

REVIEW ARTICLE

# Oral Microbial Identity: A Potential Tool for Forensic Science

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## ABSTRACT

Microbial forensics is a field that uses scientific methods to analyze the evidence of bioterrorism, bio crimes, hoaxes, or the unintentional release of a biological agent or microbial toxin to determine who is to blame. It is now possible to characterize microorganisms for various human forensic applications, including human identification, body fluid characterization, postmortem interval estimation, and bio crimes involving the tracking of infectious agents. Big applause to technological advancements, remarkably massively parallel sequencing and bioinformatics. The oral microbiome contains microbiota markers that vary between individuals, emphasizing the possibility that it is highly individualized and perhaps even particular to each person. As a result, the oral microbial evidence found at crime scenes may lead to new information. This comprehensive review will discuss the state of the art of using the oral microbiome as a signature to conclude in the forensics sector.

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## INTRODUCTION

The mouth cavity is one of the main entryways into the body and is home to hundreds of different bacterial species, fungi, and viruses. The oral cavity provides homes to various microbes, including solid surfaces and mucosal shedding (mucosa) (teeth or dentures). The habitat includes dental surfaces, the gingival crevice or sulcus, the tongue's papillae, crypts, and various keratinized (gingival and hard palate) and non-keratinized (cheeks, lips, soft palate) mucosal surfaces, contain unique ecological niches that favor

particular microbes.<sup>1</sup> One milliliter of healthy adult human saliva is thought to have about 100 million microbial cells. Every 24 hours, approximately  $8 \times 10^{10}$  germs are shed from the oral surfaces at the 750 mL/day normal salivary flow rate.<sup>2</sup> The human oral microbiome is made up of these microbes and more than 2000 different bacterial taxa, including a significant number of opportunistic diseases. Compared to the gut microbiome, the oral microbiome has received less research even though it is the second most varied microbial community

after stools and is critical in defining human health and disorders. Understanding the intricacy of oral microbiota has significantly advanced with DNA sequencing innovations.<sup>3</sup> The most significant efforts are concentrated on identifying the potential associations between oral microbiota and a broad range of systemic and oral pathological conditions, such as type 2 diabetes, cardiovascular disease, sepsis/endocarditis, as well as their known risk factors, such as obesity, chronic kidney disease, rheumatoid arthritis, Alzheimer's disease, and cancer of head and neck.<sup>4-7</sup>

Remarkably, the oral microbiome has distinctive microbial community signatures that vary from person to person, highlighting the possibility that it is highly individualizing and may be exclusive to each person.<sup>8</sup> An expert's most important task is determining the probable time since death. In both criminal and civil situations, the oral microbial remains found at crime scenes may provide insights for further investigation. The conventional manners encountered by experts are rigor mortis, algor mortis, biochemical assessments, livor mortis, supra strong reactions, etc. In this new vogue, the oral microbial signatures can be a boon for the cause of death investigation.

Thus, oral microbiomes serve as a unique signature to infer the postmortem interval in the cause of death investigations, according to the state of the art described in this comprehensive review.

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#### ORAL MICROBES

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The bacteria in the human oral cavity are the oral microbiome, oral microbiota, or oral microflora.<sup>9</sup> The Dutchman Antony van Leeuwenhoek initially discovered it using a microscope he had built to do so.<sup>10</sup> Known as the "father of microbiology," he was a trailblazer who found protists and bacteria.<sup>11</sup> In 1674, he noticed the dental plaque on his teeth and described seeing "small living animalcules prettily moving."<sup>12</sup>

An organism's genetic makeup is defined as a genome. It is the encyclopedia of DNA, including every gene in that particular body.

Oral microbiome is the collective genome of the bacteria that live in the oral cavity. It is the second largest microbial colony in humans, behind the gut. They have an incredible range of anticipated protein activities when compared to other body locations.

The human microbiome is made up of a core microbiome and a variable microbiome. All people share the same core microbiome, yet each has a different variable microbiome based on their lifestyle and physiological makeup. The complex and soft tissues of teeth and the oral mucosa are two surfaces where bacteria invade the oral cavity. The tough and soft palates, tonsils, gingival sulcus, teeth, tongue, and cheeks offer a hospitable environment for microbes to thrive. The so called bacterial biofilm is a thick layer of bacteria covering the mouth cavity's surface covers the surfaces of the mouth cavity.

The mouth cavity and related nasopharyngeal areas are among the most favorable environments for microorganisms to grow. Without significant changes, the average temperature of the mouth cavity is 37°C, which gives bacteria a stable habitat to survive. Additionally, saliva maintains a steady pH of 6.5-7, which is ideal for most bacterial species. In addition to keeping the bacteria nourished, it acts as a conduit for delivering nutrients to microorganisms.

#### *The Oral Microbiome's Makeup*

The bacterial predominance in a varied version is offered in the mouth cavity. It constantly interacts with the environment, and even research has revealed that it is susceptible to its impacts. Most of the microbiome's species are found at various locations throughout the body in a healthy state. Individuals' specific varied microbiome has developed in response to their particular lifestyle and genetic makeup.

The oral cavity's complex and diverse microbial ecology is a rich biological habitat with specific niches that offer a unique setting for the colonization of bacteria. The gingival sulcus, the tongue, the cheek, the hard and soft palates, the mouth's floor, the throat, the saliva, and the teeth are some of these niches. Due to

particular adhesins on their surface that adhere to complementary receptors on an oral surface, oral bacteria selectively colonize different textures in the mouth.

Archaea, protozoa, fungi, bacteria, and viruses make up the typical microbiome. There are limited reports on the mycobiome-fungal microbiome, while the information on a distinct microbiome is only focused on the bacteriome.

Three hundred ninety-two taxa have at least one reference genome, making the oral cavity one of the most thoroughly investigated microbiomes. The total number of genomes in the oral cavity is close to 1500. It contains over 700 different prokaryote species that have been identified. Approximately 54% of these species have official names, 14% are nameless but farmed, and 32% are only known as uncultivated phylotypes. These species are members of 185 genera and 12 phyla. Firmicutes, Fusobacteria, Proteobacteria, Actinobacteria, Bacteroidetes, Chlamydiae, Chloroflexi, Spirochaetes, SR1, Synergistetes, Saccharibacteria (TM7), and Gracilibacteria make up the 12 phyla (GN02). In healthy mouths, the oral microbial ecology is retained down to the genus level. The tongue contains a diversified microbiota that includes anaerobes due to its many papillae and sparse anaerobic locations. Despite the commonalities, the microbiome's diversity depends on the individual and the location.

The oral microbiome can vary dramatically and quickly over time, both in terms of composition and activity, and it is dynamic as the host develops. Numerous factors, including the temporal frequency of the host and diet, the response to pH changes, interactions between the bacteria, and, over a longer time horizon, gene mutations and horizontal gene transfer that confer new properties on the strain, contribute to these theaters, non - equilibrium dynamics.

***The following are the main bacterial genera that can be found in a healthy oral cavity:***

### ***Gram-positive***

1. **Cocci:** Abiotrophia, Peptostreptococcus, Streptococcus, and Stomatococcus.
2. **Rods:** Rothia, Actinomyces, Bifidobacterium, Corynebacterium, Eubacterium, Lactobacillus, Propioni-bacterium, and Pseudoramibacter.

### ***Gram-negative***

1. **Cocci:** Moraxella, Neisseria, and Veillonella
2. **Rods:** Leptotrichia, Prevotella, Seimonas, Simonsiella, Treponema, Wolinella, Campylobacter, Capnocytophaga, Desulfobacter, Desulfovibrio, Eikenella, Fusobacterium, Hemophilus.

### ***Nonbacterial Oral Cavity Members***

Numerous bacteria, including viruses, fungi, and protozoa, can be found in the oral cavity. The two most prevalent protozoa, Entamoeba gingivalis, and Trichomonas tenax, are primarily saprophytic. The most frequent fungus found in connection with the oral cavity is a member of the Candida species.

Ghannoum et al. reported 85 fungal taxa after conducting culture independent research on 20 healthy hosts. Candida, Cladosporium, Aureobasidium, Saccharomycetales, Aspergillus, Fusarium, and Cryptococcus species were the most often seen. After stool samples, the oral habitats contain the highest operational taxonomic unit-level richness in the body regarding alpha diversity. There is less alpha diversity in the cutaneous and vaginal microbiome. When samples from the same sites from different people are analyzed, the mouth sites have the lowest beta diversities. This indicates that population members share relatively comparable organisms in oral places than in other areas of the body.<sup>13</sup> Alpha diversity in taxonomy refers to variability inside the sample, while beta diversity is diversity between samples.

### ***Functions of Oral Microflora***

At both the micron and host scales, the physiology and ecology of the microbiota become intricately entwined with those of the host. The microbiome significantly impacts whether health is promoted or disease develops.<sup>14</sup> Typically, the oral microbiota exists as a biofilm. It is essential to preserve oral homeostasis, safeguard the oral cavity, and halt disease progression. For a mechanical knowledge of the significant participants, it is vital to be aware of the identity of the microbiome and the nearby neighbors with whom they frequently interact.

Critical physiological, metabolic, and immunological processes carried out by the microbial communities present in the human body include food and nutrition digestion, energy production, differentiation and maturation of the host mucosa and its immune system, regulation of fat storage and metabolism, processing and detoxification of environmental chemicals, maintenance of the immune system, and the balance between these processes.

On Microbial collaboration, an essential line of protection against invading bodies originates from oral bacteria. On the other hand, if there is an imbalance, this can have several negative repercussions on the host's health. The term "dysbiosis" is used to describe this microbial imbalance. When it happens, it may play a role in developing several diseases. This includes cirrhosis of the liver, Alzheimer's, diabetes, pancreatic cancer, and inflammatory bowel disease.

Due to the intimate relationship between the human microbiome and the immune system, dysbiosis may impact the immune system and contribute to diseases, including rheumatoid arthritis and HIV infection. Oral microbiome dysbiosis is also linked to the cardiovascular system and the emergence of atherosclerosis.

### ***The uniqueness of Oral Microbial Flora***

For the first two or three years of its existence, a human kid maintains a relatively unstable microbial environment<sup>15</sup>, which is an exceptionally long-time frame based on

what we know about microbial ecology. For instance, a recent study on bathroom surfaces showed that the microbiome had stabilized 8 to 24 hours after initial colonization.<sup>16</sup> So why does the stability of the human microbiome take so long to develop? The ecosystem, or island in biological terms, where new bacteria are settling, is continually changing, which is the most likely explanation. During the first few years of life, the human body experiences significant immunological, physiological, and endocrinological changes; as a result, of the microorganisms that live on and in it, the human body is like shifting sands.

The dynamic interaction between the human body and its microbiome during these challenging years likely influenced the evolution of features that tend to stabilize the sources of bacteria that a baby is exposed to. In this interaction, immunoglobulin A synthesis is crucial because it prevents bacteria from getting to the cellular tissue in the stomach while binding bacteria that are beneficial to the mucosal layer of the gut. This could be regarded as managing the microbial environment that interacts with the body or bacterial husbandry. Unlike plants and animals, the human body has developed mechanisms of developing a cooperative atmosphere for the bacteria that have a beneficial impact on themselves. These microorganisms aid food digestion, produce specific products like vitamin K, and defend the body from diseases. Vertical transmission from parent to offspring is one of the strategies for preserving beneficial bacteria. It offers the most plausible explanation for the earliest occurrences of individuality in the microbiome.<sup>17</sup>

Understanding how human lifestyle and habitat affect our microbial ecology is impacted specifically by the individuality of microbial metabolism discovered by Bork and colleagues using metagenomic data. Metagenomic datasets from human microbiomes showed much more variance in gene content when compared to genomes of isolated species. Therefore, compared to the genome repertoires in the public libraries, these metagenomic data caught a higher level of metabolic variety. This shows that analyzing

raw metagenomic data rather than mapping it to the known pangenome diversity can help us find greater diversity and individuality in the human microbiome.<sup>17</sup>

### ***Significance of Oral Microflora in Forensic Science***

#### **A. For Time Since Death:**

A crucial component of forensic sciences and casework is accurately estimating the time since death. According to a recent study, this might be accomplished by observing changes in the mouth's bacterial communities after death.

Finding the PMI (the amount of time since a person died) is frequently essential to a criminal investigation. Researchers have started analyzing the thanatomicrobiome to increase PMI forecast accuracy.<sup>18-19</sup> These colonies overpower the immune system after death, enabling future colonization.<sup>20</sup> According to preliminary investigations, these microbial communities may experience significant successional changes in organs that may help determine the PMI.<sup>21</sup>

Oral swab samples from each of the three donated subjects: a male and two females were collected daily throughout the various stages of decomposition. The literature review suggested that the successional alterations in bacterial taxa among the three corpses were comparable<sup>22</sup> interaction with humans and are crucial to health and disease. Bacterial populations alter after death and as the decomposition process progresses in response to the newly established environmental conditions.”

Exterior locations make it possible to sample the epinecrotic microbial community in an accessible, non-invasive way. In this sense, the oral cavity is the most important place in forensic applications, along with the skin. The skin provides information about 0 body parts with their niche of bacteria. This data thoroughly leads to the microbial population of a specific area, such as the oral cavity, which is comparable to that of other body parts and distinct from other areas simultaneously.

In a study conducted at the Southeast Texas Applied Forensic Science lab, two dead human

bodies were allowed to rot in the spring and winter seasons. The results showed that various bacterial and fungal groups form a reproducible network that emerges on a predictable time scale and can therefore be used as estimators of PMI.<sup>23</sup> The internal left buccal region, external left/right cheeks, external left/right biceps, chest, and rectum of two additional human participants were also sampled.<sup>24</sup> The microbial diversity and richness for both bodies' skin, mouth, and rectal samples were comparable as the decomposition process advanced. Microbial analysis showed that the *Acinetobacter* of the class Proteobacteria was also prevalent in both bodies during the final stages of decomposition. Researchers confirmed that communities from the mouth and oral cavity differ in the pre-bloat and end-bloat locations of decay, according to a different study by the same group.<sup>25</sup> Oral microbial specimens from three donated dead bodies demonstrated variations in bacterial composition as the decomposition process continued through fresh, bloat, active and advanced decay, and dried remains stages and had similar overall successional alterations. In the fresh stage, Firmicutes and Actinobacteria predominated, but from bloat to advanced decay, Clostridiales and Bacillaceae were the main representatives of Firmicutes.<sup>22</sup>

The similarity in the microbial progression within the various stages of breakdown is a crucial discovery, even though the sample numbers for the research listed above are smaller and more varied, as it demonstrates the feasibility of using the oral microbiome in PMI calculation. The surface communities in the ear and nasal canals of 17 cadavers were analyzed, and a k-nearest neighbor regression model was successfully developed. This model successfully predicted the true PMI to be within 55 ADD.<sup>26</sup>

Research continues by taking samples from the eyes, ears, nose, mouth, and rectum in a larger study involving 188 participants. The samples were then analyzed to demonstrate that predictive models from composition and functional profiles, in the context of each sample location, could be used to estimate PMI within the time frame of less than as well as greater than 2 days.<sup>27</sup> To predict PMI,<sup>28</sup> used



the datasets from the study stated above and machine learning methods. They discovered that the computer algorithm technique produced the highest accuracy when combining all anatomical areas. Reports suggested that the skin and the mouth cavity may be viable areas to use as the foundation for a precise microbial clock.

### B. For Personal Identification

In a manner analogous to how human DNA is routinely deposited and transferred, human microbes with their nucleic acids can be utilized to identify criminal defendants.<sup>29</sup> Furthermore, bacterial DNA is more resistant to degradation than human DNA because it is circular and well protected by peptidoglycan.<sup>8,30</sup> One advantage of using the human microbiome for personal forensic identification is that it is very individualized. This is especially true when there is little or no human DNA present. However, because every bodily habitat has a unique microbiome<sup>31</sup> the human microbiome can be used forensically to determine the source of biological traces.

In forensic practice, oral biological samples are an essential form of evidence. Police officers can identify suspects by identifying saliva and mucosa that may have been left at the crime scene in bite marks, hickeys, and stains. In forensic practice, it is necessary to determine what activity created the biological trace.<sup>32-33</sup> Because saliva and oral mucosa both include oral epithelial cells, the conventional forensic analysis based on the human cell does not concentrate on the distinctions between the two. The oral cavity, however, exhibits a variety of microbial habitats, each of which has a unique microenvironment that is inhabited by a variety of microbial communities.<sup>34</sup> In some situations of specific sexual assault, including kissing and other actions with mouth contact, the variations in the bacterial communities in saliva and buccal mucosa may be helpful in determining the source of oral biological traces. Therefore, it is essential to ascertain how the microbial communities in buccal mucosa and saliva differ. Since the human oral microbiota is just as unique as the skin microbiota, it can be used to help identify an individual, especially in cases where there is little or no human DNA,

like in bite marks and hickeys. This can help law enforcement identify potential donors by connecting oral biological traces to them.

According to numerous studies<sup>35</sup>, the human skin microbiome has the potential for forensic personal identification, but there aren't as many studies on the oral microbiome, and more need to be done in this area.

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### CONCLUSION

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Microbiomics, the study of the variety of microorganisms found in our bodies and surroundings, was created over the past ten years due to developments in genomic sequencing and bioinformatics. The evidence analyzed in this review suggests that microbiomics may be a forensically relevant and promising field with many applications, including tracing the provenance of substrates, collecting trace evidence, identifying people, and calculating PMI. These developments may enable forensic scientists to employ various microbiome data, such as those produced via thanatomicrobiome analysis, to address issues linked to criminal investigations or at least be used in conjunction with other forensic techniques.

Humans and their microbiomes engage in intricate interactions and co-adaptation processes that involve nutrition intake and result in the formation of breakdown products like metabolites during their lives. These interactions alter significantly after death, and the microbiome's dynamics and composition shift along. Understanding these colonizations, fluctuations, and antemortem microbial dynamics provides significant philosophical, methodological, and computational hurdles. Future advancements in the microbial and forensic toolset may also result from related microbiome based research in forensics and a more profound investigation of fungal and virus communities.

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