Microbiome Composition of the Sulcus Gingiva in Healthy Periodontium: Evidence from 16S rRNA Gene Sequencing Studies

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Abstract

Background: : The human oral cavity harbors one of the most diverse microbial communities in the body, with the gingival sulcus representing a specialized ecological niche adjacent to the gingival margin and tooth surface. In health, this sulcus supports a complex but balanced microbiota that contributes to immune homeostasis and prevents pathogen overgrowth. Traditional culture-based methods were limited in detecting the full breadth of microbial diversity, often overlooking fastidious and uncultivable taxa. The advent of 16S rRNA gene sequencing has revolutionized oral microbiome research by enabling high-resolution, culture-independent profiling of bacterial communities. This approach has improved our understanding of microbial ecology in the periodontal sulcus, particularly in distinguishing health-associated taxa from those related to periodontal disease.

Aim: : This article aims to synthesize current evidence from 16S rRNA gene sequencing studies on the composition of the gingival sulcus microbiome in clinically healthy individuals, highlighting dominant bacterial taxa and community structure associated with health.

Methods: A review of peer-reviewed studies was conducted, focusing on 16S rRNA gene sequencing analyses of subgingival plaque or sulcus samples from periodontally healthy subjects. Eligible studies employed next-generation sequencing platforms (e.g., Illumina MiSeq, 454-pyrosequencing) and standard bioinfo**rmatics** pipelines for taxonomic classification against reference databases such as HOMD (Human Oral Microbiome Database) and Green genes. Key metrics included alpha diversity (richness and evenness), beta diversity (community differences), and relative abundances of taxa at phylum, genus, and species levels.

Results: Across multiple 16S rRNA gene sequencing studies, the gingival sulcus microbiome in healthy individuals is characterized by high diversity and the predominance of several bacterial phyla:

- Firmicutes, Actinobacteria, Proteobacteria, Bacteroidetes, Fusobacteria, and Spirochaetes are consistently detected as dominant phyla in healthy sulcus communities.
- At the genus level, Streptococcus, Actinomyces, Corynebacterium, Veillonella, Neisseria, Haemophilus, and Capnocytophaga are frequently abundant in health, reflecting a community engaged in saccharolytic and homeostatic functions.
- Health-associated taxa tend to show higher relative abundance and prevalence compared to disease-associated pathogens such as Porphyromonas gingivalis, Tannerella forsythia, and Treponema denticola, which are rare or absent in healthy sulci.
- Alpha diversity measures in healthy subjects are generally higher than in individuals with periodontitis, suggesting that microbial richness and evenness may be protective against dysbiosis.

Beta diversity analyses demonstrate distinct clustering of microbiome profiles between healthy and diseased groups, indicating that community structure shifts markedly with inflammation and periodontal breakdown.

Conclusions: Evidence from 16S rRNA gene sequencing studies has significantly advanced our understanding of the gingival sulcus microbiome in health. A diverse and balanced community dominated by specific Firmicutes, Actinobacteria, and Proteobacteria taxa appears characteristic of periodontal health. These communities differ markedly from those found in periodontal disease, supporting the concept that microbial community composition and diversity contribute to periodontal homeostasis. Future research integrating metagenomics, metatranscriptomics, and host immune profiling will further clarify microbial functions critical for maintaining gingival health. Continued longitudinal studies are needed to define health-associated microbiome signatures that may inform preventive and therapeutic strategies. (**TCM-GMJ August 2026; 11 (3): P22-P24)**

Keywords: Microbiome Composition; Sulcus Gingiva; Healthy Periodontium; 16S rRNA Gene Sequencing

Introduction

The human gingival sulcus represents a critical interface between oral microbial communities and host immune defenses. Recent studies using 16S rRNA gene sequencing have enabled comprehensive characterization of the sulcus microbiome in health. Firmicutes, Actinobacteria, Proteobacteria, Bacteroidetes, Fusobacteria, and Spirochaetes dominate healthy sulcus microbiomes, with genera such as Streptococcus, Actinomyces, Corynebacterium, Neisseria, and Veillonella being prevalent. High alpha diversity and stable community structure distinguish health from periodontal disease. Understanding these patterns provides a foundation for mi-

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crobiome-based diagnostics and preventive strategies in oral health (4).

The oral cavity harbors one of the most complex microbial ecosystems in the human body, encompassing over 700 bacterial species. Among oral niches, the gingival sulcus the shallow groove between the tooth surface and gingival tissue is of particular interest due to its role in maintaining periodontal health. In health, the sulcus microbiome contributes to immune homeostasis, prevents colonization by pathogens, and participates in nutrient metabolism (1,2).

Traditional culture-based studies underestimated microbial diversity due to limitations in cultivating fastidious or anaerobic species. The introduction of 16S rRNA gene sequencing has transformed oral microbiology by enabling culture-independent, high-throughput profiling of microbial communities, revealing previously undetected taxa and complex inter-species relationships (6,7). This method has become the standard for defining microbial signatures associated with health and disease.

Understanding the baseline composition of the gingival sulcus microbiome in health is essential for identifying shifts associated with periodontal disease, evaluating dysbiosis, and informing targeted preventive interventions (7,10)

This review synthesizes current evidence from 16S rRNA gene sequencing studies describing the microbiome composition, diversity, and community structure of the healthy gingival sulcus, highlighting dominant taxa, diversity metrics, and health-associated patterns.

Methods

Study Selection

A literature search was conducted in PubMed, Scopus, and Web of Science for studies published between 2005 and 2025. Eligible studies profiled subgingival or sulcus microbiota in clinically healthy individuals using 16S rRNA gene sequencing. Inclusion criteria included: human subjects with no clinical signs of periodontitis or gingivitis; use of high-throughput sequencing platforms; reporting of alpha and beta diversity, and taxonomic composition. Exclusion criteria were studies solely on disease states, non-human studies, or in vitro models (1,2,4).

Sample Collection

Subgingival plaque or sulcus samples were collected using sterile curettes or paper points, minimizing contamination from saliva or supragingival plaque. Sampling aimed to capture biofilm communities directly from the sulcus (3).

DNA Extraction and Sequencing

Genomic DNA was extracted using kits optimized for oral biofilms. The 16S rRNA gene was amplified targeting hypervariable regions V1–V3, V3–V4, or V4. Sequencing platforms included Illumina MiSeq or 454 pyrosequencing, generating hundreds of thousands of reads per sample (2,3).

Bioinformatic Analysis

Sequences underwent quality filtering and chimera removal. OTUs or ASVs were identified using QIIME2, DADA2, or mothur and classified against HOMD, Greengenes, or SILVA databases. Alpha diversity (Shannon, Simpson, Chao1) and beta diversity (UniFrac distances, PCoA clustering) were calculated (4,6).

Statistical Analysis

Differences in taxonomic abundance were tested using non-parametric methods (Kruskal-Wallis), and co-occurrence analyses examined genus-level correlations. Multivariate analyses evaluated clustering by health status (3).

Results and discussion

Microbial Diversity

Healthy sulcus microbiomes display **high alpha diversity**, reflecting rich and evenly distributed microbial communities. Beta diversity analyses consistently show clustering of healthy microbiomes, distinct from periodontitis-associated communities.

Dominant Taxa

Phylum Level

Firmicutes, Actinobacteria, Proteobacteria, Bacteroidetes, Fusobacteria, and Spirochaetes dominate the healthy sulcus (9).

Genus Level

Key genera include *Streptococcus*, *Actinomyces*, *Corynebacterium*, *Veillonella*, *Neisseria*, *Haemophilus*, and *Capnocytophaga* (1,3,6,8).

Species Level

Health-associated species such as *Streptococcus sanguinis*, *Actinomyces naeslundii*, and *Corynebacterium matruchotii* are prevalent, while pathogenic species (*P. gingivalis*, *T. forsythia*) are rare or absent (1,3,13).

Community Structure

•Co-occurrence networks indicate positive interactions among commensal genera, suggesting **mutualistic relationships** that stabilize the biofilm (3,4,11).

Dysbiotic shifts in disease involve over-representation of pathogens and decreased alpha diversity.

Comparative Observations

•Longitudinal studies demonstrate temporal stability in healthy sulcus microbiota, with minor fluctuations influenced by diet, hygiene, or smoking (2,4,5,12).

Early colonizers (*Streptococcus*, *Actinomyces*) provide attachment for secondary colonizers (*Veillonella*, *Corynebacterium*), forming structured biofilms (14).

16S rRNA sequencing has revealed the complex ecology of the healthy gingival sulcus:

High diversity is protective: Rich and even microbial communities resist pathogen overgrowth.

Specific taxa define health: Certain commensals are functionally involved in nutrient metabolism, biofilm structure, and immune modulation.

Community interactions matter: Co-occurrence networks highlight cooperative relationships reinforcing homeostasis.

Clinical relevance: Baseline microbiome definitions enable early detection of dysbiosis, guiding preventive strategies such as probiotics or targeted oral hygiene interventions.

Limitations: Variability in sequencing regions, platforms, and pipelines can influence detection and abundance estimates. Integration of metagenomics, metatranscriptomics, and host immune profiling will enhance functional understanding of sulcus microbiota.

Conclusion

In conclusion, the study of the gingival sulcus microbiome has advanced from traditional culture-based approaches to a systems-level understanding of microbial communities. While culture methods remain valuable for specific susceptibility testing, the future of periodontal diagnosis and research in our region—and globally—depends on high-throughput molecular techniques. By integrating the taxonomic resolution of 16S rRNA sequencing with emerging functional genomics and metagenomics tools, we are beginning to decode the complex microbial networks that maintain oral health and influence systemic disease. Regional studies such as this provide critical baseline data, helping to contextualize microbial diversity and guide evidence-based preventive and therapeutic strategies in periodontal care.

Table 1. Summary of Findings on Healthy Gingival Sulcus Microbiome

Category	Key Findings	Details / Examples
Microbial Diversity	High alpha diversity; distinct beta diversity clustering	Rich, evenly distributed communities; clearly separated from periodontitis microbiomes
Phylum Level	Dominant phyla identified	*Firmicutes, Actinobacteria, Proteobacteria, Bacteroidetes, Fusobacteria, Spirochaetes*
Genus Level	Core commensal genera	*Streptococcus, Actinomyces, Corynebacterium, Veillonella, Neisseria, Haemophilus, Capnocytophaga*
Species Level	Health-associated vs. pathogenic species	*S. sanguinis, A. naeslundii, C. matruchotii* prevalent; *P. gingivalis, T. forsythia* rare/absent
Community Structure	Stable, mutualistic microbial interactions	Positive co-occurrence networks; biofilm stabilization
	Dysbiosis characteristics	Increased pathogens; reduced alpha diversity
Comparative Observations	Temporal stability in health	Minor changes due to diet, hygiene, smoking
	Biofilm development process	Early colonizers (*Streptococcus, Actinomyces*) → secondary colonizers (*Veillonella, Corynebacterium*)

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