

Recent Trends in Microbiological Analysis of Dental Caries

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Abstract

Dental caries is an interplay between different microbial interactions. Understanding the dynamics in a biofilm involves studies on genomics, proteomics, metabolomics, transcriptome, and metatranscriptome amongst other investigations. This review article covers various techniques and tools that are used for analyzing biofilm samples collected from an individual to isolate prominent pathogenic microorganisms.

Keywords: dental caries, microflora, genomics, proteomics, metabolomics, transcriptome, metatranscriptome.

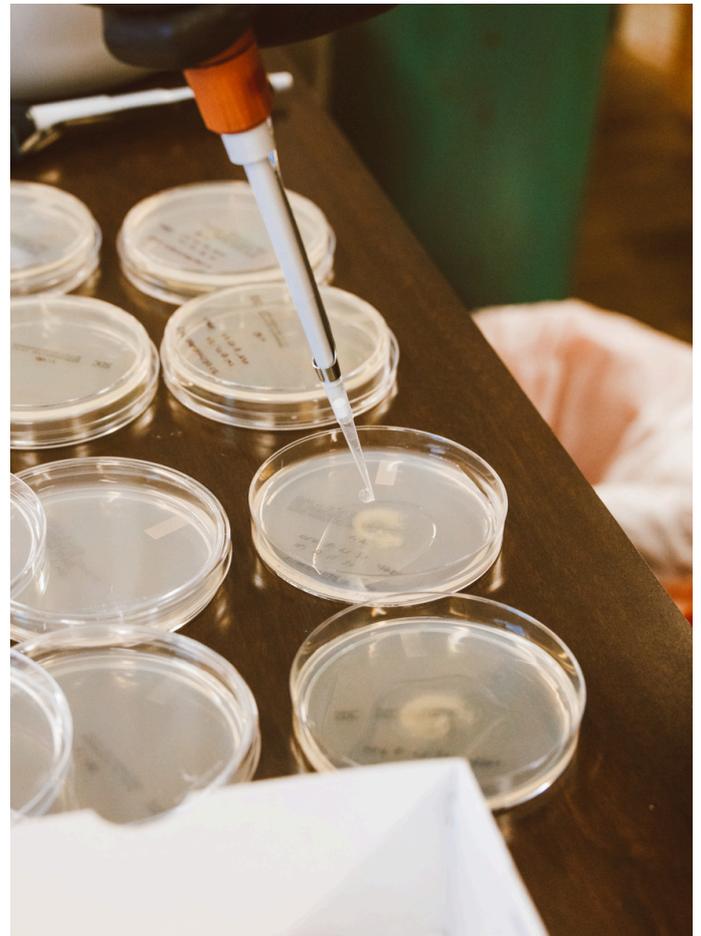
Introduction

Dental caries is the result of imbalances in biofilm metabolism. Research involving microbial communities can be categorized into: (a) Composition of microbiota and (b) Micro-environment analysis like- nutritional requirements, pH, reduction and oxidation potential, and physiology of microbial flora. Microbiological communities associated with teeth are routinely analyzed using standard culture techniques. In recent times, 16S rRNA gene cloning as well as sequencing have been used for the characterization of microbiological composition of oral biofilm, however, there are limitations associated with this method. Next-generation high throughput sequencing techniques have demonstrated a greater ability to reveal the composition as well as the metabolism of biofilm by analyzing metagenomics as well as metatranscriptomics.

The dynamics of dental caries are the total metabolic activities of dental biofilm which can be done by metabolomic analysis. Since dental carious activity is of a localized nature, sample collection from biofilm must be done from precise locations.¹

Links between biological hierarchy begin at the genome to transcriptome or proteome till metabolome. 'Genome' refers genetic makeup of any organism, 'transcriptome' refers to genes that are transcribed to mRNAs, and 'proteome' refers to the study of protein translation and is representative of the metabolism of an organism. 'Metabolome' refers to the final metabolic end-products (acidic or basic products) within a biological sample.

'Metatranscriptome' is representative of genes that are expressed in a biofilm whereas, metaproteome refers to microbiological proteins that are produced within a biofilm.



Tools for molecular microbiological analysis of oral flora

The 16S rRNA gene has been used for studying the taxonomic classification of bacterial biofilm communities associated with dental structures. However, there are overlapped results of 16S rRNA studies, due to which various other molecular techniques have been proposed for identifying microorganisms in oral bio-film.

a) Denaturing Gradient Gel Electrophoresis: This electrophoretic technique utilizes differences between partially melted Polymerase Chain Reaction (PCR)-amplified double-stranded 16S rRNA genes or gene fragments' mobility.² Different 16S rRNA gene sequences exhibit differences in melting behaviors and, hence, take up at different locations in polyacrylamide gel. Following visualization, conclusions are drawn based on differences in the presence or absence of bands. This technique has several advantages- i) It is comparatively cheaper, ii) Can be easily performed, and iii) Is reliable.

Disadvantages of this technique are- i) Its non-quantitative nature, ii) In complex plaque samples, species-specific bands cannot be determined reliably, and iii) Comparisons between different samples with multiple species on different gels are difficult. However, this is an excellent tool for initially analyzing microbial diversity as well as complexities in saliva and plaque.

b) Polymerase Chain Reaction-Based Techniques: Various advantages of these techniques are- i) These are relatively cheaper compared to other methods, ii) Can be performed relatively easily, and iii) Are reproducible. Real-time quantitative PCR (qPCR) is most commonly used due to its specificity and quantifiability.³

This method uses primers/internal probes that have been specifically designed for the 16S rRNA gene or any other gene of a particularly targeted microbial species. However, its distinct disadvantage is that no other species present in the biofilm can be evaluated.

To overcome this, multiplex qPCR techniques have been designed for simultaneously detecting three or more pathogenic organisms in a single reaction.⁴ Studies conducted by Choi et al. (2009) and Palmer et al. (2010) using qPCR have demonstrated the coexistence of *S. mutans* with *S. sobrinus* in early childhood caries.^{5,6}

c) 16S rRNA Gene Microarrays: Taxonomic microarrays are high-throughput tools for characterizing microbial population communities.⁷ In this technique, probes are used over a solid substrate like- glass. Each of the probes comprises of 16S rRNA gene sequence which is complimentary to particular species. This technique works on 16S rRNA gene amplification by use of universal primers that are labeled as well as hybridized on substrate array. In this manner, this microarray technique provides bacterial fingerprints within microbial communities located with plaque samples.⁸ Human Oral Microbe Identification microarray (HOMIM) is a taxonomy-based microarray and has been developed by the Forsyth Institute, particularly for oral microflora.⁹

This microarray helps in the detection of up to 272 bacterial species from plaque samples, most of which are presently non-cultivable. Though this technique is easy to perform, its major drawback is that it is expensive.

d) Checkerboard DNA-DNA Hybridization Technique: This 40-species-specific technique was introduced by Socransky et al. in 2004.¹⁰ It utilizes the usage of whole genomic DNA probes. Its advantages are- a) It is sensitive, b) Semi-quantitative, c) It is inexpensive when compared with other techniques, d) It is flexible in terms of selecting targets, and e) Technique variations can be easily done.¹¹⁻¹³ However, the biggest disadvantage of this technique is that due to the use of whole genomic probes, a significant amount of cross-hybridization reactions leading to incorrect quantification of species can occur.

e)Next-Generation Sequencing: 454 pyrosequencing is a Next-generation sequencing technique that differs from the Sanger method as it does not involve cloning, however, it helps in retrieving millions of sequences of partial 16S rRNA in a single sequencing step.¹⁴ This technique is relatively cheap and fast when compared to 16S rRNA gene cloning, however, they are much more expensive and the derived gene sequences are mostly of short size that do not allow one to identify species.

Metagenomic and Metatranscriptomic Approach to Identify Oral Microbial Population

Metagenomics is a tool used for accessing genetic information of entire microbial communities by isolating entire DNA content. Therefore, there is no requirement for traditional culture methods and/or PCR techniques.¹⁵ One advantage of metagenomics is the sequencing of inserted DNA can be performed afterward, thereby, providing accessibility to the entire genetic constitution of bacteria.¹⁶ Metagenomic vectors containing large-sized DNA inserts such as fosmids or artificial chromosomes carry the distinct advantage of cloning of entire operon which increases the chances of detection.

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