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Review

Anaerobic culture to detect periodontal and caries pathogens ☆

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ABSTRACT

Background: Anaerobic culture has been critical in our understanding of the oral microbiotas.
Highlight: Studies in advanced periodontitis in the 1970s revealed microbial complexes that associated with different clinical presentations. Taxonomy studies identified species newly-observed in periodontitis as *Aggregatibacter* (*Actinobacillus*) *actinomycetemcomitans*, *Campylobacter* (*Wolinella*) *rectus* and other *Campylobacter* species, and *Tannerella* (*Bacteroides*) *forsythia.* Anaerobic culture of initial periodontitis showed overlap in the microbiota with gingivitis, and added *Selenomonas noxia* and *Filifactor alocis* as putative periodontal pathogens. *Porphyromonas gingivalis* and *T. forsythia* were found to be associated with initial periodontitis in adults. The dominant microbiota of dental caries differs from that of periodontitis. The major cariogenic species are acidogenic and acid tolerant species particularly *Streptococcus mutans*, and *Lactobacillus* and *Bifidobacterium* species. Anaerobic culture of severe early childhood caries revealed a widely diverse microbiota, comparable to that observed using cloning and sequencing. The PCR-based cloning approach, however, underestimated Actinobacteria compared with culture. Only a subset of the caries-associated microbiota was acid tolerant, with different segments of the microbiota cultured on blood agar compared to a low pH acid agar. While the major caries-associated species was *S. mutans*, a new species, *Scardovia wiggsiae*, was significantly associated with early childhood caries. Higher counts of *S. wiggsiae* were also observed in initial white spot carious lesions in adolescents.
Conclusion: In periodontitis and dental caries, anaerobic culture studies of advanced disease provided a comprehensive analysis of the microbiota of these infections. Anaerobic culture highlighted the limitation of PCR with standard primers that underestimate detection of Actinobacteria.

Contents

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1. Introduction anaerobic culture of plaque biofilm samples

Dental plaque samples were first examined microscopically by van Leeuwenhoek, then by culture as methods and bacterial growth media were developed. The cultivability of dental plaque bacteria improved over time with the use of complex, blood-containing media, and with application of anaerobic methods to process and culture bacteria. Studies in the 1960s were critical to demonstrate the value of anaerobic methods, which were successfully applied to both periodontal and caries samples in the 1970s. Major investigators in anaerobic culture of plaque samples in the 1970s and 1980s were those at the Forsyth institute in the Socransky and co-workers laboratories, at the laboratories of Holdeman and Moore (Virginia Polytechnic Institute, USA), Walter Loesche (Michigan, USA), William Wade (UK), and Etsuro Hoshino (Niigata, Japan) among others.

Anaerobic cultural methods continue to expand our understanding of the oral microbiota of periodontitis and dental caries although approaches for strain identification have changed from biochemical tests to 16S rRNA sequence based identifications. This review will focus on selected anaerobic culture studies that have provided the basis of our understanding of the oral microbiota. Non-cultural molecular analyses of plaque samples, mainly based on analysis of the 16S rRNA gene, have highlighted both strengths and limitations of culture to describe the complete oral microbiota. Nevertheless, when bacteria are detected by molecular methods, the focus then becomes to devise methods to cultivate them [1] which frequently involves use of anaerobic methods.

2. Periodontitis

2.1. Advanced periodontitis

Clinically, periodontitis affects the tooth supporting structures and if left unchecked leads to tooth loosening and loss. Periodontitis is recognized by increased depth in the gingival sulcus leading to periodontal pockets, and by loss of periodontal attachment and the surrounding alveolar bone. Periodontitis is frequently associated with gingival inflammation, gingivitis, which may include spontaneous bleeding of gingival tissues.

Based on the observation that periodontal loss increased with age, in the 1960s it had been assumed that this was a slowly progressing chronic infection that was difficult to arrest. One form of periodontitis variously recognized as periodontosis, juvenile periodontitis, and currently aggressive periodontitis, however, progressed very rapidly. Permanent teeth, principally central incisors and 1st permanent molars that erupted in childhood could be lost in adolescence. Anaerobic culture of bacteria from periodontal pockets associated with aggressive periodontitis in adolescents in Socransky's laboratory in the 1970s revealed a microbiota containing bacteria not previously recognized from periodontal samples of other patients [2,3]. Further many isolates were difficult to maintain in culture. This form of rapidly destructive periodontitis in adolescents is now recognized as characterized by a microbiota frequently dominated by *Aggregatibacter actinomycetemcomitans*.

Advanced adult periodontitis sites were also found to have progressing disease as demonstrated by increasing alveolar bone loss observed from sequential radiographs. This contrasted from periodontal pockets that were not progressing and represented disease remission or repair. A second study of advanced periodontitis was undertaken in the 1970s in Socransky's laboratory. In this study adult periodontitis was defined as advanced disease with sites that either had a record of increasing alveolar bone loss on radiographs within the previous two years or young adults with very advanced periodontitis. Healthy, control sites in the same subjects (when present) had no attachment loss, and minimal if any gingival inflammation [4].

Subgingival samples were collected and processed using anaerobic methods, with prolonged incubation of samples since pilot sampling indicated that only a portion of the microbiota formed colonies with incubation times less than 10–14 days. Microbiological methods included use of prereduced anaerobically sterilized (PRAS) solutions in Hungate or roll tubes for sample processing and biochemical tests. Strain characterization relied on detection of acid end products of metabolism to define genera, and an array of fermentation and other tests for isolate speciation.

The major finding of clinical importance was that the microbiotas differed between subjects, and from that of the adolescent aggressive periodontitis. Furthermore, microbiotas of adult periodontitis could be grouped into distinct disease types. Hindsight allows renaming these disease groups from “young adult” to generalized aggressive periodontitis, “minimal inflammation” to post-antibiotics refractory periodontitis, and “moderate inflammation” to chronic periodontitis (Fig. 1). Differentiation of clinical and microbial subgroups within periodontitis led to a profound change in the description of periodontitis with the recognition of distinct and different “periodontal diseases”.

Healthy sulci microbes were dominated by gram positive species, mainly *Streptococcus* and *Actinomyces* species, although over 10% could not be identified. Gram negative anaerobic rods dominated the advanced periodontitis microbiotas (Fig. 1). The “young adult”, aggressive periodontitis group was characterized with a species tentatively identified as *A. actinomycetemcomitans* with *Bacteroides asaccharolyticus* (now *Porphyromonas gingivalis*). The “minimal inflammation”, refractory periodontitis group harbored had higher proportions of *Bacteroides melaninogenicus* subsp. *intermedius* (now *Prevotella intermedia*), *Eikenella corrodens* and unidentified isolates grouped as “fusiform *Bacteroides*” (now *Tannerella forsythia*). The third “moderate inflammation”, chronic periodontitis group was dominated by a complex of *P. gingivalis*, *F. nucleatum* and “fusiform *Bacteroides*”. As in the previous Newman studies of periodontosis [3], many isolates did not fit species recognized from the earlier studies from periodontal pockets [5]. These unidentified isolates were grouped as *A. actinomycetemcomitans*-like, “vibrio-corroders” and “fusiform *Bacteroides*”.

2.2. Species new to periodontal pockets

Characterization of the *A. actinomycetemcomitans*-like isolates included determining guanine plus cytosine (G+C) content of cells and DNA–DNA hybridizations in addition to biochemical tests. Findings confirmed that some isolates were indeed *A. actinomycetemcomitans* [6]. The *A. actinomycetemcomitans* isolates included strain Y4 from adolescent aggressive periodontitis and this isolate has become a widely used reference strain. Isolates from advancing