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Original article

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

Udijanto Tedjosasongko, Dwi Mulia Ramadhaniati and Seno Pradopo
Department of Pediatric Dentistry,
Faculty of Dental Medicine, Universitas Airlangga
Surabaya – Indonesia

ABSTRACT

Background: *Streptococcus mutans (S. mutans) bacteria mainly cause dental caries in children. These bacteria are not considered oral indigenous bacteria since they are transmitted from people around children during their deciduous teeth eruption. The detection of these bacteria can be used for dental caries prevention in children.* ***Purpose:*** *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.* ***Methods:*** *Sixteen dental plaque samples of mother-child pairs were cultured on brain heart infusion broth (BHIB) and mitis salivarius bacitracin (MSB) media until S. mutans colony isolates were obtained. Next, the isolates of S. mutans colony were introduced into the target plates of MALDI-TOF MS, and then ionized to become peptide mass fingerprint (PMF). Afterwards, the colony isolates were detected by database software. The detected S. mutans DNA 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 were detected, namely UA159, 3SN1, NFSM1, 11A1, U138, and 4SM1, one of them (UA159) was detected as serotype C.*

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

Correspondence: Udijanto Tedjosasongko, Department of Pediatric Dentistry, Faculty of Dental Medicine, Universitas Airlangga. Jl. Mayjen Prof. Dr. Moestopo No. 47 Surabaya, 60132 Indonesia. Email: udijanto@fkg.unair.ac.id

INTRODUCTION

Dental caries is an infectious disease that can transmit from one to another.[1,2] Children have high dental caries prevalence.[3,4] Dental caries is considered a significant unsolved problem. Moreover, it is a multifactorial disease since it is triggered by several interrelated factors including *Streptococcus mutans* (*S. mutans*) bacteria which are the most influential microorganisms in dental caries formation with a percentage of 45% in dental plaque.[5,6] The polysaccharide composition of *S. mutans'* extracellular layer enhances their survival rate in a very low plaque pH as to why *S. mutans* bacteria have high virulence besides being in high colonies that promote their environmental transmission.[5] Caufield et al.[7] described the concept "window of infectivity" as an early vulnerable period during which infants acquired *S. mutans* at the age between 19 and 31 months. Furthermore, another research argued that *S. mutans* colonization in the child's mouth starts with first tooth eruption and continues to grow with age.[7] However, this is closely related to the mother's oral cavity state since the mother is considered the primary child's caregiver with the highest contact frequency.[8,9]

Strain is a single colony progeny or subculture that has been isolated in pure culture. Most individuals have one *S. mutans* strain but some may have 1-4 *S. mutans* strains.[10] However, since *S. mutans* strains have similarities and differences, they may be used to identify the kinship.[11]

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On the other hand, subspecies microorganism marking based on its antigenic component is called serotype (serovar). *S. mutans* is classified into serotypes C, E, F, and K. Serotype C *S. mutans* is the most abundant in the human oral cavity with a complex rhamnose-glucose-polymer (RGP) structure which is responsible for *S. mutans* survival, attachment, and colonization in the oral cavity.[2,12,13]

Matrix assisted laser desorption ionization – time of flight mass spectrometry (MALDI-TOF MS) is an alternative detecting microorganisms' method with relative higher speed, more sensitivity and specificity, simpler work procedure, less laboratory equipment requirement, and lower cost compared to other molecular and immunological based methods.[14–16] This method has been widely used by microbiologists for several purposes including identification of strains and taxonomic microorganisms (bacteria, viruses, and fungi), and epidemiological studies in addition to detection of bioterrorism, water and food pathogens, and antibiotic resistance, etc.[15,16] MALDI-TOF MS method's principles of strains and taxonomic microorganisms' identification are based on the generated peptide mass fingerprint (PMF) by samples. The unknown organisms can be identified whether by comparing their PMF with existed database PMF or by matching their biomarker mass with proteomic databases.[16–18]

Recently, microorganism detection is conducted mainly by molecular methods such as polymerase chain reaction (PCR) followed by DNA sequencing which is considered the gold standard method in detecting and identifying microorganisms up to chromosome DNA level.[19,20] Furthermore, this method has the highest sensitivity and specificity, which is fast and accurate (100%). Therefore, this research aimed to determine the strain and serotype of *S. mutans* using MALDI-TOF MS and PCR methods on dental plaque samples taken from mother-child pairs.

MATERIALS AND METHODS

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

The research was conducted at the Institute of Tropical Disease (ITD), Universitas Airlangga. The plaque samples were taken by brushing method for maxillary and mandibular teeth surfaces, including the tongue, using sterile toothbrushes.[8] After that, the plaque was added to the brain heart infusion broth (BHIB) liquid media and incubated at 37°C for 48 hours. Thereafter, the plaque samples were cultured on mitis salivarius bacitracin (MSB) media and incubated anaerobically at 37°C for 48 hours using Gas-Pack anaerobic jars. Subsequently, a solitary colony from the incubated samples was taken carefully using a stick and cultured on the second BHIB media tubes. All tubes with *S. mutans* colonies then were incubated again at 37°C for 48 hours.

After the incubation process, the *S. mutans* colonies were re-cultured by diffusing them in a zigzag pattern on the second MSB media, in which each Petri dish was divided into 4 parts for 4 samples. The second MSB media then were incubated anaerobically at 37°C for 48 hours using Gas-Pack anaerobic jars. Next, the morphology of each sample on the second MSB media was examined under an inverted microscope (Olympus CK 128, Tokyo, Japan) to ascertain whether the emerged bacterial colonies were *S. mutans* or not. Afterward, a solitary colony was taken carefully using a stick to transfer it to the third BHIB media tubes and then was incubated at 37°C for 48 hours. After that, the tubes were vortexed to be homogeneous, and each sample was then transferred into two different sterile eppendorf tubes with a size of 2 ml micropipette. 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 eppendorf tubes samples were diluted with 3 ml of 0.45% NaCl, then introduced to the target plates, and mixed with reagents including a matrix (mixture of water and organic solvent) of acetonitrile and strong acid (Trifluoroacetate - TFA), as well as a matrix of 3,5-dimethoxy-4-hydroxycinnamic acid (Sinapic acid). Next, they were dried. After that, the target plates were inserted into the MALDI-TOF MS machine (Vitex, bioMérieux S.A, Marcy l'Étoile, France) to match the microorganisms with the software existing data. Then, the results were printed.[18]

In the PCR process, the eppendorf tube samples containing DNA were isolated with the DNA Isolation Purification Kit Wizard (Wizard® Genomic DNA Purification Kit, Promega Corporation, Singapore) to obtain the DNA extract of each sample. Next, each DNA extract was processed by the conventional PCR process that performed in 25 cycles under initial denaturation at 96°C for 2 minutes. In Each cycle, they exposed to denaturation at 96°C for 15 seconds, annealing at 61°C for 30 seconds, extension at 72°C for 1 minute, and post extension at 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] After that, DNA samples were processed by Bio-Rad T100 PCR Machine, California, USA. Subsequently, the PCR product was confirmed by 1% agarose gel electrophoresis (Mupid-2plus) on 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 the PCR product then was visualized using a translucent UV to observe whether the band was in the predetermined location or not.

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RESULTS

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

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

During the PCR process, serotype C *S. mutans* was detected since serotype C is the most dominant serotype in the human oral cavity. The results of serotype C *S. mutans* detection with PCR method were shown in Figure 1. Based on PCR process results at the 727 bp amplification site, three samples which are line 1 (An 8), line 2 (Ib1), and line 3 (Ib 4) samples were detected as serotype C *S. mutans*, while the other13 samples were not.

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

Samples	Taxon Ids	Name of the 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

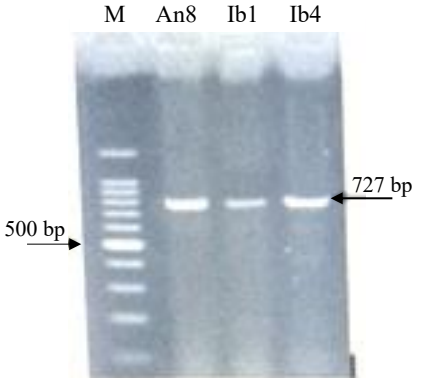

Figure 1. The results of the PCR process. Three samples were detected as serotype C *S. mutans*, i.e. An8, Ib 1, and Ib 4, while the other 13 samples were not.

DISCUSSION

The results of the *S. mutans* detection on the dental plaque samples of the mother-child pairs with MALDI-TOF MS approach showed six different strains of *S. mutans* with some samples had the same *S. mutans* strains. Gibbons et al.[11] stated that the microorganism strains show similarities and differences among individuals, that is why the strain can be used to identify the individuals' kinship. According to Klein et al.[10] humans can have 1-4 strains of *S. mutans* in their oral cavity; at least one strain in each. The strains are varied among individuals according to their geographical locations, bacterial culture conditions (not significantly different), and the sample preparation methods.

The National Center for Biotechnology Information (NCBI) in 2016 recognized 172 strains of *S. mutans*.[22] These strains have been detected from complete previous studies. For instance, *S. mutans* UA 159 with ID Taxonomy 637000288 and ID NCBI 21007 was officially announced on 1st December 2006 as a common facultative and pathogenic gram-positive cocci in the oral cavity of children with active caries , including serotype C *S. mutans* which is the sequencing status has already been studied.

Strain, according to Dijkshoorn et al,'s statement cited in Bergey's Manual of Systemic Bacteriology, is a progeny or subculture of a solitary colony isolated in pure culture.[23] Furthermore, strain is divided into several types according to certain basic properties. First of all, biotype strain, in which the strain is classified based on the biochemical or physiological structure of species. Although biotype strain is often used to describe the species' characteristics, it has not the capability to demonstrate the whole species properties. Therefore, other classifications, based on other criteria, were conducted such as morphotype strain (morphovar) in which the strain is grouped based on their morphology and serotype strain (serovars) in which the strain is classified based on antigenic structures, as well as patotype strain (patovars), or phagotype strain (fagovars) which are used sometimes to denote certain properties of strain variation.[24,25]

S. mutans is classified based on its antigenic structure (cell wall carbohydrate specificity, H_2O_2 production, bacitracin sensitivity, fermented substrate, and DNA content) into four serotypes which are: serotype C, serotype E, serotype F, and serotype K.[21,26,27] Serotype C *S. mutans* is the most common type in the human oral cavity, especially in dental plaque with a prevalence of 75-90%, since serotype C *S. mutans* has a complex RGP structure that enhances its survival, attachment, and colonization in the oral cavity.[28] Therefore, *S. mutans* serotype C was selected in this research as *S. mutans* determinant.

Substantially, bacterial identification methods include the phenotypic method in which bacteria are classified based on their profile, metabolic properties, and chemical composition and the genotypic method in which bacteria are categorized according to their genetic material (DNA). In the phenotypic method, bacteria are taken from various

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specimens to be cultured, isolated, and then detected based on taxonomic principles. The microscopic observation, in which bacteria is detected based on shape, size, group, Gram staining reaction, and motility, should be combined with the natural environment data during the bacterial identification method. Bergey's Manual of Determinative Bacteriology is an example of a guidebook that detects bacteria based on their microscopic and physiological characters. However, the phenotypic method can only distinguish genus from the same family members and cover some bacterial species, while the genotypic method has the capability to recognize the microorganism subtypes as to why modern taxonomy tend to use more complex detection method including molecular analysis.[29,30]

On the other hand, MALDI-TOF MS is considered an efficient analytical technique for detecting chemical structures in which chemical compounds are ionized into molecules based on the mass calculation of the molecule and its fragmentation pattern (m / z). This method is considered an alternative method in bacterial identification because of its advantageous characteristics including simplicity, fast result, high specificity and sensitivity, lower cost compared to the molecular and immunological method, and no laboratory requirements. Furthermore, this method is used for various purposes including identification of strain type, microbial taxonomy, bioterrorism, water and food pathogens, blood and urinary tract pathogens, and antibiotic resistance, in addition to supporting the bacterial, fungal, and viral diseases diagnosis, etc.[15,17,18]

MALDI-TOF MS is based on PMF generated by samples to detect the strain type or the microbial taxonomy. Target samples that have been trapped and dissolved in the matrix solution would be exposed to laser spectrometry to undergo an ionization process turning them into PMF. PMF is used in the detection process by comparing unknown organisms PMF with the existing database PMF or by matching the unknown microorganism biomarkers mass with proteome databases. Furthermore, matching the sample PMF pattern with the ribosome protein PMF is required to detect some particular species' microorganisms and their strain type. However, the PMF pattern matching in this method still has some limitations since the new isolates can be detected if the software database matches the sample PMF pattern. That is why the database of this method should be locally prepared for a specific taxonomy (e.g. *Streptococcus* or *Staphylococcus*) as to why geographical variations can occur among the genotype and phenotype of a microorganism.[14,17,18]

Recently, microorganisms identification tends to be more dependant on DNA sequencing approaches. For example, PCR and DNA sequencing methods become the "gold standard" in microorganism identification in modern taxonomy, defining phylogenies, and analyzing epidemiological studies ecosystem. Furthermore, this method is used for revealing bacterial evolution, constructing phylogenetic trees, tracing species diversity, and detecting new species without isolating the microorganisms. This method has many superior properties including the fast procedure, accurate result, high specificity and sensitivity reach to 100%, low vulnerability to contamination, and simultaneous detection for several microorganisms. However, this approach requires high cost, specific primary determinations, appropriate thermal cycles, and specialized expertise.[29]

Serotype C *S. mutans*, in particular, can be detected through serotype C primers wherein a specific primary PCR process will be encoded according to the nucleotide base sequence of a DNA sample. Thus, DNA samples that do not match with the nucleotide base sequence on the PCR primers will not be recognized or appeared in the amplified band. In this research, serotype C *S. mutans* were 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]. From the 16 *S. mutans* DNA samples, three samples were detected as serotype C *S. mutans* as follows: in line 1 (Child 8), line 2 (Mother 1), and line 3 (Mother 4), while the other 13 samples were not identified as serotype C *S. mutans* since serotype C primers were the only used primers. The other 13 samples may follow other serotypes, such as serotype E *S. mutans*, serotype F *S. mutans*, etc., or genetic polymorphism of previously studied *S. mutans*.[19,31]

Genetic polymorphism is a variation in the microorganism DNA structure in which a change in the nucleotide base sequence is occurred due to the insertion, addition, or subtraction of a particular base.[32] Genetic polymorphism is caused whether by the spontaneous gene mutations triggered by the normal cell function changes or by interaction with the environment. If the changes occur in only one base, referred to as point mutation, it can be inferred that the microorganism has been genetically polymorphed. Most point mutations occur as 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 follows 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. However, UA159 was the only strain that was detected as serotype C by PCR method. It is expected to use these results as a basis for further researches related to early detection of dental caries, 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 A-M, Liébana M-J, Castillo F, Martín-Platero A, Liébana J. Horizontal transmission of *Streptococcus mutans* in schoolchildren. Med Oral Patol Oral Cir Bucal. 2012; 17(3): e495-500.

DOI: 10.20473/j.djmkg.v54.i1.p52–56

3. Zou J, Meng M, Law CS, Rao Y, Zhou X. Common dental diseases in children and malocclusion. Int J Oral Sci. 2018; 10(1): 7.
4. Pierce A, Singh S, Lee J, 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: 328.
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; 365(1–2): 159–64.
7. Caufield PW, Cutter GR, Dasanayake AP. Initial acquisition of *mutans streptococci* by infants: evidence for a discrete window of infectivity. J Dent Res. 1993; 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; 163(2): 168–72.
10. Klein MI, Flório FM, Pereira AC, Höfling JF, Gonçalves RB. Longitudinal study of transmission, diversity, and stability of *Streptococcus mutans* and *Streptococcus sobrinus* genotypes in Brazilian nursery children. J Clin Microbiol. 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: 1–6.
14. Murray PR. What is new in clinical microbiology-microbial identification by MALDI-TOF mass spectrometry: a paper from the 2011 William Beaumont Hospital Symposium on molecular pathology. J Mol Diagn. 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): 791.
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: 516410.
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 (Oxford). 2020; 2020(2): 1–21.
23. Dijkshoorn L, Ursing BM, Ursing JB. Strain, clone and species: comments on three basic concepts of bacteriology. J Med Microbiol. 2000; 49(5): 397–401.
24. Prescott LM, Harley JP, Klein DA. Microbial taxonomy. In: Microbiology. 5th ed. New York: McGraw-Hill; 2002. p. 422–49.
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, S. Kaushik K, Salehi B, Bevilacqua A, Corbo MR, Antolak H, Dybka-Stępień K, Leszczewicz M, Relison Tintino S, Alexandrino de Souza VC, Sharifi-Rad J, Melo Coutinho HD, Martins N, Rodrigues CF. Advances in chemical and biological methods to identify microorganisms—from past to present. Microorganisms. 2019; 7(5): 130.
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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