**Oral Microbiome: A Comprehensive Review of its Impact on Oral and Systemic Health**

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***Oral Microbiome and Techniques for Analysis***

Researchers employ several techniques to analyse the oral microbiome, with culture-dependent and culture-independent methods offering distinct advantages and limitations. Culture-dependent methods have been conventionally used. The advantage of using these methods is that microorganisms may be identified and described according to their shape, growth patterns, and biochemical characteristics. In the past, these techniques have offered insightful knowledge on the physiology and behaviour of particular cultivable species. They can be particularly useful in identifying potential pathogens and studying their responses to different conditions. However, culture-dependent techniques have inherent biases, favouring the growth of easily culturable species while missing a substantial portion of the microbial community that is difficult to grow in the lab (1). It is difficult to cultivate many of the fastidious or anaerobic microorganisms found in the oral microbiome (2). Furthermore, the microbial makeup may change during the cultivation process, producing an imprecise depiction of the actual oral microbiome.

On the other hand, next-generation sequencing (NGS) methods, which are microbial analysis techniques independent of culture, have led to a notable advancement in the research of the oral microbiome. These advanced tools have ushered in new possibilities for conducting extensive metagenomic studies across diverse populations (3). This has led to the detailed characterization of the microbiome's structural composition and, in certain cases, the elucidation of its functional roles and implications for human health (4). As the costs associated with these techniques continue to decrease, both in terms of financial investment and computational requirements, researchers are empowered to harness their potential. This trend aligns with the ongoing expansion of microbial genetic sequence databases, further enhancing the capabilities of culture-independent NGS methods. Consequently, these techniques not only expedite analyses but also significantly amplify our understanding of previously challenging-to-study, unculturable, and rare microbiota (4). Culture-independent methods, particularly those involving DNA or RNA analysis extracted directly from samples, represent a pivotal transformation in comprehending the oral microbiome. Techniques such as 16S rRNA gene sequencing and shotgun metagenomics are commonly used tools for identification and offer distinct advantages by providing an impartial perspective of the oral microbiome. This comprehensive approach enables the identification of elusive microbial species that could potentially hold significant roles in oral health and disease dynamics (1).

Nonetheless, culture-independent methods do have their limitations. Although they excel at indicating microbial species, they cannot often furnish insights into the specific physiological characteristics and functional behaviours of individual organisms. Additionally, their increased sensitivity may result in the detection of DNA from non-viable or deceased cells, potentially inflating assessments of actual microbial activity. The emergence of NGS, a prominent subset of culture-independent methods, has revolutionised the landscape of oral microbiome analysis. NGS techniques, exemplified by Illumina sequencing, stand out for their capacity to analyse a substantial number of samples in a high-throughput manner (5,6). The data generated through NGS is both expansive and rapid, making it a cost-effective tool. NGS explores the functional potential of the oral microbiome in addition to capturing its taxonomic diversity. Consequently, NGS technologies have greatly expanded our knowledge of previously difficult-to-studied microbial species, leading to a more thorough understanding of the oral environment.

Nevertheless, RT-PCR is still the recommended technique for identifying oral cavity microorganisms, even with the benefits of NGS. RT-PCR is still the preferred technique for identifying microorganisms in the oral cavity, even with the advantages that NGS offers. This decision is explained by its inexpensiveness, speed, specificity, and low sample requirements in comparison to NGS, which requires more time and resources. (7). Although RT-PCR is frequently used by academics for regular analysis, NGS is usually chosen for in-depth studies of microbial communities. The remarkable sensitivity of RT-PCR enables the prompt and accurate identification of oral pathogens linked to a range of oral disorders, such as dental caries, periodontal diseases, and oral candidiasis. Its heightened sensitivity enhances precision in quantification and identification, making it invaluable for accurate microbial analysis (8). This method provides cost-saving benefits by reducing the need for extensive post-PCR detection procedures. Additionally, they enable the simultaneous identification of multiple pathogens within a single sample, enhancing efficiency in diagnostic and research applications (9). Other advantages include ease of quantification, reproducibility, quality control, and reduced risk of contamination (10).

The utilisation of stimulated saliva collection has emerged as a favoured approach for investigating the intricacies of the oral microbiome. By introducing specific stimuli like chewing gum or citric acid, stimulated saliva is acquired under controlled and standardised conditions, setting it apart from unstimulated saliva. This methodical stimulation ensures a consistent and reproducible sampling process, effectively minimising variations arising from factors such as circadian rhythms. Furthermore, the elevated microbial load and diversity found in stimulated saliva yield a more comprehensive representation of the oral microbiome, thereby facilitating a more insightful analysis of its composition and functions (11,12). Moreover, saliva's significance extends beyond its role in the oral microbiome. It envelops the entire oral cavity, consistently introducing its bacterial constituents into other oral samples. In essence, saliva serves as an illustrative example of a microbiome characterised by high alpha diversity but low beta diversity (13). This unique combination of attributes renders stimulated saliva collection a potent tool for comprehending the complex interaction between microbial communities within the oral ecosystem, paving the way for enhanced insights into its implications for human health.

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