemical characteristics to biotypes I or II of mutans streptococci. The four isolates were identified as *Streptococcus downei* by 16S rDNA and dextranase gene (*dex*) sequencing as well as polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) targeting *dex*. To our knowledge, this is the first report of the isolation and identification of *S. downei* from dental plaque in humans. The results suggest that *S. downei* can inhabit the human oral cavity.

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1. Introduction

Mutans streptococci (MS) are a group of oral cariogenic species which includes *Streptococcus mutans*, *Streptococcus sobrinus*, *Streptococcus cricetus*, *Streptococcus rattus*, *Streptococcus downei*, *Streptococcus macciae*, and possibly, *Streptococcus ferus* [1]. In this group of streptococci, *S. mutans* and *S. sobrinus* are the most frequently found in humans. *S. downei* was first isolated from the dental plaque of monkeys (*Macaca fascicularis*), initially designated as *S. mutans* serotype h [2], and later reclassified as an independent species by biochemical tests, serotyping, and DNA–DNA hybridization [3]. Among the oral streptococci, *S. downei* is most closely related to *S. sobrinus*. *S. downei* strains were found to be cariogenic for germ-free rats given a high-sucrose diet, and were implicated in monkey caries [3]. Recently, while studying clinical strains of mutans streptococci isolated from dental plaques of the Korean population, we detected strains of *S. downei*. Since the isolation of *S. downei* from human dental plaque has not been reported previously, their identity was confirmed by use of the methods recently described by Igarashi et al. [4,5], which were based on mutans streptococci species-specific polymerase chain reaction. In this study, *S. downei* isolates were identified using 16S rDNA sequencing and *S. downei*-specific PCR methods.
2. Materials and methods

The type strain of *S. downei*, KCTC 3634T (=ATCC 33748T), was obtained from the Korean collection for type cultures (KCTC, Daejeon, Korea). Four strains of *S. downei* (ChDC YD1, ChDC YD2, ChDC YD3, and ChDC YD4) were isolated from the dental plaque of four Korean dental patients (Table 1). All strains were cultured on a medium composed of mitis salivarius agar (Difco Laboratories, Detroit, MI, USA) supplemented with 0.0001% potassium tellurite, 0.2 units (2.8 μg ml⁻¹) of bacitracin (Sigma Chemical Co., St. Louis, MO, USA), and 20% (w/v) sucrose (CJ Co., Seoul, Korea) (MSB agar). Bacitracin was freshly prepared immediately before use. The MSB agar plates were stored at 4 °C and used within 7 days after preparation. Inoculated plates were incubated at 37 °C in a CO₂ incubator for 1–2 days.

Biochemical tests were performed to determine the biotypes of *S. downei* strains, as described by Shklair and Keene [6,7]. A phenol red broth base (BBL, Becton Dickinson Microbiology System, Cockeysville, MD, USA) was used as the basal medium for the fermentation of 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 medium was dispensed into screw cap tubes, which had previously been inoculated with the test organisms, and were read after 48 h of incubation, 0.1 ml of Nessler’s reagent was added directly to the medium, and ammonia production was indicated by the development of an orange-yellow color. The biochemical tests were repeated with cultures of the reference strains to determine the reproducibility and reliability.

The identification of mutans streptococci at the species level was performed according to the method described by Igarashi et al. [4]. In brief, the PCR reaction was performed using an AccuPower® PCR Pre-Mix (Bioneer Corp., Daejeon, Korea). The PCR primers were as follows: MSSD1467F (5'-TGG TCT GGC YRA YAT GAA AG-3') and MSSD2000R (5'-AAT ARR TTG GTT TGC TCR TC-3'). The PCR products were purified using an AccuPrep® PCR Purification Kit (Bioneer Corp.) and then digested with a HaeIII (Bio-neer Corp.) restriction enzyme. The HaeIII-digested DNA fragments were visualized after electrophoresis on a 4% NuSieve 3:1 agarose gel and stained with ethidium bromide. The length (bp) of the restriction fragments generated from the PCR products of mutans streptococci were as follows: 412 and 122 bp for *S. mutans*; 351 and 174 bp for *S. sobrinus*; 210, 174, and 141 bp for *S. downei*; 325 and 209 bp for *S. rattus*; 210, 141, 109, and 65 bp for *S. cricetus* [4].

Cloning and sequencing of 16S rDNA, as well as the *dex* gene, were performed to identify the clinical isolates at the species level. The bacterial genomic DNA was prepared using a G-spin™ Genomic DNA Extraction kit (iNtRON Co., Seoul, Korea) according to the manufacturer’s instructions. In order to amplify the 16S rDNA or *dex* gene from the bacteria, a polymerase chain reaction (PCR) was performed with the primers 27F and 1492R [9] or Sd2864F (5'-CAA GAA CAG TCA GCG CCA GT-3') and Sd3670R (5'-AAG GGT CCT CGT CTG AAT CC-3') [5], respectively. The PCR conditions were the same as described elsewhere [5,9]. The PCR products were then purified using an AccuPrep® PCR purification kit (Bioneer Corp.) and were directly ligated using the pGEM-T easy vector (Promega Corp., Madison, WI, USA). Nucleotide sequencing was performed using the dyeoxy chain termination method with a Big Dye Terminator Cycle Sequencing Kit (Applied Biosystems, Foster City, CA, USA) and an ABI PRISM 310 Genetic Analyzer (Applied Biosystems). The ChDC-GEM-F (5'-TTC CCA GTC ACG ACG TTG TAA AA-3') and ChDC-GEM-R (5'-GTT TGG AAT TGT GAG CGG ATG ATA AC-3') primers were used for the nucleotide sequencing of 16S rDNA and *dex* gene. The additional sequencing primers for 16S rDNA were as follows: Seq-F1 (5'-TCC AGT GGA GCC AGC AG-3'), Seq-R2 (5'-GAC TAC CAG GGT ATC TAA TCC-3'), and F16 (5'-TAG ATA CCC YGG TAG TCC-3'). All sequences were compared with a similar sequence of the reference organism by BLAST interrogation of the GenBank database (National Center for Biotechnology Information).

### Table 1

<table>
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<tr>
<th>Strains</th>
<th>Origin</th>
<th>GenBank Accession No.</th>
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<td>AY942561 (99.1) AY986766 (99.3)</td>
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<td>Female (15, 9)</td>
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<td>ChDC YD3</td>
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<td>AY942563 (99.4) AY986768 (99.9)</td>
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<td>ChDC YD4</td>
<td>Female (13, 7)</td>
<td>AY942564 (98.9) AY986769 (99.3)</td>
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</tbody>
</table>

*a* (%): Percent identity of the nucleotide sequence of the 16S rDNAs from each strain compared with that of the *S. downei* type strain (ATCC 33748T), which were obtained using the ClustalV method and DNAStar software (DNAStar Inc., Madison, WI, USA).

*b* (%): Percent identity of the nucleotide sequence of *dex* (807 bp) of each strain compared with that of the *downei* type strains (ATCC 33748T), which were obtained using the ClustalV method and DNAStar software (DNAStar Inc.).

**ChDC**: Department of Oral Biochemistry, College of Dentistry, Chosun University.
3. Results and discussion

The PCR-RFLP profile resulting from the HaeIII-digested 525 bp fragment of dex genes revealed three subfragments (210, 174, and 141 bp) for each strains tested, and the restriction patterns turned out to be the same for both human and monkey S. downei isolates (Fig. 1). The percent identity of the nucleotide sequences of the 16S rDNA of the four strains of human S. downei isolates with that of the S. downei-type strain (ATCC 33748\textsuperscript{T}) was >98.9% (Table 1 and Fig. 2(A)).

The data from PCR performed using S. downei-specific primers (Sd2864F and Sd3670R) showed that the species-specific PCR products were amplified from all four isolates, as well as from the type strain (Fig. 3).

In order to confirm the specificity of the PCR, the amplicons of dex (807 bp) were cloned and sequenced; the data showed that the amplicons for the four isolates had either a 99.1% (ChDC YD1, YD2, and YD4) or 99.3% (ChDC YD2) identity with that of the type strain (Table 1 and Fig. 2(B)).

The biotyping data showed that the four S. downei isolates were either biotype I or II, which are both quite unlike biotype VI of the type strain (Table 2). The hydrolysis of arginine is of particular interest as this is a phenotypic characteristic previously restricted to S. rattus within the mutans streptococci. Although it is unclear why these phenotype differences exist, it is believed that they may be due to differences in the oral environments of hosts, e.g., eating habits.

![Fig. 1. dex gene PCR-RFLP profiles of S. downei strains. The PCR products amplified with primers MSSD1467F and MSSD2000R were digested with HaeIII, and then electrophoresed in 3% agarose gel. Lanes: 1, S. downei ATCC 33748\textsuperscript{T}; 2, S. downei ChDC YD1; 3, S. downei ChDC YD2; 4, S. downei ChDC YD3; 5, S. downei ChDC YD4; S, size marker (100 bp DNA ladder).](https://academic.oup.com/femsle/article-abstract/249/2/323/569805)

![Fig. 2. Dendrogram based on the alignment of the nucleotides of the 16S rDNA (A) and dex (B) of four clinical isolates of S. downei and representative mutans streptococci species. The strains studied are designated by their culture collection accession number, followed by the GenBank accession number. The tree was inferred from the similarity values obtained using the ClustalV method and DNAStar software (DNAStar Inc.). The distance between two strains or species is obtained by adding the lengths of the connecting horizontal branches using the scale at the bottom of the figure (percentage of sequence difference).](https://academic.oup.com/femsle/article-abstract/249/2/323/569805)

![Fig. 3. Specific dex gene PCR identification of S. downei strains. The PCR products amplified with primers Sd2864 and Sd3670R were electrophoresed in 1.2% agarose gel. Lanes: 1, negative control (sterilized water); 2, S. downei ATCC 33748\textsuperscript{T}; 3, S. downei ChDC YD1; 4, S. downei ChDC YD2; 5, S. downei ChDC YD3; 6, S. downei ChDC YD4; S, size marker (1 kbp DNA ladder).](https://academic.oup.com/femsle/article-abstract/249/2/323/569805)
In conclusion, four strains of *S. downei* were isolated from the dental plaque of humans. To our knowledge, this is the first report of the isolation of this species from the human oral cavity, which implies that *S. downei* may somehow take part in the development of dental caries in humans.

**Acknowledgment**

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**Table 2**

<table>
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<tr>
<th>Strains</th>
<th>Mana</th>
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<th>Melb</th>
<th>Argc</th>
<th>Biotype</th>
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<tr>
<td>ATCC9 33748T</td>
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<td>–</td>
<td>–</td>
<td>+</td>
<td>–</td>
<td>VI</td>
</tr>
<tr>
<td>ChDC* YD1</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>–</td>
<td>I</td>
</tr>
<tr>
<td>ChDC YD2</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>–</td>
<td>–</td>
<td>I</td>
</tr>
<tr>
<td>ChDC YD3</td>
<td>+</td>
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<td>+</td>
<td>+</td>
<td>–</td>
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<tr>
<td>ChDC YD4</td>
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<td>+</td>
<td>+</td>
<td>II</td>
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</table>

* Man, fermentation of mannitol.
* Sor, fermentation of sorbitol.
* Raf, fermentation of raffinose.
* Mel, fermentation of melibiose.
* Arg, hydrolysis of arginine.
* ATCC, American Type Culture Collection.
* ChDC, Department of Oral Biochemistry, College of Dentistry, Chosun University.

**References**


