Journal of Dentistry & Oral Disorders

Open Access

Austin Publishing Group

Research Article

Overview of Method for Detecting of *Streptococcus mutans* and *Lactobacillus* in Saliva

Sounah SA[1]* and Madfa AA[1-3]

[1]Departement of Restorative and Prosthodontics, College of Dentistry, University of Sciences and Technology, Yemen

[2]Department of Conservative Dentistry, Faculty of Dentistry, Thamar University, Yemen

[3]Department of Restorative Dental Science, Collage of Dentistry, Hail University, Kingdom of Saudi Arabia

***Corresponding author:** Sabah A Sounah, Department of Restorative and Prosthodontics, College of Dentistry, University of Sciences and Technology, Sana'a, Yemen

Received: January 02, 2020; **Accepted:** February 05, 2020; **Published:** February 12, 2020

Abstract

Saliva provides some real potential in evaluating dental caries risk. Lack of saliva predisposes the development of atypical or unusual dental decay, i.e., cervical, incisal or in cusps tips, as well as radicular lesions. This is due to that saliva contains various microbes and host biological components that could be used for caries risk assessment. This review focuses on the research topics that connect on some methods used for detecting of *Streptococcus mutans* and *Lactobacillus* in Saliva.

Keywords: *Streptococcus mutans*; *Lactobacilli*; Caries risk; Evaluation methods

Introduction

More than 700 species in the oral environment have been detected which make the oral flora one of the most complex microbial communities in the human body [1,2]. Saliva acts as an oral circulating fluid for the spread of bacteria and acts as a reservoir for bacterial colonization [2].

Previous studies have shown an increase in the ratio of MS to LB in saliva, which is related to the increase in caries initiation and progression [3]. Higher levels of salivary MS were found in subjects with multiple surface repair; in addition, it was possible to continue caries activity compared to those without caries and without recovery [4]. On the other hand, data on the possible association between salivary LB levels and dental caries onset is less convincing [5]. However, once dental caries begins, the level of LB is significantly increased [6]. This may reflect the owner's sugar consumption. Therefore, the level of *Lactobacillus* in saliva may be indirectly related to the progression of caries [7]. Therefore, there are no absolute values, such as high or low values, which explain the predicted thresholds for saliva MS and LB. For example, Krasse and Fure, et al. suggest that 105 MS per ml of saliva can be considered high value for people with only a few teeth and no fillings. However, for people with many restorations, 106 may not be a very high value [8]. For LB, the count of 104 CFU / mL in saliva was considered low; while 106 CFU / mL in saliva was considered high [9]. Some studies on the high value sensitivity and probability of SM or LB in individuals with rickets are different from 44% to 71%, which is below their specificity of 56% to 100%. This may mean that the chances of individuals with no new caries or dental caries decrease the value of these species [10,11]. Several methods have been used to detect mutans streptococci and lactobacilli from oral samples, for example; direct microscopy, cultivation, enzyme test, monoclonal antibodies (mAbs), Enzyme-Linked Immunosorbent Assays (ELISA), culture methods and species-specific DNA probes (22-26). However, most of these methods are time-consuming, laborious, and relatively unspecific [24]. Four detecting and evaluating methods will be discussed in this review.

Culture-Based Methods

These methods are a common approach to identify the proportion of salivary MS and LB on selective media. A selective medium based on "the Mitis Salivarius Bacteriacin agar (MSB) was described by Gold, et al. for MS, which were found to be resistant to bacitracin [9]. However, the shortest shelf life of (MSB), the longest one, is a major limitation. This is especially inconvenient when the board is used in a clinical setting. Mitis Salivarius Bacitracin Broth (MSBB) was developed by Matsukubo et al. In 1981, it had a longer shelf life. The concentration of bacitracin and sucrose in the medium was chosen to obtain the distinct characteristics of the colonies and to make them have good adhesion to the glass [12].

In 1940, Snyder described a simple colorimetric method for indirect determination of LB counts in saliva [13]. Moreover, in 1951 a further perfection? in the cultivation of LB had introduced by Rogosa, et al. that was an improved selective medium" [14]; It allows for the growth of wide range of oral LB and remains the basis for modern diagnostic of saliva LB testing.

Dip-slide methods

Compared to traditional agar plate technology, the dip-slide test has been found to be "a reliable method for detecting MS and LB levels in saliva [15,16]. Recently, all commercial dip-slide methods have determined the proportion of MS in saliva by using bacitracin to inhibit the growth of all other S. gingivalis on MSB medium other than MS. Currently, the most commercially available kit for detecting LB saliva is the Rogosa-based medium" [17].

Molecular methods

DNA-based sensitive methods, including "checkerboard DNA-DNA hybridization, genomic fingerprinting, 16S rRNA gene cloning and sequencing, or TRFLP for identification and classification of dental caries [18,19]. In addition, Polymerase Chain Reaction (PCR) can detect large numbers of microorganisms in saliva and provide

Citation: Sounah SA and Madfa AA. Overview of Method for Detecting of *Streptococcus mutans* and *Lactobacillus* in Saliva. J Dent & Oral Disord. 2020; 6(1): 1122.

accurate measurements of known cariogenic species in saliva [20]. Real-time quantitative polymerase chain reaction (qPCR) technology is more sensitive to the counting of salivary SM than traditional culture-based methods [21]. Polymerase Chain Reaction-Denaturing Gradient Gel Electrophoresis (PCR-DGGE) analysis and species identification can be used as a community-based molecular technique and allow the study of oral bacterial community structures associated with severe caries [22]. In addition, DNA and RNA released by microorganisms are also present in saliva because Streptococcus 16S rRNA / rDNA is specified in the liquid phase of saliva [23,24]. Therefore, by directly detecting 16S rRNA / rDNA and without bacterial cell separation, there is a new possibility of detecting oral pathogens from the liquid phase of saliva.

High-throughput DNA sequencing technology and rapid expansion of bacterial genomic data are now a practical way to identify essential microbial populations in saliva [24]. This high-throughput sequencing is expected to help distinguish potential cariogenic species that may not be detected using currently available technologies such as 16S rRNA analysis [25].

In addition, another technique for the detection of cariogenic bacteria in saliva is the Monoclonal Antibody (MAb) technology. A variety of bacteria present unique surface protein and polysaccharide structures on the cell surface. With MAbs that can be generated for these structures, the corresponding bacterial species can be detected with very high specificity and sensitivity. These antibodies can be attached to various detection systems, such as fluorescent, colorimetric or aggregating reagents" [26].

Real-Time Quantitative Polymerase Chain Reaction (QPCR)

In order to quantify bacterial species in biological samples, qPCR was introduced as a new method for rapid detection and enumeration of bacterial species [27]. Specific primer sequences for a particular bacterial DNA gene can be used to quantify a target bacterium by amplifying a double-stranded DNA product. During the PCR cycle, the amplified DNA product is bound by a fluorescent DNA dye, allowing detection of the relative amount of DNA copies. In addition to overcoming the inability to detect non-viable bacteria in culture methods, high sensitivity and specificity are the main advantages of qPCR [28-30]. Therefore, concerns about the transport and storage of clinical samples will not be as critical concerns as conventional bacterial cultures [30]. Subsequently, for epidemiological studies in large populations, simple sample processing can be a benefit of qPCR, for example, samples can be stored on ice or frozen immediately and analyzed later [28,30].

As reported by Psoter et al., qPCR is more sensitive than conventional PCR and indicates that this technique is suitable for oral epidemiological studies [31]. This test can be used to reveal low numbers of target bacterial species with detection limits as low as 25-100 cells [21,32,33]. The presence of specific primers specific for a particular bacterial gene increases the specificity of qPCR. Primers also offer considerable benefits for qPCR because it accurately distinguishes between SM and *S. sobrinus* for accurate dental caries risk prediction and effective caries prevention programs [33].

In addition, real-time quantitative PCR assays also allow for the relative or absolute quantification of bacteria of interest, including species that cannot be cultured or that are difficult to culture in vitro. Another advantage over conventional PCR or endpoint PCR is that the assay does not require post-PCR manipulation of the PCR product. Therefore, this can reduce the chance of legacy contamination of the product [28,29]. qPCR requires less laboratory work and time to get results from various types of samples [29].

Many previous studies support the use of PCR and qPCR to detect and quantify MS, a cross-sectional study by Okada et al. Using PCR to study intraoral distribution and longitudinal studies of children with MS? In 2005, it was reported that PCR is suitable and effective in MS detection [32,33]. In addition, Loyola-Rodriguez, et al. proposed that PCR is comparable to conventional bacterial culture on MS agar and MSB agar as a tool for the detection and identification of MS [34]. To date, some studies have used qPCR to identify MS in oral samples, such as plaque and saliva samples. It is also used to study the correlation between MS detection and tooth status, i.e. caries status; caries, missing, filling index of primary or permanent teeth (dmf or DMF), or caries activity; innocent and carious activity [35-37].

Perspectives

We expect that ongoing innovative research and development will have a significant impact on dental caries prediction and control. We envision that in the future, treating dental caries will be an evidence-based dental practice emphasizing the triple-pronged approach of early detection, effective and sustainable treatment, and prevention. Specifically, detection of microbial and host-related caries risk factors can become routine. This approach will help clinicians to reinforce the concept of dental caries as an infectious process and will facilitate immediate, evidence-based treatment decisions.

References

1. Aas JA, Paster BJ, Stokes LN, Olsen I, Dewhirst FE. Defining the normal bacterial flora of the oral cavity. J Clin Microbiol. 2005; 43: 5721-5732.
2. Paster BJ, Boches SK, Galvin JL, Ericson RE, Lau CN, Levanos VA, et al. Bacterial diversity in human subgingival plaque. J bacteriol. 2001; 183: 3770-3783.
3. Klock B, Svanberg M, Petersson LG. Dental caries, *mutans streptococci*, *lactobacilli,* and saliva secretion rate in adults. Community dentistry and oral epidemiology. 1990; 18: 249-252.
4. Leroux BG, Martin JA, White BA. Identification of adult populations at high risk for dental caries using a computerized database and patient records: a pilot project. J Public Health Dent. 2000; 60: 82-84.
5. Tanzer JM, Livingston J, Thompson AM. The microbiology of primary dental caries in humans. Journal of dental education. 2001; 65: 1028-1037.
6. Klock B, Krasse B. Microbial and salivary conditions in 9 to 12 year old children. European Journal of Oral Sciences. 1977; 85: 56-63.
7. Mcgrady J, Butcher WG, Beighton D, Switalski LM. Specific and charge interactions mediate collagen recognition by *oral lactobacilli*. J Dent Res. 1995; 74: 649-657.
8. Krasse B, Fure S. Root surface caries: a problem for periodontally compromised patients. Periodontology 2000. 1994; 4: 139-147.
9. Gold W, Preston FB, Blechman H. The nature and amounts of bound glucose in dental plaque. J Periodontol. 1973; 44: 263-268.
10. Pienihäkkinen K, Salivary *lactobacilli* and yeasts in relation to caries increment: Annually repeated measurements versus a single determination. Acta Odontol Scand. 1988; 46: 57-62.
11. Krasse B, Biological factors as indicators of future caries. International dental

journal. 1988; 38: 219-225.

12. Matsukubo T, Ohta K, Maki Y, Takeuchi M, Takazoe I. A semi-quantitative determination of *Streptococcus mutans* using its adherent ability in a selective medium. Caries Res. 1981; 15: 40-45.

13. Snyder ML, A simple colorimetric method for the estimation of relative numbers of *lactobacilli* in the saliva. Journal of Dental Research. 1940; 19: 349-355.

14. Rogosa M, Mitchell JA, Wiseman RF. A selective medium for the isolation and enumeration of oral *lactobacilli.* J Dent Res. 1951; 30: 682-689.

15. Alaluusua S, Alaluusua S, Jouko Savolainen, Helena Tuompo, Lisa Gronroos. Slide-scoring method for estimation of *Streptococcus mutans* levels in saliva. European Journal of Oral Sciences. 1984; 92: 127-133.

16. Ensen B, Bratthall D. A new method for the estimation of mutans *streptococci* in human saliva. J Dent Res.1989; 68: 468-471.

17. Armas M, A new dip-slide method for the counting of salivary *lactobacilli*. Proc Finn Dent Soc. 1975; 71: 31-35.

18. Hommez G, Verhelst R, Claeys G, Vaneechoutte M, De Moor RJ. Investigation of the effect of the coronal restoration quality on the composition of the root canal microflora in teeth with apical periodontitis by means of T-RFLP analysis. Int Endod J. 2004; 37: 819-827.

19. Socransky S, Smith C, Martin L, Paster BJ, Dewhirst FE, Levin AE."Checkerboard" DNA-DNA hybridization. Biotechniques. 1994; 17: 788-792.

20. Akiyama T, Miyamoto H, Fukuda K, Sano N, Katagiri N, Shobuike, et al. Development of a novel PCR method to comprehensively analyze salivary bacterial flora and its application to patients with odontogenic infections. Oral Surg Oral Med Oral Pathol Oral Radiol Endod. 2010; 109: 669-676.

21. Childers NK, Hsu KL, Manmontri C, Momeni S, Mahtani H, Gary R, et al. Real-time quantitative polymerase chain reaction for enumeration of *Streptococcus mutans* from oral samples. Eur J Oral Sci. 2011; 119: 447-454.

22. Li Y, Yihong Li, Yulin Ge, Page w Caufield. Genetic profiling of the oral microbiota associated with severe early-childhood caries. Journal of clinical microbiology. 2007; 45: 81-87.

23. Gu F, Li Y, Zhou C, Wong DTW, Ho CM, Qi F, et al. Bacterial 16S rRNA/rDNA profiling in the liquid phase of human saliva. Open Dent J. 2009; 3: 80.

24. Cephas KD, Kathleen A, Scot E, Brandon S, Kelly S, Juhee, et al. Comparative analysis of salivary bacterial microbiome diversity in edentulous infants and their mothers or primary care givers using pyrosequencing. PloS one. 2011; 6: 23503.

25. Yang F, Zeng X, Ning K, Liu KL, Lo CC, Wang W, et al. Saliva microbiomes distinguish caries-active from healthy human populations. ISME J. 2012; 6: 1.

26. Jaffe C, Bennett E, Grimaldi Jr, McMahon-Pratt D. Production and characterization of species-specific monoclonal antibodies against Leishmania donovani for immunodiagnosis. J Immunol. 1984; 133: 440-447.

27. Hsu KL, Osgood RC, Cutter GR, Childers NK. Variability of two plaque sampling methods in quantitation of *Streptococcus mutans*. Caries Res. 2010; 44: 160-164.

28. Kishi M, Abe A, Kishi K, Ohara-Nemoto Y, Kimura S, Yonemitsu M. Relationship of quantitative salivary levels of *Streptococcus mutans* and S. sobrinus in mothers to caries status and colonization of mutans streptococci in plaque in their 2.5 year old children. Community dentistry and oral epidemiology. 2009; 37: 241-249.

29. Price R, Viscount HB, Stanley MC, Leung KP. Targeted profiling of *oral bacteria* in human saliva and *in vitro* biofilms with quantitative real-time PCR. Biofouling. 2007; 23: 203-213.

30. Yoshida A, Suzuki N, Nakano Y, Kawada M, Oho T, Koga T. Development of a 5 nuclease-based real-time PCR assay for quantitative detection of cariogenic dental pathogens *Streptococcus mutans* and *Streptococcus sobrinus.* J Clin Microbiol. 2003; 41: 4438-4441.

31. Organization WH, Oral health surveys basic methods. 4th edn Geneva World Health Organization.1997; Baseline Characteristics of PAES Dental Program Participants. (377).

32. Hamada S, Slade HD. Biology, immunology, cariogenicity of *Streptococcus mutans.* Microbiological reviews. 1980; 44: 331.

33. Hata S, Hata H, Miyasawa-Hori H, Kudo A, Mayanagi H. Quantitative detection of *Streptococcus mutans* in the dental plaque of Japanese preschool children by real-time PCR. Letters in applied microbiology. 2006; 42: 127-131.

34. Loyola-Rodriguez JP. Distribution of *Streptococcus mutans* and *Streptococcus sobrinus* in saliva of Mexican preschool caries-free and caries-active children by microbial and molecular (PCR) assays. Lett Appl Microbiol. 2007; 32: 121-126.

35. Nurelhuda NM. Caries experience and quantification of *Streptococcus mutans* and *Streptococcus sobrinus* in saliva of Sudanese schoolchildren. Caries research. 2010; 44: 402-407.

36. Ge Y, Caufield PW, Fisch GS, Lia Y, et al. *Streptococcus mutans* and *Streptococcus sanguinis* colonization correlated with caries experience in children. Caries Res. 2008; 42: 444-448.

37. Teanpaisan R, van Loveren C, de Soet J, de Graaff J, ten Cate JM. Longitudinal study of the presence of mutans *streptococci* and *lactobacilli* in relation to dental caries development in 3–24 month old Thai children. J Dent Res. 2007; 57: 445-451.