

## Application of 16s RRNA in Identifying Oral Microflora-A Review of Literature

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**Abstract:** The microflora within the oral biofilm is quite complex. Determination of the microbial populations responsible for oral infections is even more difficult. Dental plaque is said to be a complex microbial biofilm consisting of different bacterial species in the human oral cavity. But unfortunately no single species has been identified as a primary pathogen. Microbiological diagnosis is important in the rationale for treatment planning in patients with oral diseases. The methods commonly used for the identification of periodontopathogens of dental plaque include direct microscopy, immunological techniques, Deoxyribonucleic acid (DNA) probe technology, DNA probes have also been offered as a commercial service and Polymerase Chain Reaction (PCR)-based methods. Culturing technique or DNA approaches when used for studying bacterial etiology of periodontitis, will target only the known species. PCR techniques records only the presence of the bacteria in the sample but not qualitative. PCR amplification of the bacterial 16S ribosomal ribonucleic acid (rRNA) gene using universal bacterial primers would help to identify the presence of bacterial DNA in the oral cavity of the patients. Advances in molecular biology have permitted us to perform the study of microbial communities and obtain improved information of bacterial diversity from the oral cavity. This technology has evolved beyond the research and developed into clinical applications. Further research and studies should be performed using 16S rRNA gene analyses to examine the bacterial profile of oral micro flora responsible for oral diseases.

**Key words:** Oral Microflora • Polymerase Chain Reaction (PCR) • 16S rRNA

### INTRODUCTION

Molecular biological methods using 16S ribosomal ribonucleic acid (rRNA) gene sequences are commonly used for identifying and classifying bacteria. rRNA sequences are mainly used in ranking biological phylogenetic nomenclature including that of microorganisms [1]. 16S rRNA is a type of RNA that plays a major role in synthesis of protein [2]. As the mechanism of protein synthesis doesn't vary much from one organism to another, the RNA that assists the protein production also doesn't vary much. The variations there are occur in predictable locations. It is observed that the nucleotide sequences of some portions of the 16S

ribosomal deoxyribonucleic acid (rDNA) are highly sealed. However, other regions of this gene are hypervariable [3]. 16S rRNA identifies organisms by comparing certain locations on a 16S rRNA molecule with a database of previously identified organisms whose 16S rRNA mark is known. 16S rRNA is located in the major groove in an upper portion of the rRNA. Since the 16S rRNA contains very well-conserved regions among biological species, which makes the comparison of 16S rRNA sequences possible in studies of molecular evolution. 16S rRNA sequences also enable the identification microorganisms because the 16S rRNA contains variable sequences that change according to different species [4].

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More than one 16S rRNA sequences may exist in a single bacterium. It is considered to be fast and better alternative to other methods of bacterial identification [5]. Along with its use identifying the bacteria, 16S sequencing can also be used to re-categorize the bacteria into new species [6].

**Oral Microflora:** It is known that the microflora within the oral biofilm is quite complex. In addition to this determination of the microbial populations responsible for oral infections is even more difficult. Bacteria present in the oral cavity are both, gram positive and gram negative. In diseases, like bacterial endocarditis [7], pneumonia [8, 9], osteomyelitis [10], low birth weight [11] and cardiovascular diseases [12, 13] definite oral bacterial type are associated. Dental plaque is said to be a complex microbial biofilm with numerous bacterial species in the human oral cavity. Specific organisms have been associated with oral diseases. Dental caries is observed to be associated with *Streptococcus mutans* and *Lactobacilli* [4, 14]. In subgingival plaque, *Porphyromonas gingivalis* (*P. gingivalis*), *Tannerella forsythia* (*T. forsythia*) and *Aggregatibacter actinomycetemcomitans* (*A. actinomycetemcomitans*) have been strongly associated with periodontal disease [15]. Evidence supports that advancement and severity of periodontitis especially among older population is influenced by the anaerobic gram-negative bacterium and *P. gingivalis* [16, 17]. *A. actinomycetemcomitans*, gram-negative bacteria is usually associated with the aggressive periodontitis [18, 19]. These bacteria are usually seen in the periodontal pockets and attacks the periodontal tissues [20-22]. From the available literature it is understood that the bacterial plaque is one of the etiologic agent in chronic periodontitis [23]. Knowledge of the ecological relationships among bacterial species can direct investigations on the critical bacterial interactions.

But unfortunately no single species has been identified as a primary pathogen. Microbiological diagnosis is important in the rationale for treatment planning in patients with periodontitis. Few of the epidemiological studies conducted to identify periodontal bacteria had used RNA gene probes and DNA fingerprinting techniques [1]. It was reported that the molecular biological techniques are almost equivalent, if not superior, to other bacterial cultural methods [24]. Direct microscopy, immunological techniques, DNA probes and PCR-based methods are mainly used for recognizing the pathogens present in dental plaque.

Culturing technique or DNA approaches when used for studying oral bacterial profiles, will target only the known species. PCR techniques records only the presence of the bacteria in the sample but not qualitative [17]. However, the PCR based method have been reported to be simple and also provide rapid results [25]. The difference in the two methods; one based on culture and the other on molecular biology were notified by Pratten *et al.* [26]. 16S rRNA genes have been used to investigate the composition samples from the human oral cavity. By designing PCR primers complementary to conserved regions of the rRNA gene and subsequent cloning and sequencing of the PCR products, the 16S rRNA gene sequences are compared with other known 16S rRNA sequences to identify the bacteria species. Fixed 16S rDNA sequences provide PCR primers which facilitate the collection of large amounts of DNA for sequence analysis of species-specific variable regions [3]. Around 700 orally derived 16S sequences are deposited in GenBank. Primers specific for the fimbrial gene of *P. gingivalis* is available and a PCR assay is usually done to detect *P. gingivalis* cells in pure culture [27]. PCR has been employed to detect the leukotoxin gene of *A. actinomycetemcomitans* [28].

Oral *treponemes* are frequently secluded from the periodontal pockets of humans [29]. These gram-negative, anaerobic, motile, helical rods have also been associated with periodontal inflammation [7]. Literature shows that 16S rRNA gene sequencing is used to identify these organisms from oral cavity.

Previous studies using 16S rRNA found bacterial species in the 'red complex' to be strongly linked to periodontal disease [30] and this complex consisted of *P. gingivalis*, *B. forsythus* and *T. denticola*. and also reported increased bacterial variety in plaque among individuals diagnosed with poor oral condition [31]. Olson *et al.* [14] reported significant variation in the bacterial profiles between subjects with high and low grade oral infections. Inflammation of peri-implant hard and soft tissues caused by bacterial biofilms can result in implant failure. Studies have been performed to analyze the peri-implant and periodontal microflora using gene analysis in patients with clinical signs of gingivitis or mucositis [32, 33]. This study was able to demonstrate the bacterial diversity of the sulcular flora at inflamed tissues of implants and teeth, the high bacterial diversity of natural teeth compared with implants and different bacterial compositions at implant and teeth sites in the same individual.

Preza *et al.* [34] in his study analyzed the diversity and site of microflora among elderly by use of a 16S rRNA gene-based microarray reported that the bacterial flora appears site-specific for different oral niches and subject-specific bacterial profiles were not evident. 16S rRNA technique has been used to identify the various bacterial compositions and their action on implant surfaces placed in the mouth [35]. Many *in vivo* and *in vitro* studies [36] suggest that the composition of the supragingival bacterial community around healthy teeth differs from that around implants. Studies in peri-implantitis patients have used the 16SrRNA method to assess the presence and the variants of *Archaea* in the subgingival biofilm [33].

**Limitation of 16S RRNA Technique:** Although 16S rRNA sequencing is being used widely for bacterial identification, there are no specific guidelines for using the technique and interpreting the sequence data. As no threshold values are available, one of the major drawback in the interpretation of sequence data is concerned with the assigning the bacterial species in response to the likeness of search results. It is been reported that this technique may not be useful when two different bacterial species share almost the same 16S rRNA sequence [37]. The use of various software packages is also restricted due to the options of bacterial types available within the database.

## DISCUSSION AND CONCLUSION

Advances in molecular biology have permitted us to perform the study of microbial communities and obtain improved information of bacterial diversity from the oral cavity. This technology has evolved beyond the research and developed into clinical applications. Microbiological diagnosis is essential in the rationale for treatment planning for periodontitis. PCR amplification of the bacterial 16S ribosomal rRNA gene using bacterial primers would help to determine the bacterial DNA in the oral cavity. This is followed by cloning and sequencing of the PCR amplicons. Depending on the data of nucleic acids obtained from PCR amplification of the bacterial 16S rRNA, the bacterial variants would be investigated. The DNA sequences are analyzed by Genetyx-Win software. The partial 16S rRNA gene sequences are used to determined identity or nearest phylogenetic position. For recognition of closest species, the consent sequences

would be match up with 16S rRNA gene sequence in GenBank databases using the National Center for Biotechnology Information. Furthermore, the relation between the obtained bacteria information and clinical parameters could be analyzed.

The 16S rRNA can thus be used for phylogenetic analysis as well as for the species-specific detection of bacteria. On review of published literature it was observed that this technique being independent of culture has been able to expose greater diversity than what was perceptible with culturing. To have a better understanding of oral biofilm communities and oral disease processes, it is important to identify the microbiota involved. These data obtained from such analysis reinforce the association of microbes with oral diseases, as suggested for chronic periodontitis aggressive periodontitis, infected root canals and peri-implantitis. Further research and studies can be performed using 16S rRNA gene analyses to examine the bacterial profile of oral microflora responsible for oral diseases.

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