Streptococcus mutans detection on mother – child pairs using matrix assisted laser desorption ionization – time of flight mass spectrometry and polymerase chain reaction

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ABSTRACT

Introduction: *Streptococcus mutans (S. mutans) bacteria mainly cause dental caries in children. These bacteria are not considered as oral indigenous bacteria since they are transmitted from people around children during eruption of their deciduous teeth. The detection of these bacteria can be used as a prevention of dental caries in children.* ***Objective:*** *To determine the strain and serotype of S. mutans by using Matrix Assisted Laser Desorption Ionization - Time of Flight Mass Spectrometry (MALDI-TOF MS) and Polymerase Chain Reaction (PCR) on dental plaque samples taken from mother-child pairs.* ***Method:*** *Sixteen dental plaque samples of mother-child pairs were cultured on Brain Heart Infusion Broth and Mitis Salivarius Bacitracin media until S. mutans colony isolates were obtained. Next, the isolates of S. mutans colony were put on the target plates of MALDI-TOF MS, and then ionized to become Peptide Mass Fingerprint. Afterwards, the colony isolates were detected by database software. The DNA of detected S. mutans then was extracted by using conventional 727 bp PCR (serotype C).* ***Results:*** *Six strains of S. mutans were detected by MALDI-TOF MS method. Five samples were classified into UA159, two samples were 3SN1, two samples were NFSM1, two samples were 11A1, two samples were U138, two samples were 4SM1, and one sample was classified into another bacterium. Five out of 16 samples were detected by PCR as serotype C (UA159).* ***Conclusion:*** *Six strains of S. mutans was detected, namely UA159, 3SN1, NFSM1, 11A1, U138, and 4SM1, among them one strain (UA159) was detected as serotype C.*

Keywords: *MALDI-TOF MS; mother-child pairs; PCR; Streptococcus mutans*

INTRODUCTION

Dental caries is an infectious disease that can transmit from one to another.[1,2] The high prevalence of dental caries was found in children.[3,4] It is considered as a significant problem still unsolved. Dental caries is also known as a multifactorial disease since it is caused by several interrelated factors. One of the factors is *Streptococcus mutans* (*S. mutans*) bacteria considered as the most influential microorganisms in dental caries development, 45% of which were found in plaque.[5,6] Polysaccharide composition on their surface layer is known to make *S. mutans* survive in a very low pH of plaque, thus improving the survival and virulence of those bacteria. *S. mutans* bacteria with their high virulence and number of colonies also allow the bacteria to transmit to another environment.[5] Caufield et al suggests that an early period of "window of infectivity" is considered as a vulnerable period for children infected with *S. mutans*, ranging from age 19 up to 31 months. Another research also argues that *S. mutans* colonization in the mouth of a child starts to form from the first tooth eruption and then increases as getting older.[7] However, this is closely related to the state of the mother's oral cavity because the mother is the primary caregiver with the highest contact frequency.[8,9]

Strain is a progeny or subculture of a single colony isolate in pure culture. In average, one strain of *S. mutans* can be found in each individual, but may also be 1-4 strains of *S. mutans*.[10] There may be similarities as well as differences between them so that the strains can be identified to see their kinship.[11] Subspecies marking of a microorganism based on its antigenic component, moreover, is called as serotype (serovar). *S. mutans* is classified into serotypes C, E, F, and K. Serotype C *S. mutans* is the most commonly found serotype in the human oral cavity and has a complex Rhamnose-Glucose Polymer (RGP) structure that is very useful in survival, attachment, and colonization of *S. mutans* in the oral cavity.[2,12,13]

Matrix Assisted Laser Desorption Ionization - Time Of Flight Mass Spectrometry (MALDI-TOF MS) is a method chosen as an alternative in detecting a microorganism because of its relatively fast, sensitive, specific, simpler work procedure and lower cost rather than other molecular and immunological-based methods, moreover, it also requires less laboratory equipment.[14,15,16] This method has widely been used by microbiologists for several purposes, such as identification of strains and taxonomic types of microorganisms (bacteria, viruses and fungi), epidemiological studies, detection of bioterrorism, detection of water and food pathogens, detection of antibiotic resistance, etc.[15,16] Principles of the identification of strains and taxonomic types of microorganisms by MALDI-TOF MS method is based on Peptide Mass Fingerprint (PMF) generated by samples. This PMF is used as the basis of the identification by

comparing the PMF of unknown organisms with the PMF contained in the database or by matching the mass of biomarker microorganisms that have not been identified with proteomic databases. [16,17,18]

At this time, the detection of a microorganism is mostly conducted by molecular methods, such as Polymerase Chain Reaction (PCR) followed by DNA sequencing as the main method that becomes "gold standard" in detecting and identifying a microorganism since microorganisms can be detected up to chromosome DNA level.[19,20] This method is also known to have the highest sensitivity and specificity, which is fast and accurate to 100%. Therefore, this research was aimed to determine the strain and serotype of *S. mutans* by using MALDI-TOF MS and PCR on dental plaque samples taken from mother-child pairs.

MATERIALS AND METHODS

Samples used in this research were dental plaque taken from 16 subjects in Jagiran Tambaksari area in Surabaya. The subjects were eight pairs of mother and child, the children were younger than 2 years old. The subjects were healthy, and did not consume antibiotics and corticosteroid drugs. The mothers had the DMF-T index of more than 2.7, and were willing to participate in this research by signing the informed consent. The ethical clearance certificate was given by the ethical clearance committee of Faculty of Dental Medicine, Universitas Airlangga (No. 77/KKEPK.FKG/VI/2016).

The research was done at Institute of Tropical Disease (ITD), Universitas Airlangga. The plaque sample was taken by brushing method using sterile toothbrush on maxillary and mandibular teeth surfaces, including tongue[8]. The plaque then was added to the *Brain Heart Infusion Broth* (BHIB) liquid media to be incubated at a temperature of 37 °C for 48 hours. Thereafter, the plaque samples were prepared to be cultured on *Mitis Salivarius Bacitracin* (MSB) media and then incubated anaerobically at 37°C for 48 hours using anaerobic jars filled with gas pack. Subsequently, solitary bacteria in the incubated samples were taken carefully using a stick to be cultured on the second BHIB media. All of the second BHIB tubes containing *S. mutans* colonies then were incubated again at 37°C for 48 hours.

After the incubation process was completed, the colonies of *S. mutans* were re-cultured by diffusing them in zigzag pattern on the second MSB media, in which each Petri dish was divided by 4 parts for 4 samples. The second MSB media then were incubated anaerobically at 37°C for 48 hours with anaerobic jars filled with gas pack. After that, each sample on the second MSB media was examined for its morphology under an inverter microscope (Olympus CK 128, Tokyo, Japan) to ascertain whether bacterial colonies emerged were *S. mutans* or not. Afterwards, a single colony was collected with the stick carefully to transfer to the tubes containing the third BHIB media and then incubated at 37°C for 48 hours. Next, the tubes were vortexed to be homogeneous, and then each sample was transferred into two different sterile eppendorf tubes with each micropipette sized 2 ml. One eppendorf tube was used as a sample for the MALDI-TOF MS process,[17] while the other eppendorf tube was used as a sample for the PCR process.

In the MALDI-TOF MS process, the samples in the eppendorf tubes were diluted with 3 ml of 0.45% NaCl, then placed in the target plates, and mixed with reagents, such as matrix solution (water and the organic solvent mixture) containing acetonitrile and strong acid (Tri Fluoro Acetate-TFA), as well as a matrix of 3,5-dimethoxy-4-hydroxycinnamic acid (sinapinic

acid). After that, they were dried. The target plates then were inserted into the MALDI-TOF MS (Vitex, bioMérieux S.A, Marcy l'Étoile, France) machine to match the microorganisms with the existing data in the software. Next, the results were printed.[18]

In the PCR process, the samples in the eppendorf tubes containing DNA were isolated with the DNA Isolation Purification Kit Wizard (Wizard® Genomic DNA Purification Kit, Promega Coorporation, Singapore) to obtain the DNA extract of each sample. Subsequently, the DNA extract of each sample was processed by conventional PCR performed in 25 cycles under the initial denaturation of 96°C for 2 minutes. Each of the cycles then got the denaturation of 96°C for 15 seconds, the annealing of 61°C for 30 seconds, the extension of 72°C for 1 minute, and the post extension of 72°C for 10 minutes, using SC-F primer pairs (CGG AGT GCT TTT TAC AAG TGC TGG) and SC - R (AAC CAC GGC CAG CAA ACC CTT TAT) at site 727 bp.[21] DNA samples then were processed by Bio-Rad T100 PCR Machine, California, USA. Subsequently, the PCR product was confirmed by 1% agarose gel electrophoresis (Mupid-2plus) performed at a voltage of 90 Volts for 30 minutes. After the electrophoresis process was completed, the gel was soaked into a 2% Ethidium Bromide immersion solution for 30 minutes, and then the PCR product was visualized using a translucent UV to see whether the band was in a predetermined location or not.

RESULTS

There were 16 samples of dental plaque taken from the mother-child pairs. After those samples were cultured on BHIB and MSB media, their morphology was tested using an inverter microscope (Olympus CK 128, Tokyo, Japan). The results showed round or ovoid single cubes with a diameter of 1-2 µm arranged as chains.

The pure *S. mutans* isolates were processed on the Vitex machine, (bioMérieux S.A, Marcy l'Étoile, France) with MALDI-TOF MS method to detect *S. mutans* strains. Based on the detection of *S. mutans* strain with MALDI-TOF MS method, the results were obtained in Table 1.

Based on the results of this study, 16 samples were identified to have six strains of *S. mutans*. Five samples, Mother 1, Child 1, Mother 4, Mother 8, and Child 8, had *S. mutans* UA159. Two samples, Mother 2 and Child 2, had *S. mutans* 3SN1. Two samples, Mother 3 and Child 3, had *S. mutans* NFSM1. Two samples, Mother 5 and Child 5, had *S. mutans* 11A1. Two samples, Mother 6 and Child 6, had *S. mutans* U138. And, two samples, Mother 7 and Child 7, had *S. mutans* 4SM1. Meanwhile, one sample, Child 4, had *S. gordonii* IE35.

During the PCR process, serotype C *S. mutans* was detected since serotype C is the most abundant serotype in the human oral cavity. The results of the serotype C *S. mutans* detection with PCR method showed in Figure 1. Based on the results of the PCR process at the 727 bp amplification site, there were three samples, namely line 1 (An 8), line 2 (Ib1), and line 3 (Ib 4) samples detected as serotype C *S. mutans*, while 13 other samples were not detected as serotype C *S. mutans*.

Table 1. The results of *S. mutans* strain detection with MALDI-TOF MS method

Samples	Taxon Ids	Name of organism
Mother 1	637000288	*S. mutans* UA159
Child 1	637000288	*S. mutans* UA159
Mother 2	2558860343	*S. mutans* 3SN1
Child 2	2558860343	*S. mutans* 3SN1
Mother 3	2558860323	*S. mutans* NFSM1
Child 3	2558860323	S. mutans NFSM1
Mother 4	637000288	*S. mutans* UA159
Child 4	2639762775	*S. gordonii* IE35
Mother 5	2558860348	*S. mutans* 11a1
Child 5	2558860348	*S. mutans* 11a1
Mother 6	2558860328	*S. mutans* U138
Child 6	2558860328	*S. mutans* U138

Mother 7	2558860342	*S. mutans* 4SM1
Child 7	2558860342	*S. mutans* 4SM1
Mother 8	637000288	*S. mutans* UA159
Child 8	637000288	*S. mutans* UA159

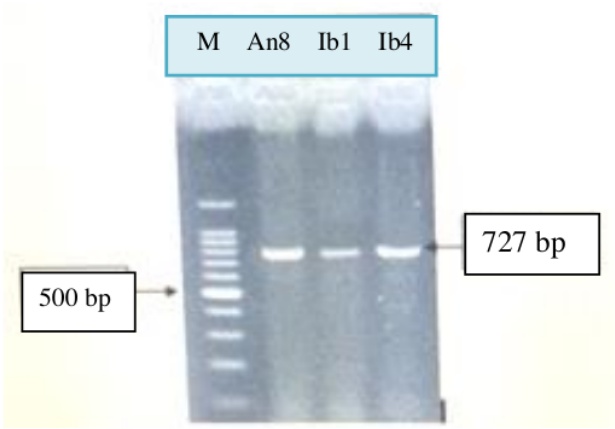

Figure 1. The results of the PCR process. Three samples detected as serotype C *S. mutans*, i.e. An8, Ib 1, and Ib 4, while 13 other samples not detected.

DISCUSSION

Based on the results of the *S. mutans* detection on the dental plaque samples of those mother-child pairs with MALDI-TOF MS approach, there were six different *S. mutans* strains. However, there were some samples with the same *S. mutans* strains. According to a research conducted by Gibbons et al,[11] the strain of a microorganism in an individual can be similar with or different from others, thus, the strain can be used to identify the kinship of individuals.[11] In average, each individual, according to Ine et al,[10] has one strain of *S. mutans* in his oral cavity, yet he can also have 1-4 strains of *S. mutans*. The diversity of strains from one individual to another is influenced by their geographical locations, bacterial culture conditions (but not significantly different), or sample preparation methodologies.

Based on the data of the National Center for Biotechnology Information (NCBI) in 2016,[22] there were 172 *S. mutans* strains detected. These strains have been detected from previous studies with complete information. For instance, *S. mutans* UA 159 with ID Taxonomy 637000288 and ID NCBI 21007 found in some samples of this research was officially announced on December 1, 2006 as facultative and pathogenic gram-positive cocci commonly found in the oral cavity of children with active carries, including *S. mutans* with serotype C already having sequencing status.

Strain, according to Dijkshoorn et al,[23] statement cited in Bergey's manual of Systemic Bacteriology, is a progeny or subculture of a single colony isolate in pure culture. Strain also has several types identified with certain basic properties. First, biotype strain grouping a bacterial strain based on the biochemical or physiological structure of a species and its properties is often used to describe the species, but the biotype strain is unable to show all the strain properties of a species. Therefore, subspecies marking of a microorganism conducted based on the other components is divided into morph type strain (morphovar) grouping of bacterial strains based on their morphology, serotype strain (serovars) grouping bacterial strains based on antigenic structures, as well as patotype strain (patovars), or phagotype strain (fagovars) sometimes used to denote certain properties of strain variation [24,25]

S. mutans is classified into four serotypes based on its antigenic structure (carbohydrate specificity on its cell wall, h_2O_2 production, sensitivity to bacitracin, fermented substrate, and its DNA content), namely serotype C, serotype E, serotype F, and serotype K. [21,26,27] Serotype C *S. mutans* is the most common type found in the oral cavity of humans, especially dental plaque with a prevalence of 75-90%. Since serotype C *S. mutans* has a complex Rhamnose-Glucose Polymer (RGP) structure that is very useful in survival, the process of attachment and

colonization in the oral cavity.[28] Therefore, *S. mutans* serotype C was selected in this research to detect *S. mutans*.

In general, bacterial identification methods can be divided into phenotypic method based on their profile, metabolic properties, and chemical composition as well as genotypic method based on genetic material (DNA). Phenotypic method in detecting bacteria is started by taking bacteria from various specimens to be cultured, isolated, and then detected based on taxonomic principles. The detection of a bacterium is microscopically based on shape, size, group, Gram staining reaction, and motility. The microscopic observation combined with natural environment data is essential for detecting bacteria. Bergey's Manual of Determinative Bacteriology is a guidebook for detecting bacteria based on microscopic and physiological characters. Bacterial identification using phenotypic method can only distinguish genus from family and species of some bacteria, while genotypic method is used to see subtype of a microorganism so that taxonomic principles used become modern taxonomy, the use of complex detection method, including molecular analysis.[29,30]

On the other hand, MALDI-TOF MS is a useful analytical technique for detecting chemical structures in which chemical compounds are ionized into molecules based on the mass calculation of a molecule and its fragmentation pattern (m / z). This method can be used as an alternative method to detect bacteria because of some advantages, such as easy process, fast result, high specificity and sensitivity, lower cost than the molecular detection and immunologically based method, and no laboratory requirement. This method also has several uses, such as for detecting strain type and microbial taxonomy, detecting bioterrorism, detecting pathogens of water and food, detecting blood pathogens and urinary tract, supporting diagnosis of bacterial, fungal and viral diseases, detecting antibiotic resistance, and etc.[15,17,18]

MALDI-TOF MS as an alternative method of detection of strain type and microbial taxonomy based on PMF generated by samples. Samples that have been trapped by the matrix and dissolved with the matrix solution are subjected to laser spectrometry so that the target sample undergoes ionization and turns into a PMF. PMF is used as the basis of detection by comparing the PMF of unknown organisms with the PMF contained in the database or by matching the mass of unknown microorganism biomarkers with proteome databases. Matching the sample PMF pattern with the ribosome protein PMF is required to detect the microorganism of a particular species and its strain type. However, the matching of the PMF pattern in this method may still have a limitation since the identification of new isolates can be detected if the database within the software matches the sample PMF pattern so that in this method a database

should be locally prepared for a specific taxonomy (e.g. *Streptococcus* or *Staphylococcus*) in which geographical variations can cause variations in the genotype and phenotype of a microorganism.[14,17,18]

In recent years, the detection of microorganisms has led to DNA sequencing. PCR and DNA sequencing method becomes "gold standard" in detecting a microorganism in modern taxonomy, defining phylogenies and analyzing an ecosystem for epidemiological studies. This method is also useful for revealing bacterial evolution, constructing phylogenetic trees, tracing species diversity, and detecting new species without isolating the microorganisms. There are several other advantages of this method, such as fast procedure, accurate result, specificity and sensitivity reaching 100%, not easily contaminated procedure, and simultaneous detection for several microorganisms. Nevertheless, this method requires high cost, specific primary determinations, appropriate thermal cycles, and specialized expertise.[29]

Serotype C *S. mutans* can specifically be detected through serotype C primers wherein a specific primary PCR process will encode according to the nucleotide base sequence of a DNA sample. Thus, DNA samples that do not fit the nucleotide base sequence on the PCR primers will not be detected or will not appear in the amplified band. In this research, there were serotype C *S. mutans* detected with SC-forward primers (CGG AGT GCT TTT TAC AAG TGC TGG) and SC-reverse primers (AAC CAC GGC CAG CAA ACC CTT TAT) at the amplification region of 727 bp.[19] In those 16 *S. mutans* DNA samples, three samples were detected as serotype C *S. mutans*, found in line 1 (Child 8), line 2 (Mother 1), and line 3 (Mother 4), while 13 other samples were not detected as serotype C *S. mutans*. This may occur since only serotype C primers were used. The other 13 samples might be detected as *S. mutans* with other serotypes, such as serotype E *S. mutans*, serotype F *S. mutans*, etc., or genetic polymorphism occurred in *S. mutans* studied.[19,31]

Genetic polymorphism is a variation or change in the basic structure (DNA) of a microorganism in which there is a change in the nucleotide base sequence caused by insertion, addition, or subtraction of a particular base.[32] Polymorphism occurs because of spontaneous gene mutations triggered by changes in normal cell function or due to interaction with the environment. The occurrence of changes in only one base or often referred to as point mutations has been said to be a genetic polymorphism. Point mutations that often occur are substitutions of G - C (Guanin - Cytosine) or A - T (adenine - thymine).

In conclusion, there were six strains of *S. mutans* detected by MALDI-TOF MS method, namely five samples of *S. mutans* UA159, two samples of *S. mutans* 3SN1, two

samples of *S. mutans* NFSM1, two samples of *S. mutans* 11A1, two samples of *S. mutans* U138, and two samples of *S. mutans* 4SM1, among them one strain (UA159) was detected as serotype C by PCR method. These results then are expected to be used as a basis for further research for early detection in dental caries prevention, identification of new *S. mutans* isolates, and epidemiological studies.

REFERENCES

1. Mattos-Graner RO, Li Y, Caufield PW, Duncan M, Smith DJ. Genotypic diversity of mutans streptococci in Brazilian nursery children suggests horizontal transmission. J Clin Microbiol. 2001; 39(6): 2313–6.
2. Baca P, Castillo AM, Liébana MJ, Castillo F, Martín-Platero A, Liébana J. Horizontal transmission of streptococcus mutans in schoolchildren. Med Oral Patol Oral Cir Bucal. 2012; 17(3).
3. Zou J, Meng M, Law CS, Rao Y, Zhou X. Common dental diseases in children and malocclusion. Int J Oral Sci. 2018; 10: 7.
4. Pierce A, Singh S, Lee JH, Grant C, Cruz de Jesus V, Schroth RJ. The Burden of Early Childhood Caries in Canadian Children and Associated Risk Factors. Front Public Heal. 2019; 7(November).
5. Okada M, Soda Y, Hayashi F, Doi T, Suzuki J. PCR detection of Streptococcus mutans and S. sobrinus in dental plaque samples from Japanese pre-school children. J Med Micro. 2002; 51(5): 443–7.
6. Jiang Q, Yu M, Min Z, Yi A, Chen D, Zhang Q. AP-PCR detection of Streptococcus mutans and Streptococcus sobrinus in caries-free and caries-active subjects. Mol Cell Biochem. 2012; .
7. Caufield PW1, Cutter GR DA. Initial acquisition of mutans streptococci by infants: evidence for a discrete window of infectivity. J Dent Res. 1993; Jan;72(1): 37–45.
8. Tedjosasongko U, Kozai K. Initial acquisition and transmission of mutans streptococci in children at day nursery. ASDC J Dent Child. 2002; 69(3): 284–8, 234–5.
9. Hameş-Kocabaş EE, Uçar F, Kocataş Ersin N, Uzel A, Alpöz AR. Colonization and vertical transmission of Streptococcus mutans in Turkish children. Microbiol Res. 2008; .
10. Ine M, Pereira AC, Gonc RB. Longitudinal Study of Transmission, Diversity, and Stability of. Society. 2004; 42(10): 4620–6.
11. Gibbons RJ, Cohen L, Hay DI. Strains of Streptococcus mutans and Streptococcus sobrinus attach to different pellicle receptors. Infect Immun. 1986; 52(2): 555–61.
12. Pieralisi FJS, Rodrigues MR, Segura VG, Maciel SM, Ferreira FBA, Garcia JE, Poli-Frederico RC. Genotypic Diversity of Streptococcus mutans in Caries-Free and Caries-Active Preschool Children . Int J Dent. 2010; 2010: 1–5.
13. Damé-Teixeira N, Arthur RA, Parolo CCF, Maltz M. Genotypic diversity and

virulence traits of streptococcus mutans isolated from carious dentin after partial caries removal and sealing. Sci World J. 2014; 2014.

14. Murray PR. What Is new in clinical microbiologymicrobial identification by MALDI-TOF mass spectrometry: A paper from the 2011 William Beaumont Hospital symposium on molecular pathology. J Mol Diagnostics. 2012; 14(5): 419–23.
15. Singhal N, Kumar M, Kanaujia PK, Virdi JS. MALDI-TOF mass spectrometry: An emerging technology for microbial identification and diagnosis. Front Microbiol. 2015; 6(AUG): 1–16.
16. Croxatto A, Prod'hom G, Greub G. Applications of MALDI-TOF mass spectrometry in clinical diagnostic microbiology. FEMS Microbiol Rev. 2012; 36(2): 380–407.
17. Clark AE, Kaleta EJ, Arora A, Wolk DM. Matrix-Assisted laser desorption ionization-time of flight mass spectrometry: A fundamental shift in the routine practice of clinical microbiology. Clin Microbiol Rev. 2013; 26(3): 547–603.
18. Bilecen K, Yaman G, Ciftci U, Laleli YR. Performances and reliability of Bruker microflex LT and VITEK MS MALDI-TOF mass spectrometry systems for the identification of clinical microorganisms. Biomed Res Int. 2015; 2015(Ivd).
19. Childers NK, Osgood RC, Hsu K-L, Manmontri C, Momeni SS, Mahtani HK, Cutter GR, Ruby JD. Real-time quantitative polymerase chain reaction for enumeration of Streptococcus mutans from oral samples. Eur J Oral Sci. 2011; 119(6): 447–54.
20. Gilbert K, Joseph R, Vo A, Patel T, Chaudhry S, Nguyen U, Trevor A, Robinson E, Campbell M, McLennan J, Houran F, Wong T, Flann K, Wages M, Palmer EA, Peterson J, Engle J, Maier T, Machida CA. Children with severe early childhood caries: Streptococci genetic strains within carious and white spot lesions. J Oral Microbiol. 2014; 6(1): 1–11.
21. Shibata Y, Ozaki K, Seki M, Kawato T, Tanaka H, Nakano Y, Yamashita Y. Analysis of loci required for determination of serotype antigenicity in Streptococcus mutans and its clinical utilization. J Clin Microbiol. 2003; 41(9): 4107–12.
22. Schoch CL, Ciufo S, Domrachev M, Hotton CL, Kannan S, Khovanskaya R, Leipe D, McVeigh R, O'Neill K, Robbertse B, Sharma S, Soussov V, Sullivan JP, Sun L, Turner S, Karsch-Mizrachi I. NCBI Taxonomy: A comprehensive update on curation, resources and tools. Database. 2020; 2020(2): 1–21.
23. Dijkshoorn L, Ursing BM, Ursing JB. Strain, clone and species: Comments on three basic concepts of bacteriology. Vol. 49, Journal of Medical Microbiology. 2000. p.

397–401.

24. Prescott, Lansing M; Klein, Donald A; Harley JP. Microbial taxonomy.pdf. New York: McGraw-Hill; 2002.
25. Maki Y, Sakayori T, Hirata S, Ishii T, Tachino A. Monitoring caries risks before the window of infection and later caries increment: a caries prediction study on rapid detection of Streptococcus mutans using monoclonal antibodies. Bull Tokyo Dent Coll. 2014; 55(1): 19–23.
26. Nakano K, Lapirattanakul J, Nomura R, Nemoto H, Alaluusua S, Grönroos L, Vaara M, Hamada S, Ooshima T, Nakagawa I. Streptococcus mutans clonal variation revealed by multilocus sequence typing. J Clin Microbiol. 2007; 45(8): 2616–25.
27. Nakano K, Nemoto H, Nomura R, Homma H, Yoshioka H, Shudo Y, Hata H, Toda K, Taniguchi K, Amano A, Ooshima T. Serotype distribution of Streptococcus mutans a pathogen of dental caries in cardiovascular specimens from Japanese patients. J Med Microbiol. 2007; 56(4): 551–6.
28. Tabchoury CPM, Sousa MCK, Arthur RA, Mattos-Graner RO, Del Bel Cury AA, Cury JA. Evaluation of genotypic diversity of Streptococcus mutans using distinct arbitrary primers. J Appl Oral Sci. 2008; 16(6): 403–7.
29. Franco-Duarte R, Černáková L, Kadam S, Kaushik KS, Salehi B, Bevilacqua A, Corbo MR, Antolak H, Dybka-Stępień K, Leszczewicz M, Tintino SR, de Souza VCA, Sharifi-Rad J, Coutinho HDM, Martins N, Rodrigues CF. Advances in chemical and biological methods to identify microorganisms—from past to present. Microorganisms. 2019; 7(5).
30. Sato T, Matsuyama J, Kumagai T, Mayanagi G, Yamaura M, Washio J, Takahashi N. Nested PCR for detection of mutans streptococci in dental plaque. Lett Appl Microbiol. 2003; 37(1): 66–9.
31. Kazemtabrizi A, Haddadi A, Shavandi M, Harzandi N. Metagenomic investigation of bacteria associated with dental lesions: A cross-sectional study. Med Oral Patol Oral y Cir Bucal. 2020; 25(2): e240–51.
32. Williams JGK, Kubelik AR, Livak KJ, Rafalski JA, Tingey S V. DNA polymorphisms amplified by arbitrary primers are useful as genetic markers. Nucleic Acids Res. 1990; 18(22): 6531–5.

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